BIOCHEMICAL RESPONSE OF ACTIVATED SLUDGE PROCESSES TO ORGANIC SHOCK LOADS

By

KUSUG KOMOLRIT

Bachelor of Engineering
Ilinois Institute of Technology
Chicago, Illinois
1960

Master of Engineering
University of Illinois
Urbana, Illinois
1962

Submitted to the Faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
May, 1965

BIOCHEMICAL RESPONSE OF ACTIVATED SLUDGE

PROCESSES TO ORGANIC SHOCK LOADS

Thesis Approved:

Thesis Adviser

Dean of the Graduate School

ACKNOWLEDGEMENTS

Much of the material presented in this thesis is drawn from experimental work carried on under a Research Grant (WP - 75) from the Division of Water Supply and Pollution Control, United States Public Health Service, entitled "Responses of Activated Sludge to Organic Shock Loads." Permission to use the data is gratefully acknowledged and appreciated.

The author wishes to express sincere appreciation to Dr. A. F. Gaudy, Jr., advisor, and Dr. E. T. Gaudy for their valuable counsel, guidance, encouragement and assistance without which the work could not have been satisfactorily completed. Special thanks are due to all members of the Bio-engineering laboratory staff for assistance and for supplying additional data. The help of Mrs. Dian Gephardt, who assisted with some of the analyses, is gratefully acknowledged.

I am very grateful to the Thai Government for awarding me a scholarship during my entire tenure of study in the United States. And lastly I owe a great deal to my dear wife, and my daughter, without whose patient understanding, love, encouragement and assistance this thesis would never have been brought to completion.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

A. Nature and Importance of the Problem

Water is a strategic element in the economy of the nation and is related in some degree to every major activity of life. With the ever growing population and the increasing use of water for industry and irrigation, the question of conserving this vital national resource has proved to be of utmost importance to the future of the nation. According to the late President John F. Kennedy in a message to Congress in February 1961 (1):

> "Our nation has been blessed with a bountiful supply of water; but it is not a blessing we can regard with complacency. We now use, over 300 billion gallons of water a day, much of it wastefully. By 1980 we will need 600 billion gallons a day .....
>
> " Pollution of our country's rivers and streams - as a result of our repid population and industrial growth and change-has reached alarming proportions.... To meet all needs-domestic, agricultural, industrial, recreational- we shall have to use and reuse the same water, maintaining quality as well as quantity. In many areas of the country we need new sources of supply, but in all areas we must protect the supplies we have. Current corrective efforts are not adequate.... Industry is lagging far behind in its treatment of wastes."

It may be discerned that pollution abatement is being given serious consideration and attention due to the realization of this alarming problem. It is not only a problem affecting the health of the people; it also affects the nation in other ways, for example the detrimental effects of pollution on wildlife, recreation, fish industries and other water uses. During the past few years the budgets for water pollution abatement have been sky-rocketing. Altogether from 1953 to 1963 federal funds available for water pollution control in the Public Health Service increased more than a hundred times from slightly over a million dollars in 1953 to more than $120 million in 1963; a total expenditure of $820 millions, a new record for sewage treatment facility construction in U. S. municipalities, was spent in 1963 and will have to continue at this pace for some time in order to cope with the problem, according to the recent statement released by the U. S. Public Health Service (2). It can be readily seen that the main efforts chiefly concern an increase in the number of waste treatment plants. However, it should also be realized that it will be quite advantageous and more economical to improve the functional efficiency and reliability of the existing plants as well as improving the design of future plants. These aims can be achieved only when the designing engineers and plant operators have gained better insight into operational criteria and various basic concepts concerning the kinetics and mechanisms of the purification processes. One biological treatment process which has been widely used by both municipalities and industries is the "activated sludge" treatment process. This may be due to the facts that it has relatively high

efficiency, requires less space for treatment of the same waste quantity, and provides a high degree of operational flexibility.

During recent years, there has been a great increase in efforts to promote cooperation between municipalities and industries in joint treatment of their wastes. Joint treatment exists to-day and will undoubtly continue at an increased pace, since many advantages have been gained from the combined treatment (1, 3, 4). Some of these are listed below:

1. Lower construction and equipment costs because much duplication can be eliminated.

2. Less operating cost since more waste will be treated at a lower rate per unit of volume.

3. Since the operator of a combined treatment plant usually receives higher pay than separate domestic plant operators, better trained people should be attracted to operational positions

4. Municipalities can apply for state and/or federal funds for plant construction, whereas private industry is not eligible to receive these benefits.

5. Responsibility is placed with one specific owner.

6. Mixture with municipal wastes adds essential nutrients to industrial wastes which otherwise may have to be added at an extra expense in order to enhance suitable environmental conditions for microbial growth.

However, many problems have also arisen from combined treatment; the most important factor contributing to such problems is the

character of the industrial waste water reaching the disposal plant which may cause a rapid change in environmental conditions as well as highly deleterious effects to the bacteria and other organisms which act as purifying agents. Rapid changes in the environment may be referred to as "shock loadings" to a biological treatment system (5). Among the type of environmental changes which may be included as shock loads are:

1. The quantitative shock load, which may be envisioned as a rapid change in BOD loading or in hydraulic loading to the system.

2. The toxic shock load, which involves an influx of wastes which contain certain toxic components or heavy metals that disrupt the established physiological condition of the microbial population.

3. The qualitative shock load, which involves a change in the chemical structure of the substrate or the structural configuration of the carbon source to which the sludge has been acclimated. Experience has shown that these three types of shock load can readily upset the operation as well as the efficiency of the biological treatment process.

The quantitative shock and the toxic shock are normally considered to be the primary representatives of the shock load phenomenon. The qualitative shock load, even though it is equally pertinent, has been given less attention. It is thought that due to the heterogeneity of the microbial population in the waste treatment process, all waste components can be concurrently removed during the purification, and this also tends to negate the need for acclimation.

Recent findings by Gaudy and his associates (6, 7), however, have indicated that sequential removal of organic carbon sources or waste components in a heterogeneous population such as exists in activated sludge can occur and that the presence of one compound in a waste can block the removal, as well as prevent acclimation to, some substrates. In view of these and further findings in our laboratory, it is believed that this aspect of the qualitative shock load can become very important and requires serious consideration especially where joint treatment of municipal and industrial wastes is contemplated. The aim of the present studies is to gain a better insight and understanding into such qualitative shock loading phenomena; hopefully, as a result, various design formulations and operational criteria for the biological treatment plant can be improved.

B. Purposes of the Study

1. To determine the extent of interference between waste components under a severe shock loading condition by employing various defined synthetic wastes with their organic components chosen from carbohydrates and polyalcohols. The shock load condition is accomplished by rapidly injecting one waste component into a waste system while its sludge population is actively metabolizing another carbon compound. The responses of the system are to be determined by observing the course of removal of each component.

2. To determine the effect of cell age on the responses of the activated sludge. Old cell sludge developed by a feed-and-draw batch process is to be employed in this study. A shock loading procedure

similar to that described above for young cells is used so that the results can be compared with those for the young cells.

3. To determine the extent of substrate interactions in the steady state continuous flow activated sludge process. In this phase of the study, waste components which had been found to interact in the batch studies were chosen; these include the glucose-sorbitol and glucose-glycerol systems. The system, after being acclimated to one carbon source, is then shock loaded by disrupting its steady state condition with another carbon source, together with or without the acclimated carbon source. Different shock loading conditions which include various concentrations, detention times, and methods of application are employed in the study. The systems are also observed under the carbon-limited condition as well as the nitrogen-limited condition.

It is hoped that the results of the present study will contribute in significant measure toward the understanding and elucidation of the effects of qualitative shock loading on the biological waste treatment process and will provide a basis for predicting its reponse to scuh shock loads or to fluctuations in the chemical nature of the influent waste.

CHAPTER II

LITERATURE REVIEW

A. Activated Sludge Process and Its Recent Development

The treatment of waste water by the activated sludge process has been widely practiced for over a half-century and considerable progress had been made in improving its operational as well as its functional efficiency. Recent developments have included the use of greater loading factors and provision for greater operational flexibility both of which result in reduction in the space requirement and in construction costs.

The conventional activated sludge process was developed at a time when treatment of domestic sewage was the major consideration; attempts to apply the process to industrial wastes or to mixtures of both, which contain abnormal amounts of soluble and readily oxidizable organic matter, often met with failure and much of this difficulty has been cited as due to lack of understanding that the process was fundamentally biochemical in character rather than a mere application of physical principles (8). In general the loading for conventional activated sludge process is fixed at approximately 0.5 lb. BOD (5 days) per lb. of aeration solids, or at approximately 35 lb. BOD per 1000 cu. ft. of aeration capacity (9). However, many limitations and difficulties have been

encountered in both operation and design of such conventional processes, which include: limited BOD loadings, high initial oxygen demand, tendency to produce bulking sludge, the need for high sludge recirculation ratios for wastes with high BOD, high solids loadings on final clarifier and high air requirements (8). During recent years various modifications of the conventional process have been advanced in order to overcome these disadvantages. Among these are "tapered aeration" systems, "step aeration" systems, "biosorption" systems, the "Kraus" process, the "completely mixed" system and the "extended aeration" or "total oxidation" processes. In general these modifications have stemmed from observation of the operational characteristics of existing full scale plants and continued laboratory research, which has resulted in better understanding of the kinetics and mechanisms of substrate removal. The impact of the overloading placed upon treatment plants by industrial wastes has also played an important role in bringing about improved design. Organic loadings as high as 400 lb. of BOD (5-days) per 1,000 cu. ft. per day through the modification of flow patterns and improvement of aeration devices have been reported by Kraus (10).

Busch and Kalinske (11) have cited ideal conditions required for optimum activity in the activated sludge process. These are listed as follows:

a. Maintenance of a young flocculent sludge in the logarithmic stage of growth

b. Maintenance of log-growth state by controlled sludge wastage

c. Continuous loading of organisms

d. Elimination of anaerobic conditions at any point in the oxidative treatment.

One of the recently developed activated sludge processes in which the above principles are incorporated is the "completely mixed" activated sludge process, which McKinney (12, 13) has defined as the process in which the untreated wastes are instantaneously mixed throughout the entire aeration tank. He pointed out that, in effect, the organic load on the aeration tank is uniform from one end to the other end and this results in a uniform oxygen demand and biological growth; furthermore, the aeration tank of the "completely mixed" activated sludge system operates as a surge tank which levels out variations in the organic strength of the raw wastes so that shock loads due to variations in the concentration of organic substrates do not have as much shock effect on the microorganisms in the aeration tank as in the conventional system. McKinney et al. (13)have also reported, "Theoretical relationships and field operations have confirmed that with the complete-mixing activated sludge process it is possible to take a waste of any organic strength and produce an effluent of any organic strength in a single stage unit when the unit is properly designed." Busch and Kalinske (11) have reported an average treatment efficiency of 89 percent with BOD loadings up to 350 lb. per 1,000 cu. ft. of aeration volume for the "Aero-Accelator" pilot plant, which is essentially a completely mixed activated sludge process. The process has been successfully applied to treatment of various types

of industrial wastes such as highly alkaline cotton textile wastes, antibiotic wastes with 7,000 mg/l BOD, phenolic wastes with 2,000 mg/l phenol, and textile dye wastes with 150 mg/l BOD (13, 14). An economic advantage of the completely mixed activated sludge system over conventional activated sludge or trickling filter systems has been reported by various investigators (15, 16). The completely mixed activated sludge system has been quite extensively reviewed here because it is felt that the system employed in this study falls into this category.

Another type of activated sludge process which also has recieved considerable attention is the contact stabilization process (short period sewage aeration and long period sludge reaeration). Using this method, Zablatsky, et al. (17) reported its capacity as at least twice that permissible under standard aeration procedures. Conversion of many sewage plants from the conventional process to the contact stabilization process have been made successfully (18, 19). It has also been claimed that such modification has provided a greater flexibility of operation and better protection against shock loadings imposed by industrial waste discharges.

In addition to the search for better understanding of the kinetics of the process, great efforts also have been made to elucidate further the biochemical and biophysical mechanisms of the activated sludge processes. There has been a great concern over nutritional and cultural requirements for rapid purification and maintenance of optimum sludge activity. Nutritional aspects were

generally overlooked until the recent increase in industrial expansion and the required treatment of industrial wastes either separately or jointly with municipal sewage. Many industrial wastes are deficient in the required inorganic constituents or essential nutrients whereas municipal sewage is not. Most of the nutritional studies have centered around optimum nitrogen and phosphorus concentrations required in relation to the pollutional strength of the waste which is normally measured in terms of BOD. Sawyer (20) suggested that where it was desirable to produce a biological growth or activated sludge with maximum nitrogen and phophorus content, the ratio of 5-day BOD to nitrogen and phosphrous should be maintained at 17 to 1 and 90 to 1 respectively; but when it is desired to accomplish stabilization of waste with the minimum amount of mineral nutrients the ratio of 5-day BOD to nitrogen and phosphorus can be increased to 32 to 1 and 150 to 1 respectively. A BOD:N:P ratio of 100:5:1 in a waste has been recommended by Eckenfelder and O'Connor (21) as adequate for nutritional requirements. Helmer, et al. (22) reported that when deficiencies of nitrogen become too great the sludges tend to have poor settling and filtering characteristics.

In the study by Symons and McKinney (23), regarding the biochemistry of nitrogen in the synthesis of activated sludge, it was found that a decrease in the nitrogen in the system was usually accompanied by a build-up of biological solids which was not metabolically degradable, since it was found that this material was not utilized during a long period of endogenous respiration

and was slowly accumulated in the sludge mass throughout the run when the systems were operated with no sludge wasting. Microscopic examination of such sludge with Alcian blue stain revealed that it possessed a high extracellular polysaccharide content which was not a constituent of cell protoplasm. Gaudy and Engelbrecht (24) have also reported that for systems deficient in nitrogen the increase in biological solids concentration was largely due to an increase in carbohydrate content as measured by the anthrone test whereas in growing systems the increase was mainly due to an increase in protein content of the sludge. They also found that in the nitrogen deficient system a greater portion of the substrate removed was channeled into synthesis.

The results of these studies seem to contradict the general belief that the activated sludge system could be operated as a total self-oxidation unit and thus there would be no sludge removal required. In the studies of the "total oxidation" activated sludge process using dry skim milk as a source of organic matter, Kountz and Forney (25) confirmed that total endogenous oxidation or aerobic digestion of activated sludge was not possible and found that about 20 to 25 percent of the new activated sludge produced remained unoxidized. About 58 percent by weight of the ultimate influent oxygen demand was converted to new activated sludge in a continuous flow system. By employing radioisotopic techniques, Washington and Symons (26) found that volatile solids accumulated at about 10 to 15 percent of the ultimate BOD of the waste when the carbon source was

fatty acid or carbohydrate in nature, but less sludge accumulated for amino acids; they also concluded that the accumulated biologically inert mass was also mainly polysaccharide in nature.

Morgan (27) reported that the synthesis of protein in activated sludge units was integrated with the Gibbs (hexose monophosphate) pathway as well as the Embden Meyerhof (glycolysis) pathway; most of the amino acids were oxidized via the citric acid cycle and some of the components of this cycle were then used to produce other amino acids which were incorporated into protein. This concept is, however, essentially the "nitrogen pool" concept which has already been reported in the area of basic biological science (28). Morgan has also reported further that urea seemed to serve as a better nitrogen donor than even the simplest amino acids.

Aside from the economic aspects, excessive amounts of nitrogen and phosphorus in the effluent are not desirable since both elements enhance excessive growth of weeds and algae in the receiving streams. Ludzack and Ettinger (29) recently have suggested a modification of the activated sludge process, called "semi-aerobic" operation, as a method of removing excess nitrogen from sewage. In this process aeration liquor and return sludge were recycled under semi-aerobic conditions; nitrates were then reduced to nitrogen and were stripped out of the system. It should be noted, however, that such operation would bring about many operational difficulties and would require close engineering controls to avoid upsetting the metabolic patterns of the treatment processes, resulting in reduction of removal efficiency.

The activity and reactive capacity of various sludges also can vary widely depending on handling and acclimatization conditions as well as the sludge age (30). Too little air or too much carbon source, especially as sugars, can enhance the production of floc composed largely of Sphaerotilus which can cause sludge "bulking" (31, 32). The chemical composition of the substrate is also an important factor in determining the performance of the system. Genetelli and Heukelekian (33) using substrates of various chemical compositions reported that BOD removal efficiency as well as sludge yield per lb. of BOD removed varied with substrates employed. The sludge yield per lb. of BOD for glucose was significantly higher than that for casein and egg albumin. Studies by Engelbrecht and McKinney(34) on activated sludge developed on a variety of pure chemical compounds indicated that activated sludges developed on structurally related chemical compounds had similar morphological appearance and produced similar biochemical changes; the chemical structure of the organic matter fed to the activated sludge was concluded to be the controlling factor in predomination of microorganisms as well as in the biochemical changes. The work of Ludzack and Ettinger (35) demonstrated that the chemical structure of substrates was an important factor in determining the degree of biodegradability of a compound in biological treatment process, and the structure of a compound to which the cells were already acclimated also determined the success of "cross acclimation" of an activated sludge population to another compound. Malaney (36) reported that it was possible to train a

"normal" activated sludge to utilize aniline (aminobenzene) as sole source of carbon, and this acclimated sludge possessed the ability to oxidize a wide variety of compounds structurally related to aniline. Further studies by Gaudy (37) on various types of carbohydrates have shown that acclimation to one substrate may automatically confer acclimation to another compound, depending upon the structural similarity of the compounds, however, he has noted that utilization of compounds to which the sludge is acclimated may suppress utilization of another compound requiring an induction period, even though the induction may proceed concurrently with utilization of the acclimated substrate.

Wide variations in microbial population in different phases of growth were also reported by Jasewicz and Porges (38) in their studies on dairy waste. They found that during the assimilative phase, 74 per cent of the organisms were of the genus Bacillus or Bacterium while only 8 per cent of the sludge in the endogenous phase was composed of these organisms. The endogenous sludge contained 42 per cent of the proteolytic organisms, Pseudomonas and Alcaligenes, and 48 per cent of the saccharolytic organisms, Flavobacterium and Micrococcus. Recent studies by Prakasam and Dondero (39) showed that the activated sludge developed on sorbitol synthetic waste medium such as employed in this study consisted mainly of coliforms.

In the recent survey completed by the Municipal and Industrial Waste Treatment Subcommittee of the National Technical Task Committee on Industrial Wastes (40), it has been suggested that one of the needs for knowledge on combined treatment is the limiting ratios of industrial

wastes to sewage for the various industrial wastes that may be treated satisfactorily and economically in biological treatment processes as well as the possible interactions between waste components. Knowledge regarding these aspects, at the present time, is rather limited, since most of the research efforts on shock loadings have centered on determining the effects of common toxic wastes and heavy metals on the standard biological treatment processes. Quirk (41) reported a laboratory study on joint treatment of a composite waste, which provided valuable information regarding the feasibility, stability and design criteria for the treatment of the composite waste. Jones et al. (42) reported that in textile processing and finishing plant waste treatment, addition of 7 to 10 per cent domestic waste water was required for satisfactory biological treatment.

Rather direct studies concerning the possible synergistic and antagonistic effects between waste compounds have been reported recently by Gaudy and his associates (6, 7, 43, 44) in which the presence of one component could prevent utilization and acclimation to another waste component. In addition, they have also shown that the physiological condition of the sludge (operationally defined as sludge age) can play an important role in controlling such phenomena.

B. Metabolic Control Mechanisms

Very recent investigations in the basic fields have shown that several mechanisms of metabolic control may be operative in bacteria. Since the activated sludge process is essentially a biological process it can be anticipated that such metabolic control mechanisms also may

operate in activated sludge systems. It is appropriate to review the current state of knowledge in this area which may have serious ramifications to shock loading considerations. Since the beginning of the present century, striking differences in the enzymic activities of bacteria grown in different environment were noted, and the significance of these observations for growth were well recognized. It was found that the ability of bacteria to ferment a sugar often depended on prior growth on that sugar. It was postulated that growth on a given carbon source could cause formation of a special enzyme capable of fermenting that carbon source. These enzymes were named "adaptive enzymes"; but now they are usually called "inducible enzymes". Furthermore, it was also found that some carbon sources such as glucose were readily metabolized no matter what medium was used for growth of the bacteria. Enzymes involved in this sort of fermentation are termed "constitutive enzymes". According to Pardee (45), however, even glucose fermentation is not a completely fixed property of the bacteria but varies depending on prior growth conditions; constitutivity appeared to be an idealized extreme response of enzyme formation to nutritional conditions.

Several investigators studied the effect of glucose on the utilization of other sugars and found that glucose could exert striking blockage of metabolism of various compounds; this phenomenon has been termed the "glucose effect". The "glucose effect" was first defined by Epps and Gale (46) who observed that the formation of amino acid deaminases was inhibited by glucose. Monod (47) observed the phenomenon of diauxic growth in Escherichia coli and Bacillus

subtilis grown on medium containing glucose and certain other carbon compounds and postulated that glucose prevents the formation of enzymes essential for the degradation of other carbon sources. Recent findings by Magasanik (48), however, have indicated that such an inhibitory effect is not entirely specific for glucose; compounds closely related to glucose such as gluconic acid, mannitol, or galactose, which can serve as a ready source of metabolic intermediates and energy can also cause a similar effect. In another line of studies by Roberts and his associates (49) employing radioactive carbon sources, it was found that bacteria will utilize exogeneous metabolites provided in the medium in preference to making them *de novo*. Such preferential utilization of exogeneous metabolites was noted among various groups of compounds such as amino acids, purines and pyrimidines. Several investigations have also shown similar occurrences, and it is felt that such phenomena are not limited only to a few bacterial species but seem to be of widespread occurrence in a wide variety of organisms including yeasts, molds, and even in the living cells of higher animals.

So far two types of mechanisms have been generally cited as the major controls of such phenomena; these exert their influence by regulating the flow of metabolites through the metabolic pathways. These two mechanisms are generally referred to as "repression" and "feed-back inhibition". Repression is generally defined as a mechanism capable of controlling of the flow of metabolites through a pathway by decreasing the rate of enzyme formation which otherwise would result in over-production of end products as well as the

temporarily unnecessary enzyme(s); "feed-back inhibition" (also referred to as "end-product inhibition" and "negative feed-back inhibition") is defined as the control mechanism which exerts its influence by inhibiting the function or activity of an early enzymatic step of the pathway that would produce the temporarily unnecessary end product(s). Thus the difference between "repression" and "feed-back inhibition" primarily lies in the fact that the former involves the inhibition of enzyme synthesis and hence controls the amount of enzyme(s) produced, whereas the latter involves the immediate control of enzyme activity. Both mechanisms actually exist side by side in a biological system and are interconnected. The feed-back inhibition machanism has been assumed to be a relatively rapid mechanism for maintaining the intracellular supply of low molecular weight metabolites at a constant level. This mechanism can rapidly stop functioning temporarily, when the level of the pathway's eventual end product is high and yet it can retain its capacity to release the inhibition as the level of the end product decreases. Repression, however, is thought to be a rather sluggish mechanism for controlling the flow of metabolites and is thought to have as its main purpose the conservation of the cell's capacity for protein synthesis (50). Although these two mechanisms appear to be quite closely related in providing economical and efficient controls for living systems, various studies in the basic biological field tend to recognize and report them as separated entities. Krebs (51), however, stated that, "Whether suppression and repression should be regarded as fundamentally different is perhaps a matter of opinion."

For repression in catabolic pathways, Jacob and Monod (52) have proposed that glucose, or its derived products, might act as the repressor, exerting its effect at the gene level by preventing the formation of the messenger ribonucleic acid that conveys information to the ribosome for the assembly of enzyme molecules. Cohen and Monod (53) proposed that enzyme-like factors (permeases) which catalyze entry and control concentration of metabolites between the cells and their environment, also might be subjected to such regulation. Presently the induction of enzyme(s), which is the first step required for acclimation of microorganisms to substrate, also is thought to act by reversing the action of an intracellular repressor. Pardee (54) has generalized the relationship between induction and repression as being dependent on the balance between inducer and repressor. This provides a flexible system for the regulation of enzyme synthesis. The amount of enzyme synthesized would depend on both the supply of substrate and the concentration of end product present in the cell. Substrate would be utilized at a high rate when available but only when an excess of the end product was not already present. The repressor also could be a compound at the end of a metabolic pathway, rather than the direct product of the enzyme reaction itself. Furthermorer, Pardee also observed that not only one enzyme but several enzymes of the pathway could be repressed by a repressor. It should be noted that if such induction and repression mechanisms are so closely related, from the applied standpoint such mechanisms could play an important role in controlling the acclimation of activated sludge to various waste

components. Jacob and Monod (52) have viewed the analogy between the repression and induction through the activation of a cytoplasmic component or an "aporepressor". According to their hypothesis repression occurs when the aporepressor is converted to its active form (i. e., the form which prevents the operation of the enzyme-forming system) by combining with the appropriate end product. In the case of induced enzyme formation these workers have postulated a slightly different mechanism, in that it involves the action of another factor called the "primary product" which by itself is the active repressor and interacts with the enzyme-forming system; however, upon combining with the inducer, such a repressor becomes inactive and the enzyme forming system is free to function. According to Moyed and Umbarger (50), a more unified view of the regulation of both inducible and repressible enzyme synthesis has been proposed by Magasanik by extending the analogy between induction and repression which was previously proposed by Jacob and Monod. In this hypothesis, Magasanik (48) has suggested a term "catabolite repression" so as to emphasize the major role of catabolites in the controlling of such phenomena; in his hypothesis he has viewed the aporepressor as a primary gene product which would become active repressor only when it has combined with a low molecular weight molecule or a catabolite arising from the breakdown of an energy source; the inducer would then exert its role by specifically inhibiting the conversion of an aporepressor to an active repressor. Thus the catabolite product plays the controlling role analogous to that played by end products of biosynthetic pathways. According to

Moyed and Umbarger (50), however, there is not enough evidence at present to permit a decision between the two views. Since the catabolite(s), which is a breakdown product in the energy yielding pathway, seems to be a major control in enzyme synthesis rather than the glucose itself, Magasanik (48) then suggested the term "glucose effect" be replaced by the term "catabolite repression" since it would better generalize the expression of such phenomenon. He has also postulated that the reason glucose can exert so potent a repressive effect is that it is generally more rapidly metabolized than other carbon sources. He has reasoned that in general glucose could be degraded via two metabolic pathways, one involving the constitutive series of enzymes responsible for the degradation of glucose via triose phosphate to pyruvate and another inducible series of enzymes which catalyzes the rapid dissimilation of glucose via gluconic acid. With the aid of these two independent mechanisms, glucose, could produce, very rapidly, various precursors which are required for production of most essential cell building blocks such as amino acids, purine and pyrimidine mucleotides. The intermediary metabolites could be formed from glucose at a rate more than sufficient to saturate the capacity of the cell to convert them to the immediate precursors of the proteins and nucleic acids. Hence, a cell growing on a mixture of glucose and another less rapidly metabolized carbon compound would not profit from making the enzymes required for degradation of such a less rapidly metabolized compound. He therefore postulated that the degradation of such compounds would only increase the supply of

metabolites which were already in abundant supply due to the rapid degradation of glucose. The manufacture of enzyme(s) required to degrade these compounds would be gratuitous since the cell does not require them at all and would impose an additional burden on the protein synthesizing machinery of the cell. This postulation, although simple to understand, does not provide any sound definitive understanding of the real regulating mechanisms of the cells, such as what cellular control mechanism is so sensitive to the metabolite level and how such a mechanism can be regulated by the metabolite level.

Mandelstam's recent studies (55) have shown that the rate of utilization of a carbon source is of primary importance in establishment of repression; by controlling the growth rate of the cells in continuous flow systems, through limitation of several essential nutrients, he has also shown that synthesis of even constitutive enzyme(s) can be repressed and that carbon sources normally used at very slow rates can exert repression in cells which are growing very slowly. He has postulated that two types of repressors may be in operation, one of which is specific and whose synthesis is controlled by a gene as has been proposed by Jacob and Monod; and the second type which is the carbon-source repressor and has not been genetically characterized but can be synthesized through the metabolic process. Further studies by McFall and Mandelstam (56) have indicated that accumulation of a metabolic intermediate can lead to repression of only those enzymes which would be involved in production of that same intermediate from another carbon source, therefore, they have proposed the term "metabolite repression" for this type of control mechanism,

since the key intermediate may be formed by either synthesis or degradation. This mechanism is not identical with genetic repression.

Kornberg and his associates (57) have reported the occurrence of a metabolic shift from one metabolic cycle to another which is governed largely through a combination of feedback inhibition and repression mechanisms. MacQuillan and Halvorson (58) in studies on yeasts have reported that glucose at low concentration showed a stimulatory effect on synthesis of β-glucosidase whereas at high concentrations it caused repression of such enzyme synthesis. Gorini and Maas (59) have found that the level of concentration of an inhibitor required for maximal feedback inhibition seems to be lower than that needed to give maximal repression. Hence the levels of inhibitor concentration also seem to be a significant factor in regulating these control mechanisms.

In recent studies, Gaudy and his associates (6, 7, 43, 44) have shown that the utilization of a carbon source may be immediately blocked if a second carbon source is added to the medium, even though the cells have been previously acclimated to the former carbon source and thus should possess functioning enzyme systems required for its utilization. They have reasoned that this rapid control would appear to be possible only through immediate inhibition of the function of the enzyme(s) and could be, in a manner, analogous to feed-back inhibition. By analogy with metabolite repression, it was postulated that such control acts through accumulation of common intermediates(s), favoring utilization of the more rapidly metabolizable carbon source, and would possibly function only for

combinations of carbon sources which do produce common intermediates(7,43).

C. Modes of Response of Activated Sludge to Shock Loadings

As has been previously pointed out, there are three types of shock loadings which may affect an activated sludge process; they are the quantitative, the qualitative and the toxic shock load. However, the present study will be primarily limited to qualitative shock load aspects i. e., conditions involving changes in the chemical structure of the carbon sources. However, often both qualitative and quantitative shock loads are closely related. In the present study when the system was subjected to a qualitative shock load, it was also automatically subjected to an increase in BOD loading (which is one of the distinguishing characteristics of the quantitative shock load), since, in general the new substrate was introduced without simultaneous reduction in the quantity of the substrate which the system had been receiving prior to the shock.

According to Gaudy (37), there are three possible means by which the heterogeneous population such as exists in activated sludge may successfully respond to a qualitative change of the incoming waste.

1. Selection of species or a shift in predominance: This type of response is essentially in accord with the principle of Darwinian theory, which implies that only those microbial species which are best suited to a particular waste or environment will survive and predominate, and those which cannot readily adapt themselves to the new incoming waste will be ultimately eliminated. However, due to

the heterogeneity of the population, pollution control engineers generally anticipate that in the activated sludge process there will always exist microbial species which can efficiently utilize the incoming wastes. It should be noted that this type of response mechanism would involve selection of an entirely new bacterial population or activated sludge mass for every change in waste character, hence it is apparent that such a response mechanism would be relatively slow in coping with the qualitative shock load problem, and would not be very efficient in cases involving high fluctuation in the quality of incoming wastes.

2. A shift to alternate metabolic pathways: This type of response to changes in waste character would involve intracellular shift of the metabolic pathway of the biological sludge mass to another pathway which is best suited to utilize a new substrate or new incoming waste component.

3. Induction of enzymes: This type of response would involve an intracellular induction of the necessary enzyme(s) required to convert the new organic carbon source into intermediary products which can be readily utilized via the already existing metabolic pathways. From knowledge in the area of basic biological science, it is readily recognized that this type of response is a most crucial mechanism for all microorganisms for their survival and they must possess the ability which would enable them, to some extent, to adjust to variety of substrates. However, the synthesis of induced enzymes is dependent upon the presence of specific inducers which normally would be the

new carbon source or a closely related compound. Historically, induced enzymes were first called "adaptive enzymes", a practice which is now being discarded because of the teleological implications of the term adaptive (60). It has been also noted that the control of the synthesis of induced enzymes is genetically determined and a specific specie cannot induce all the required enzymes for utilizing all type of substrates, since the genetic apparatus responsible for synthesis of any specific enzyme by a cell must have been a constitutive part of that cell.

According to Gaudy (37), in a heterogeneous population such as exists in an activated sludge, it is very unlikely that any one of these three types of response would occur independently but the total response of such a population to the qualitative shock load would be a combination of the effects of all the three types which would occur simultaneously but somewhat interdependently. Both changes in predominances and shifts to alternate metabolic pathways may be dependent upon the ability to synthesize inducible enzymes. Thus, the "acclimation" of an activated sludge, to which pollution control engineers have frequently referred, is actually the resultant of the effects of the three types of response as described above and it is very likely that it would mainly depend on enzyme induction. As has been discussed previously, according to recent findings in the basic sciences it is now thought that induction is essentially a reversal of repression. If this is the case repression would actually then be the key control mechanism in the acclimation process. It is

possible that a delay in acclimation of an activated sludge to a waste component can be caused by repression which prevents the induction of an enzyme(s) required to metabolize the new incoming waste component as well as by suppression which prevents the function of an existing enzyme(s) capable of utilizing the new waste component in the absence of the inhibitory component. Although these basic regulating mechanisms have not yet really been elucidated, they undoubtedly can play an important role in determining the response of the biological treatment process to shock loading.

CHAPTER III

THEORETICAL CONCEPTS

A. Concepts of Steady State Kinetics

Since the continuous flow activated sludge employed in this study was operated as a completely mixed system under steady state conditions, it is appropriate to review some of its theoretical kinetic concepts and the relationships between various parameters which can affect its operation.

The steady state (also stationary state) can be defined as the condition prevailing in the culture vessel when the bacterial population is maintained at a constant density and at a definite log growth rate. Such a steady state condition can normally be accomplished by either internal control employing some device which measures cell density in the aerator or growth tube directly, or by external control through the regulation of the flow rate and the concentration of limiting nutrients or growth factors. The externally controlled system has been widely accepted in practice due to its greater convenience of operation and less complex instrumentation. A wide variety of substances has been employed as the controlling growth factor in externally controlled systems. Such substances must be

required by the microorganisms and must exhibit a region of concentration where growth rate is dependent on concentration; among such substances are (61):

a. a required amino acid

b. one of the organic carbon sources, e. g. glucose, glycerol etc.

c. the nitrogen source, e. g. ammonia

d. one of the inorganic salts, e. g. phosphate or sulfate.

In general the growth equation for a continuous flow system can be expressed as (62):

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

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

x is the concentration of organisms

μ is the specific growth rate and is dependent on the substrate concentration maintained in the vessel.

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

The above equation is obtained from consideration of the mass-balance principle which simply implies that the rate of increase in the bacterial population in the system is equal to the rate of increase in the population due to its growth less the rate of decrease which is due to the "dilute out" rate from the system. It can be seen that if the growth rate (μ) is greater than the dilute out rate (D) the concentration of organisms will increase while if the dilute out rate (D) is greater than the growth rate (μ) the culture will

be washed out of the culture vessel. Therefore, the steady state can be attained only when the specific growth rate (μ) is exactly equal to the dilution rate (D), and the bacterial concentration (x) is constant and $\frac{dx}{dt}$ is zero. Since the dilution rate (D) is controlled by the feed inflow rate (Q) and the volume of the growth vessel (V), their general relationships under steady state condition can be summed up as:

$$\mu = D = \frac{Q}{V} \quad \text{.....(II)}$$

Since the volume of the growth vessel (V) is constant for a particular system, it can be seen that under steady state conditions the growth rate (μ) is mainly controlled by the feed inflow rate (Q) as long as the value of μ does not exceed the maximum growth rate (μ_m).

The specific growth rate (μ), however, is also dependent on the substrate concentration maintained in the growth vessel. According to Herbert and his associates (62), Monod has proposed the following relationship,

$$\mu = \mu_m \left(\frac{S}{K_s + S} \right) \quad \text{.....(III)}$$

where:

S is the substrate concentration in the growth vessel

μ_m is the maximum growth rate constant or the maximum value of μ at saturation level of substrate

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

From this relationship it can be seen that the growth rate of the microorganisms will always vary and be controlled by the concentration of substrates present in the system, and would vary from zero (when there is no substrate) to μ_m (the maximum limit when substrate is present in very large amount). In the system existing in a completely mixed activated sludge process under normal loading conditions, the ratio of supplied organics to microorganisms is relatively low and this always reflected in the low concentration level of substrate present in the system. As a result, the growth rate of the cells would be only a fraction of the maximum growth rate (μ_m). In a steady state condition, the concentration level of the substrate remaining in the system will be constant and will also be controlled by the dilution rate. By combining equation (II), which signifies a steady state condition, with the growth rate equation (III), the concentration of substrate (S) in a system operated under a completely mixed steady state condition then may be obtained:

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

It should be noted that in a completely mixed steady state condition, the concentration of substrate in the system(s) is also the substrate concentration in the effluent, and is independent of the inflow substrate concentration (S_r) according to equation (IV).

The corresponding concentration for bacterial population density (X) in the steady state reactor may be represented by:

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

in which Y is the yield constant and can be defined as the ratio of the weight of bacteria formed to the weight of substrate consumed. It should be realized that these latter two equations are only applicable to a system which is at a steady state equilibrium and cannot be applied to a system when its steady state has been disrupted or until a new steady state equilibrium has been attained. It is to be realized that, however, the growth rate equation as proposed by Monod, equation (III), is not limited only to a steady state condition. When a steady state condition is disrupted, as due to shock load conditions employed in this study which affect the concentrations of substrates in the system, it is seen that the system growth rate is no longer controlled by dilution rate but can increase, and may follow the Monod growth rate equation which is dictated by the substrate concentration level present in the system at that time. The mathematical relationships between various parameters under such shock loading conditions, however, would be quite complicated and would vary greatly with time.

For a system operated in a steady state condition, its critical dilution rate (D_c) which represents the maximum dilution rate above which the microorganisms will be completely washed out of the system (at such a dilution rate the substrate removal will be zero, i. e. $S = S_r$) can be derived from equation (IV):

$$D_c = \mu_m \left(\frac{S_r}{K_s + S_r} \right) \quad \text{.....(VI)}$$

The values of Y, K_s and μ_m can be obtained from batch experiments. However, from the results of batch study (63), it was found that for the activated sludge process comprised of heterogeneous population the values of Y, K_s and μ_m were not constant and varied considerably depending on physiological and environmental conditions. However, in order to be able to demonstrate and approximately evaluate various relationships in the continuous flow system the following values from batch studies were selected: $Y = 0.60$; $\mu_m = 0.51\ hr^{-1}$ and $K_s = 50$ mg/l for a glucose system. The relationship between substrate concentration in the system (S) and the bacterial concentration(x) at any value of dilution rate (D) for various inflow substrate concentrations (S_r) can be predicted by employing the equations which have been previously given. Such relationships are presented in Figure 1. These curves show the effects of dilution rate and concentration of influent substrate on the concentrations of bacterial population and of residual substrate in the reactor and in the effluent. It is to be noted that according to the prediction shown in Figure 1, the dilution rate does not significantly affect the system until its value exceeds $0.375\ hr^{-1}$ (or an inflow rate of 15 ml/min.) after which the efficiency of substrate removal is sharply reduced as indicated by the increase in the residual substrate in the effluent and the corresponding decrease in the concentration of microorganisms. At a dilution rate of approximately $0.5\ hr^{-1}$, corresponding to a flow rate of 20 ml/min. or a 2-hour detention time, it is predicted that the bacterial population will be completely washed out.

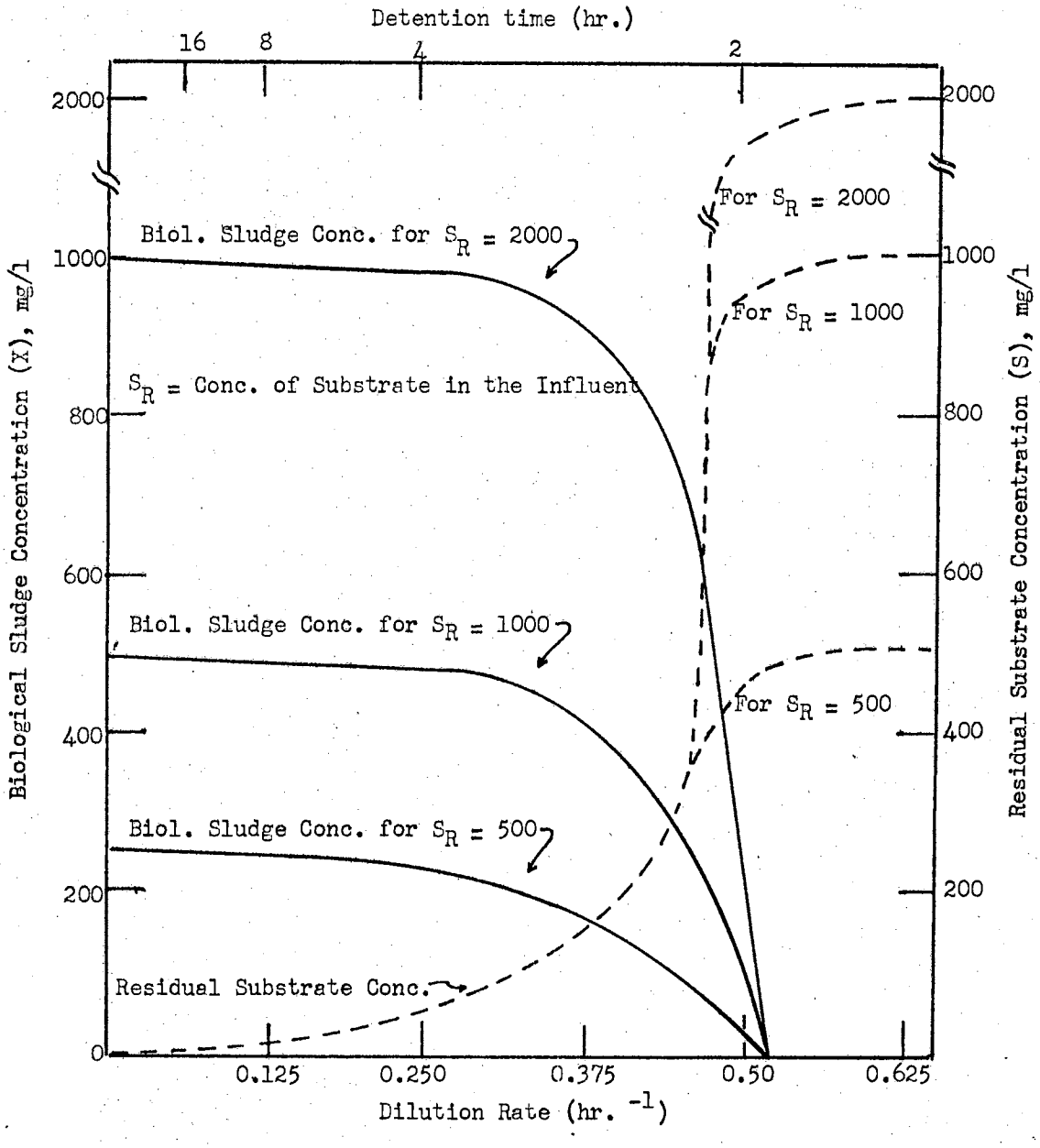

FIGURE 1 STEADY STATE RELATIONSHIPS IN A COMPLETELY MIXED ACTIVATED SLUDGE SYSTEM SHOWING THE EFFECTS OF DILUTION RATE AND CONCENTRATION OF INFLOW SUBSTRATE ON BIOLOGICAL SLUDGE AND RESIDUAL SUBSTRATE CONCENTRATIONS IN THE REACTOR AND THE EFFLUENT

For the studies to be herein reported, three dilution rates, 0.0625 $hr.^{-1}$, 0.25 $hr.^{-1}$ and 0.50 $hr.^{-1}$ corresponding to detention times of 16, 4 and 2 hours respectively, were employed. From the results of the studies on the continuous flow system by the author it was noted that at 16-hour and 4-hour detention times, the hydraulic loading effect or flow rate was not (as predicted) significant. The concentration level of the biological solids was approximately 50 per cent of the applied organic loading concentration and the residual substrate concentration was generally less than 10 per cent of the loading concentration. However, at the 2-hour detention time, the concentration level of the biological solids that could be maintained in the system was greatly reduced (less than 20 per cent of the organic loading) and there was a very high residual substrate concentration remaining in the effluent. Hence, it is apparent that the above predictions as derived from the results of batch studies serve quite well to demonstrate relationships between various parameters.

B. The Kinetics of Increase in Substrate Concentrations due to Shock Loadings in Steady State Systems

In the studies reported herein, two methods of administering shock loadings were employed; one is referred to as "gradual" and the other as "immediate". A gradual shock loading to a system was accomplished by rapidly changing the inflow feed of the system from one type of substrate to a new substrate but without changing the

hydraulic flow rate. It should be noted that the increase in the new substrate concentration in the reactor due to such shock load was not immediate because the system was completely mixed and under continuous flow conditions. The equation which represents such kinetics can be expressed as:

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

which implies that the rate of increase in the substrate in the system is equal to the rate of increase of substrate due to its inflow less the rate of decrease of substrate due to its outflow,

Where C_o = concentration of the substrate in the inflow

C = concentration of the substrate in the outflow at any time "t" after introduction of the new substrate.

D = either the inflow or the outflow rate which is the same since the volume of the reactor vessel is constant.

Upon integration of the equation the following relationship is obtained:

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

The validity of this equation was checked experimentally employing methyl-red dye. The experimental results and the theoretical values are compared in Figure 2. From these results, it can be concluded that the system employed in this study had approached ideal mixing conditions and the derived equation representing the kinetics of gradual shock loading is valid.

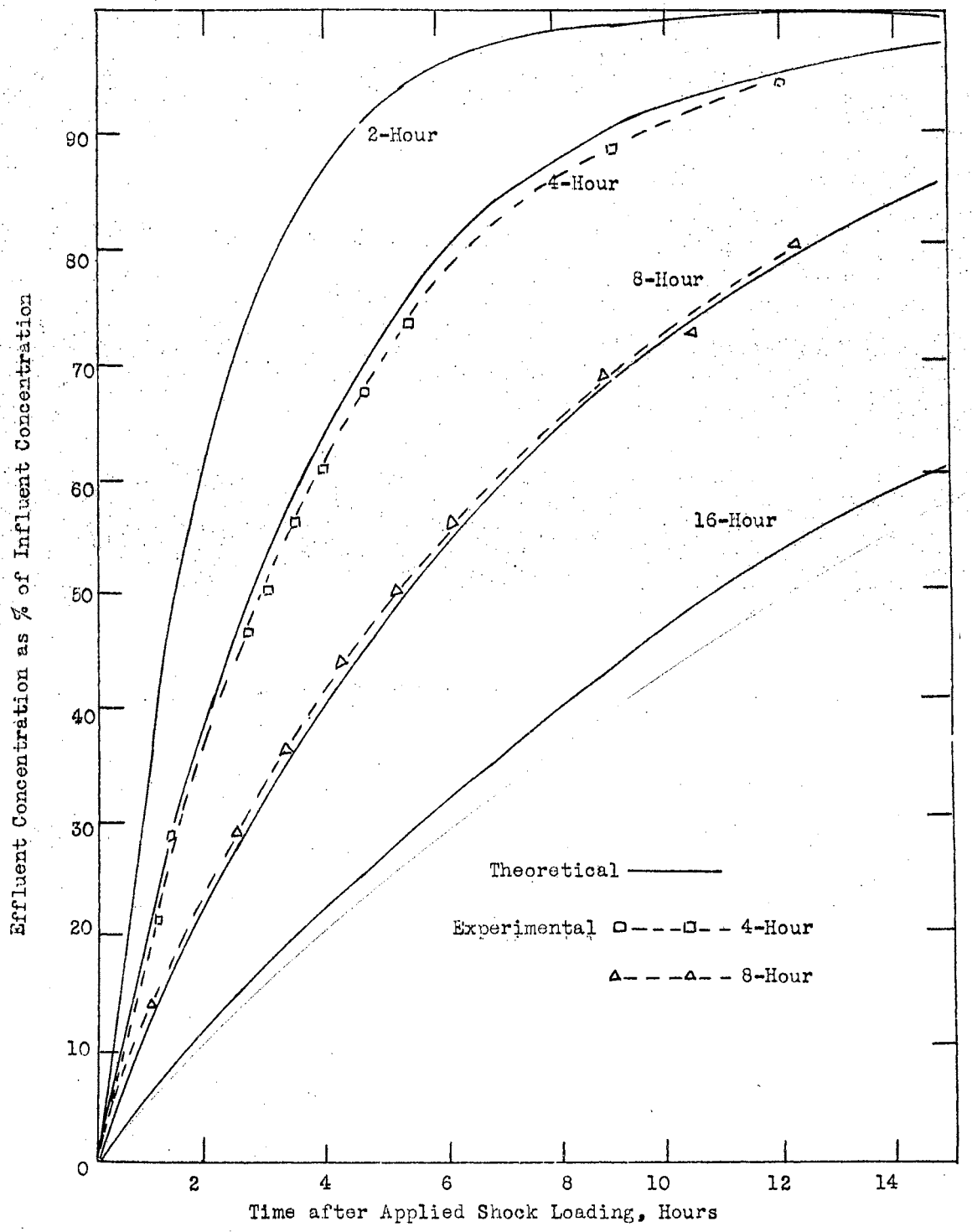

FIGURE 2 INCREASE IN THE SUBSTRATE CONCENTRATION OF THE SYSTEM DUE TO GRADUAL SHOCK LOADING AT VARIOUS DETENTION TIMES AS DETERMINED BY THE METHYL-RED DYE TEST

In contrast, the "immediate" shock load was achieved by rapidly injecting a concentrated solution of the shock compound into the mixed liquor aerator; as a result the initial concentration of the shock compound was immediately brought up to a specific level.

CHAPTER IV

EXPERIMENTAL EQUIPMENT AND PROCEDURE

A. Batch Activated Sludge Systems

1. Severe Shock Loading Studies

a. Young Cells: Activated sludges were developed from an initital sewage seed taken from the primary clarifier effluent of the municipal waste water treatment plant at Stillwater, Oklahoma. Activated sludges were developed using either a sugar or a sugar alcohol as the sole source of organic carbon. The constituents of the synthetic growth medium were: the organic carbon scurce (one of the following compounds - sorbitol, mannitol, dulcitol, glycerol or ribose), 5000 mg/l; 1.0 M potassium phosphate buffer, pH 7.0, 30 ml/l; $(NH_4)_2SO_4$, 1000 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; $FeCl_3 \cdot 6H_2O$, 1.0 mg/l; $MnSO_4 \cdot 1H_2O$, 20 mg/l; $CaCl_2$, 15 mg/l; tap water, 100 ml/l; and distilled water to volume.

The purpose of employing such rather high concentrations of all constituents was primarily to insure the development of a large population and to prolong the log growth phase. The seeded synthetic waste was aerated for 24 hours. After 24 hours, 50 ml of the mixed liquor were transferred to one liter of freshly prepared synthetic

growth medium of the same compositions as that described above. This procedure was followed for three days. On the fourth day after 16 hours of incubation, cells were harvested by centrifugation and washed once in 0.05 M phosphate buffer, pH 7. The washed cells were then resuspended in fresh medium containing the same carbon source on which they had been growing. However, in this new medium which was used in the experimental run the concentration of salts was reduced to one-half and buffer concentration was reduced to one-third of that in the original growth medium. This was done because the organic carbon source was also reduced to 1000 mg/l or less depending on the particular experiment. It was felt that such high inorganic salts were not necessary; also it was desirable to keep a somewhat constant balance between inorganic and organic components in the synthetic waste. Aeration was begun, and samples were withdrawn for measurement of substrate removal and biological solids production at various time intervals. After substrate removal was well under way, a small volume of another carbon source (in highly concentrated solution) was rapidly introduced into the system. Thus the system received a qualitative shock load while it was rapidly metabolizing the carbon source to which it was acclimated. The biochemical response was examined by continued sampling and analyses for the specific carbon sources as well as for total COD removal. The methods of analysis employed are described in detail in the "Methods of Analysis" section. In all cases a unit which did not receive the shock was maintained as a control. In some

experiments two identical one-liter units were set up, one of which was shocked; the one which did not receive the shock was maintained as a control. In some cases a single unit was divided into two parts immediately prior to administering the shock; one part was maintained as a control. For experiments in which oxygen uptake was measured, 40 ml of mixed liquor were placed on the Warburg apparatus (140 ml reaction flasks) and at an appropriate time, the shock compound was tipped into the mixed liquor from the side arm.

b. Old Cells: This type of sludge was also initially started from the municipal sewage seed and was maintained as a batch activated sludge for a period of at least 21 days prior to use in any specific experiment. The constituents of the synthetic waste employed were: the organic carbon source (either sorbitol or mannitol), 1000 mg/l; 1.0 M potassium phosphate buffer, pH 7.0, 10 ml/l; $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot 1H_2O$, 10.0 mg/l; $CaCl_2$, 7.5 mg/l; tap water, 100 mg/l; and distilled water to volume. The total volume of the mixed liquor of the system was maintained at 1.5 liters. Prior to daily feeding, 500 ml of mixed liquor were wasted and the remaining 1000 ml settled for one hour, after which 500 ml of supernatant were wasted. The total volume was again made up to the original 1.5 liters with appropriate constituents to maintain initial concentrations at the same level as described above. The system was then aerated at an air flow rate of 4000 ml/min at room temperature until the next feeding period. After 21 days

or more of such daily wasting and feeding, the sludge was harvested at 18 hours after the last feeding, washed once in 0.05 M phosphate buffer and employed in the experiments. This type of sludge was normally of a dark brown color and exhibited rapid flocculating and settling characteristics upon cessation of aeration.

The experimental protocol in determining the response of the "old cells" to shock loadings was the same as that decribed previously for the "young cells". For non-proliferating cell studies, however, the nitrogen source, i. e. $(NH_4)_2SO_4$, was omitted from the experimental synthetic waste medium.

B. Continuous Flow Activated Sludge Systems

1. Description of Apparatus, Standard Wastes and Operations

a. Apparatus: A sketch of the bench scale activated sludge unit employed in this study is shown in Figure 3. The aeration volume of the activated sludge reactor was 2.5 liters but the actual effective volume of the mixed liquor under aeration was approximately 2.4 liters due to the displacement of the mixed liquor by the diffused air. The feed line to the system was regulated by a liquid flow meter pump for which the discharge volume could be set at a definite rate (0-20 ml/min). The outlet of the effluent line was made of bent glass tubing, of which one end was submerged about three inches in the mixed liquor and the other end was left open at the liquid level. It was found that a greater consistency between the waste effluent and the mixed liquor inside the unit could be attained by such an outlet arrangement, since it was intended to operate the system as closely as possible to ideal continuous flow completely

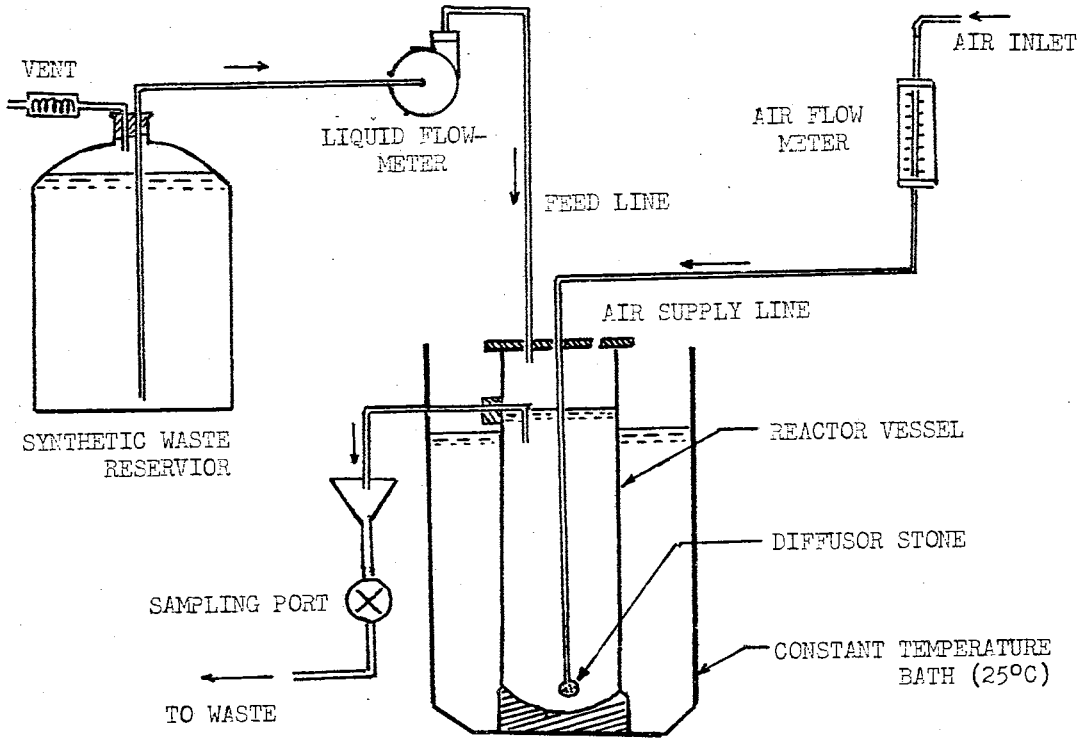

FIGURE 3 SCHEMATIC DRAWING OF THE BENCH-SCALE CONTINUOUS FLOW COMPLETELY MIXED ACTIVATED SLUDGE SYSTEM WITH NO SLUDGE RECYCLING

mixed steady state conditions. The air was supplied to the system at a rate of approximately 4000 ml/min. The temperature of the system was maintained at 25°C by the use of a thermostat-controlled constant temperature water bath.

b. Standard Synthetic Wastes for Organic Carbon-Limited Systems. The synthetic wastes were made up of specific organic carbon sources and inorganic salts. The amount of inorganic salts used per 1000 mg/l of carbon source were: $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot 1H_2O$, 10.0 mg/l; $CaCl_2$, 7.5 mg/l; tap water, 100 ml/l; 1.0 M potassium phosphate buffer (140 gm/l $K_2HPO_4 \cdot 3H_2O$, 52.7 gm/l KH_2PO_4), 10 ml/l; and distilled water to volume. These concentrations of inorganic salts and buffer were used for 1000 mg/l of carbon source and they were increased (or decreased) proportionally according to concentrations of the carbon source used. This was done 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.

c. Standard Synthetic Wastes for Nitrogen-Limited Systems. The synthetic waste for the nitrogen-limited system was made up with essentially the same constituents and concentrations as described previously for the carbon source-limited systems except that the amount of $(NH_4)_2SO_4$ was considerably reduced and varied for each specific experiment. It was found from the growth rate studies with batch units that at least 150 mg/l of $(NH_4)_2SO_4$ would be

required for 500 mg/l of carbon source (glucose or sorbitol) in order to produce a normal sludge growth (see Appendix A). Thus systems for which the ratio of nitrogen as $(NH_4)_2SO_4$ to organic carbon is less than 150 to 500 were regarded as nitrogen-limited systems. For convenience and clarity in reporting the exact amount of nitrogen employed in each experiment on a nitrogen-limited system, the amount of nitrogen will be given as each experiment is presented in "Experimental Results."

2. Experimental Protocol

The standard wastes were made up in a 20-liter bottle. Normally three to four systems of identical concentrations were set-up and operated concurrently for each set of experiments. Initially each system containing the standard waste was inoculated with sewage seed and was operated under batch conditions for about 24 hours. Thereafter, the systems were operated under continuous flow conditions at a fixed hydraulic flow rate for a minimum of three days prior to setting up the shock loading condition.

The detention period in the aerator was regulated by the feed inflow rate, i. e. the rate of the feed would be controlled at 20 ml/min. for the two-hour detention period, since each activated sludge aerator had an effective volume of 2,400 ml; for four-hour and sixteen-hour detention periods the inflow rate would be fixed at 10 ml/min and 2.5 ml/min respectively.

Normally, during the period of acclimation and equilibration, samples of the effluents were collected at one to two day intervals and were analyzed for residual substrate in order to be certain that a steady state condition had been attained prior to the introduction of the shock loading. However, in some cases, when a control unit was also operated concurrently during the shock loading experiment, it was felt that the checking procedure was not necessary and it was therefore omitted. In general, it was found that a steady state equilibrium was reached quite readily within three days.

Just prior to an experiment, the contents of all units operated with the same feeding protocol were pooled, completely mixed and again placed back into each system. This was done in order to insure that each unit had exactly the same sludge quality prior to the shock loading so that slight differences in responses to the shock loadings could not be attributable to slight differences in sludge activities or predominant microbial species. At times significant differences in the appearances of the sludges and their settleability were observed even though the same feeding protocol was followed.

The shock loadings were introduced by immediately shifting the influent feed to a new standard waste of different waste characteristics. The differences in waste characteristic which were employed in these experiments were mainly increases in concentration of the organic carbon sources, or complete changes to entirely new organic carbon sources of differing structure,

or combinations of the new and the previous organic carbon sources in various proportions and concentrations. For convenience in considering the results obtained under various conditions, the changes in concentration and composition of the waste influents which were employed as shock loadings and the various shock loading conditions will be described in detail in the presentation of each experimental result.

The experimental procedures for the nitrogen-limited systems were essentially the same as those for the organic carbon-limited experiments. The main difference in procedure was the maintaining of the same nitrogen content, i. e. the $(NH_4)_2SO_4$ concentration, in the influents as had been employed prior to shock loading whereas other constituents, such as the organic carbon sources, various salts and buffer, were increased proportionally.

C. Methods of Analysis

In these studies, the biochemical response of each system was examined by periodic sampling and subsequent analysis for specific carbon sources as well as for total substrate removal during the time course of the experiment. Biological solids were determined using the membrane filter technique (Millipore Filter Corp., Bedford, Mass., HA 0.45 μ). In some experiments, however, the biological solids concentration was determined by measuring the optical density and converting to the corresponding biological solids concentration using correlation curves which had been

previously determined experimentally. Total substrate removal was measured using the standard COD test (64). Sorbitol, mannitol, dulcitol and glycerol were measured using a modification of the periodate chromotropic acid test which was recommended by Neish(65), (see details in Appendix B). For measurement of lactose, galactose, and glucose, the anthrone test for determination of carbohydrates as suggested by Gaudy (66) was employed, while for ribose the orcinol-ferric chloride test as given by Fernell & King (67) was used. For the experiment employing mixtures of ribose and glucose, the concentration of glucose was determined by subtraction of COD due to ribose as determined by the orcinol test, from the total COD since both ribose and glucose react with anthrone. In the continuous flow studies, however, the Glucostat test (Worthington Biochemical Corp., Freehold, New Jersey), which is quite specific for glucose, was also employed in order that these results could be compared with those obtained using the anthrone test.

In some experiments, the protein and carbohydrate contents of the sludge were also measured using the biuret and anthrone test respectively; the procedures for these tests, applied to activated sludges, have been described in detail by Gaudy (66).

For convenience in comparing the rates of removal of different organic carbon sources with one another and with the rate of total COD removal, the concentration of each organic compound as obtained by its specific test was converted to COD using the stoichiometric relationships shown in Table I.

TABLE I

STOICHIOMETRIC RELATIONSHIPS BETWEEN CONCENTRATIONS AND COD VALUES OF CARBOHYDRATES AND POLYALCOHOLS

Carbohydrates

mg/l lactose	x	384/342	=	mg/l lactose COD
mg/l glucose	x	192/180	=	mg/l glucose COD
mg/l galactose	x	192/180	=	mg/l galactose COD
mg/l ribose	x	160/150	=	mg/l ribose COD

Polyalcohols

mg/l sorbitol	x	208/182	=	mg/l sorbitol COD
mg/l mannitol	x	208/182	=	mg/l mannitol COD
mg/l dulcitol	x	208/182	=	mg/l dulcitol COD
mg/l glycerol	x	112/92	=	mg/l glycerol COD

The COD values for the above compounds were determined experimentally by the author using the standard COD test; they have also been reported previously by Gaudy and Engelbrecht (5). It was found that the experimental values in all cases agreed quite well with the calculated theoretical values; the differences were well within ± 5% of the theoretical values. Hence, it can be concluded that the application of theoretical factors in conversion of the above substrate concentrations to their corresponding chemical oxygen demand (COD) values is valid. The purpose of such a conversion was to enable one to compare all substrate removal data on the same basis.

CHAPTER V

RESULTS

A. Batch - Operated Systems

To facilitate presentation of the experimental results, all curves representing the "shocked" systems, are designated by the first letter in the names of the compounds employed in that particular experiment, e. g. for glucose shock loaded to a system metabolizing sorbitol, the curve is labelled G+S, while for lactose shock loaded to a system metabolizing sorbitol the curve is designated as L+S.

The curves shown for all polyalcohol substrates in both the control and the shocked systems were plotted from periodate analyses computed as COD. All carbohydrates with the exception of ribose and glucose in one experiment, were measured by the anthrone test and computed to COD values. This plotting procedure was followed for all results obtained under batch-operated studies.

1. Effects of Glucose Shock Loading on Various Types of "Young Cell" Hexitol-Acclimated Sludge.

Figure 4 shows the results of an experiment in which a young sorbitol-acclimated sludge was initially fed sorbitol; the course of substrate removal was followed for 3.5 hours. At this time the

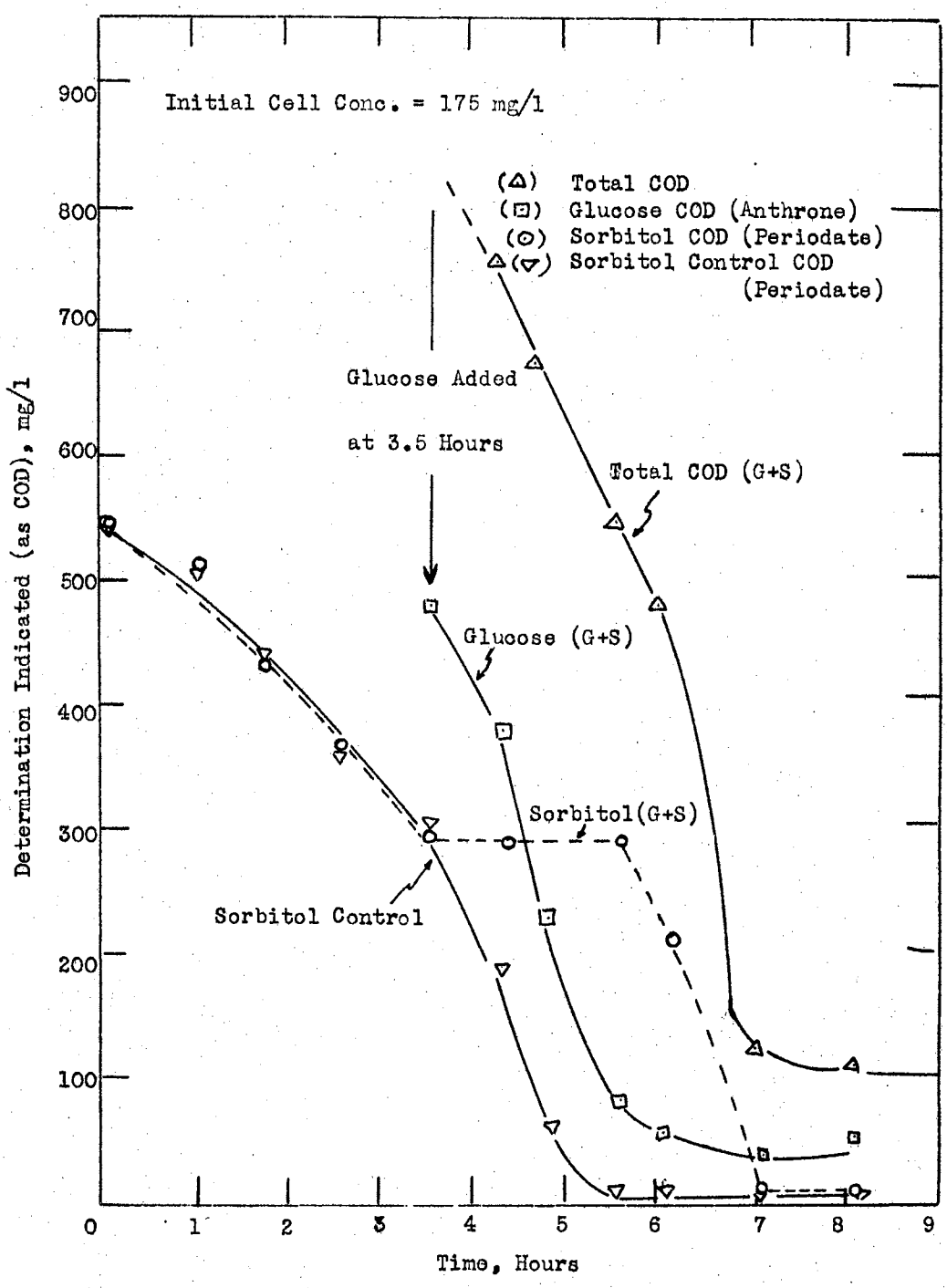

FIGURE 4 RESPONSE OF SORBITOL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLUCOSE

system was shock loaded with glucose. It can be seen that immediate cessation of sorbitol removal ensued as a result of the glucose shock load. Sorbitol removal in the shocked system did not begin again until after all the glucose had been exhausted. It should be noted that the shock load effected sequential substrate removal but that this was not apparent in the curve for total COD removal. The added glucose was removed immediately and at a faster rate than was the sorbitol itself even though the sludge had been previously acclimated to sorbitol. The shift in the utilization of carbon sources in this case seems to operate very rapidly since no break in the general shape of the total COD removal curve was observed.

Results of experiments in which young mannitol and young dulcitol acclimated cells were subjected to the same severe glucose shock loading condition are shown in Figure 5 and Figure 6, respectively. The results, in general are similar to those in the case of sorbitol, i. e. the removal of both hexitols, to which the cells were acclimated and which they were actively metabolizing, was severely retarded and suppressed upon introduction of the shock substrate glucose. Such results can be readily observed by comparing the hexitol removal curves for the control systems to those of the shocked systems. It is also evident that the continued rapid removal of the total COD immediately after the shock was primarily due to glucose metabolism from a comparison of the glucose removal curves with the total COD removal curves of the shocked systems. There appear to be some differences between the inhibitory effect of glucose on the course of dulcitol removal

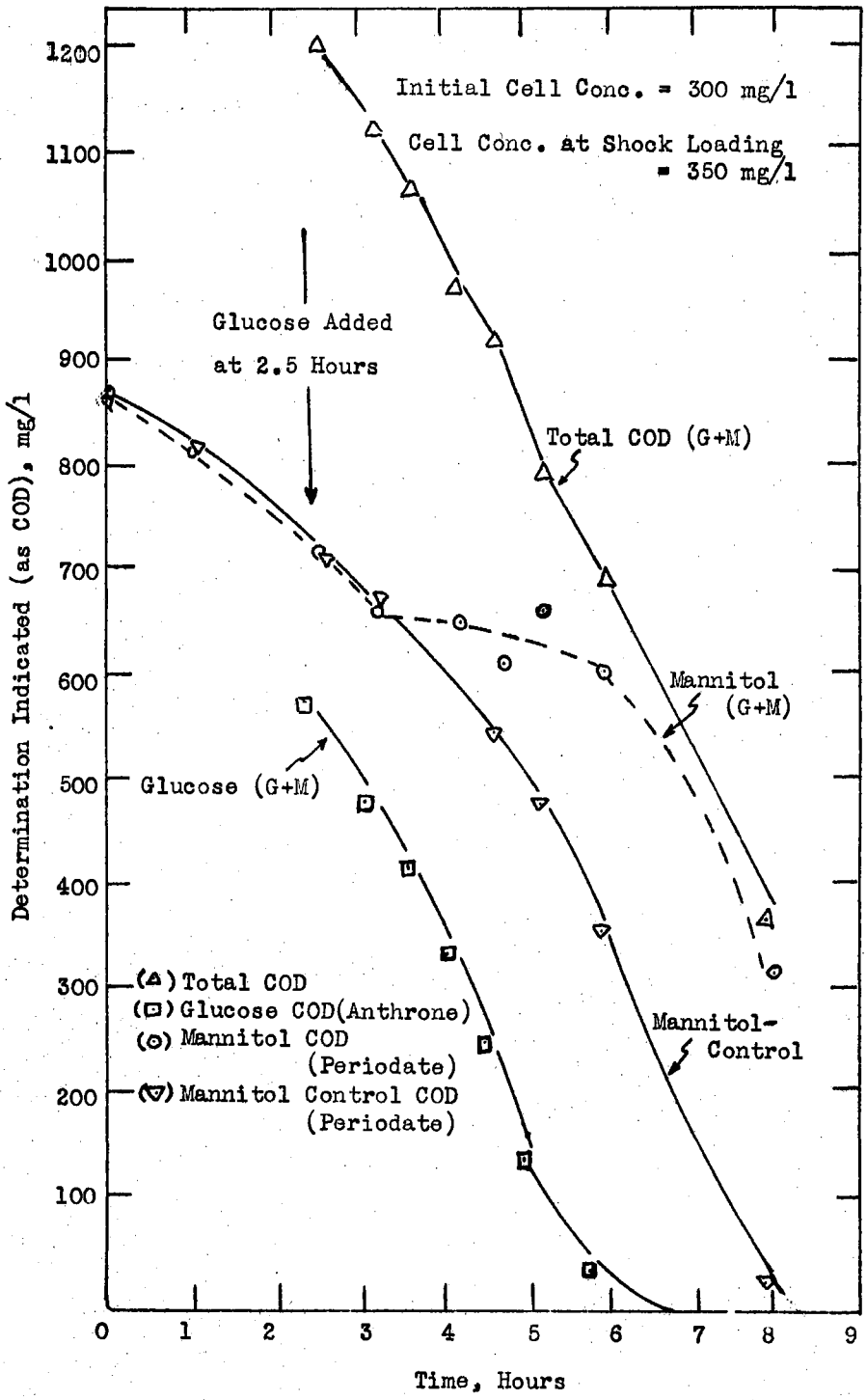

FIGURE 5 RESPONSE OF MANNITOL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLUCOSE

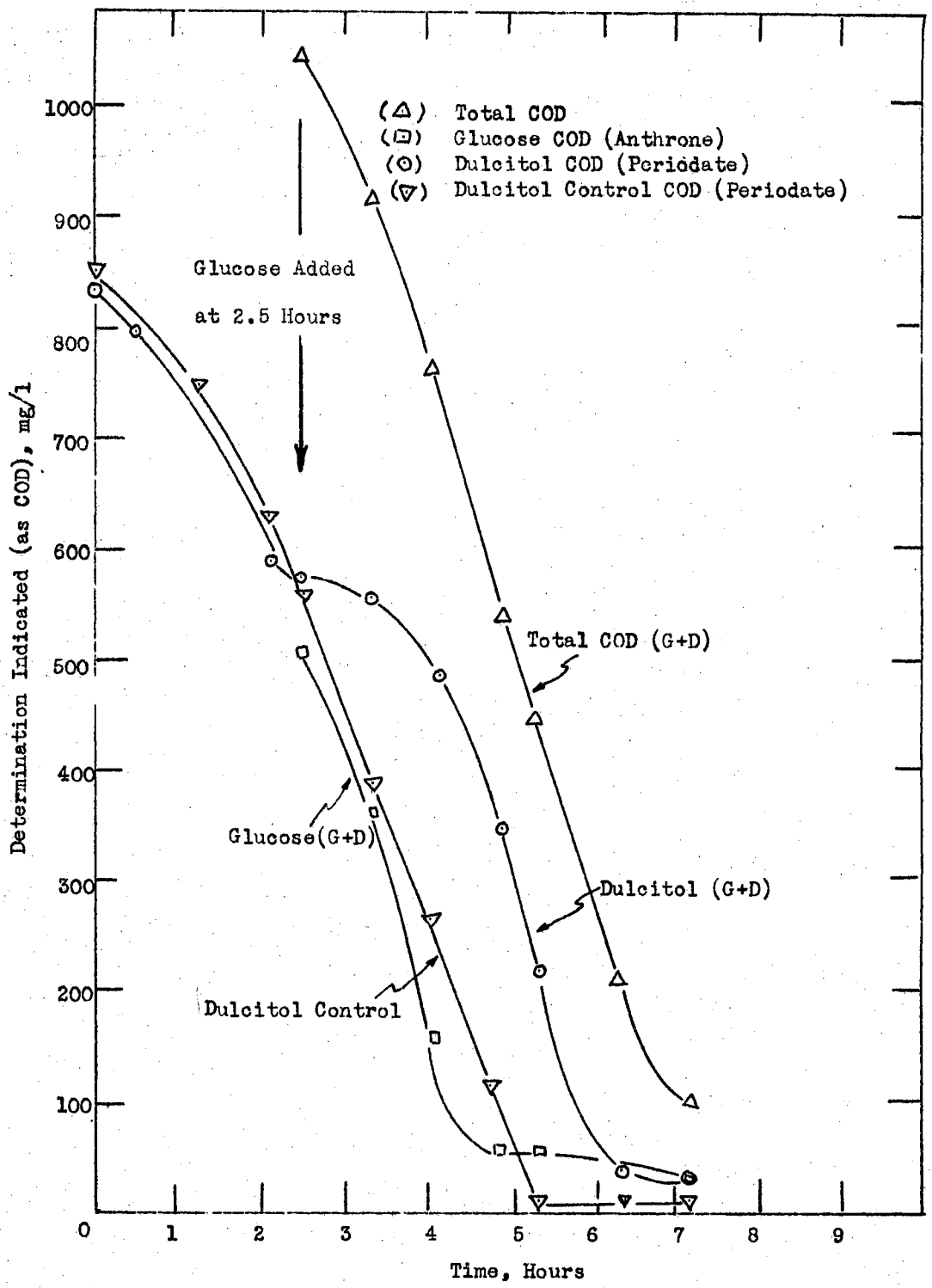

FIGURE 6 RESPONSE OF DULCITOL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLUCOSE

and that on sorbitol or mannitol removal, since the effect of glucose did not seem to be as prolonged as for the latter substrates. It should be noted, however, that in the dulcitol acclimated system, glucose was removed at a very rapid rate and to a very low level within less than two hours. This was a relatively shorter length of time than in the other two cases, and thus glucose affected the course of dulcitol removal for a shorter period. It is apparent, however, in that all cases the removal of hexitols was severely retarded upon introduction of glucose and was not initiated again until glucose was exhausted or reduced to a very low level. The initiation of glucose utilization was immediate and it was rapidly metabolized at the expense of hexitols and at a relatively faster rate than the hexitols.

2. Effects of Galactose and Lactose Shock Loadings on "Young Cell" Sorbitol-Acclimated Sludge

Galactose and lactose, both of which are structurally related to glucose and are also carbohydrates, were chosen as shock compounds in order to determine whether the suppressing effect is limited only to glucose. The effect of shock loading with galactose on the removal of sorbitol is shown in Figure 7. It is seen that a partial blockage of sorbitol metabolism was brought about by the introduction of the galactose shock load. From these results it is evident that the population required no acclimation to galactose; however, galactose was removed at a slower rate by the sorbitol-acclimated population than was glucose by the other sorbitol acclimated cells (see Figure 4). From Figure 7, it is discerned

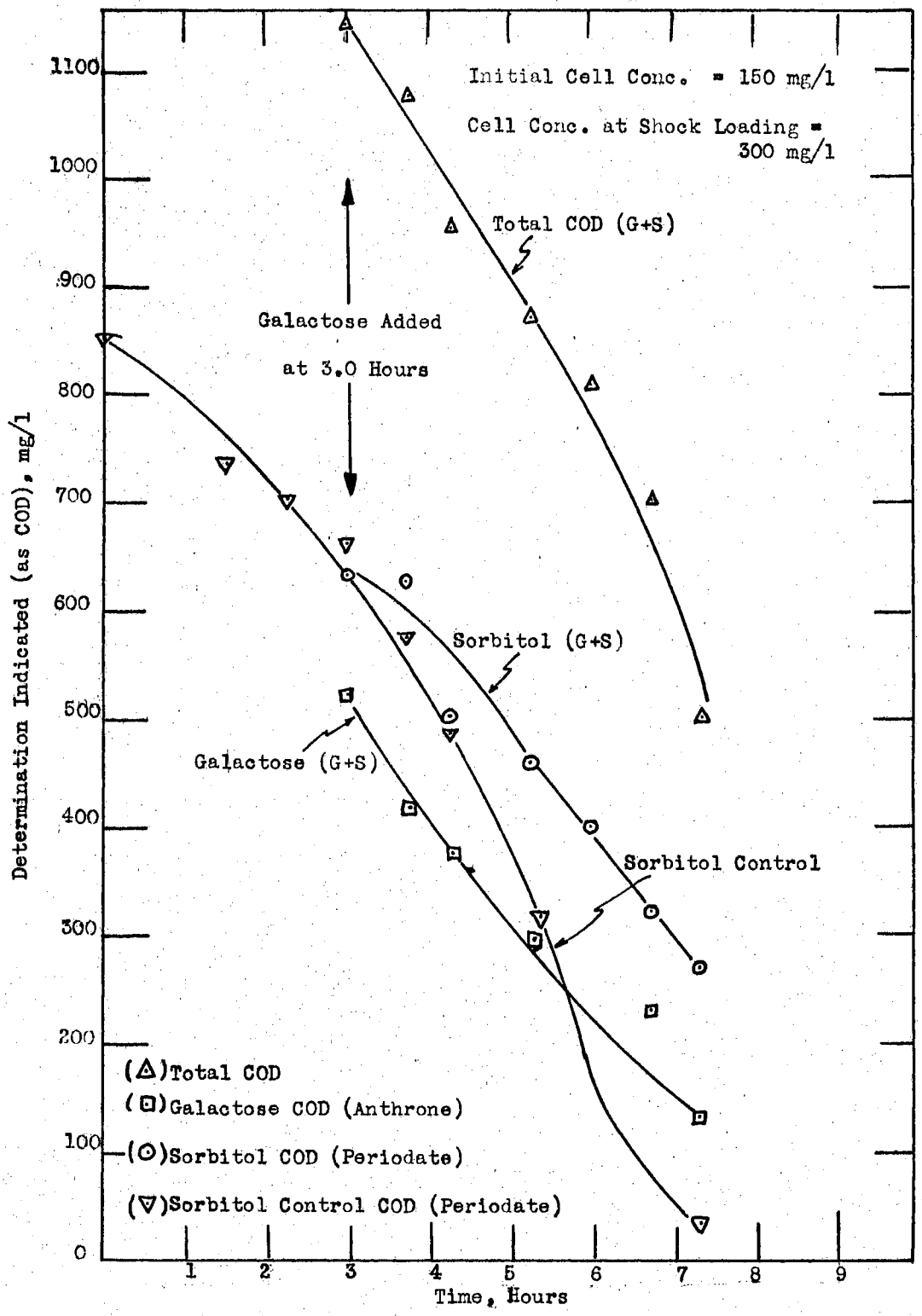

FIGURE 7 RESPONSE OF SORBITOL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GALACTOSE

that sorbitol and galactose were removed concurrently, but that the introduction of galactose seriously altered the rate of sorbitol removal as shown by comparison of the rates of sorbitol metabolism in the shocked and the control systems.

The preceding data have shown the effects of severe shock loadings of glucose and galactose on the course of sorbitol metabolism. It would, therefore, be interesting to determine the effect of a severe shock loading with lactose, which consists of glucose and galactose joined in β-1, 4 galactoside linkage. Figure 8 shows the results of an experiment in which a sludge metabolizing sorbitol was shocked with lactose. It is seen that lactose has absolutely no effect on sorbitol removal and that lactose removal was not initiated until sorbitol had been metabolized. The slow initiation of lactose metabolism is also reflected in the curve for total COD removal which for almost the entire course of the experiment, is of the same general shape as the sorbitol removal curves in both the control and the shocked systems. At a later stage in the experiment, the lactose analysis indicated slightly less than 500 mg/l lactose (as COD), whereas the total COD in the system was 700 mg/l. Since lactose responds only slightly to the periodate test and had already been accounted for, and hexitols do not respond to anthrone, the results shown in Figure 8 yield evidence that non-carbohydrate or non-hexitol intermediate products which could be oxidized by dichromate might have been released in the latter stages of sorbitol removal.

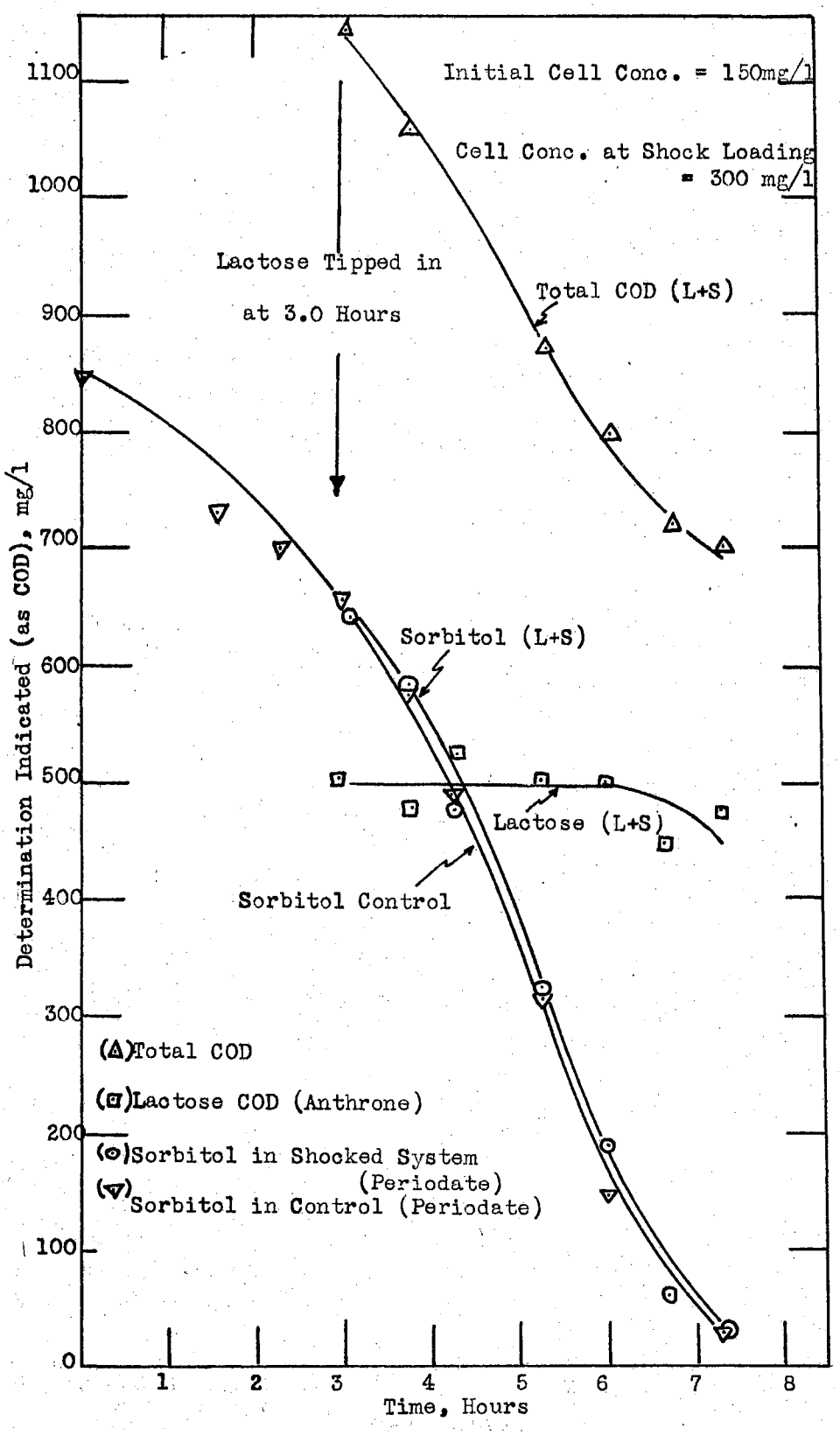

FIGURE 8 RESPONSE OF SORBITOL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH LACTOSE

Figure 9 shows oxygen uptake data for identical portions of sorbitol-acclimated cells, three of which were shock loaded at precisely the same time with 500 mg/l each of glucose, galactose and lactose. It is seen that the delayed removal of lactose is reflected in the oxygen uptake curve which parallels the sorbitol control; in both the glucose and galactose shocked systems the oxygen uptake curves continue to increase, indicating that both glucose and galactose can be readily metabolized by the sorbitol acclimated sludge. However, there appears to be no apparent effect on respiration rate upon introduction of glucose and galactose to the systems until after 2.5 hours. At this time the uptake rate in the control system had declined presumably due to the exhaustion of sorbitol in both the sorbitol control and the lactose shocked systems. The preferential utilization of either glucose or galactose in the early course of the experiment could not be inferred from the oxygen uptake data.

3. Substrate Interactions between Selected Compounds

This phase of the study was undertaken in order to determine the extent of substrate interactions between various compounds of differing molecular structure which were less closely related structurally and metabolically than those employed in the previous cases. Three organic carbon sources, glucose, ribose and glycerol, were selected for the study. When a population actively metabolizing the carbohydrate ribose was shocked with glucose, the effect was not an immediate blockage of ribose (see Figure 10). Removal of ribose in the control and in the shocked systems proceeded at the same rate for approximately two hours after the shock, while glucose metabolism

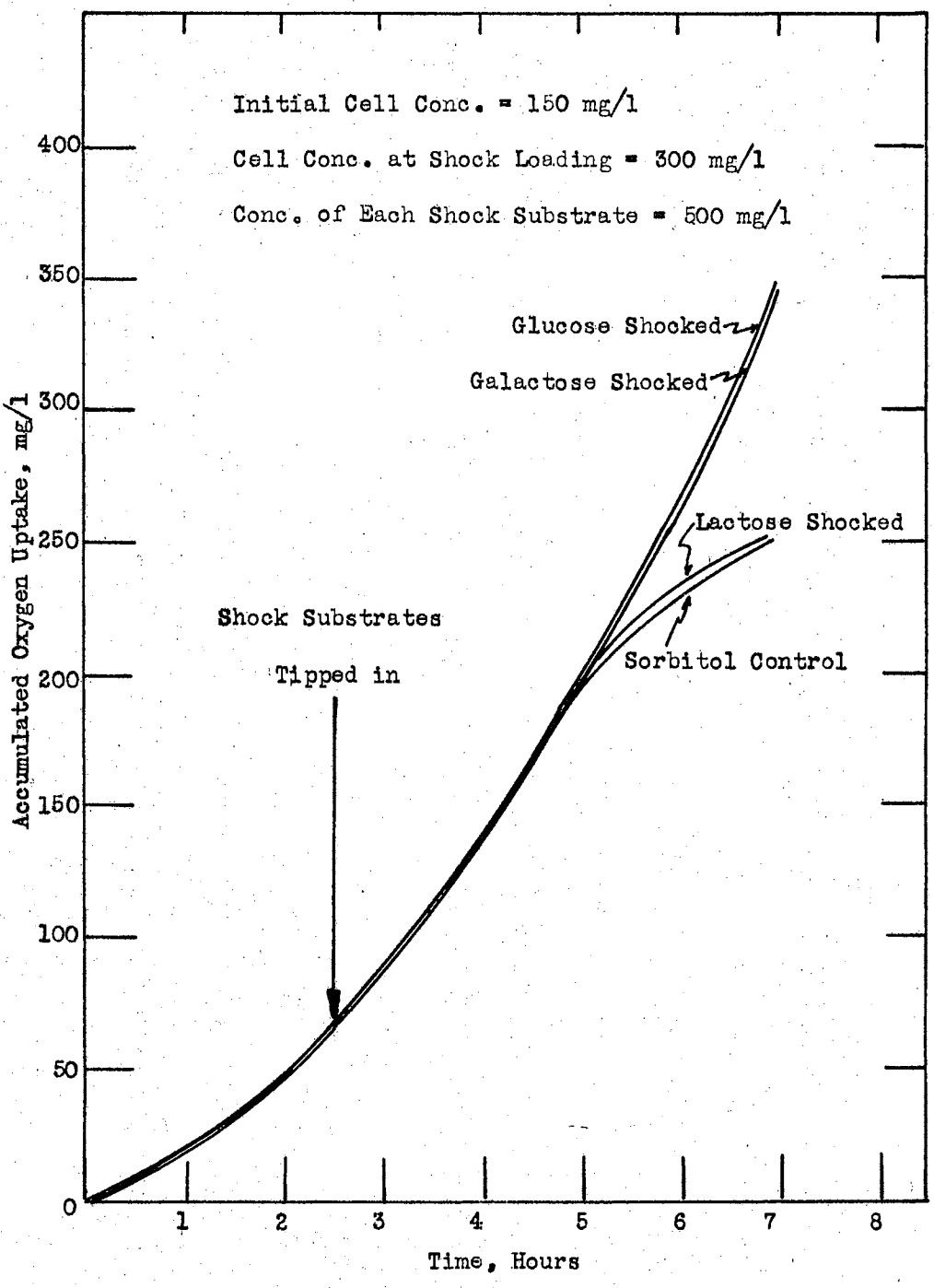

FIGURE 9 RESPONSE OF SORBITOL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITIONS, TO SHOCK LOADING BY INDICATED COMPOUNDS

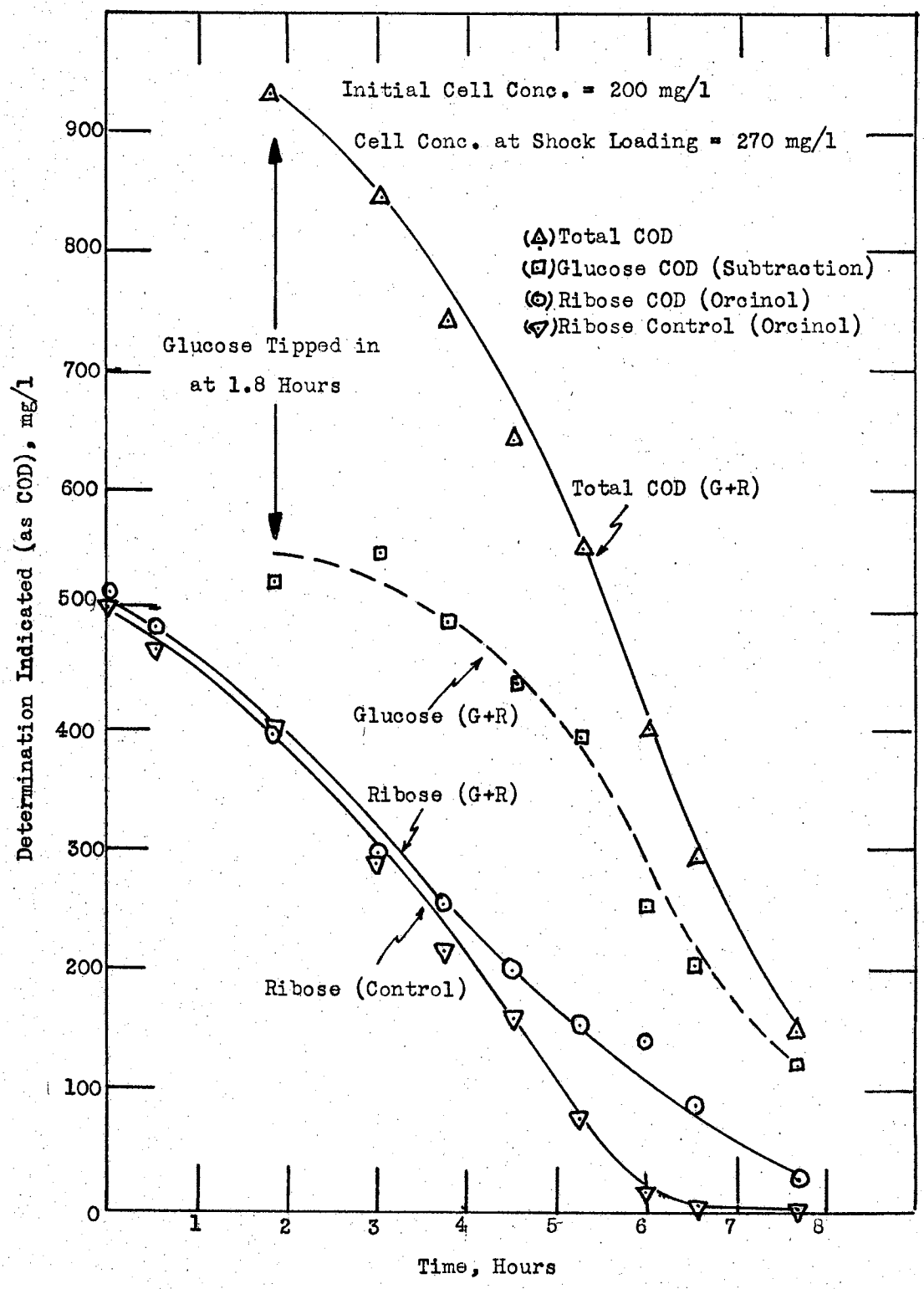

FIGURE 10 RESPONSE OF RIBOSE-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLUCOSE

started at a slow rate and gradually increased. By comparing ribose removal in the control and in the shocked system it can be seen that as glucose metabolism increased, ribose removal was increasingly retarded.

When a system consisting of another portion of the same ribose-acclimated population was shocked with glycerol while actively metabolizing ribose, the response was somewhat different. These results are shown in Figure 11. Here, it is seen that the course of ribose removal in the control and in the shocked system are parallel, even though the shock compound, glycerol, was initially removed at a relatively faster rate than was glucose in the figure previously shown. Both substrates were removed concurrently; active metabolism of ribose did not appear to be deterred by the initiation of glycerol removal.

The effect of shock loading with ribose on a sludge actively metabolizing glycerol is shown in Figure 12. The introduction of ribose had no effect on the course of glycerol removal. Acclimation to ribose proceeded at a fairly rapid pace. It can be seen from the total COD curve that the overall course of carbon source depletion in the system was controlled by two relatively independent and sequential rates, that for glycerol removal and that for ribose.

4. Effects of Sludge Age on Shock Loading Responses

In all the preceding cases of this study, young cell populations were used. The procedures for their development have been described in detail in the experimental protocol. It was important to study the response of the sludge developed under operational conditions which

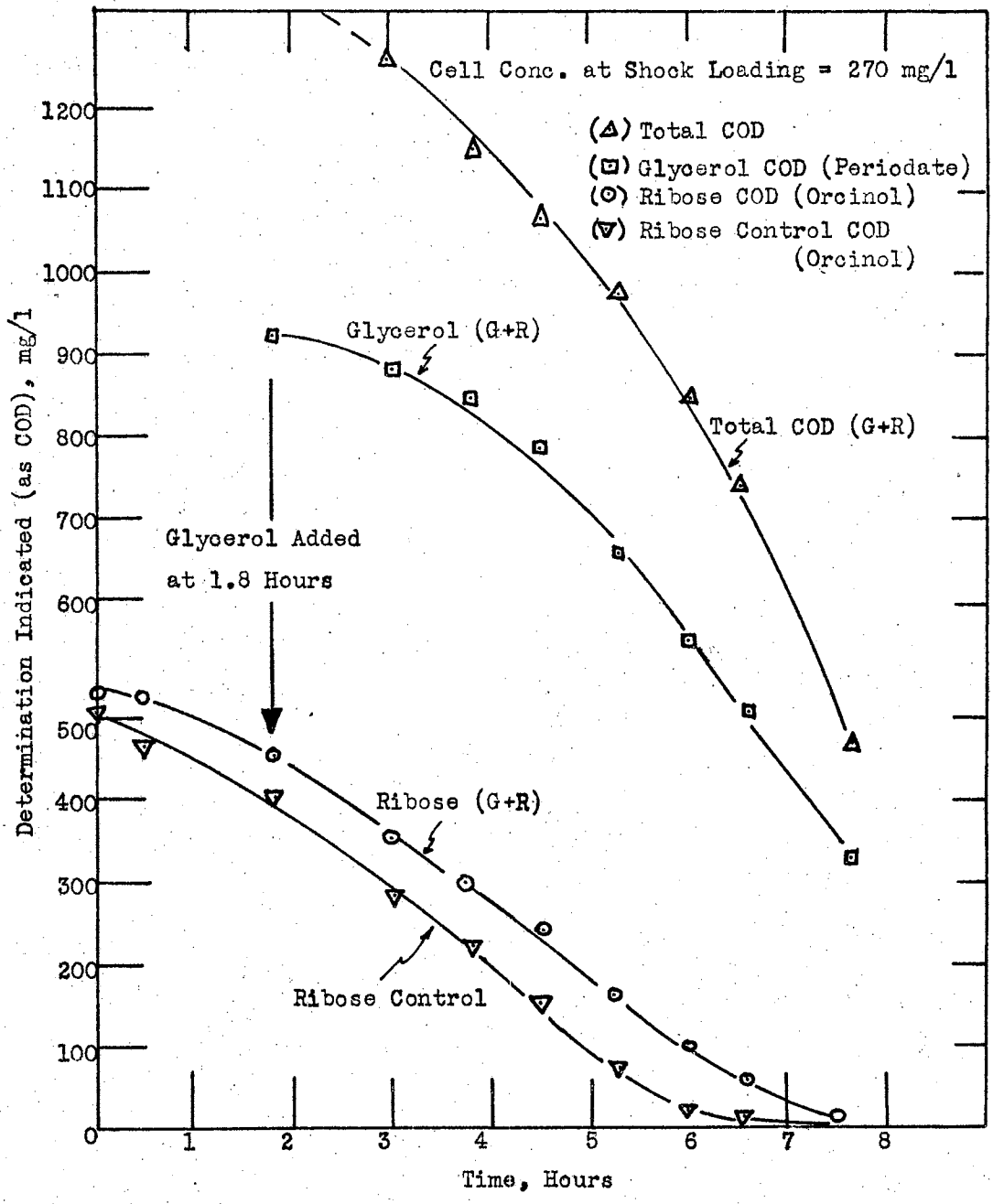

FIGURE 11 RESPONSE OF RIBOSE-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLYCEROL

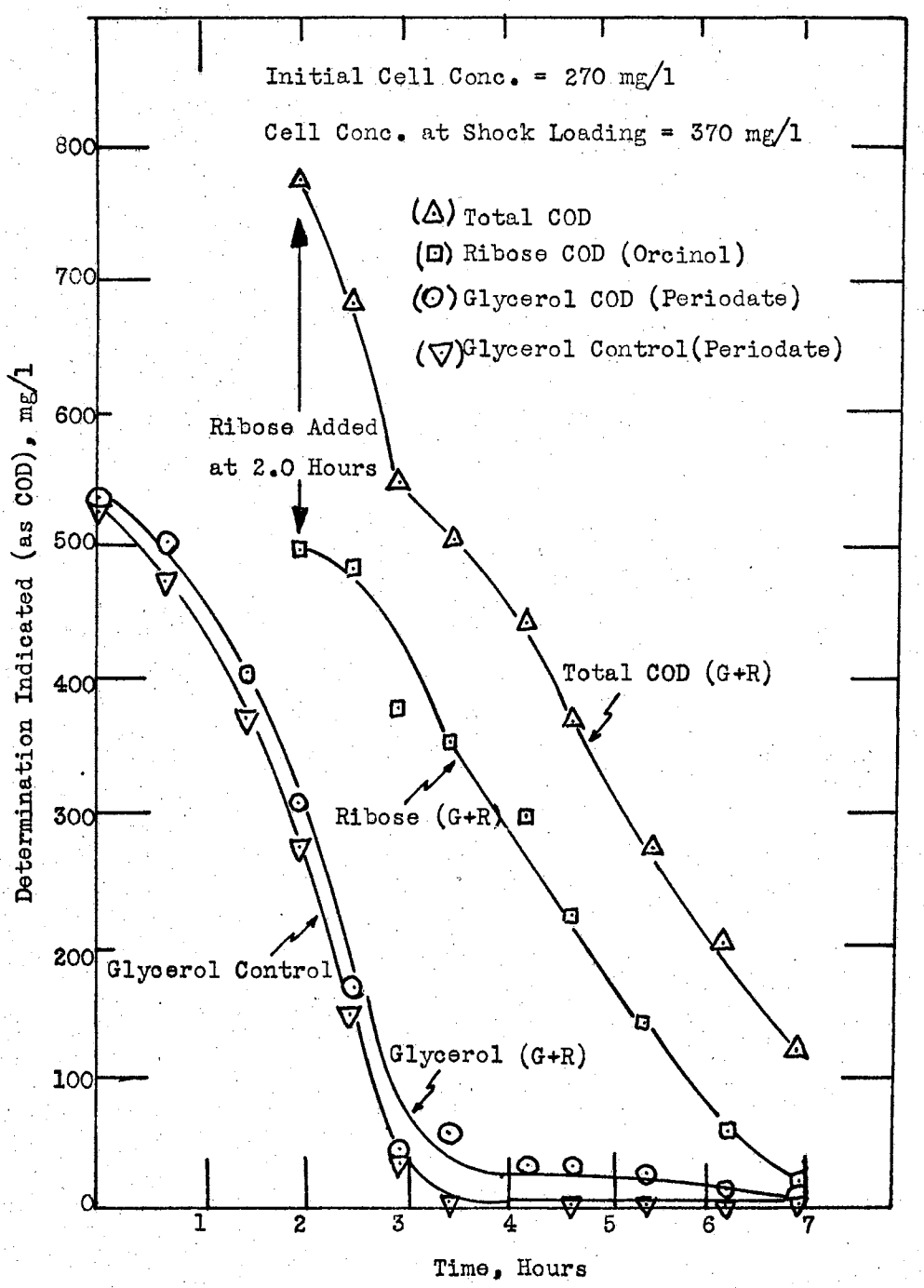

FIGURE 12 RESPONSE OF GLYCEROL-ACCLIMATED SLUDGE, YOUNG CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH RIBOSE

yielded sludge masses designated as "old cell" population. Such populations normally had greater settleability and were characterized by brownish or dark brown color. The responses of the old cell populations were studied under two conditions, i. e., under growth conditions, in which the supply of nitrogen was in excess, and under non-proliferating conditions, in which the supply of exogenous nitrogen source was omitted completely from the medium.

a. Growth Conditions: Results employing the old cell sorbitol acclimated sludge are shown in Figure 13. It is seen that sorbitol removal was not blocked upon introduction of glucose as in the case of the "young cell" sludge (Figure 3). In contrast, glucose was initially removed at a slower rate and required a short acclimation period but afterward continued to be removed at an increasing rate. By comparison of sorbitol removal in the control and in the shocked system, it is discerned that after initiation of glucose metabolism the sorbitol metabolism rate was increasingly retarded, but both substrates were concurrently removed.

The competitive effect of glucose is not in evidence for the old cell mannitol-acclimated system (Figure 14). Mannitol removal proceeded at the same rate in both the shocked and in the control system. As with the old cell sorbitol-acclimated system, glucose was removed concurrently with the compound to which the cells were previously acclimated and which they were actively metabolizing prior to the shock, and a lag in glucose metabolism occurred which seem to be more pronounced than in the sorbitol system. It should be noted

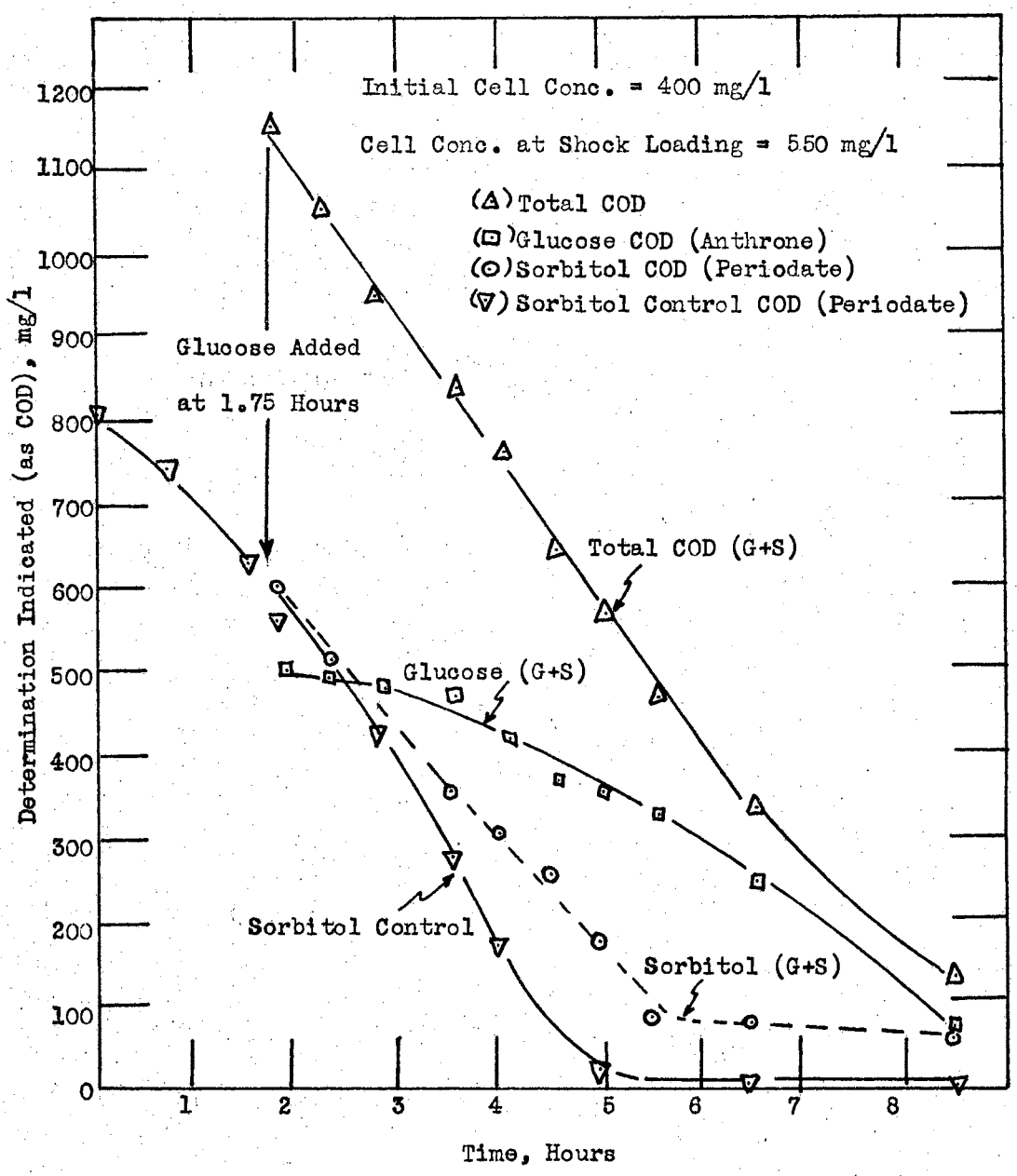

FIGURE 13 RESPONSE OF SORBITOL-ACCLIMATED SLUDGE, OLD CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLUCOSE

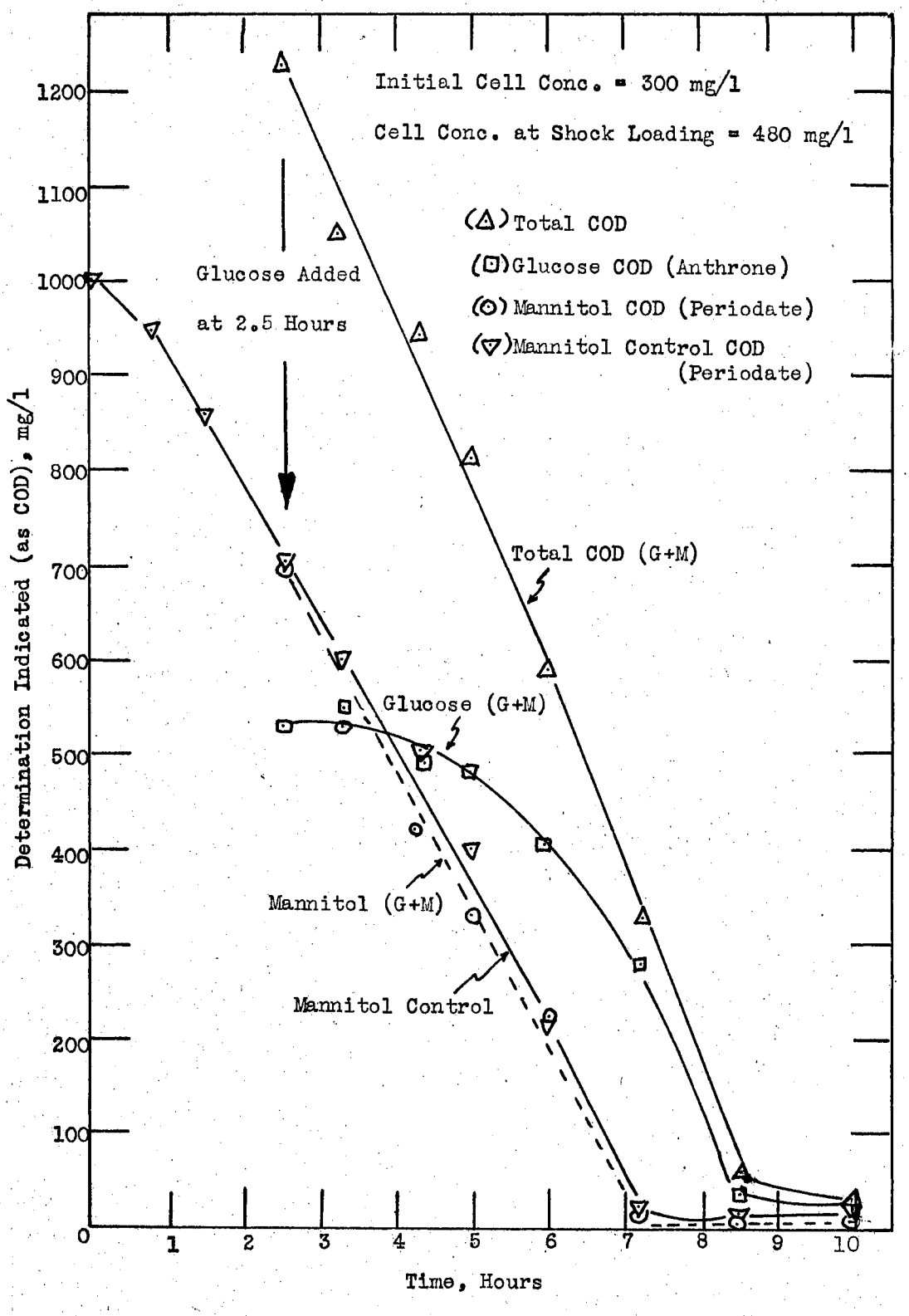

FIGURE 14 RESPONSE OF MANNITOL-ACCLIMATED SLUDGE, OLD CELLS UNDER GROWTH CONDITION, TO SHOCK LOADING WITH GLUCOSE

that eventhough the biological solids, at the time the shock loadings were applied, were higher for the old cell systems than for the young cell systems, the total COD removal for these systems was slower than that of the corresponding young cell systems and substrate removal proceeded in accordance with zero order kinetics.

b. Nonproliferating Conditions: Results of an experiment under nonproliferating conditions for an old cell sorbitol-acclimated system are shown in Figure 15. It is seen that, as in the old cell growth system, concurrent removal of both substrates occurred after the shock load. A comparison of the sorbitol removal curves in the control and the shocked system shows that glucose did not seriously affect the rate of sorbitol metabolism. The results also show that the cells had the ability to metabolize glucose but at a much slower rate than sorbitol.

However, such was not the case for the mannitol-acclimated sludge (Figure 16). Glucose was not removed at all during the experimental period. It is seen that the course of mannitol removal in the control and in the shocked system was identical, indicating that glucose had absolutely no effect on the shocked system. The cells had apparently lost the capacity for oxidative assimilation of glucose.

B. Continuous Flow Systems

1. Responses of Sorbitol-Acclimated Systems to Glucose Shock Loadings

a. Responses to Gradual Shock Loading: Glucose and sorbitol were selected for these studies because the inhibitory effect of glucose

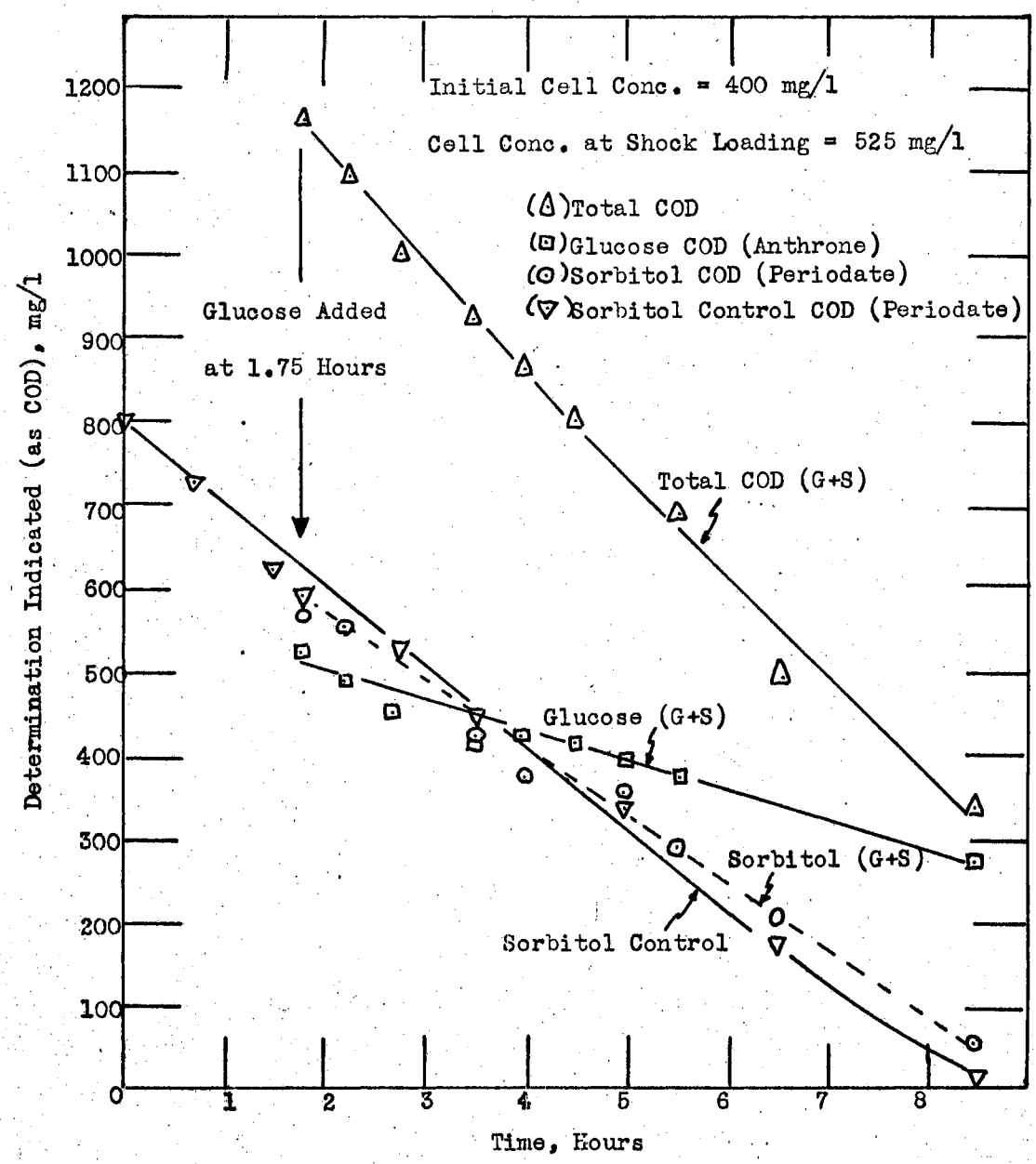

FIGURE 15 RESPONSE OF SORBITOL-ACCLIMATED SLUDGE, OLD CELLS UNDER NONPROLIFERATING CONDITION, TO SHOCK LOADING WITH GLUCOSE

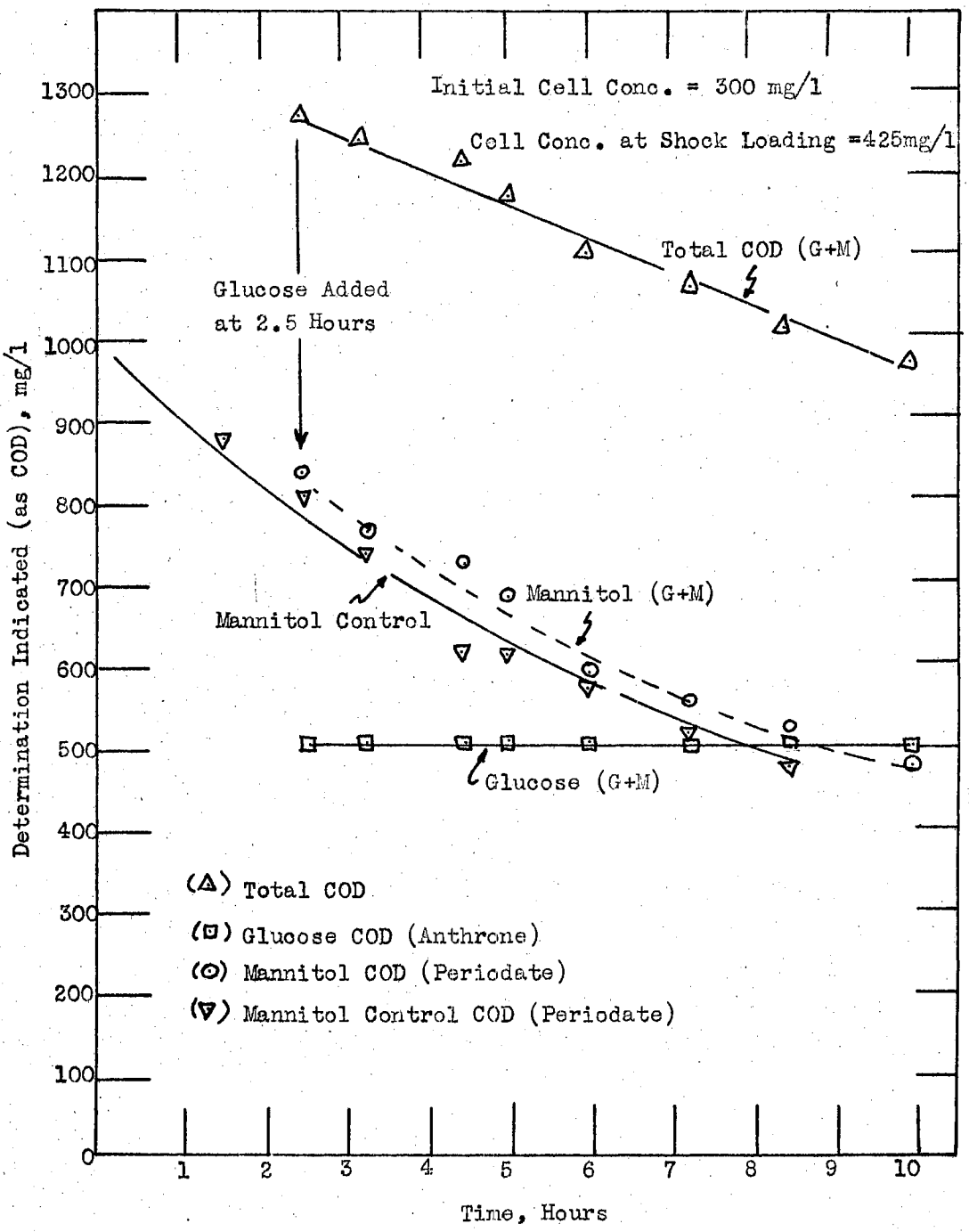

FIGURE 16 RESPONSE OF MANNITOL ACCLIMATED SLUDGE, OLD CELLS UNDER NONPROLIFERATING CONDITION, TO SHOCK LOADING WITH GLUCOSE

on sorbitol acclimated sludge has already been shown in the batch-operated systems. It was, therefore, interesting to determine whether the same phenomenon would be observed under completely-mixed continuous flow conditions which simulate more closely the actual operational conditions at waste treatment plants. A 4-hour detention period was employed in this portion of the study.

Figure 17 shows the general response of a continuous flow activated sludge to shock loading when the influent waste was changed from 1000 mg/l sorbitol to 1000 mg/l glucose. A control system which was fed 1000 mg/l sorbitol as previously was also run concurrently in order to obtain comparisons between the two systems. It can be seen that there was no significant difference in the effluent characteristics of the two systems. All of the parameters determined remained relatively constant throughout the experimental period even though the quality of the influent waste of the shocked system had been completely changed. The biological solids protein also indicated no significant fluctuation. Both glucose and sorbitol in the effluent remained at a very low level indicating over 95 per cent removal for both substrates. It should be noted, however, that the values of residual total COD remaining in both systems during the entire period of the experiment were over 100 mg/l; this could not be accounted for as either glucose COD or sorbitol COD. Such residual COD also had been noted previously in batch-operation studies. This could not be solely attributable to the interference of inorganic salts in the waste medium, since several times the COD of the waste medium consisting of all inorganic salts but without organic carbon source was determined and it was found that the residual COD, due to the inorganic salt interference, never exceeded

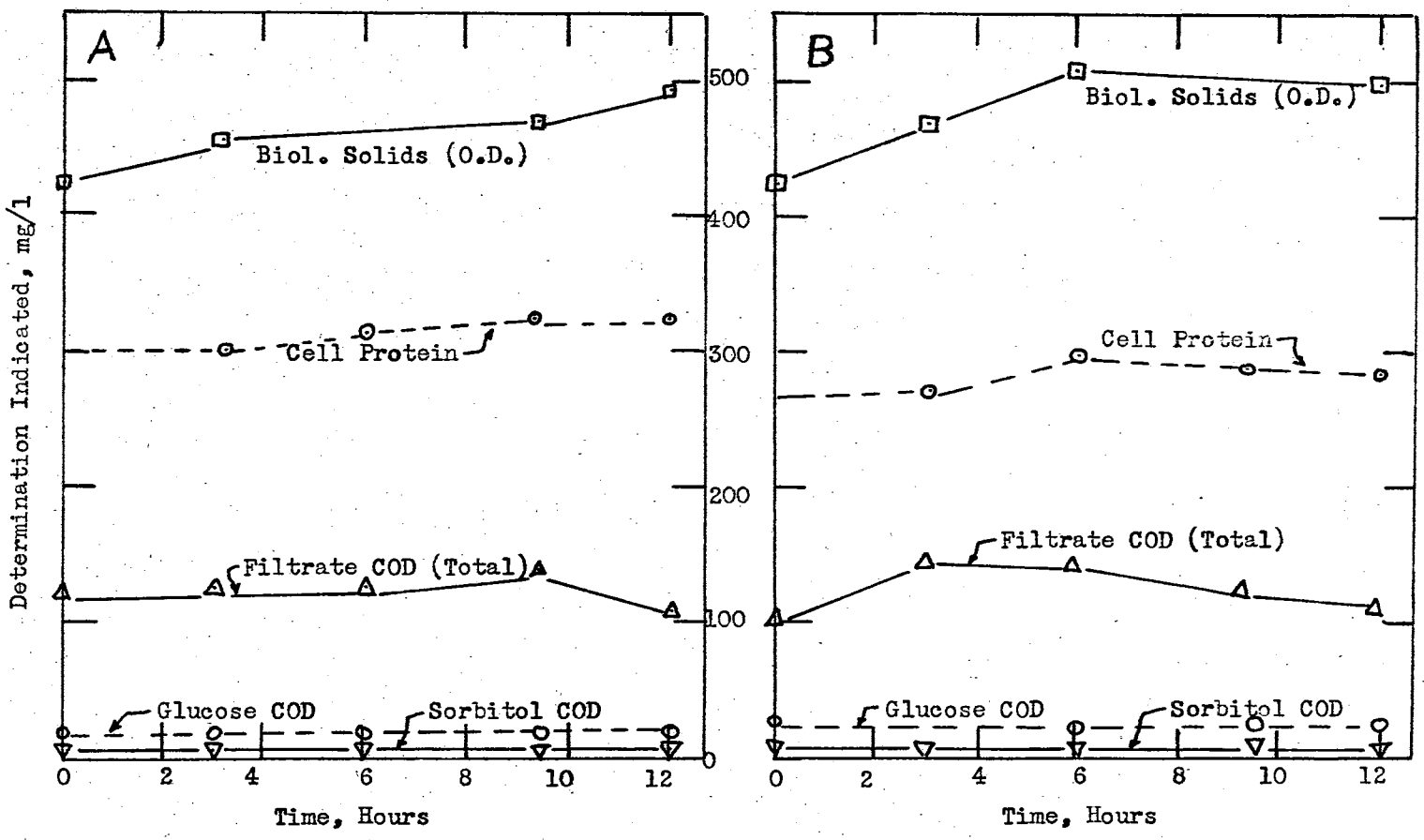

FIGURE 17 SHOCK LOAD RESPONSE IN A STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME. A, AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 1000 MG/L GLUCOSE. B, CONTROL SYSTEM FED WITH 1000 MG/L SORBITOL

50 mg/l and generally was only about 30 mg/l. Therefore, these results tend to indicate that the cells may release an intermediate(s) or end product(s) which does not react to either the periodate or the anthrone test. The control system was fed only with sorbitol but analysis of the effluent indicated a slight reaction with the anthrone test. This value indicated approximately 30 mg/l as glucose COD. However, it is not implied that glucose was produced by the sorbitol metabolizing system. This finding may be interpreted as a result of the production of small amounts of compounds which react with anthrone. Furthermore, it was noted that sorbitol itself also reacted slightly in the anthrone test by yielding a brownish color which interfered with colorimetric determination. However, these interferences did not significantly affect the results, since they were of comparatively low value.

In another set of experiments, the influent waste composition was changed from 1000 mg/l sorbitol to 750 mg/l sorbitol plus 250 mg/l glucose, a second system was changed from 1000 mg/l sorbitol to 250 mg/l sorbitol plus 750 mg/l glucose. This was done in order to determine whether the ratio of the concentration of interacting substrates had any significant effect on the shock load response. The results are shown in Figure 18. It can be seen that these changes did not have any effect on the system responses; both glucose and sorbitol were removed quite readily without any change in either biological solids or protein level; significant amounts of total COD in the effluent filtrate were noted as in the previous cases, but these were the same for both systems.

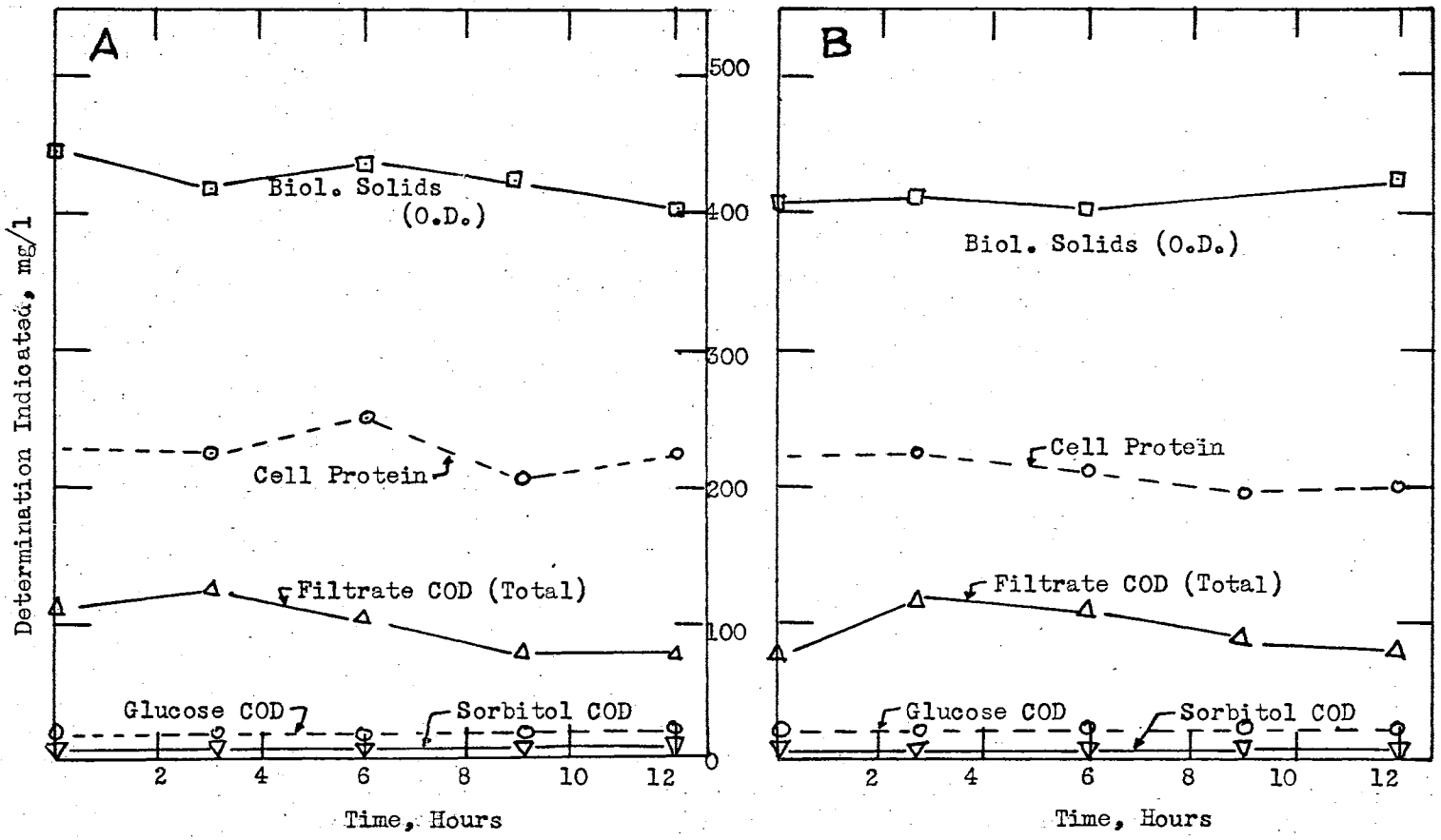

FIGURE 18 SHOCK LOAD RESPONSE IN A STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME. A, AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 750 MG/L SORBITOL PLUS 250 MG/L GLUCOSE. B, FROM 1000 MG/L SORBITOL TO 250 MG/L SORBITOL PLUS 750 MG/L GLUCOSE

It should be realized that for all cases which have been reported thus far, the systems were subjected only to qualitative shock loading since the total organic carbon source concentration remained at the same level (1000 mg/l) before and after the shock load. From the results obtained thus far, it is apparent that when the systems were subjected to only a qualitative shock load no deleterious effect on the systems resulted.

Figure 19 shows the shock-load responses of systems which were subjected to both qualitative and quantitative shock loads. In Figure 19-A the system was operating in the steady state on 500 mg/l sorbitol; it was then shock loaded by changing the waste influent composition to 500 mg/l sorbitol plus 500 mg/l glucose. It can be seen that even though the feed concentration was increased two-fold there was apparently no significant effect on the removal efficiency as measured by the levels of glucose COD and sorbitol COD in the effluent. There was slight increase in the level of total filtrate COD shortly after administering the shock loading, which was not registered as either glucose COD or sorbitol COD. A significant change in the biological solids concentration occurred. It increased fairly rapidly at a rate which paralleled the rate at which the glucose shock load was applied. The curve labelled "applied glucose shock loading" (dotted line) represents the calculated cumulative concentration of glucose in the aeration vessel if it were not removed by the activated sludge. This would theoretically be equal to the concentration of the influent glucose after a due period of time (cf. Figure 2).

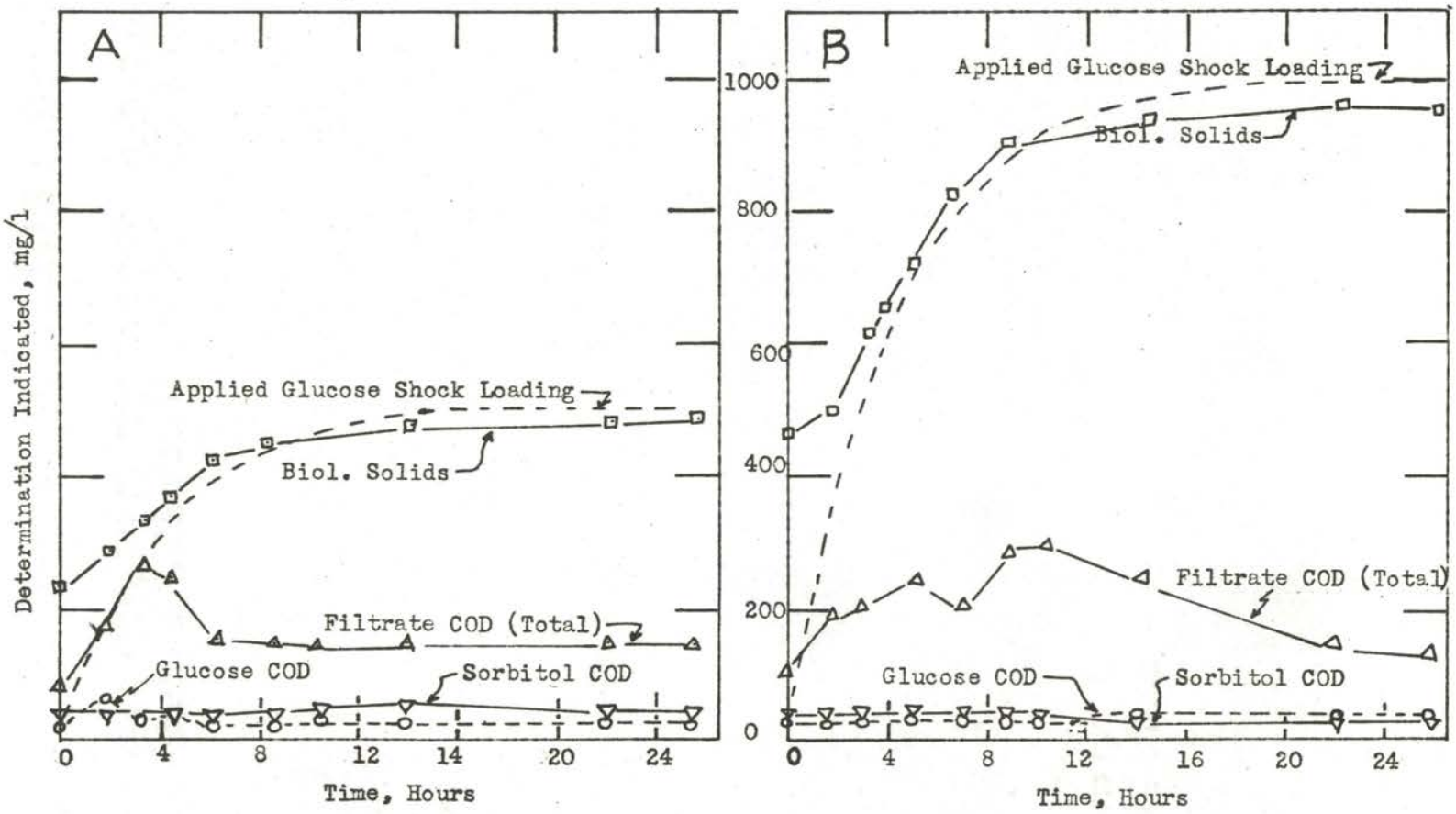

FIGURE 19 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION: A, FROM 500 MG/L SORBITOL TO 500 MG/L SORBITOL PLUS 500 MG/L GLUCOSE. B, FROM 1000 MG/L SORBITOL TO 1000 MG/L SORBITOL PLUS 1000 MG/L GLUCOSE

Figure 19-B shows the response of a system which had been operating at a level of 1000 mg/l sorbitol and was shock loaded by changing the influent waste to 1000 mg/l sorbitol plus 1000 mg/l glucose. A comparatively high increase in total filtrate COD can be noted and response time of almost 16 hours was required before effluent quality returned to its former level. However, there was no noticeable change in either glucose or sorbitol levels in the effluent which tends to indicate that the system could metabolize both compounds readily.

Figure 20 shows the results when sorbitol acclimated sludge at 1500 mg/l sorbitol waste influent concentration was shock loaded with 1500 mg/l sorbitol plus 1500 mg/l glucose. Again there was a substantial increase in the total filtrate COD (as measured by dichromate oxidation), but only a slight increase in sorbitol COD was noted for a very short period immediately after the shock load was applied; the level of glucose remained practically unchanged. It may be noted that when a high loading was applied, a proportionally higher amount of intermediates appeared to be released. From the results obtained thus far it is discerned that these systems have great capability to accept the glucose shock loading if the increase of glucose concentration in the influent is about the same level as that of the sorbitol concentration formerly employed, that is, if the total concentration of carbon source is increased two fold. This statement holds for glucose and sorbitol COD removal but not for the total filtrate COD removal. The efficiency of total filtrate COD removal seems to be greatly reduced as the loadings increase.

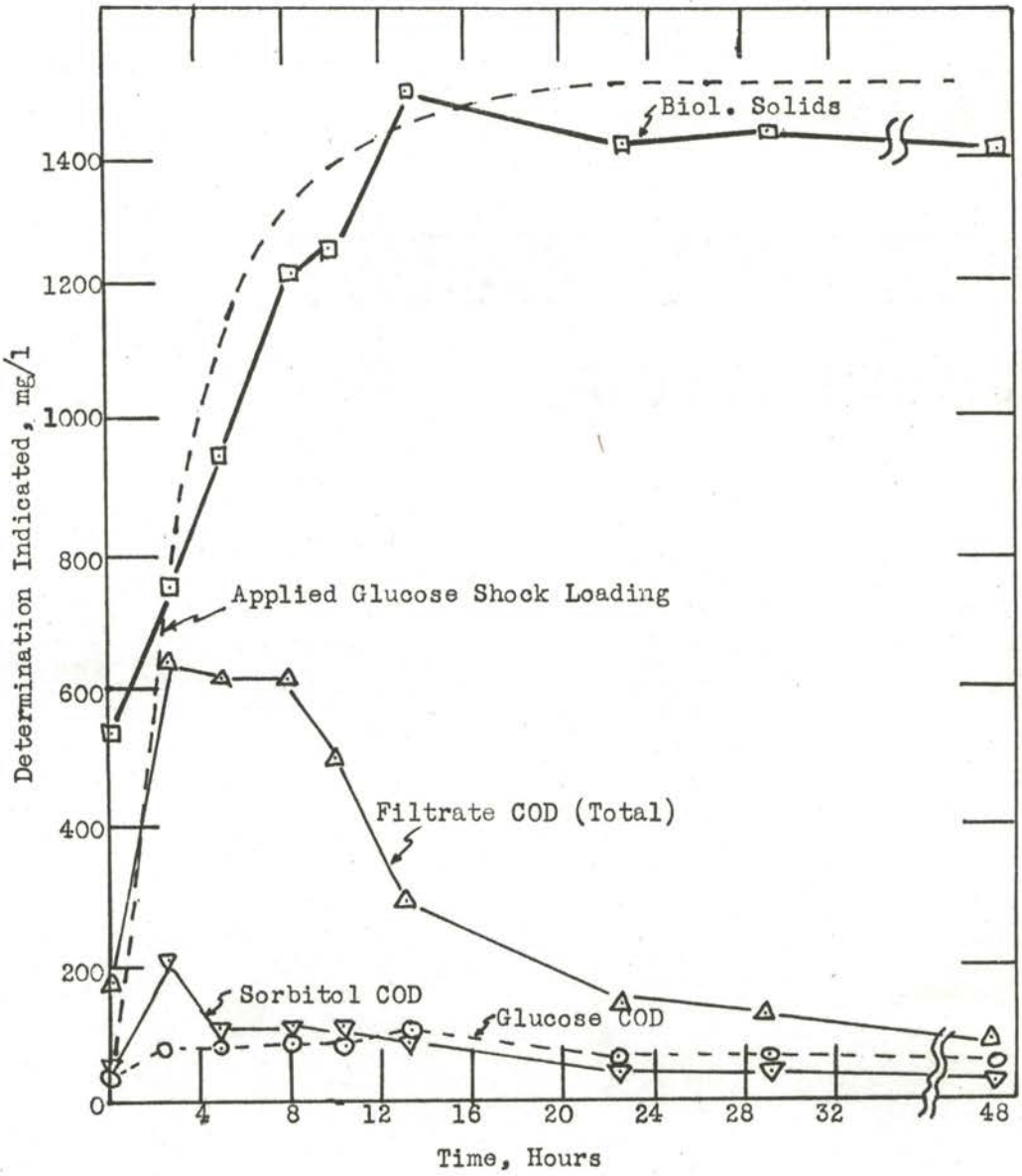

FIGURE 20 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1500 MG/L SORBITOL TO 1500 MG/L SORBITOL PLUS 1500 MG/L GLUCOSE

It was of interest then to determine the response when a sorbitol acclimated system was subjected to only a sorbitol quantitative shock load and to compare this response with combined shock loads as well as a pure glucose shock load. The response of a system in which the influent concentration was changed from 1500 mg/l sorbitol to 3000 mg/l sorbitol is shown in Figure 21. It is seen that when the system was shock loaded with twice the sorbitol concentration on which it had been operating, the system could not readily cope with such a shock loading and sorbitol was passed out in the effluent. After about 24 hours, however, the system recovered and both the COD and the sorbitol in the effluent were again reduced to a low level. At this time a new level in biological solids had been attained indicating that the system had established a new steady state equilibrium to cope with the increased loading condition. A considerable amount of intermediates in the effluent can also be noted as indicated by the differences between the total COD and sorbitol COD in the filtrate.

A shock load response which occurred when the influent was changed from 1500 mg/l sorbitol to 3000 mg/l glucose was shown in Figure 22. It can be seen that the glucose shock load could be readily metabolized by the sorbitol acclimated sludge. A significant increase in total filtrate COD was noted at an early stage but this was reduced to about 250 mg/l after approximately 10 hours. There was no significant increase in glucose COD after the shock even though the system was supplied solely with glucose, a slight increase in sorbitol COD at approximately two hours after the shock can be interpreted as interferences by intermediates which might react slightly with

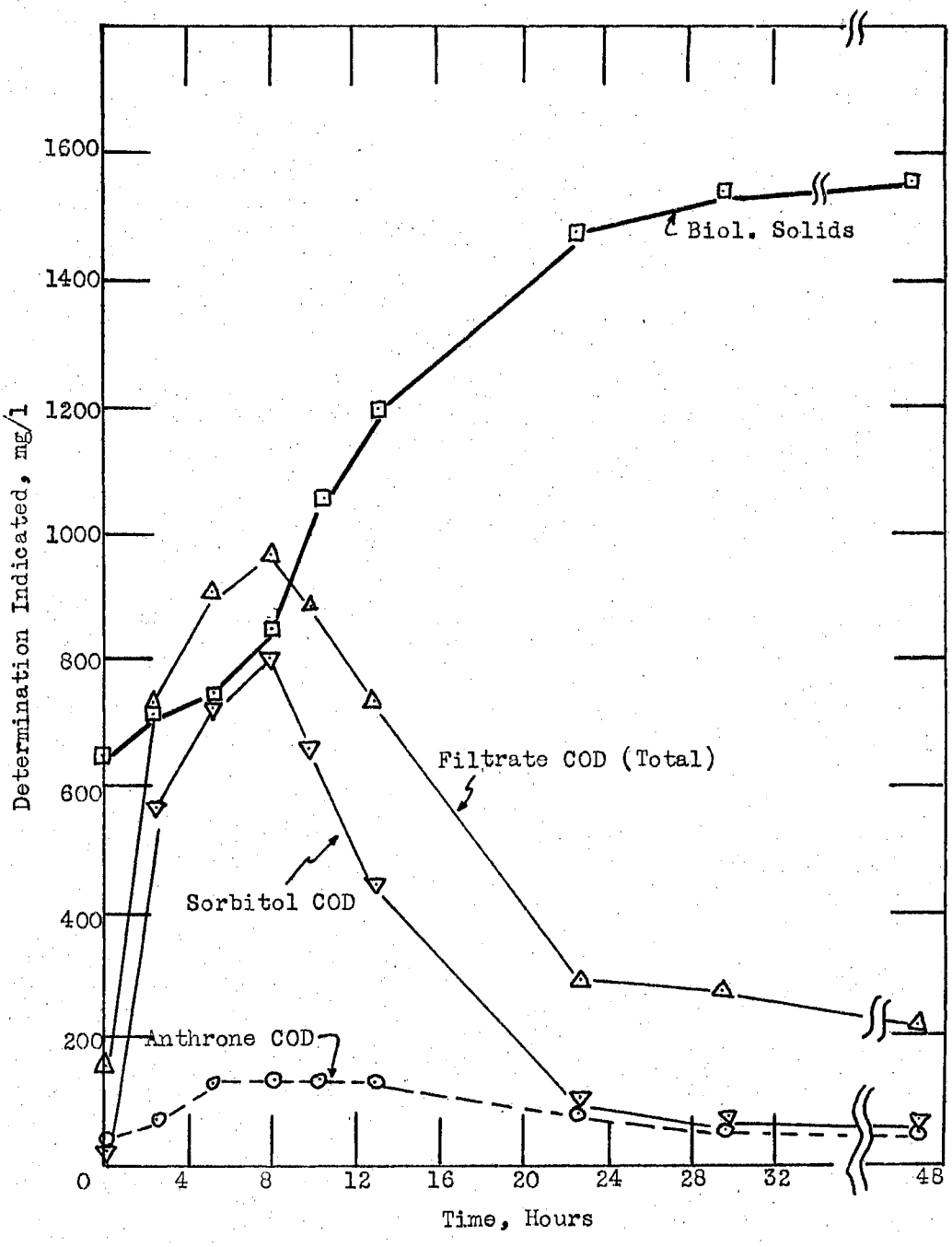

FIGURE 21 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1500 MG/L SORBITOL TO 3000 MG/L SORBITOL

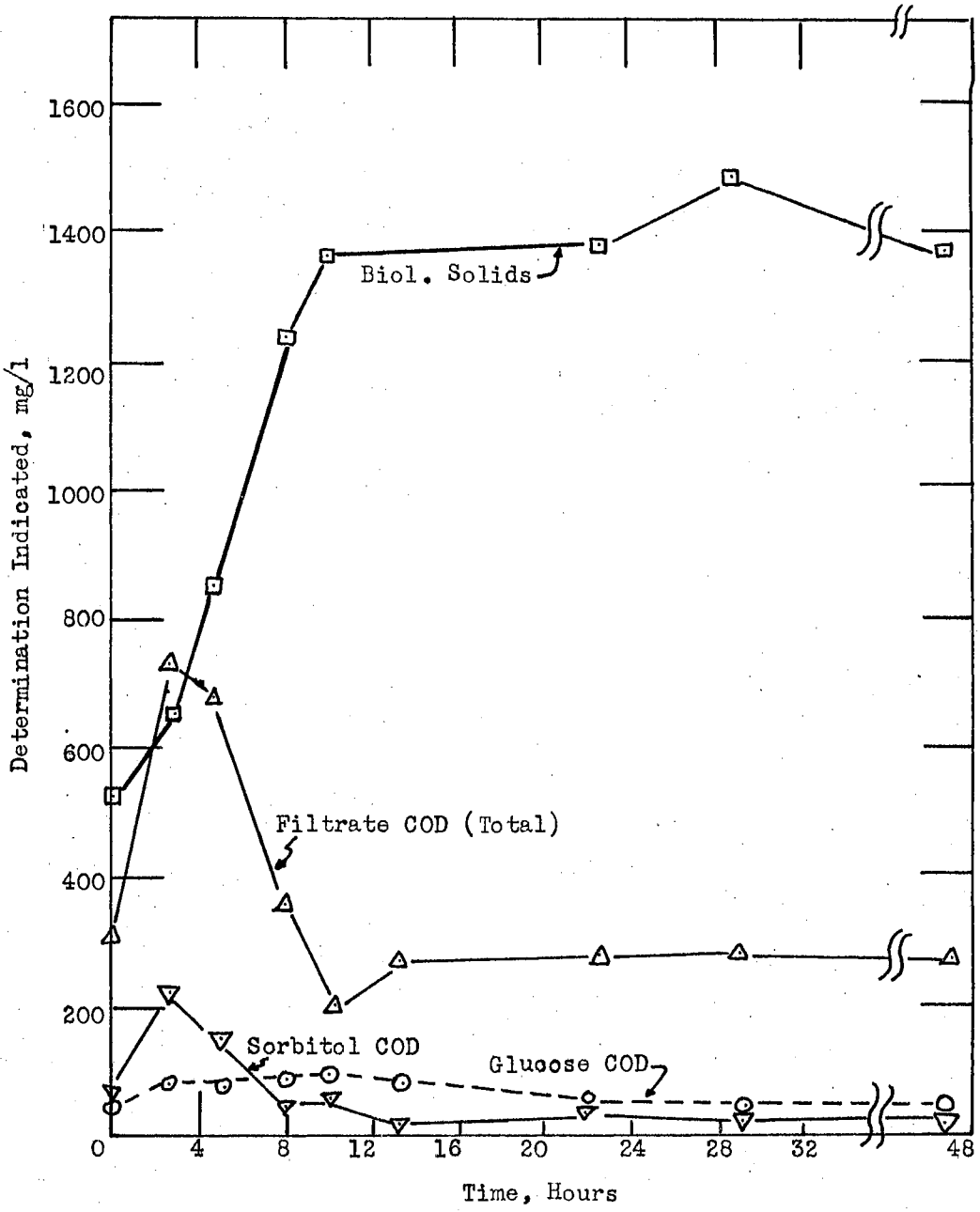

FIGURE 22 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1500 MG/L SORBITOL TO 3000 MG/L GLUCOSE

the periodate test.

The difference between the initial biological solids concentrations of the two systems shown in Figure 21 and 22 may be due to some fluctuations occurring in the sampling process. However, significant differences in the responses to glucose and sorbitol shock loading of the two systems were apparent and it is very unlikely that these could be due to the differences, if any existed, in the initial biological solids levels. By comparing the results shown in Figure 21 and Figure 22 it can be seen that glucose not only could be utilized readily by the sorbitol-acclimated sludge but the rate at which it was metabolized was even faster than the rate of sorbitol utilization, even though the sludge had been previously acclimated to sorbitol. It can be noted that less total filtrate COD was passed out in the effluent in the glucose shock-loaded system and the time at which a new equilibruim was reached for the glucose shocked system was much shorter; in addition, the growth rate of the biological solids was also apparently faster in the glucose shocked system than in the sorbitol shocked system. However, there appeared to be a greater amount of intermediates released in the glucose shocked systems as can be seen from the differences between the total filtrate COD and the corresponding substrate COD in each system.

The response of a system which was subjected to a greater proportional shock loading is shown in Figure 23. In this case the system was acclimated to 500 mg/l sorbitol; the shock was then applied by changing the waste influent from 500 mg/l sorbitol to 750 mg/l sorbitol plus 750 mg/l glucose. The control systems, in which the

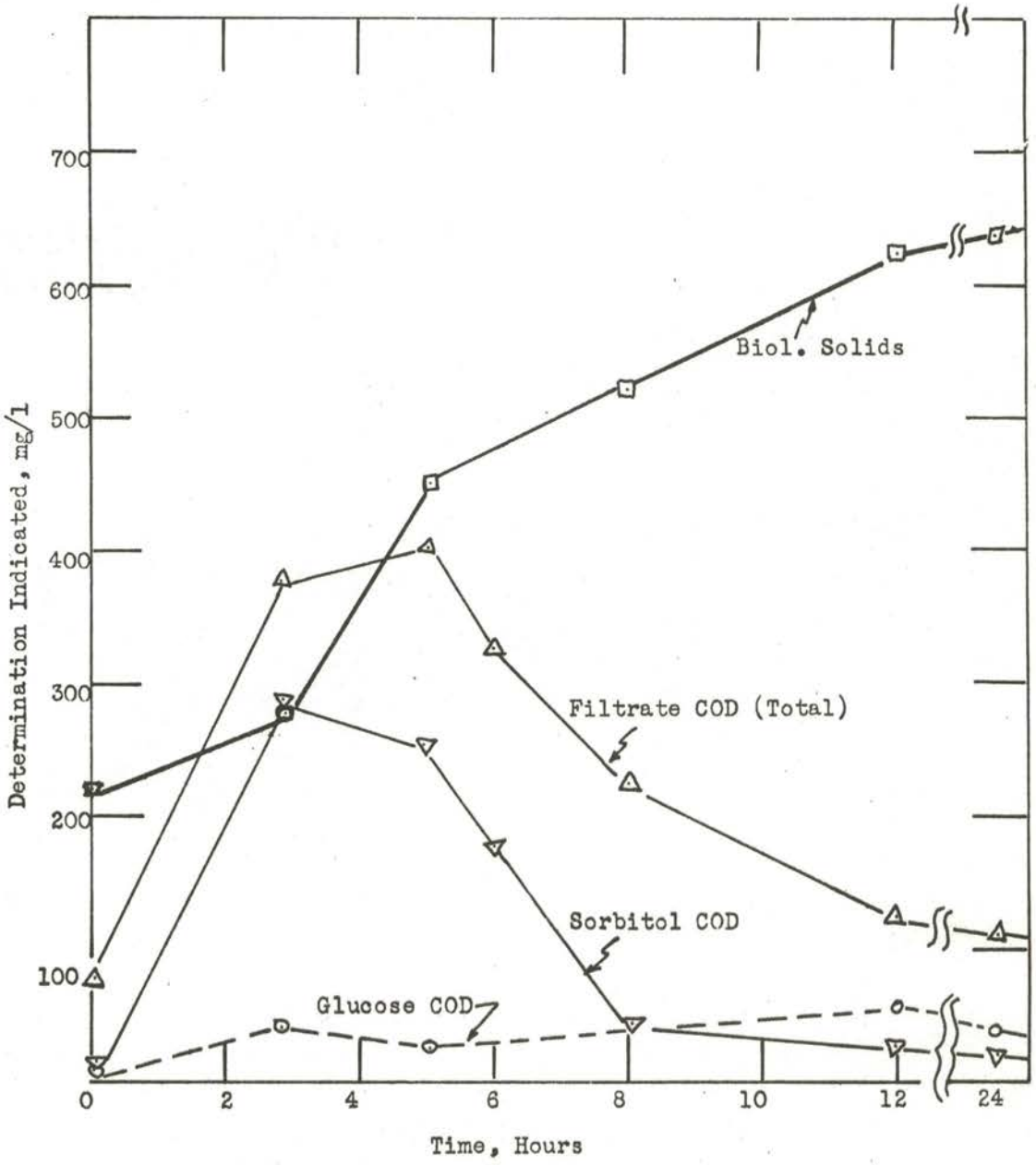

FIGURE 23 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE FROM 500 MG/L SORBITOL TO 750 MG/L SORBITOL PLUS 750 MG/L GLUCOSE

influent wastes were changed from 500 mg/l sorbitol to 750 mg/l sorbitol and to 750 mg/l glucose, were also run concurrently and these results are shown in Figure 24. Comparing results in Figures 23 and 24, it is evident that when both glucose and sorbitol were present together in the waste influent as a shock load, the glucose exerted its inhibitory effect on sorbitol removal. There was no significant increase in the sorbitol level when the system was fed with sorbitol only (see Figure 24-B), but when both substrates were fed together sorbitol was only partially removed and for a time was passed out in the effluent. However, after approximately 8 hours, the sorbitol level in the combined shock-load system again returned to a low level. The glucose concentration remained at a relatively low level even though both glucose and sorbitol were fed to the system in equal concentrations. Actually the system was shock loaded with only 250 mg/l sorbitol but with 750 mg/l glucose, since the system had been operated in a steady state condition with 500 mg/l sorbitol prior to shock loading.

In order to gain a more complete picture of the responses of the systems to qualitative as well as quantitative shock loads, experiments in which the shock-load concentration were applied at three times the previous concentration were also made; these results are shown in Figure 25. It can be seen that when a system acclimated at 500 mg/l sorbitol was shock loaded with 1500 mg/l sorbitol the system could not successfully respond to the increase until the biological solids had increased to a higher level. A considerable amount of sorbitol was released in the effluent for almost 12 hours.

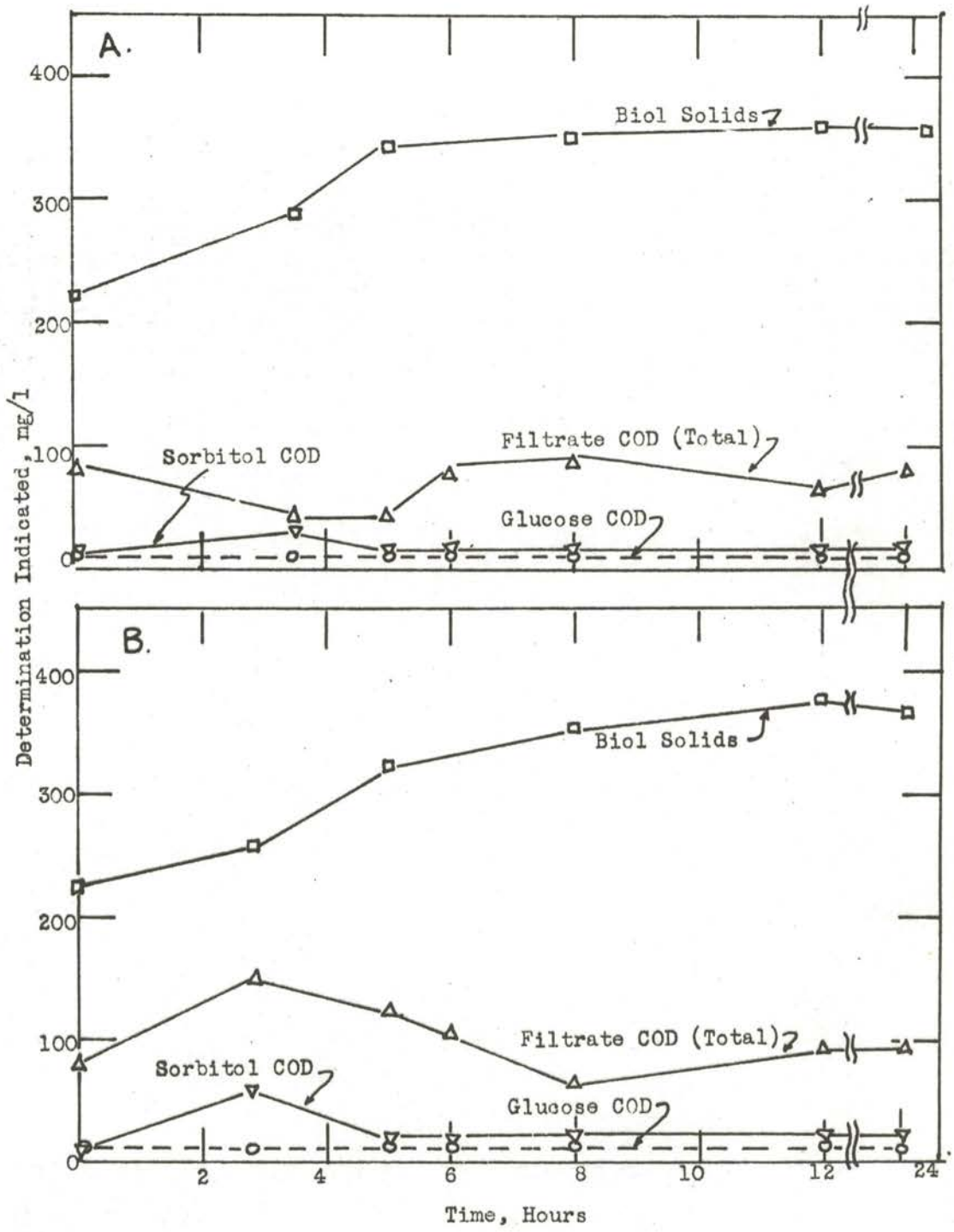

FIGURE 24 RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION. A, FROM 500 MG/L SORBITOL TO 750 MG/L GLUCOSE. B, FROM 500 MG/L SORBITOL TO 750 MG/L SORBITOL

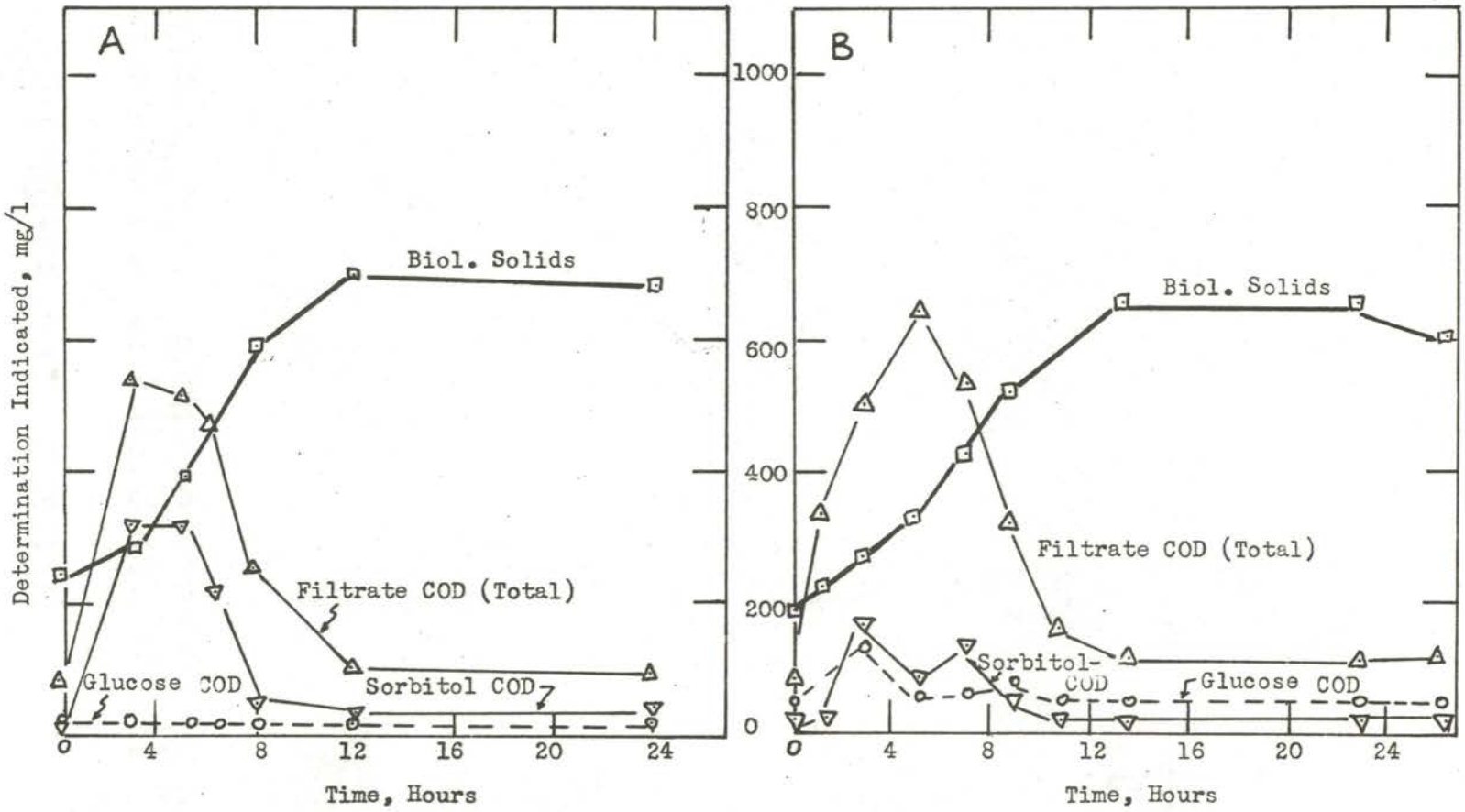

FIGURE 25 RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION. A, FROM 500 MG/L SORBITOL TO 1500 MG/L SORBITOL. B, FROM 500 MG/L SORBITOL TO 1500 MG/L GLUCOSE

The shock load response of a sorbitol acclimated system operating at 500 mg/l of sorbitol, to a shock load of 1500 mg/l of glucose is shown in Figure 25-B. It can be seen that there was also a significant increase in the total filtrate COD of the system which can be interpreted as intermediates or end products released into the medium, since this COD cannot be accounted for as either sorbitol COD or glucose COD. By comparing the differences between the total COD and the corresponding substrate COD for each systems it is also obvious that a greater amount of intermediates was released due to the glucose shock loading.

Another experiment in which the system was also shock loaded with three times its previous influent concentration is shown in Figure 26. In this case the influent waste composition was changed from 1000 mg/l sorbitol to 1500 mg/l sorbitol plus 1500 mg/l glucose. For comparison one can refer to Figure 19-B (as a control system). This system was also shock loaded with 1500 mg/l sorbitol plus 1500 mg/l glucose but this amounted to only two times its previous influent concentration, and the system responded quite successfully as very little glucose COD or sorbitol COD in the effluent could be noted. In the case shown in Figure 26, however, there was a definite increase in sorbitol concentration in the effluent and the system reached a new equilibrium about 14 hours after the shock. The sorbitol concentration in the effluent reached a peak of 700 mg/l at approximately 7 hours. It is interesting to compare these results with the results obtained in Figure 25-A in which a system with only half the initial biological solids concentration as that shown in

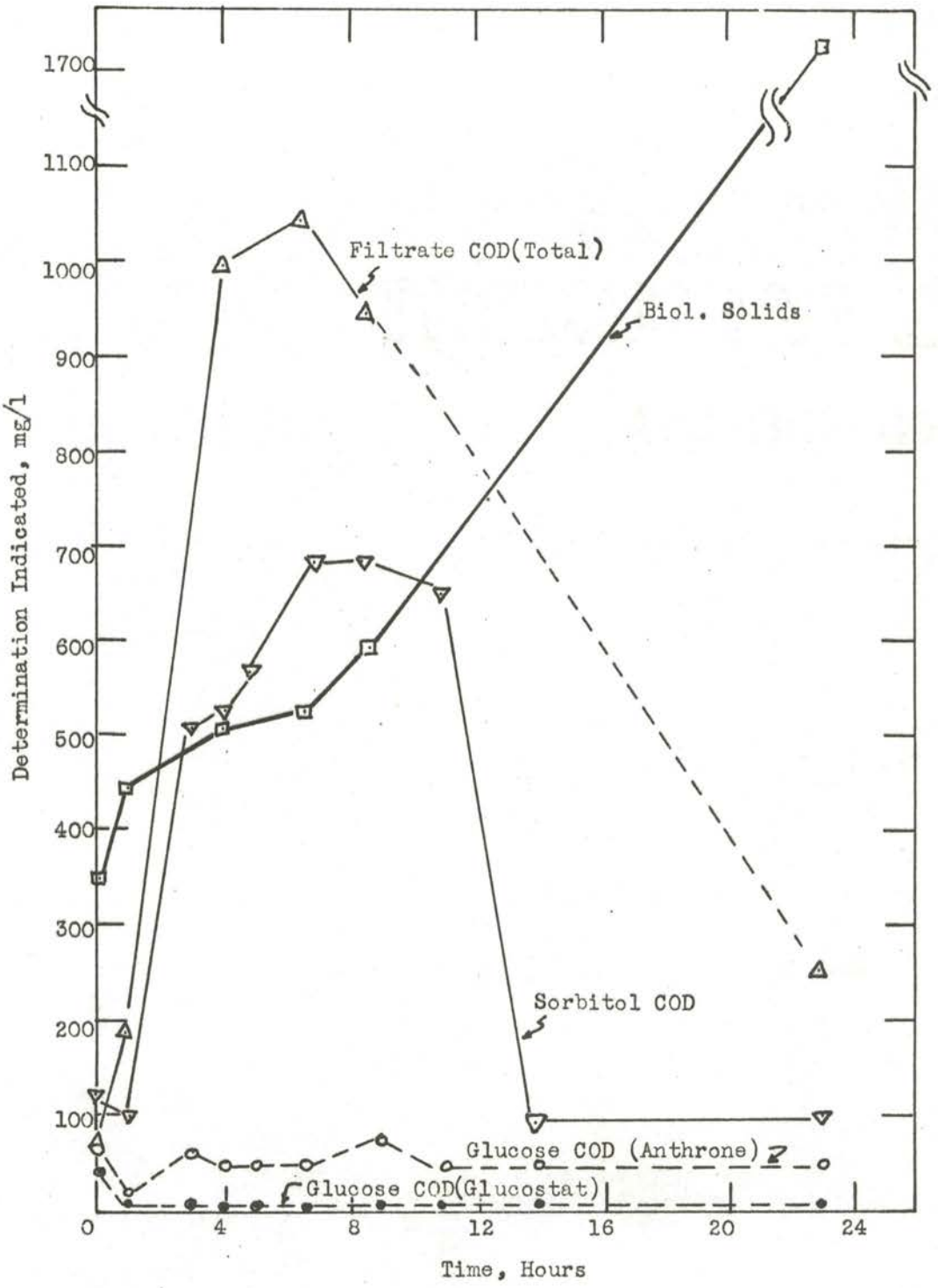

FIGURE 26 SHOCK LOAD RESPONSE IN THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 1500 MG/L SORBITOL PLUS 1500 MG/L GLUCOSE

Figure 26, was also shock loaded with 1500 mg/l sorbitol. The results indicated only approximately 300 mg/l sorbitol were released at the maximum point and a new equilibrium was attained within 12 hours after shock loading. It is obvious from such a comparison that the presence of glucose interfered with sorbitol removal. The Glucostat test, which is more specific for glucose than the anthrone test, was also run in order to determine the actual glucose concentration present in the filtrates and to compare this with the results obtained by the anthrone test. It found was that the glucose concentrations as obtained by the Glucostat test were lower than those of the anthrone test, indicating that not all the glucose as measured by the anthrone test is actually glucose. But such differences were comparatively small and do not in any way affect the interpretation of the results reported herein.

Figure 27-A shows the results of a system in which the waste influent was changed from 500 mg/l sorbitol to 500 mg/l sorbitol plus 500 mg/l glucose. It can be seen that there was a slight increase in the glucose level at the initial stage and a significant increase in sorbitol level for 7 hours. This experiment thus gave a different shock-load response than that found previously for a similar system (Figure 19-A). In the former case a system acclimated to 500 mg/l sorbitol could respond to 500 mg/l sorbitol plus 500 mg/l glucose more readily and no sorbitol or glucose appeared in the effluent. It should be noted, however, that the initial biological solids concentration present in the experiment of Figure 19-A was higher than that shown in Figure 27-A; furthermore the response of the

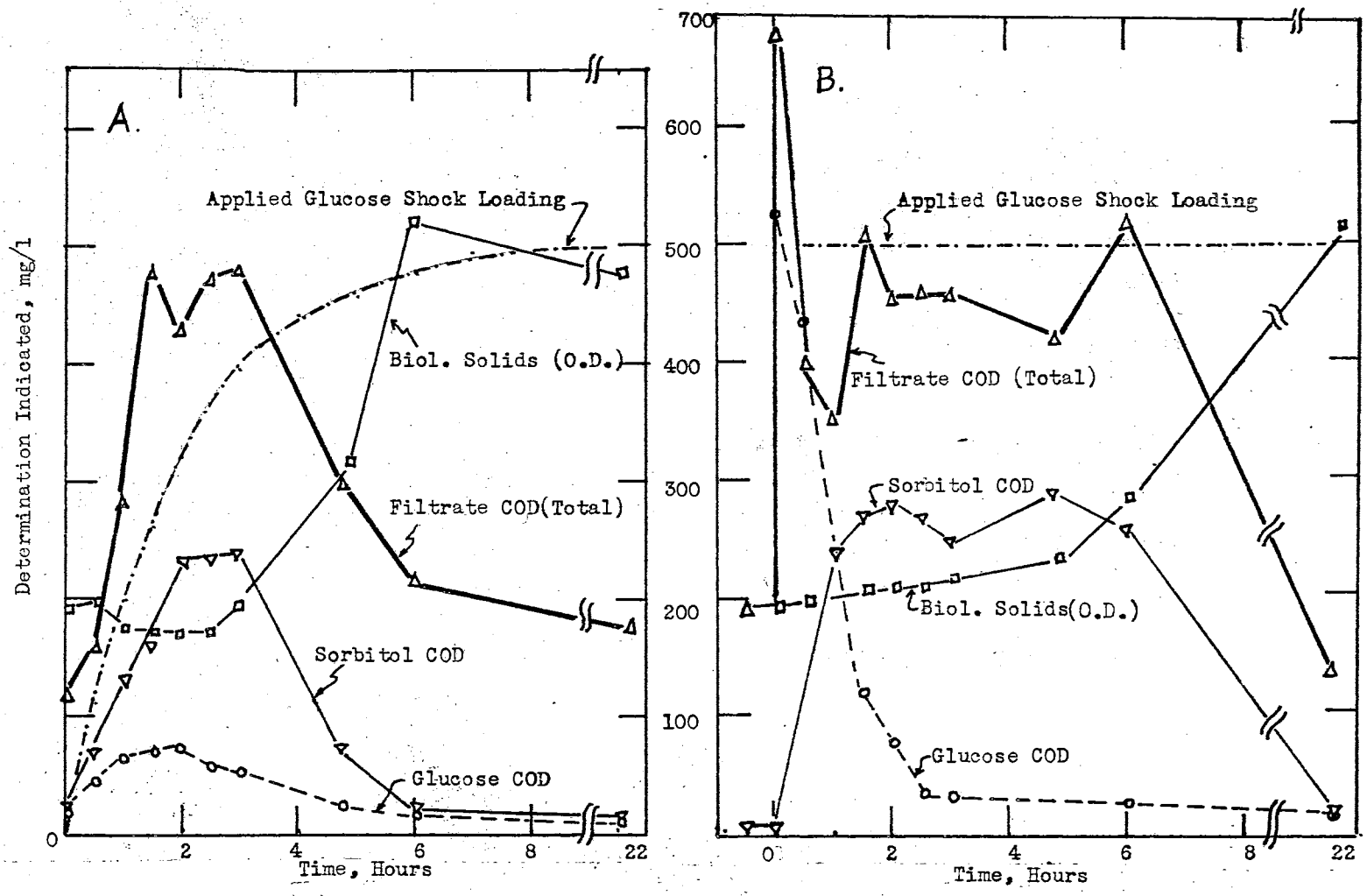

FIGURE 27 RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 500 MG/L SORBITOL TO. A, 500 MG/L SORBITOL PLUS 500 MG/L GLUCOSE, B, 500 MG/L SORBITOL PLUS 500 MG/L GLUCOSE ALSO IMMEDIATELY SHOCKED WITH 500 MG/L GLUCOSE

sludge to shock loading and its activity as shown in Figure 27-A was relatively much slower than that of Figure 19-A as can be observed from the sludge growth rate. Also some differences in the morphology of the sludges were noted; the sludge of the system shown in Figure 27 appeared to be more flocculent. Such differences may be due to the differences in predominating species.

b. Responses to Immediate Shock Loading: The system responses due to an "immediate" glucose shock loading are shown in Figure 27-B and Figure 28. The immediate shock load was accomplished by immediately injecting a concentrated solution of glucose into the mixed liquor aerator; as a result the initial concentration of the shock compound was immediately brought up to a specific level. The system shown in Figure 27-B initially was identical to the system of Figure 27-A since they were mixed prior to shock loading (see Materials and Methods). In the experiment shown in Figure 27-B the influent waste was changed from 500 mg/l sorbitol to 500 mg/l sorbitol plus 500 mg/l glucose and in addition glucose was immediately injected into the system so as to increase the level of glucose concentration in the system instantaneously to 500 mg/l. Again it can be seen that the rate of increase in biological solids in the system was relatively slow; however, glucose was rapidly removed (within 3 hours). It should be noted that the increase in the sorbitol concentration in the effluent was more rapid and that a much longer time was required for the return to a low equilibrium value, in comparison with the system shown in Figure 27-A. From these results, it is obvious that

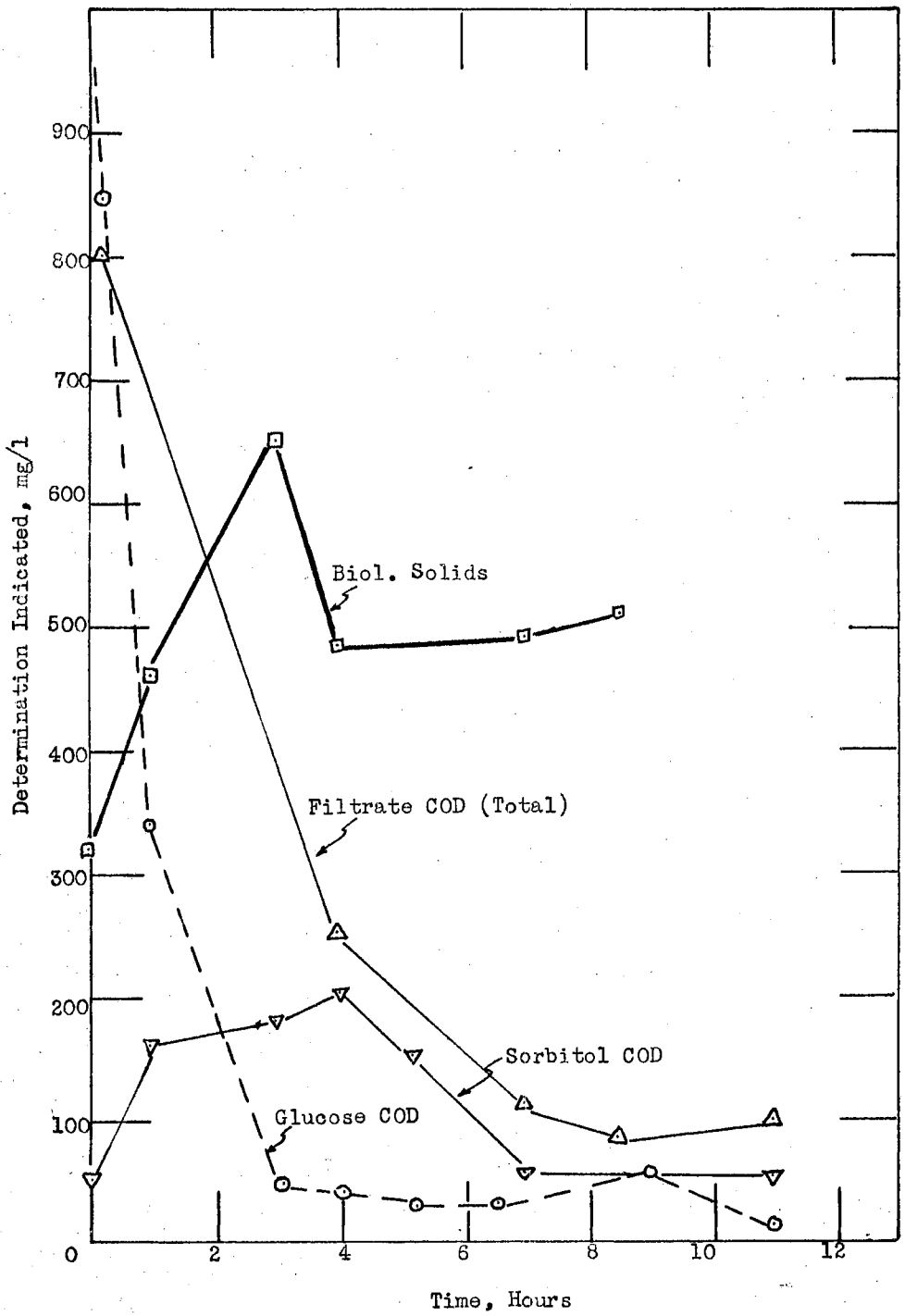

FIGURE 28 SHOCK LOAD RESPONSE IN THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME CONTINUOUSLY FED WITH 1000 MG/L SORBITOL THEN IMMEDIATELY SHOCKED WITH 1000 MG/L GLUCOSE

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.

Another immediate glucose shock loading experiment is shown in Figure 28. In this case, the system was acclimated to 1000 mg/l sorbitol for about three days, then glucose was rapidly injected into the system in order to bring the initial glucose concentration to 1000 mg/l, while the influent waste concentration was maintained at 1000 mg/l sorbitol as previously. It can be seen that glucose was rapidly removed, within no more than 3 hours. This time period also corresponds to a period of rapid increase in the biological solids and rapid decrease in total filtrate COD. However, it is apparent that there was an increase in the sorbitol level, indicating that glucose was removed at the expense of sorbitol. After 6 hours, sorbitol again was removed by the activated sludge to the same level as before the shock loading. The biological solids rapidly increased to a maximum at approximately 3 hours, then decreased, which indicates that the added glucose was rapidly metabolized and converted to biological solids. After glucose exhaustion the biological solids growth rate was reduced and the dilute out rate became greater, resulting in the drop in the biological solids level. The initial biological solids concentration (at zero time) as shown in Figure 28 appeared to be comparatively low in comparison to all the previous cases which were also fed with 1000 mg/l sorbitol influent. It was suspected that this could be due to an error in solids determination or in the sampling process, since normally the biological solids concentration level was

found to be between 400 to 500 mg/l for 1000 mg/l sorbitol feed (cf. Figure 17, 18, 19).

c. Effects of Detention Time: The experiments in continuous flow reported thus far were conducted at a 4 hour detention period. In the experimental results to follow, detention periods other than four hours were employed in order to determine what effect this variable might have on the shock-load responses of the systems. Results for an experiment is which a 2-hour detention time was employed are shown in Figure 29. It can be seen that at this detention period, the biological solids concentration which could be maintained in the system was relatively low. The system was initially maintained with an influent concentration of 1000 mg/l sorbitol; the biological solids level was only about 150 mg/l, which represents only about 15 per cent of the feed. The total filtrate COD of the effluent also remained relatively high (about 500 mg/l residual COD) indicating that the system was operated at the transition zone close to the dilute out point, which has been previously discussed under "Theoretical Concepts". It was rather difficult at times to maintain the system at its true equilibrium at this high dilution rate. It was found that a change of dilution rate from a 4-hour detention time to a 2-hour detention time resulted in an almost complete wash-out of the biological sludge population after 24 hours, and a new population mass was again established after a few days. It is possible that at the 2-hour detention time the system became very highly selective and only those organisms with a growth rate higher than 0.5 hr^{-1} could survive; presumably these organisms were not predominant at a lower

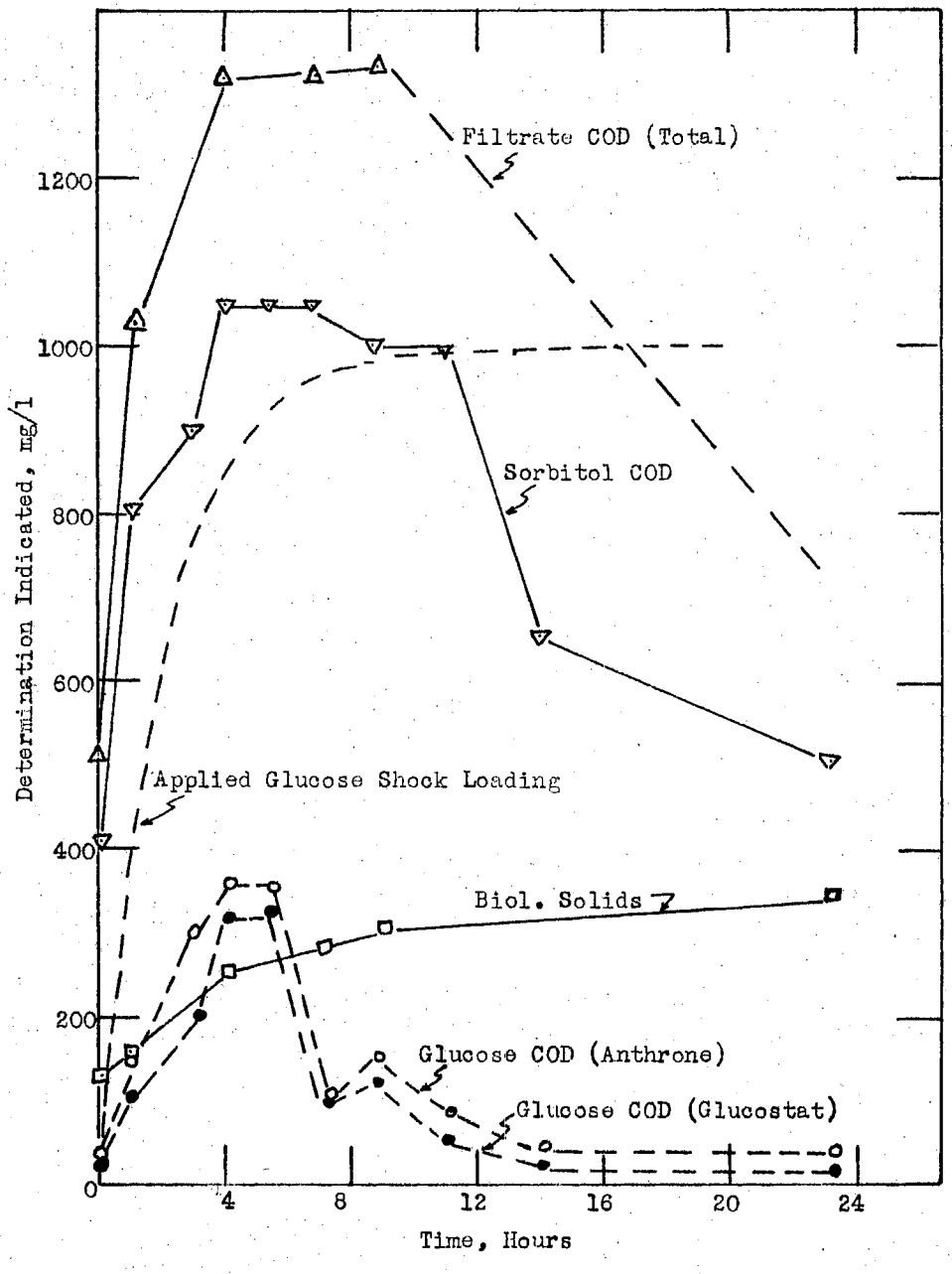

FIGURE 29 SHOCK LOAD RESPONSE IN THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 2-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 1000 MG/L GLUCOSE PLUS 1000 MG/L SORBITOL

dilution rate. Figure 29 shows the shock load response of the system which had been operating at a 2-hour detention period with 1000 mg/l sorbitol and was shock-loaded by changing the influent waste to 1000 mg/l sorbitol plus 1000 mg/l glucose. If such a shock loading condition had been applied to a system operated at a 4-hour detention time, both substrates would have been removed quite readily according to the results shown in Figure 19-B. However, for the 2-hour detention time it was found that there was an increase in the levels of both glucose and sorbitol in the effluent. The glucose level was again reduced to a low value after about 8 hours but the sorbitol level remained at over 1000 mg/l for almost 12 hours after the shock loading, indicating that at the early stage of shock loading, sorbitol metabolism was totally replaced by glucose metabolism, and sorbitol could not be removed until after glucose had been reduced to a very low level, which was after approximately 10 hours. The rate of increase in biological solids was relatively slow, due to the high dilute-out rate. The Glucostat test, which is quite specific for glucose, was also employed in this case to determine whether the glucose concentration determined by the anthrone test was due mainly to glucose or an intermediate(s) or end product(s) which might also give positive test with anthrone. The results of the Glucostat test indicate that the glucose as determined by the Glucostat reagent was slightly less than the glucose as determined by the anthrone test; however, the major portion of the glucose COD as determined by anthrone was glucose. The differences were normally well under 50 mg/l glucose COD. Hence, this finding helps

to confirm that glucose determined by the anthrone test was sufficiently valid and did represent the actual glucose in the systems.

The shock-load response of a system which was operated at a 16-hour detention time is shown in Figure 30. In this case the system was originally fed with 500 mg/l sorbitol, then was shock loaded with influent waste of 750 mg/l sorbitol and 750 mg/l glucose. It can be discerned that there was no significant increase in total filtrate COD, nor in glucose or sorbitol. The biological solids concentration of the system, however, increased in an amount relative to the increase in applied glucose shock-loading, indicating that the system could cope with the shock loading quite successfully. It is seen that the initial biological solids concentration of all the systems operated at a 16-hour detention time were relatively low and represented only approximately 30 per cent yields. It is possible that such low yields could be due to the effects of the long detention time which was employed. It is interesting to compare these results with those shown in Figure 23 in which the system was also acclimated to 500 mg/l sorbitol waste and shock-loaded with 750 mg/l sorbitol plus 750 mg/l glucose, but was operated at a 4-hour detention period. In the latter case a significant increase in the sorbitol level in the effluent was observed. It should be noted that for the 16-hour detention period the rate of applied glucose shock-loading was much slower than that of the 4-hour detention period, hence the system was provided with a longer period to adjust its growth rate to cope with the shock loadings.

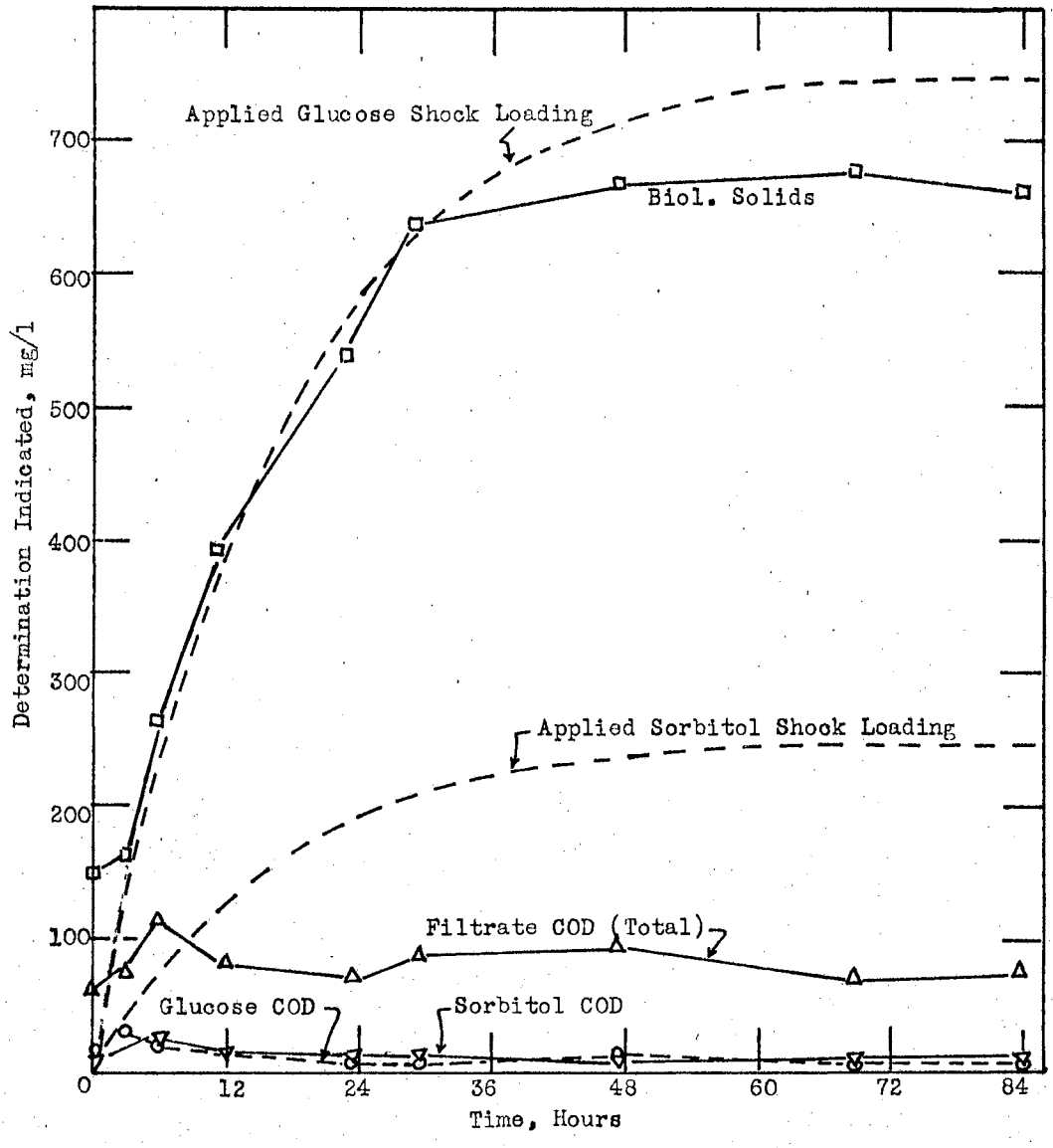

FIGURE 30 SHOCK LOAD RESPONSE IN THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 16-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 500 MG/L SORBITOL TO 750 MG/L SORBITOL PLUS 750 MG/L GLUCOSE

Another system in which the waste concentration was increased three fold, from 500 mg/l sorbitol to 1500 mg/l sorbitol, is shown in Figure 31. By comparison with the results shown in Figure 25, again it can be noted that the 16-hour detention period has provided the system with a greater capability for successful response to the shock loading, as less sorbitol appeared in the effluent. At the 16-hour detention time there was a slight increase in the total filtrate COD as well as the sorbitol COD for approximately 10 hours but the peak values were much less than those shown in Figure 25.

It was of interest to determine whether the system operated at the 16-hour detention time could respond to qualitative as well as quantitative shock loads of higher magnitude without releasing either glucose or sorbitol in the effluent. The results of such an experiment are presented in Figure 32. In this case the system was originally operated at the 16-hour detention time and was fed with 500 mg/l sorbitol; the system was then shock-loaded with six times its original influent concentration by changing the influent waste composition to 1500 mg/l glucose and 1500 mg/l sorbitol. The control system which was shock loaded with only 1500 mg/l sorbitol is shown in Figure 32-B. It can be seen that when glucose and sorbitol were present together in the waste influent, glucose was again preferentially utilized at the expense of sorbitol. After approximately 24 hours the sorbitol again was being reduced to a low level and there was a correspondingly high level of biological solids in the system. There appeared to be a considerable increase in the amount of intermediates released, which can be noted from the significant

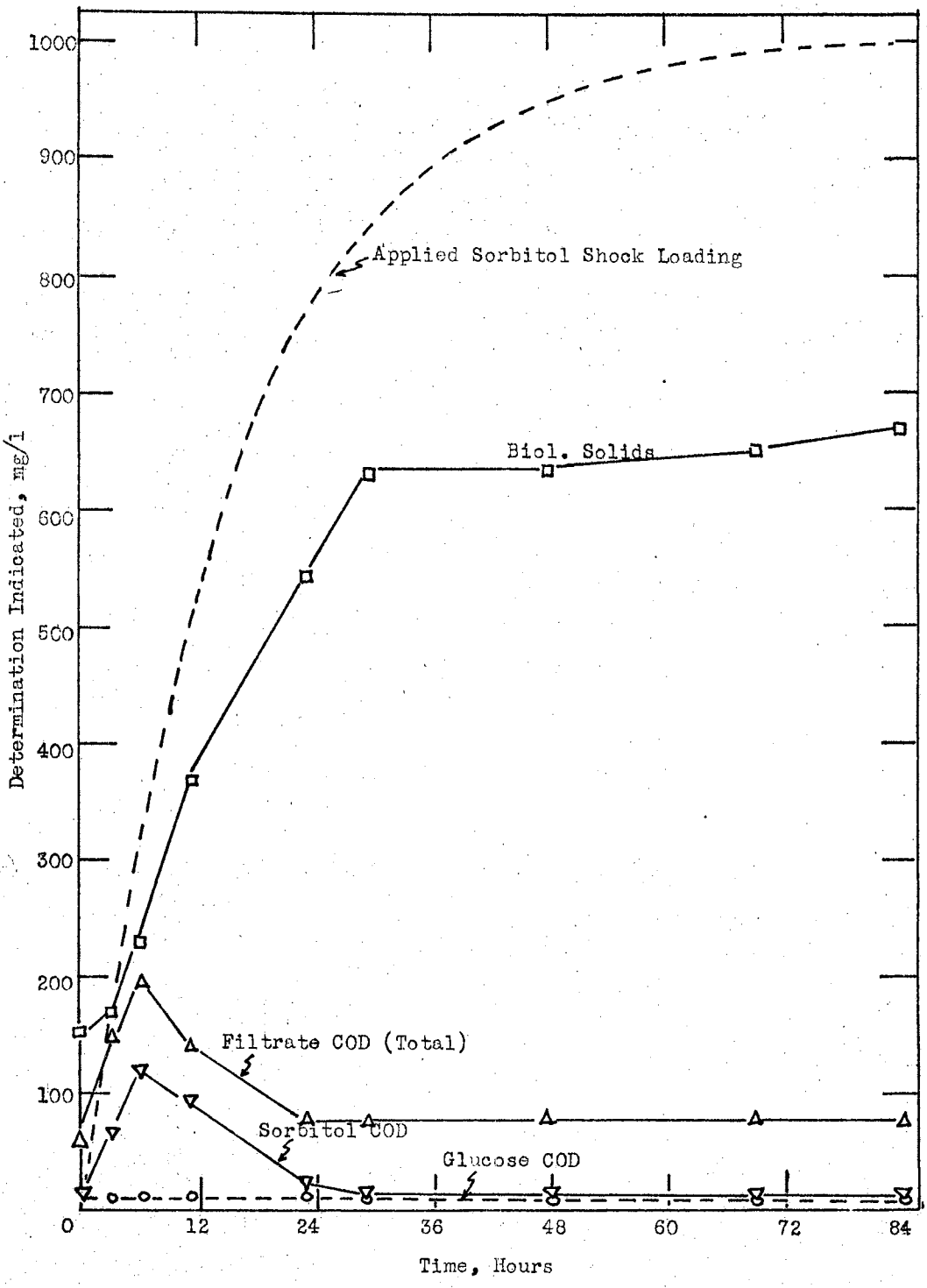

FIGURE 31 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 16-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 500 MG/L TO 1500 MG/L SORBITOL

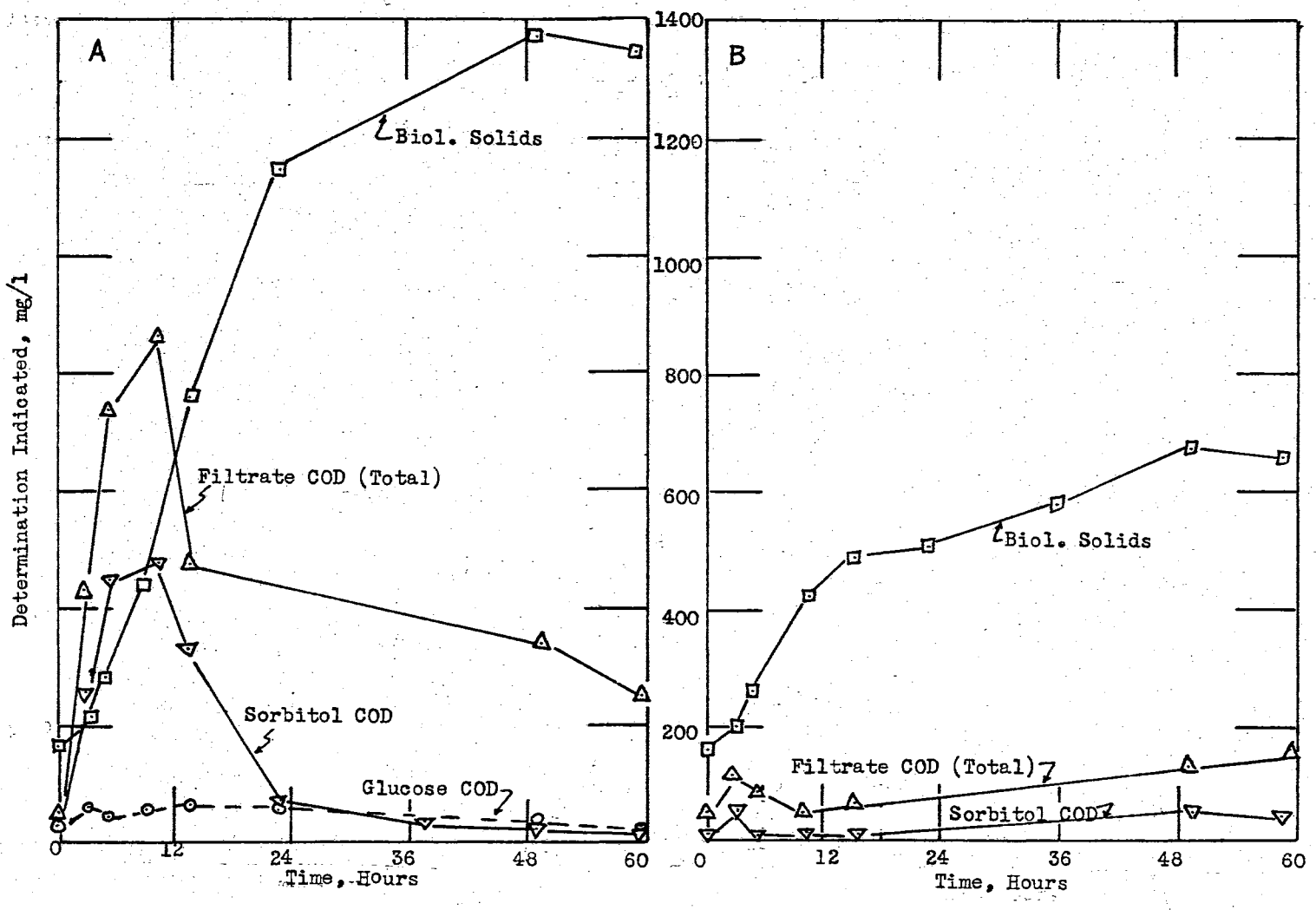

FIGURE 32 SHOCK-LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 16-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION. A, FROM 500 MG/L SORBITOL TO 1500 MG/L SORBITOL PLUS 1500 MG/L GLUCOSE. B, FROM 500 MG/L SORBITOL TO 1500 MG/L SORBITOL (CONTROL)

differences between the total filtrate COD and the sum of sorbitol COD and glucose COD. By comparing with the control system (Figure 32-B), it seems possible that the released intermediates were derived mainly from glucose since in the control system the difference between the filtrate total COD and the sorbitol COD was much less than that shown in Figure 32-A. However, it is not known what other effects glucose may have on sorbitol metabolism besides blockage of the sorbitol uptake i. e. the data are not sufficient to eliminate the possibility that glucose may cause intermediate to be released during sorbitol metabolism.

d. Effect of Nitrogen Deficiency on Shock Load Responses: In this portion of the study, experiments were conducted to determine the modes of response of continuous flow steady state systems under nitrogen deficient conditions. From growth rate studies in which the supply of nitrogen was varied (see appendix A), it was established that approximately 150 mg/l $(NH_4)_2SO_4$ would be required to supply sufficient nitrogen to metabolize 500 mg/l sorbitol under normal growth conditions in a batch system. Taking 0.7 as an approximate factor to convert the substrate concentrations to corresponding BOD values as that employed by Gaudy et al. (69), it was found that the minimum BOD:N ratio as obtained in this case would be approximately 10:1 which is much lower than the recommended limit of 20:1 as suggested by many investigators (20, 21, 22). Based on the results obtained in the present studies, those systems in which the ratio of nitrogen as $(NH_4)_2SO_4$ to carbon source is less than 150:500 can be regarded as nitrogen-limited systems.

Figure 33 shows the response of a nitrogen-limited system when it was shock-loaded with glucose plus sorbitol. The system was operated at a 4-hour detention period with the supply of nitrogen in the influents of both the shocked and the control systems maintained at 300 mg/l $(NH_4)_2SO_4$. Based upon the limit of nitrogen which has been established, the supply used in this experiment would only be sufficient for 1000 mg/l of either glucose or sorbitol. Therefore, in the shocked system, when the waste influent was changed from 1000 mg/l sorbitol to 1000 mg/l sorbitol plus 1000 mg/l glucose the system was disproportionally overloaded with organic carbon source. It is evident from the results shown for the shocked system that the level of biological solids protein remained relatively constant and at about the same level as that in the control system, indicating that the system could not synthesize protein to respond to the shock load. However, the carbohydrate content, expressed as glucose, increased to more than twice the cellular carbohydrate content of the control system. There was a slight increase in the sorbitol level in the shocked system shortly after the shock but it decreased after about 6 hours. The residual total filtrate COD which was higher in the shocked system than in the control cannot be accounted for as either glucose COD or sorbitol COD. The biological solids in the shocked system rose sharply in a manner related to the rate at which the glucose shock loading was applied. From these results, it can be stated that the system could respond quite successfully at this level of nitrogen supply, even though the cells' ability to

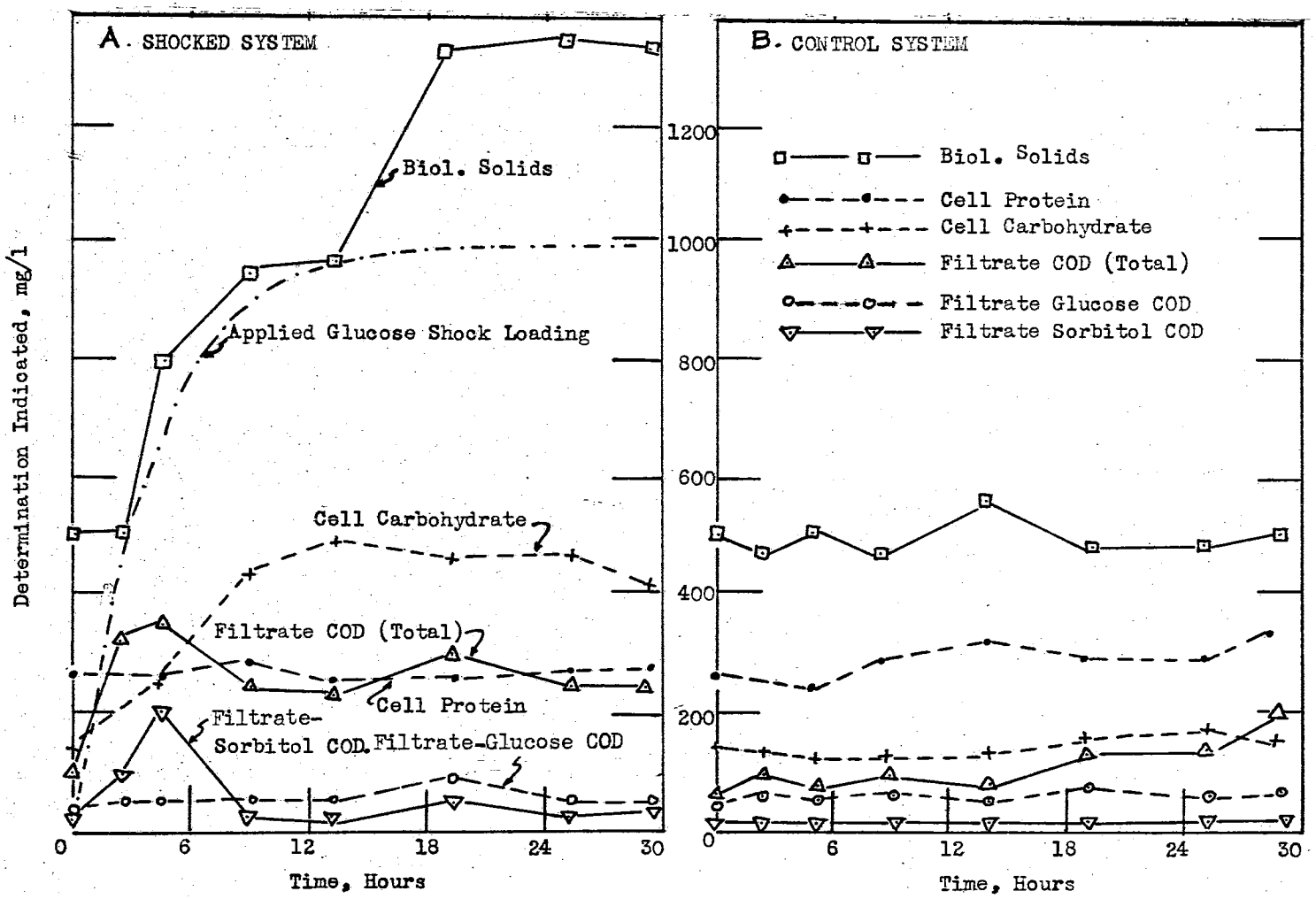

FIGURE 33 A, SHOCK LOAD RESPONSE IN A NITROGEN-LIMITED STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4 HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 1000 MG/L SORBITOL PLUS 1000 MG/L GLUCOSE. B, CONTROL SYSTEM FED WITH 1000 MG/L SORBITOL

synthesize protein was limited. It should be noted that the yield of biological solids was almost 70 per cent of the supply of organic carbon source which was considerably higher than the yield obtained under normal growth conditions (cf. Figure 19-B) which was only about 50 per cent of the supplied organic carbon concentration.

Another test of the response of systems under nitrogen-limited conditions is shown in Figure 34. In this instance, two systems were fed with 1000 mg/l sorbitol and 300 mg/l $(NH_4)_2SO_4$ as nitrogen source, then were shock loaded with 2000 mg/l sorbitol influent without increasing the supply of nitrogen; in addition to the sorbitol shock load, one system also received an immediate shock by injection of glucose which instantaneously increased the glucose concentration in the system to 1000 mg/l (Figure 34-A). It can be seen that in both systems there were rapid increases in the biological solids as well as high total filtrate COD in the effluents. Even after 24 hours of shock loading the effluent still contained a high total filtrate COD which could not be accounted for as either glucose COD or sorbitol COD, indicating that sorbitol itself could produce high concentrations of metabolic intermediates under nitrogen-limited conditions. An important observation which can be made from the results of this study is that the system which was shock-loaded with additional glucose showed a much greater increase in the sorbitol level in comparison to the control system in which no glucose was added. It is apparent that glucose interfered with sorbitol metabolism and that glucose was metabolized preferentially at the expense of sorbitol.

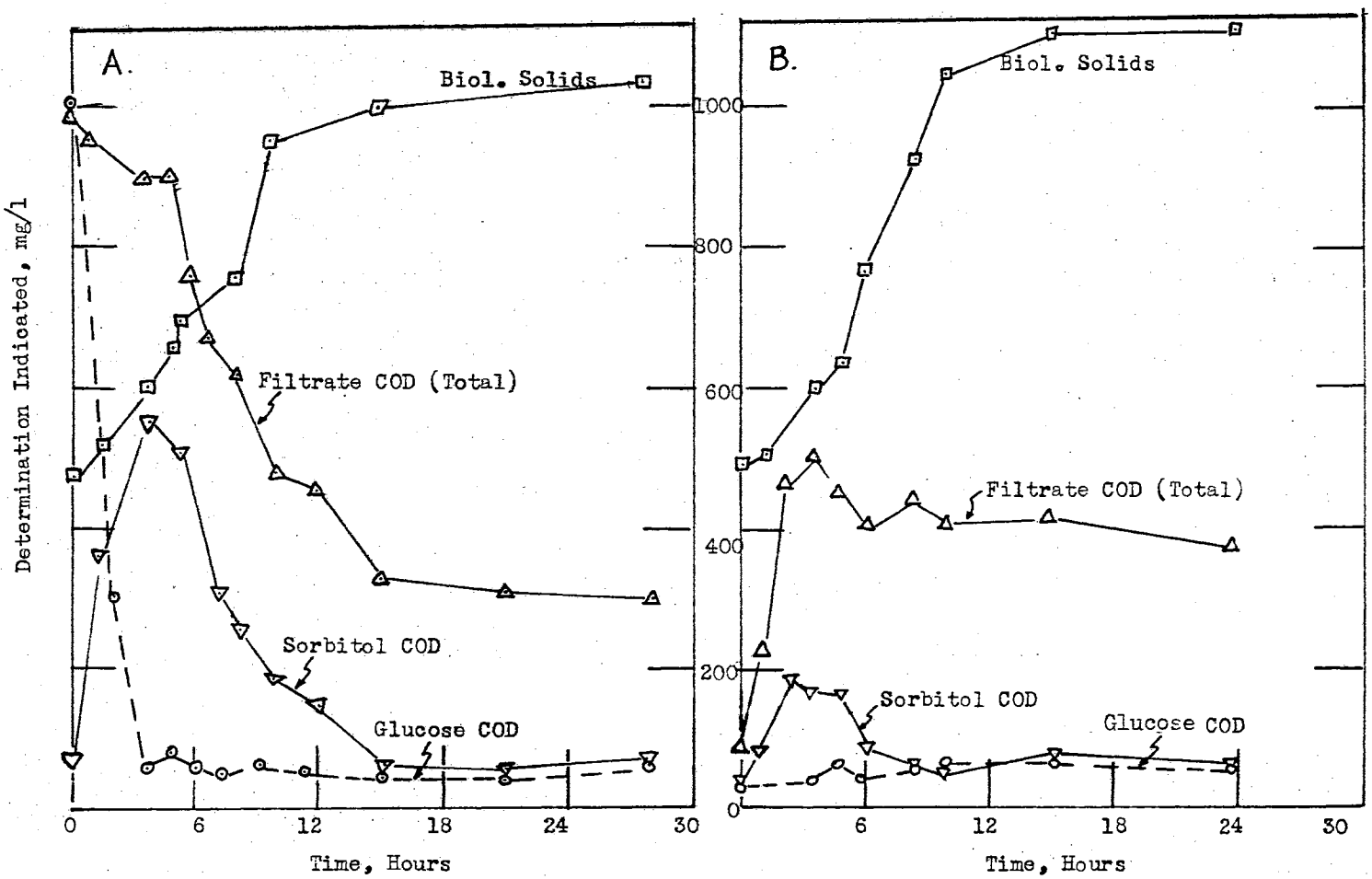

FIGURE 34 SHOCK LOAD RESPONSE IN A NITROGEN-LIMITED STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME. A, AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 2000 MG/L SORBITOL THEN IMMEDICATELY INJECTED WITH 1000 MG/L GLUCOSE. B, SAME AS A BUT GLUCOSE INJECTION WAS OMITTED

Figure 35 shows the results obtained with a nitrogen deficient system which was previously fed with 1000 mg/l sorbitol and 300 mg/l $(NH_4)_2SO_4$ supplied as nitrogen source. The system was then shock-loaded with waste influent consisting of 1500 mg/l sorbitol plus 1500 mg/l glucose while maintaining the supply of nitrogen at 300 mg/l $(NH_4)_2SO_4$. It can be seen that glucose was readily removed and sorbitol was only partially removed. The results shown in Figure 34-B in which a system also acclimated to 1000 mg/l sorbitol, was shock-loaded with an even greater amount of sorbitol (2000 mg/l), indicate the peak of sorbitol concentration in the effluent was less than 200 mg/l. However, in the present case over 600 mg/l sorbitol was released even though the system was shock-loaded with only 1500 mg/l sorbitol. Hence, it is obvious that the presence of glucose prevented the removal of sorbitol. Even after 28 hours the system still could not respond successfully; the sorbitol level remained at about 500 mg/l and the total COD of the filtrate was approximately 1000 mg/l. It should be noted that for similar shock-loading conditions, but with an ample supply of nitrogen (see Figure 26), the system could respond readily within 15 hours and yielded only 250 mg/l of total filtrate COD in the effluent. It is obvious that the amount of nitrogen supplied had limited the system efficiency in the present case.

A more rigorous test involving preferential glucose removal is shown in Figure 36. In this case a system was originally fed an influent waste of 1000 mg/l sorbitol and 150 mg/l $(NH_4)_2SO_4$ as nitrogen source yielding a BOD:N ratio of approximately 20:1.

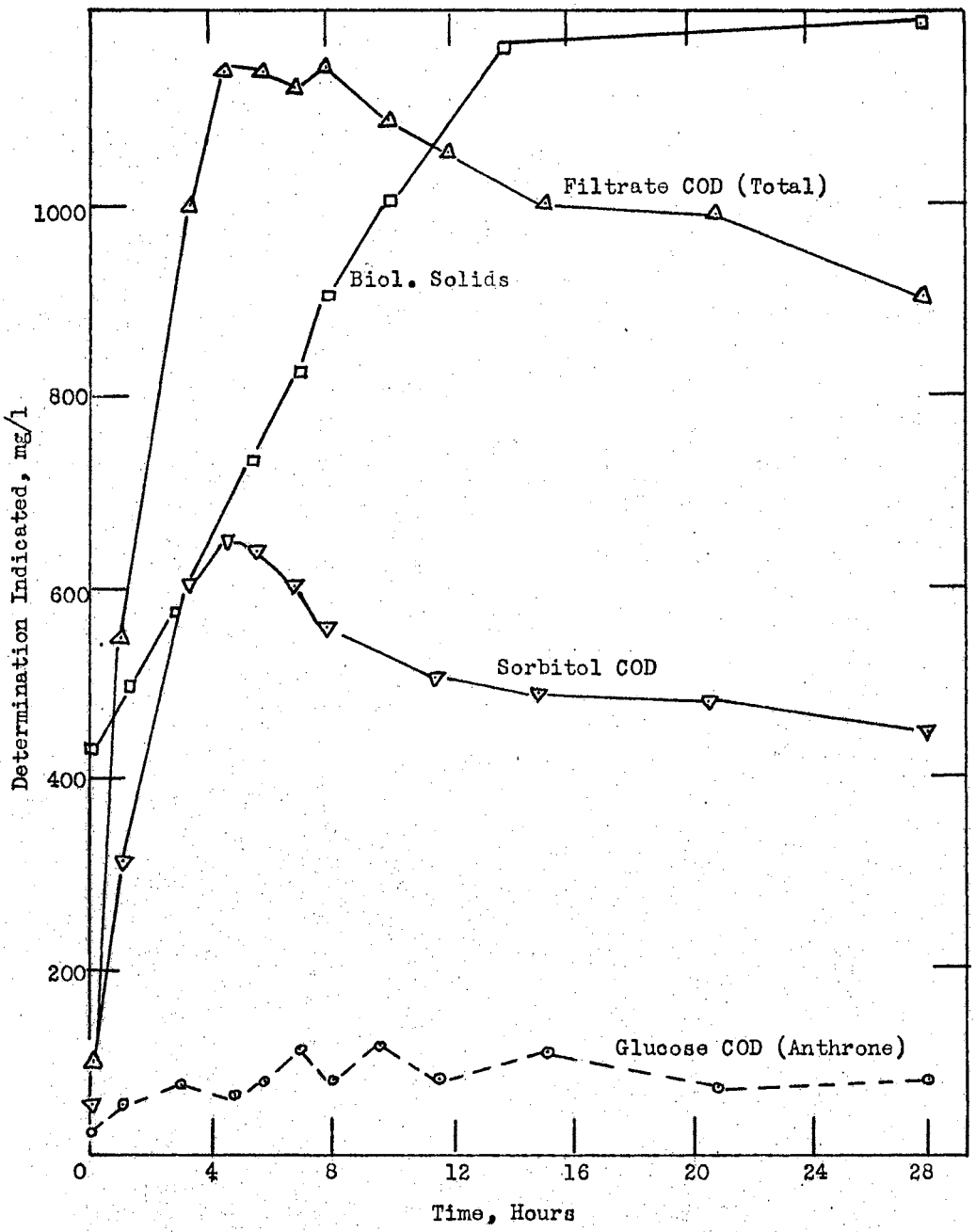

FIGURE 35 SHOCK LOAD RESPONSE OF THE NITROGEN-LIMITED STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 1500 MG/L SORBITOL AND 1500 MG/L GLUCOSE

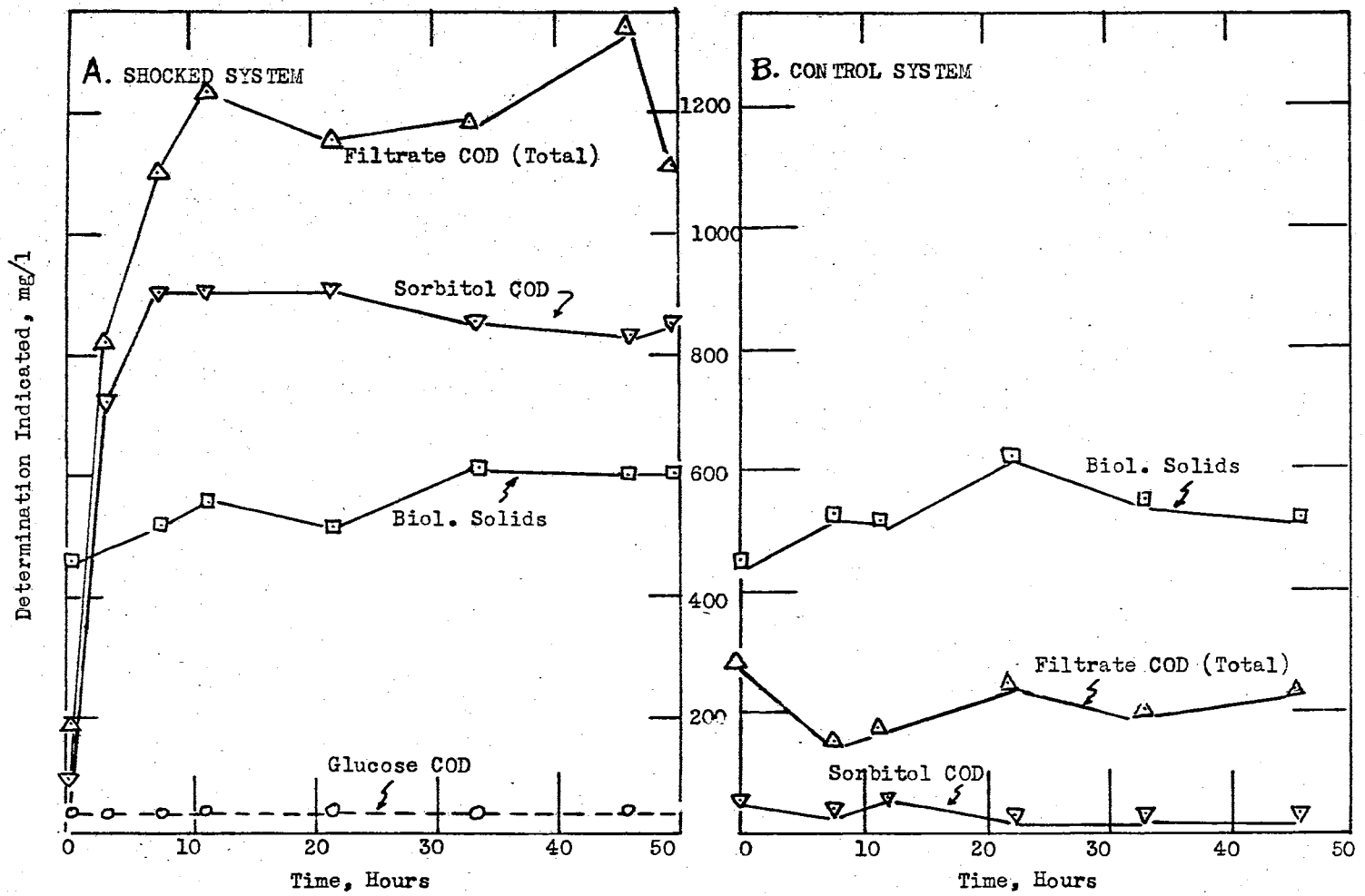

FIGURE 36 SHOCK LOAD RESPONSE IN THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME WITH SEVERE NITROGEN DEFICIENCY. A, AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L SORBITOL TO 1000 MG/L SORBITOL PLUS 1000 MG/L GLUCOSE. B, CONTROL SYSTEM (NO CHANGE) FED WITH 1000 MG/L SORBITOL

The shock load was then applied by changing the influent composition from 1000 mg/l sorbitol to 1000 mg/l sorbitol plus 1000 mg/l glucose. For comparison another system in which the influent remained at 1000 mg/l was maintained as a control system. It can be seen that in the shocked system only one-half of the total supplied substrate, apparently mostly glucose, was removed and the remaining substrate consisted mainly of sorbitol. It can be seen that less than 40 mg/l glucose, as determined by the anthrone test, remained in the effluent. However, in the control system (Figure 36-B) in which no glucose was added in the influent, the sorbitol concentration remained at a very low level, less than 50 mg/l during the entire experimental period. It is, therefore, obvious that the glucose replaced or inhibited sorbitol metabolism. In both systems the biological solids levels remained at approximately 500 mg/l indicating that in the shocked system the supply of nitrogen had definitely limited the total growth of the sludge mass. Even after a period of 50 hours after shock loading, the system did not show any tendency to remove more sorbitol. In both systems, analyses of the effluent indicate considerable differences between the total filtrate COD and the COD due to sorbitol and glucose which can be attributed to metabolic intermediates released into the medium.

2. Responses of Glycerol-Acclimated Systems to Glucose Shock Loadings

In previous studies under batch operation by Krishnan(68), it was found that glucose also could exert inhibitory effects on glycerol removal. The purpose of this portion of the study was to determine

the extent of such effects under continuous flow steady state conditions which are more akin to the actual operation of the waste treatment process and to compare results with those of the glucose-sorbitol systems. Detention periods of four hours were employed for all glycerol-acclimated systems herein reported.

The response of a system acclimated to 1000 mg/l glycerol influent which then was shock-loaded with 1000 mg/l glycerol plus 500 mg/l glucose as waste influent is shown in Figure 37. It is seen that the system could readily respond to this shock loading since the levels of the total filtrate COD, the glucose COD and the glycerol COD in the effluent were not significantly changed in comparison to those exhibited by the control system (Figure 37-B)

A greater shock load was employed in the experiment shown in Figure 38. In this case the systems were also initially fed 1000 mg/l glycerol; the shock was then applied by changing the waste influent of one system to 1000 mg/l glycerol plus 1000 mg/l glucose (Figure 38-A), and changing the other system to 1000 mg/l glycerol plus 2000 mg/l glucose (Figure 38-B). It is seen that even with increases in influent loading of two and three times the original influent loading by addition of glucose, the systems still could respond readily to such shock loading. There were increases in the glycerol level in both systems in the early stage after changing the waste flow but both systems recovered readily within about four hours. The level of the total filtrate COD in the effluent of the system shock-loaded with 2000 mg/l glucose was considerably higher than than that in the system shocked with 1000 mg/l glucose, especially during the first few hours after shock loading began. Since the

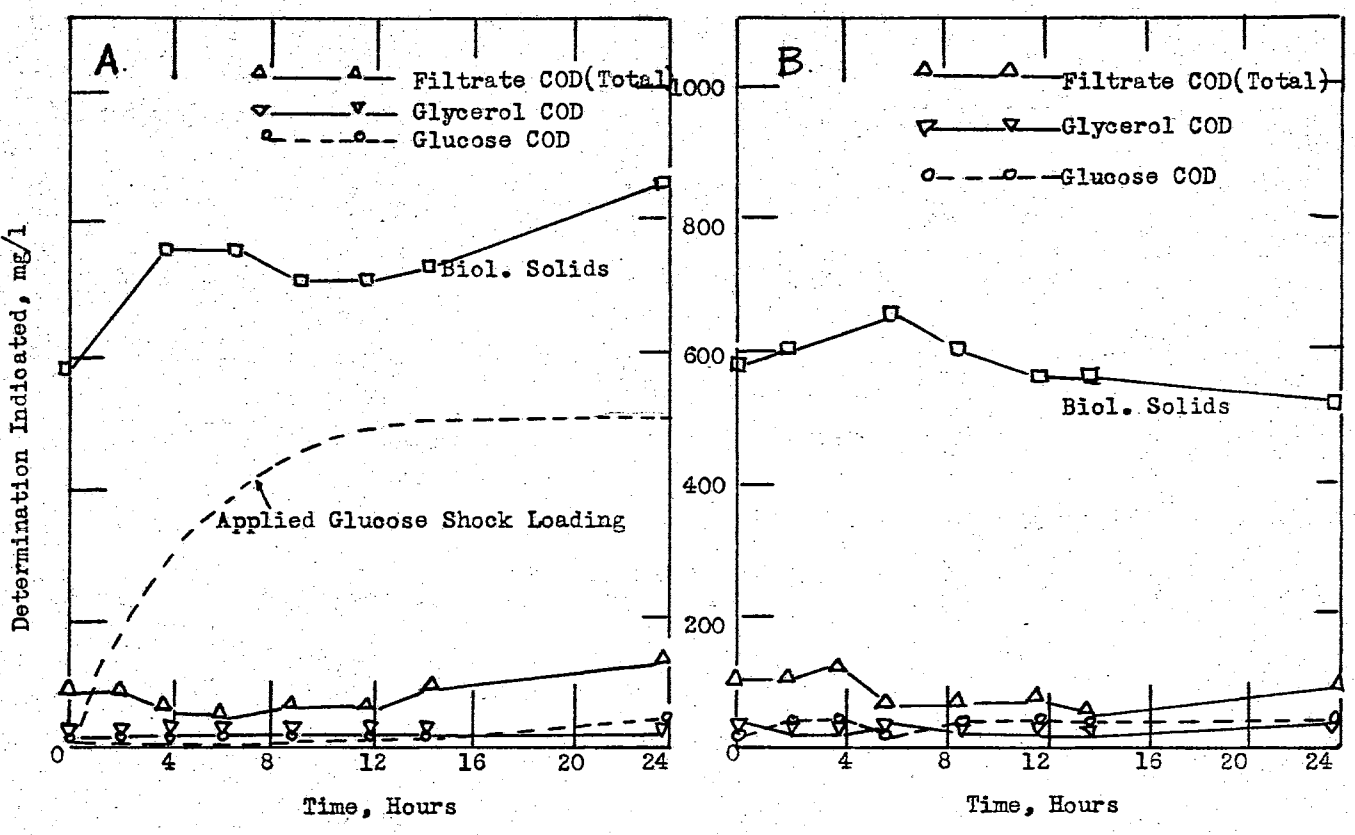

FIGURE 37 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME. A, AFTER CHANGING INFLUENT WASTE FROM 1000 MG/L GLYCEROL TO 1000 MG/L GLYCEROL PLUS 500 MG/L GLUCOSE. B, CONTROL SYSTEM

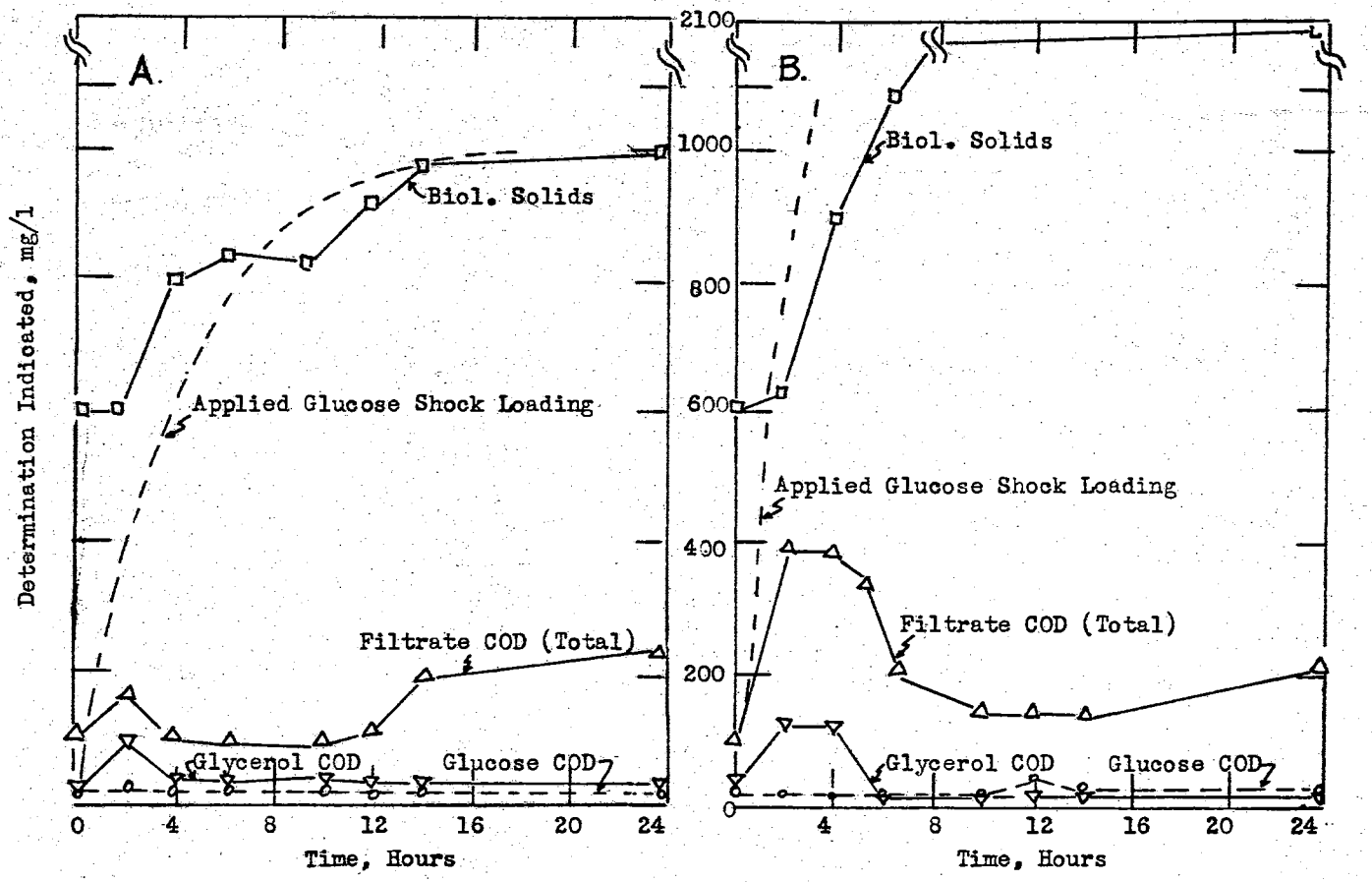

FIGURE 38 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE. A, FROM 1000 MG/L GLYCEROL TO 1000 MG/L GLYCEROL PLUS 1000 MG/L GLUCOSE. B, FROM 1000 MG/L GLYCEROL TO 1000 MG/L GLYCEROL PLUS 2000 MG/L GLUCOSE

difference between the two systems was only the applied concentration of glucose, the larger amount of the total filtrate COD could be attributable only to the release of metabolic intermediates upon the rapid utilization of glucose. It should also be noted that for the system shock-loaded with 2000 mg/l glucose, a slightly higher concentration of glycerol was released in the effluent. The slight increase in the total filtrate COD in the later stage of the experiment could be due to fluctuations in the system performance.

The shock load response of systems which were acclimated to a lower concentration of glycerol and subjected to high glucose shock loadings are shown in Figure 39. These systems were initially acclimated to 500 mg/l glycerol; one system was then shock-loaded with 500 mg/l glycerol plus 1000 mg/l glucose as waste influent (Figure 39-A) whereas another system was shock-loaded with 500 mg/l glycerol plus 1500 mg/l glucose influent (Figure 39-B). It is seen that there were increases in both glucose and glycerol levels in the early stage of shock loading. For the system shock-loaded with 1000 mg/l glucose, both glucose and glycerol returned to low levels after about 5 hours. For the system which was shock-loaded with 1500 mg/l glucose, the glucose concentration rose sharply but was reduced to a low level within less than 8 hours. The glycerol COD level, however, was reduced at much slower rate even though only 500 mg/l of glycerol was fed in the influent. It is apparent that the presence of glucose interfered with the rate of removal of glycerol. Moreover, the system shock-loaded with 1500 mg/l glucose

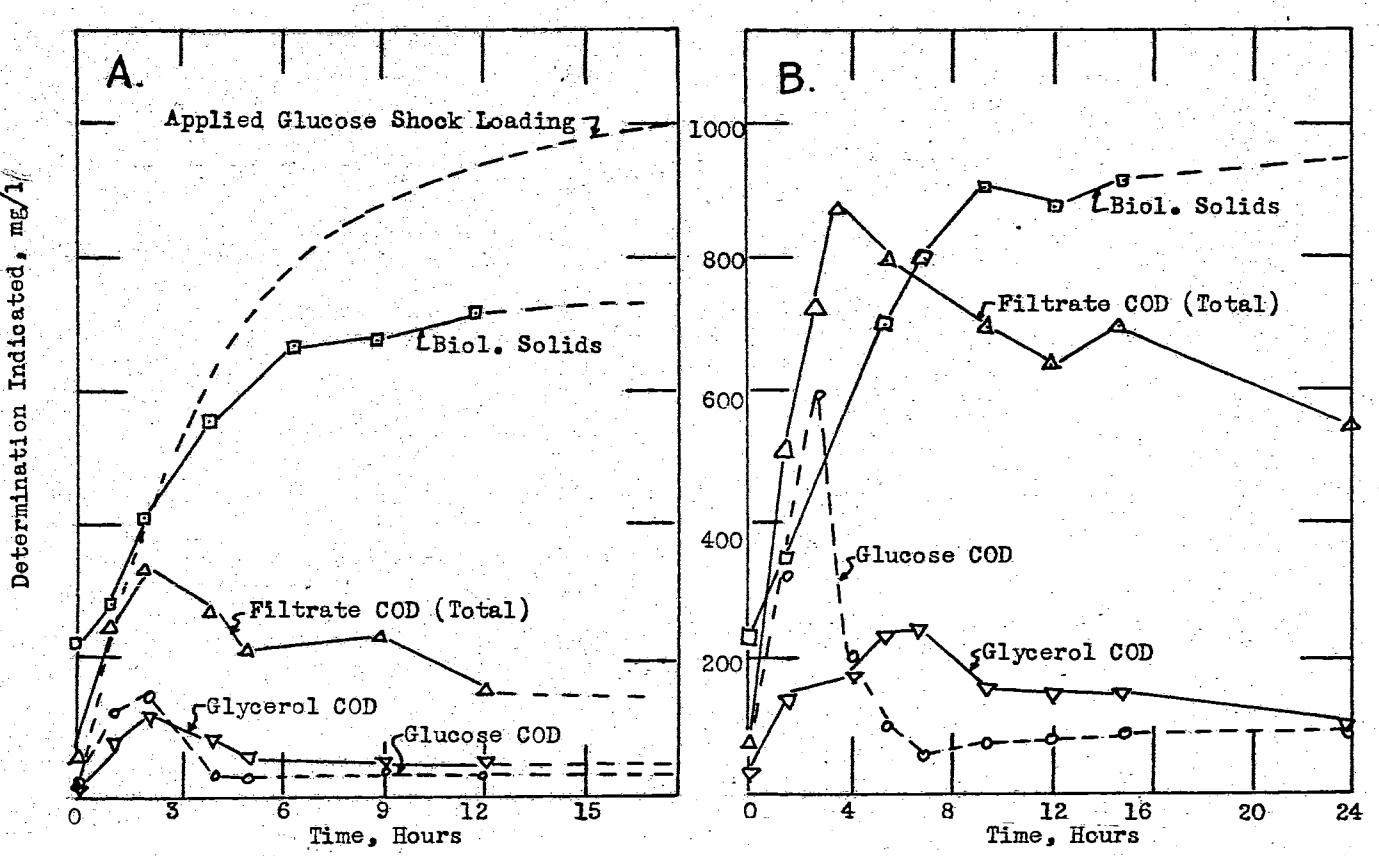

FIGURE 39 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE. A, FROM 500 MG/L GLYCEROL TO 500 MG/L GLYCEROL PLUS 1000 MG/L GLUCOSE. B, FROM 500 MG/L GLYCEROL TO 500 MG/L GLYCEROL PLUS 1500 MG/L GLUCOSE

(Figure 39-B) showed a substantial amount of COD remaining in the filtrate which was being reduced at a very slow rate. The response of the systems as measured by increase in biological solids was also immediate, and the cells apparently did not require any acclimation to glucose.

It was felt that a more rigorous test to determine whether glucose is utilized in preference to glycerol by glycerol-acclimated cells could be obtained under a severe nitrogen-limited condition. Results for such an experiment are shown in Figure 40. In this case two identical systems were set up; both were acclimated to 1000 mg/l glycerol as waste influent with 150 mg/l $(NH_4)_2SO_4$ supplied as nitrogen source. One system was then shock-loaded by changing the influent feed to 1000 mg/l glycerol plus 1000 mg/l glucose while another system was maintained as a control to be used for comparison. It can be readily seen that in the presence of glycerol as a sole carbon source, the system removed about 80 per cent of the glycerol supplied while about 200 mg/l of glycerol remained in the effluent. However, when both glucose and glycerol were fed concurrently to the system, glucose metabolism almost entirely replaced glycerol metabolism. The systems were maintained for a period of 50 hours after the shock; still there was no indication that the glycerol level in the shocked system would be reduced. These results definitely show a clear-cut preference for glucose over glycerol by the activated sludge system even though the population was previously acclimated to glycerol and both substrates were applied in equal amounts (1000 mg/l of each). The biological solids

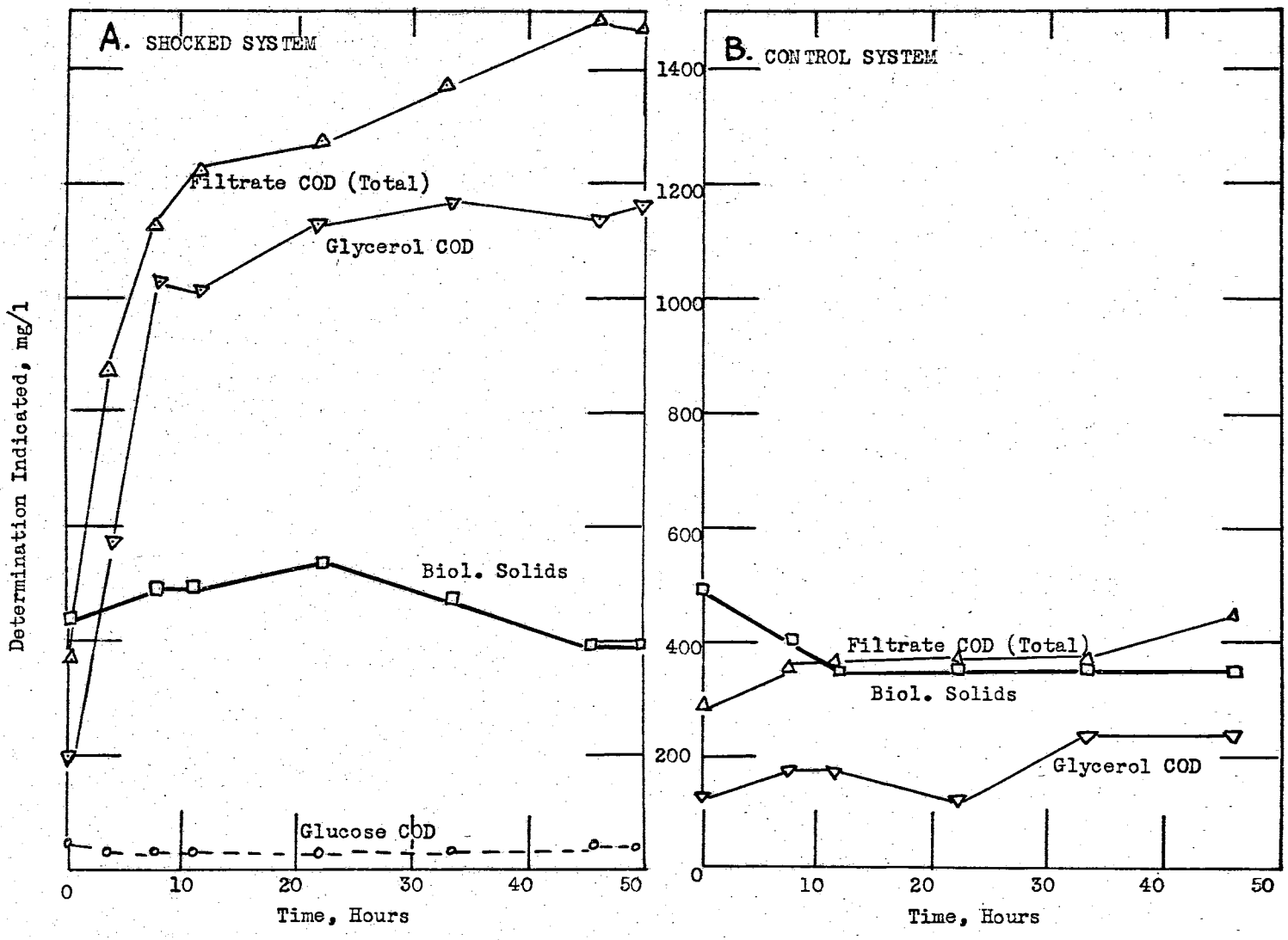

FIGURE 40 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME WITH SEVERE NITROGEN DEFICIENCY. A, AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L GLYCEROL TO 1000 MG/L GLYCEROL PLUS 1000 MG/L GLUCOSE. B, CONTROL SYSTEM (NO CHANGE) FED WITH 1000 MG/L GLYCEROL

in both systems remained relatively constant and both remained at about the same level indicating that the supply of nitrogen had limited growth.

The modes of response of the glycerol-acclimated system under nitrogen-limited conditions when it was shock-loaded with glucose are shown in Figure 41. The nitrogen supplied in this case was fixed at 300 mg/l $(NH_4)_2SO_4$ which would only suffice for balanced removal of 1000 mg/l of carbon source, according to the results obtained from the batch studies. It can be seen that the system could respond quite successfully when it was shock loaded with 1000 mg/l glycerol plus 1000 mg/l glucose even though the ratio of nitrogen to substrate was reduced by one half. The protein content of the sludge remained at a relatively constant level and at about the same level as the control unit, whereas the carbohydrate content of the sludge increased sharply to about three times its level prior to shock loading. The biological solids level increased to over 1600 mg/l indicating that a substantial portion of the supplied substrates were synthesized into cell constituents. The levels of both glucose and glycerol remaining in the filtered effluent of both systems were under 50 mg/l indicating that both substrates were readily removed by the systems. The total COD in the filtrate of the shocked system, however, still remained over 200 mg/l. It was noted that the sludge under this condition was highly gelatinous which presented much difficulty both in centrifugation and filtration due to its light weight and tendency to clog the filter. The biological solids level near the end of the

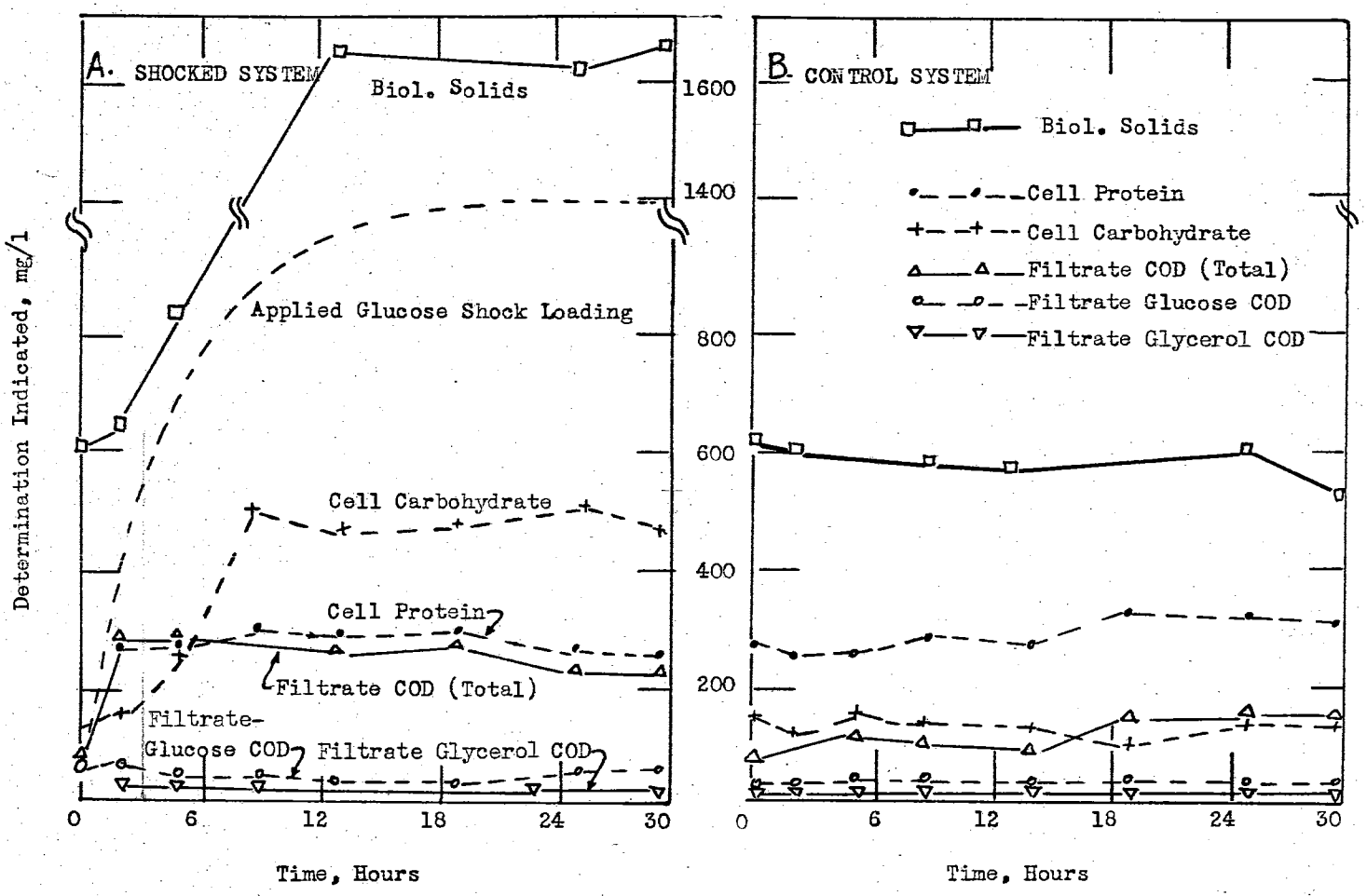

FIGURE 41 A, SHOCK LOAD RESPONSE IN A NITROGEN-LIMITED STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE FROM 1000 MG/L GLYCEROL TO 1000 MG/L GLYCEROL PLUS 1000 MG/L GLUCOSE. B, CONTROL SYSTEM FED WITH 1000 MG/L GLYCEROL

experiment was unusually high and could be due to errors caused by inorganic salts occluded with the gelatinous mass.

3. Responses of Glucose-Acclimated Systems to Sorbitol Shock Loading

The response of a glucose-acclimated system to sorbitol shock loading is shown in Figure 42. The system was initially acclimated to 1000 mg/l glucose as waste influent with an ample supply of nitrogen as employed for a normal growth system (see Experimental Protocol). The system was then shock-loaded by changing the waste influent from 1000 mg/l glucose to 1000 mg/l sorbitol. It should be noted that the shock load as applied in this case was primarily the qualitative load. It is seen that the system could not respond readily. The sorbitol level in the shocked system increased rapidly in the early stages but began to decrease after about four hours. The biological solids of the shocked system also decreased in the early stages but recovered after about four hours. Approximately eight hours after shock loading the full transition had occurred and the system could readily remove sorbitol.

Experiments in which the systems were shock-loaded with combinations of both glucose and sorbitol are shown in Figure 43. The systems were initially acclimated to 1000 mg/l glucose influent before they were shock-loaded. The waste influent of one system was then changed from 1000 mg/l glucose to 500 mg/l glucose plus 500 mg/l sorbitol (Figure 43-A). In the other system (Figure 43-B) the influent was changed from 1000 mg/l glucose to 1000 mg/l

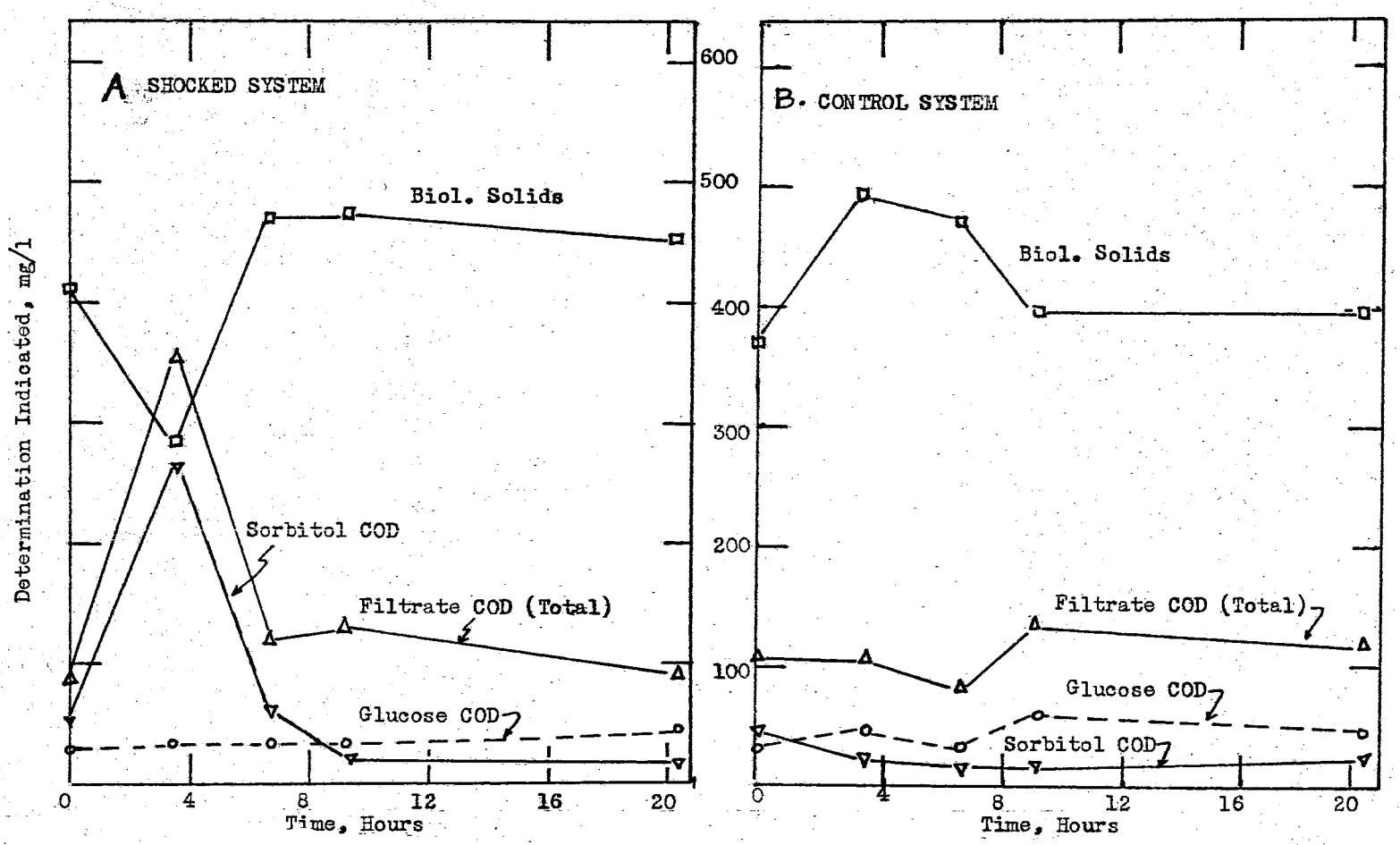

FIGURE 42 A, SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION FROM 1000 MG/L GLUCOSE TO 1000 MG/L SORBITOL. B, CONTROL SYSTEM FED WITH 1000 MG/L GLUCOSE

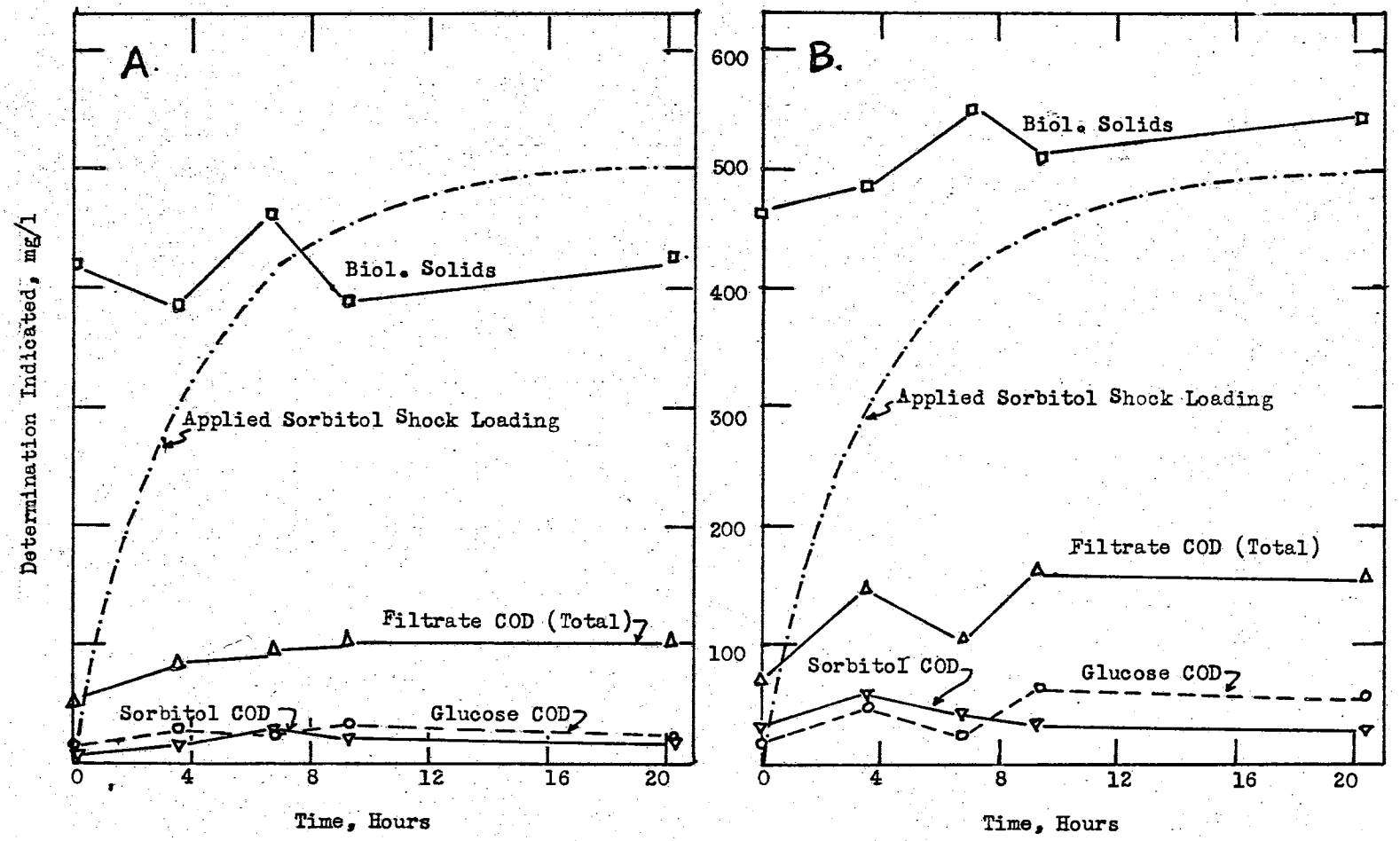

FIGURE 43 SHOCK LOAD RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 4-HOUR DETENTION TIME AFTER CHANGING INFLUENT WASTE COMPOSITION. A, FROM 1000 MG/L GLUCOSE TO 500 MG/L GLUCOSE PLUS 500 MG/L SORBITOL. B, FROM 1000 MG/L GLUCOSE TO 1000 MG/L GLUCOSE PLUS 500 MG/L SORBITOL

glucose plus 500 mg/l sorbitol. It is seen that both systems responded quite readily to such changes since both glucose and sorbitol levels in the effluent still remained at a relatively low level after shock loading. The system which was shock-loaded with 1000 mg/l total substrates (Figure 43-A) indicated a slightly lower total COD in the filtrate and the concentration of the biological solids was also slightly lower. There were considerable fluctuations in the biological solids level shown in this case which could be attributable to some difficulties encountered in the sampling processes.

CHAPTER VI

DISCUSSION

A. Batch-Operated Systems

1. Substrate Interactions in "Young Cell" Populations.

The purpose of this portion of the studies was to gain a better insight into substrate interaction phenomena with the hope that as a result such phenomena could be readily predicted and understood on the basis of biochemical principles. The studies were made under conditions of "severe" shock loading in which shock compounds were introduced while the cells were actively metabolizing other carbon sources. It was felt that such conditions would be more akin to the way in which the shock might be applied in actual practice in waste water treatment. Substrate interactions should be readily observable as changes in the removal course of each waste component.

It can be readily seen from results of the present study that suppressing effects between compounds do not seem to be limited to combinations of only a few compounds and that there are varying degrees of suppressive or repressive response which are possible, depending upon the compounds employed. From results such as these it would appear that general patterns are beginning to evolve from which it may eventually be possible to predict the response of a system to a particular shock load.

A general metabolic flow chart for compounds of current interest is shown in Figure 44. Sorbitol, mannitol and dulcitol are metabolized generally via similar routes. All of these hexitols have been reported to be metabolized primarily by two routes depending on the microbial species. In one reaction sequence, oxidation at the free hexitol level yields hoxose, which is further phosphorylated and enters the oxidative pathway as phosphorylated hexose. Another route involves initial phosphorylation of the hexitol which is then oxidized, thus also yielding the phosphorylated hexose such as fructose 6-phosphate, or glucose 6-phosphate (70). Recent studies have also found that acclimation to one type of polyalcohol, in many bacterial species, can automatically confer acclimation to others (71, 72, 73), this also indicates that all of these hexitols are closely related in the way in which they are biochemically assimilated. Glucose, galactose and fructose also enter the same metabolic pathway at the level of phosphorylate hexoses. Hence, it may be discerned that all the hexitols and hexoses under study are metabolized via closely related pathways which may yield common intermediary metabolite(s). In the previous studies, it has been also shown that fructose possesses an inhitory effect similar to that of glucose (7). By analogy with metabolite repression, as proposed by Mandelstam (55), it can be reasoned that glucose, fructose and galactose exert their suppressive effects by causing an immediate build-up of intermediary metabolic product(s). The suppressing effect of glucose and fructose was quite potent; galactose, however, appeared to possess a lesser inhibitory effect. It should be noted

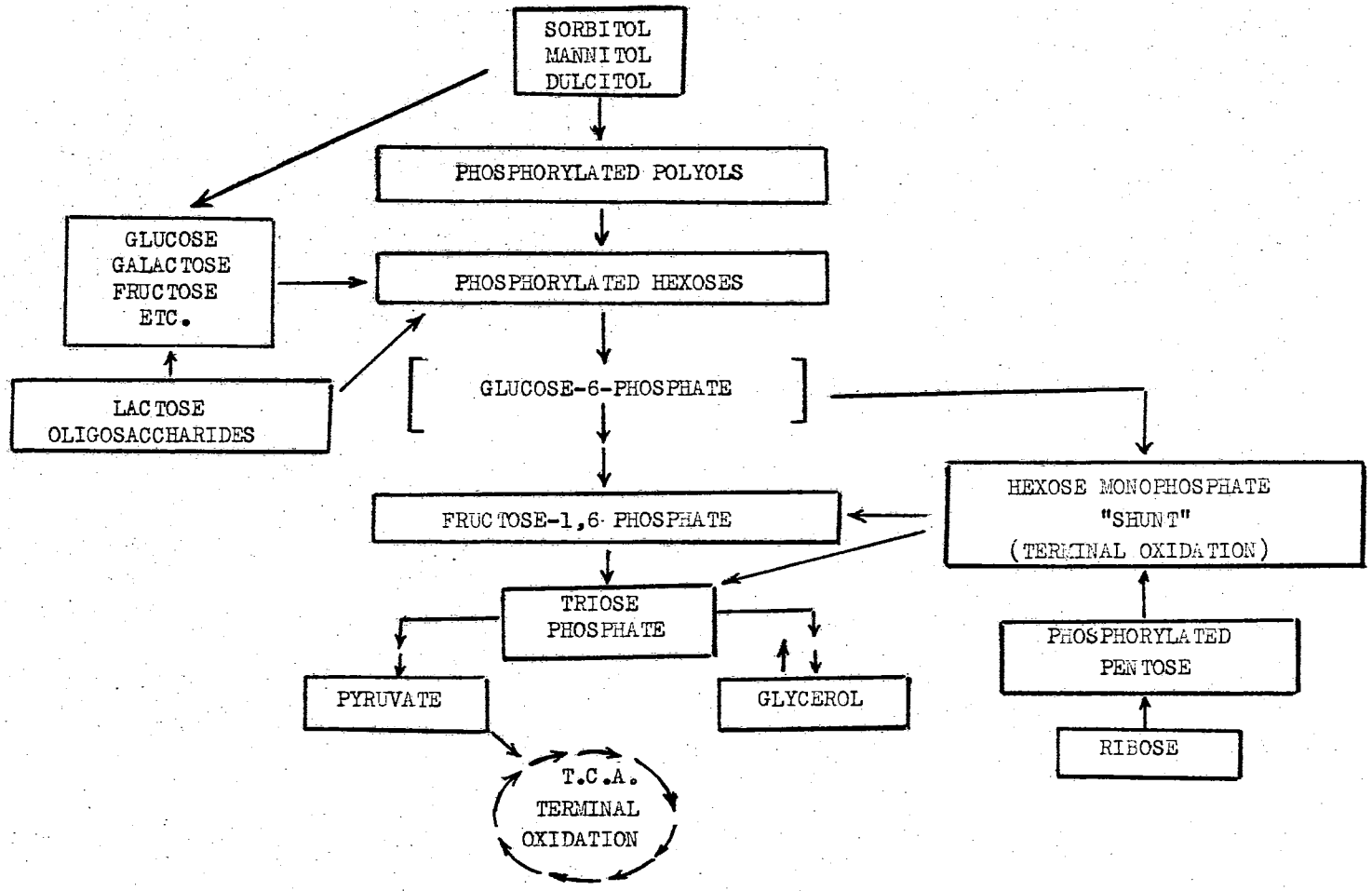

FIGURE 44 GENERALIZED METABOLIC FLOW CHART FOR VARIOUS CARBOHYDRATES AND RELATED SUGAR ALCOHOLS

that glucose and fructose can be utilized by the sorbitol-acclimated sludge at a much faster rate than galactose (cf. Figure 4, and 7). These observations seem to be in agreement with those found by Neidhart and Magasanik (74) in their studies with Aerobacter aerogenes, from which they concluded that the degree of repression exerted by a compound depended upon the rate at which that compound supported growth. Since they found that, while glucose caused a complete cessation of induced enzyme formation, several other compounds such as galactose and glycerol could also produce partial inhibitions, they attributed such differences to the greater rate at which glucose could support growth. Hence, in the present studies it is also possible that the faster rate at which glucose was utilized may be a significant factor which controls the extent of its suppressing effects. Thimann (74) has stated that the repressive effect of a compound seems to be dependent on the rate at which its intermediary catabolites are being synthesized as well as being utilized. On a similar basis, glucose and fructose may be metabolized by the activated sludge at a rate which can give rise to a rapid build-up of a metabolite that can be utilized at a relatively slower rate, thus setting the suppressing mechanism in full operation. On the contrary, galactose may give rise to a lower level of such metabolite(s) and thus not allow the build-up of the metabolite(s) to a critical level that would set the control mechanism into full operation. Lactose did not seem to have any effect on sorbitol metabolism, even though it is composed of glucose and galactose joined in a β-galactoside linkage.

The metabolism of lactose would involve an induction of the enzyme β-galactosidase, which is required to cleave such a linkage. It is quite interesting to note that both glucose and galactose have been shown to have suppressing effects on sorbitol metabolism but when the two compounds are joined together they have absolutely no effect. This result is predictable in occordance with the principle of repressive or suppressive theory: that is, lactose lies outside of the metabolic flow path, not within it. Furthermore, it is possible that sorbitol repressed the synthesis of galactoside permease and/or of β - galactosidase.

When a ribose-acclimated sludge was shocked with glucose (Figure 10), it was found that there was no immediate effect upon ribose metabolism. Ribose metabolism proceeded in a manner similar to that found for the control, but it was noted that glucose in the shocked system was initially metabolized at a slower rate than was ribose. As the rate of glucose metabolism increased, the rate of ribose metabolism decreased in comparison to that in the control. Since glucose removal was initially slow (an apparent acclimation period was required), there was little opportunity to build-up a sufficient concentration of the supposed critical intermediate(s). Alternatively, the specific intermediate required for feed back control may not be common to the pathways for these two compounds since ribose is normally known to be metabolized via the hexose monophosphate shunt instead of the glycolytic pathway, while glucose may be metabolized through either. The fact that a shock loading of glycerol (Figure 11)

had no effect on ribose metabolism, even though the glycerol was metabolized at a faster rate than glucose by ribose-acclimated sludge, could be interpreted as an indication that any key intermediate which might be involved in the control mechanism lies above the triose level. This is also borne out by the results obtained when a glycerol-acclimated sludge was shocked with ribose (Figure 12).

Although the precise control mechanisms determining the occurrence of sequential substrate removal are by no means known, the results of the present study have contributed in significant measure to its further elucidation, and general patterns have been established. From this pattern it can be predicted that, for example, if mannose and sorbose were used as shock compounds, both would have suppressing effects on sorbitol as well as mannitol metabolism. It would be quite interesting to see whether such a prediction is correct. From the practical standpoint, such a pattern would provide a basis for predicting response of the treatment process to such types of qualitative shock loads, if the characteristics of the incoming waste can be classified.

2. Effects of Sludge Age

From the results of the present studies, it can be seen that the responses of the old cell sludge to qualitative shock loading are quite different from the previously discussed young cell sludge, indicating that the physiological condition operationally defined as "sludge age" plays an important role in controlling the extent of this inhibitory effect. Glucose, which is normally considered to be

readily utilizable by almost all microbial species, since the enzymes required for oxidative assimilation of glucose are thought to be constitutive, was not readily degradable by either sorbitol or mannitol-acclimated old sludge. It is possible that glucose permease, required by the cells for glucose uptake, or an initial enzyme step required to bring glucose into the Embden-Meyerhoff pathway was absent in the old cell sludge and had to be induced. This reasoning is borne out from the results which indicated that the old cell sludge under growth condition was more responsive to glucose shock loading than that under nonproliferating condition. In the case of the old mannitol-acclimated sludge, the system did not respond at all but still possessed the ability to utilize mannitol to which it had been previously acclimated. From knowledge in the area of biochemistry, as has been pointed out previously, both sorbitol and mannitol are metabolized via a pathway closely related to glucose through the Embden-Meyerhoff-Parnas pathway, both polyalcohols entering at the level of fructose or fructose 6-phosphate. Hence it cannot be argued that glucose could not be metabolized readily by the old polyalcohol-acclimated sludge because enzymes in the oxidative pathway were absent in the old sludge. However, it is possible that only those which were required for initial conversion of glucose into the already existing pathway could be absent and had to be induced. Thimann (75) has pointed out that the cell age of a bacterial population might involve changes in the permeability of the cell membrane to a substrate and noted that very

limited knowledge was available with regard to the effects of this physiological phenomenon. It is apparent that the sludge under growth conditions in which an ample amount of nitrogen was present could respond more readily to such a qualitative shock load. These observations also help to support the previous findings which had been reported by Gaudy (37) in which he had concluded that the successful response of an activated sludge to qualitative shock loading could be accomplished only when it was accompanied by a readily available nitrogen source. It is believed that such a requirement is attributable to the need for de novo synthesis of enzyme, and only that portion of the population possessing the genetic capability for inducing the required enzyme(s) can respond (37). Hence, such enzyme synthesis might be required in the cases of old sorbitol and mannitol-acclimated sludge herein reported with repect to their responses to the glucose shock loading.

However, it should be noted that after the acclimation to glucose had been set in operation, an inhibitory effect of glucose on sorbitol-acclimated cells could be observed as a retardation of the sorbitol removal rate in the shocked system (Figure 13); this was not observed in the old mannitol-acclimated cells (Figure 14). Mandelstam (55) has shown that the ability of different carbon sources to repress synthesis of inducible enzymes varies with the rate at which they are metabolized and with the growth rate of the cells. While these observations apply to enzyme repression, they may also be applicable to the phenomenon studied herein, thus a

decrease in the rate at which glucose can be metabolized by old cells might prevent the accumulation of an intermediate necessary for operation of the control mechanism. Mandelstam reported further that even constitutive enzymes can be repressed under the proper physiological conditions. The 24-hour feeding cycle for a prolonged period of time, as used herein for development of the old cells, may impose sufficient limitations upon cell growth to bring about such repression.

While operational conditions leading to promulgation of older sludges enhanced concurrent rather than sequential substrate removal under the severe shock loading conditions employed, it is to be emphasized that this possible means of providing an engineering control can give rise to other serious problems. An increase in the age of sludge is normally accompanied by a decrease in the sludge metabolic activity or the rate of substrate removal per unit weight of sludge, and a longer acclimation period is required in order to metabolize new compounds in the waste stream. In addition, it was seen from the results of the mannitol-acclimated old cell study shown in Figure 14 (non-proliferating) that a new and somewhat unexpected phenomenon can occur in that the cells cannot respond at all to the shock compound. From the results of previous studies (43), it was found that young cell populations possess more flexibility and can readily respond to a qualitative shock loading even under nonproliferating conditions but are subjected to the suppressive effects of the shock compounds. From an engineering standpoint, these findings should play an important role and be a decisive factor in

determining the design and operational criteria of biological waste treatment processes.

B. Continuous Flow Systems

1. Glucose Shock Loadings to Sorbitol-Acclimated Systems.

a. General Responses: It should be realized that the activated sludge systems as employed in this study were operated under steady-state, continuous flow, completely mixed conditions. Hence, their growth rates were controlled primarily by the dilution rates. According to the steady state concept, the sludge growth rate of a system would be equal to the system dilution rate provided that the system is maintained under steady state conditions. Hence, at a 2-hour detention period, the growth rate of the system would be equal to 0.5 hour^{-1}, for 4-hour and 16-hour detention times the growth rates would be 0.25 hour^{-1} and 0.125 hour $^{-1}$ respectively. It is evident that the population of the system operated under such a steady state would always be in a constant state of the log growth phase regardless of the detention period employed (within practical limits). It was noted that all the sludges developed at various detention times in this study were not flocculent and had no ability to settle. Their physiological characteristics were similar to those observed in the young cell populations which were developed in the previous batch studies. Therefore, according to the definition which has been suggested previously, the sludge population obtained in these continuous flow systems may be classified as "young cell" sludge.

From observations made during these continuous flow studies, it was noted that at times the characteristics of the systems, such as their substrate removal efficiency and biological solids level were considerably different even with the same feeding protocols. The biological solids in one system tended to be more flocculent than in the other. Such variations could be due to differences in predominance in the two systems as well as to some experimental difficulties which were encountered. It was noted that at times there were difficulties in maintaining a system in an ideal steady state condition and in maintaining homogeneous mixing between the effluent and the mixed liquor inside the tank. Large floc particles which had been dislodged from a diffusor or from the side wall growth of a reactor had a tendency to remain within the tank and did not pass out in the effluent, thus causing a deviation from a true steady state condition. However, attempts were made to overcome these difficulties as much as possible by continuous care and frequent cleaning of the air diffusor as well as the reactor in order to eliminate the effect of side wall growth.

From the results indicated in Figure 17 through Figure 22 it is apparent that the systems had the capability of accepting shock loading to some extent. Under a 4-hour detention period it is seen that the system was capable of accepting an influent organic shock load concentration equal to about twicethe load which had been applied previously at its steady state equilibrium. For example, the system which was previously acclimated to 500 mg/l of sorbitol was capable of accepting a shock load influent of 500 mg/l sorbitol

plus 500 mg/l glucose or a total influent concentration of 1000 mg/l (Figure 19-A): the system previously acclimated to 1000 mg/l sorbitol was capable of accepting a shock load influent of 2000 mg/l of the combined carbon sources (Figure 19-B). Similarly the system acclimated to 1500 mg/l sorbitol was also capable of accepting a total shock load influent of 3000 mg/l of the combined carbon source (Figure 20). These conclusions, however, are based on the system efficiency as determined by the levels of residual glucose and sorbitol which were released in the effluent, not on the basis of the residual total filtrate COD.

By comparing the results shown in Figures 20, 21, and 22 it is readily seen that glucose seemed to be removed at a faster rate than sorbitol even though the systems had been previously acclimated to sorbitol. When the system was acclimated to 1500 mg/l sorbitol and was then shock-loaded with 3000 mg/l sorbitol as shown in Figure 21, there was a considerable increase in sorbitol level in the effluent for almost 24 hours before attaining a new equilibrium. For similar systems which were shock loaded with 1500 mg/l sorbitol plus 1500 mg/l glucose (cf. Figure 20) or with 3000 mg/l glucose (Figure 22), the systems recovered more readily, within 15 hours, and within an even shorter period for the system shock-loaded solely with glucose. The levels of both sorbitol and glucose for the latter two systems were definitely lower than for the system shock-loaded solely with sorbitol. It is obvious that sorbitol was removed at a slower rate than was glucose. On a quantitative basis, the system was less capable of accepting sorbitol shock loads.

The differences between the residual total filtrate COD and the residual sorbitol COD plus glucose COD seemed to increase as the loading increased. These differences were apparently greater when glucose was involved, and could be attributed only to some metabolic intermediate(s) which had been released by the activated sludge upon utilization of either glucose or sorbitol. This observation, however, does not seem to be unique only to the study herein reported, but had also been noted previously by the author in his batch-study report (44) as well as by other investigators (39, 76). The study of release of metabolic intermediates, however, is not a major purpose of this investigation; therefore, the discussion concerning this aspect will be limited even though a more detailed study certainly would be valuable to the water pollution control field. Furthermore, it was felt that the occurrence of this phenomenon would not affect the findings herein reported since specific tests for each waste component were also employed and the presence of the intermediate(s) did not seem to interfere with the specific tests employed.

From the results shown in Figure 17 through Figure 20, it is apparent that the sorbitol-acclimated system could readily respond to glucose shock loading and no deleterious effects due to glucose could be observed even when the total influent waste concentration had increased to twice its previous loading. From the results shown in Figure 17 and Figure 18, it is obvious that the system would be

able to accept various combinations of glucose and sorbitol applied solely as qualitative shock loading i. e. when there is no change in the total concentration of organic carbon source in the influent. There was no change in the level of biological solids or in total cell protein in these cases. This indicates that no acclimation to glucose was required; otherwise both total solids and protein levels would have been initially decreased due to the continuous diluting out of cells by the hydraulic loading. Furthermore, there was no significant change in the levels of total filtrate COD, sorbitol COD or glucose COD in the effluent, which also indicates the successful response of the systems to such qualitative shock loading.

The results shown in Figures 19 and 20 indicate that the sorbitol-acclimated system could also respond to qualitative as well as quantitative glucose shock loading to a certain extent. Under a 4-hour detention period, the allowable increase in shock loading concentration in the influent was approximately equal to the concentration as applied prior to shock loading with some exceptions which will be discussed later. The systems responded to the shock loading by rapid increases in the sludge population density. It is evident that the growth rate of the system, operated under the steady state condition, was not at its maximum but was limited by the supplied organic carbon source which was in turn controlled by the system hydraulic loading rate. Upon disruption of such a steady state by increasing the supply of the influent organic carbon source, the system could respond quite readily by a rapid increase in its

population growth rate. Under this condition, the system may act somewhat like a batch-operated system with respect to the substrate removal rate as well as the biological sludge growth rate. But after a period of time, the applied organic shock load concentration in the system leveled off, and again the organic carbon source became the growth limiting factor; a new equilibrium was finally attained and the growth rate was again equal to the hydraulic dilution rate. It should be realized that the actual system growth rate, as the result of disruption of a steady state condition by organic shock loading, would be the sum of the apparent growth rate and the hydraulic loading rate of the system. The true growth rate of the system would be dependent on the substrate concentration according to the Monod growth rate equation which has been previously discussed. In all the cases mentioned thus far there was no apparent harmful effect of glucose shock-loading on the sorbitol-acclimated systems; the successful responses of the systems can be attributed to the ability of the sludge to increase the population density in the systems.

When a system was subjected to a greater quantitative shock loading, such as a tripling of the steady state influent concentration the system could not readily respond to such an increase. A portion of its applied carbon source was released in the effluent, as can be noted by the increases in both total filtrate COD and sorbitol COD (cf. Figure 23, 25, 26); however, no significant amount of glucose COD was released into the effluent, even though both glucose and sorbitol were applied in equal amounts in the system shock loading

influent. Theoretically, for all the cases just mentioned the systems were shock-loaded with more glucose than sorbitol since all systems had been previously acclimated to sorbitol prior to the introduction of shock loadings. By comparing the results in Figure 23 and Figure 24, it is evident that such systems could remove the sorbitol component readily in the absence of glucose but when given the choice between the two substrates, glucose was preferentially metabolized at the expense of sorbitol. The results shown in Figure 26, which represented a higher organic loading concentration, also comfirmed that glucose is preferentially metabolized. All the systems, however, could again remove all the sorbitol after a period of time had elapsed during which biological solids concentration in the systems had reached a higher level. By comparing the results shown in Figure 20 and Figure 26, it is obvious that the initial state of the system, more specifically the ratio between the concentration of biological solids and of the applied organic carbon source, determines the ability of a system to respond to shock loading. When the system was acclimated to 1500 mg/l sorbitol, then shock-loaded with 1500 mg/l sorbitol plus 1500 mg/l glucose, neither glucose nor sorbitol appeared in the effluent (cf. Figure 20), but when the system was acclimated to only 1000 mg/l sorbitol, then was shock loaded with the same influent concentration (1500 mg/l sorbitol plus 1500 mg/l glucose), a considerable amount of sorbitol was released for over 12 hours before it was again removed. From these results, it may be reasoned that when the biological solids concentration of the

system is maintained at a high level, the system can remove both glucose and sorbitol as rapidly as they are applied and therefore, no residual glucose or sorbitol will remain in the effluent. However, when the biological solids concentration of the system is at a lower level, the population growth cannot provide removal of all the substrates as rapidly as they are applied, and under this condition the sludge population will selectively metabolize glucose, a readily available energy source, rather than sorbitol. After glucose is exhausted, then the system would shift to utilize sorbitol. This postulation is also borne out in the results which will be discussed later.

The results shown in Figure 27-A seem to contradict those shown previously in Figure 19. In both cases the systems were acclimated to 500 mg/l sorbitol and were shock-loaded with 500 mg/l sorbitol plus 500 mg/l glucose. The results indicate that in the system shown in Figure 27-A a considerable amount of sorbitol appeared in the effluent for almost 12 hours before it could be completely removed. The differences in these two systems could be primarily due to differences in their sludge activities since the sludge population shown in Figure 27-A appeared to be more flocculent and less active as could be observed from the rate of increase in its biological solids. The differences in sludge properties between these two cases may be attributable to differences in the initial seed as has been reported by Genetelli and Heukelekian (33). But regardless of the differences in sludge quality and activity of the systems, it is also evident that glucose is always utilized preferentially to sorbitol and as

long as glucose still remains in the system, the level of sorbitol will not be decreased.

When a system was subjected to an immediate glucose shock loading such as shown in Figure 28, the sorbitol concentration in the effluent also increased rapidly and was not decreased until glucose had been exhausted. When a system was subjected to both gradual and immediate shock loadings, the time required for sorbitol in the effluent to be again completely removed was more prolonged than when it was subjected solely to one type of shock load (cf. Figure 27-A and Figure 27-B). It is apparent that the concentration of glucose present in the system is an important factor in controlling the manifestation of substrate interactions. Under a severe shock loading condition, when a greater amount of glucose is applied, a greater amount of sorbitol appears in the effluent and a longer period of time is required to reach a new equilibrium. However, the effects of substrate interactions will not be apparent when shock loading is applied within the limits of the ability of the system to accept shock loads.

b. Effects of Detention Time

As has been previously discussed, the sludge of all systems operated under a steady state continuous flow condition as employed in this study is in a log growth phase and has the characteristics of a "young cell" population. When the system was operated at 2-hour detention period, the steady state cell concentration was relatively low and there was a concomitant high residual of organic

carbon source in the effluent. It is also evident that the ability of a system to accept a gradual shock load will increase as its detention time increases. At a 2-hour detention time, the system could not remove a shock loading influent of twice its previous influent concentration (cf. Figure 29). At a 4-hour detention time, the system could respond more readily with an increase to double its previous influent concentration (Figure 19), and at a 16-hour detention time the system could accept more than three times its previous steady state concentration (Figure 30 and 31) as measured by the levels of glucose and sorbitol released in the effluent. It should be realized also that the rate of increase in applied glucose concentration due to a gradual shock loading to a system is greater as its detention time is decreased. For example, the concentration of applied glucose shock load in the system would reach 90 per cent of its influent concentration within less than 5 hours for a 2-hour detention time, but would require about 10 hours for a 4-hour detention time and over 40 hours for a 16-hour detention time. (cf. Figure 2). It is obvious that the effect due to gradual shock loading by an increase in the influent concentration will be greater for the system with a lower detention period; in addition, the biological solids level that can be maintained in the system will also be reduced due to a smaller detention period.

The substrate interaction between glucose and sorbitol was quite apparent when the system was operated at 2-hour detention period (cf. Figure 29). The introduction of glucose caused a rapid increase in the sorbitol concentration in the effluent. This may be due to the

fact that the biological solids concentration in the system was rather low, coupled with the relatively rapid increase in the concentration of glucose in the system. Upon introduction of glucose, the sludge population shifted to metabolism of glucose and left sorbitol to be released in the effluent. However, the system was not able even to respond to all the glucose shock loading, and a portion of glucose also appeared in the effluent. One important point which should be noted is that sorbitol was not again utilized until glucose had been removed to a low level. Obviously, the glucose concentration in the system during an early stage of shock loading had prevented sorbitol metabolism, and the glucose inhibition was not released until the system could first cope with the increase in glucose shock loading by increasing the sludge level.

When the system was acclimated to 500 mg/l sorbitol influent at a 4-hour detention period and was shock-loaded with 750 mg/l sorbitol plus 750 mg/l glucose, which represented an increase to three times its previous influent concentration, there was a significant amount of sorbitol, but no glucose, appearing in the effluent for approximately 8 hours (cf. Figure 23). However, when the system operated at a 16-hour detention period, also acclimated to 500 mg/l sorbitol influent and shock-loaded with 750 mg/l sorbitol plus 750 mg/l glucose, no sorbitol or glucose appeared in the effluent (cf. Figure 30). But when the same system was shock loaded with 1500 mg/l sorbitol plus 1500 mg/l glucose, which represented a six-fold increase in the influent concentration, again considerable sorbitol but not glucose was released in the effluent for over

12 hours before the system could again recover (cf. Figure 32). It can be noted that in no case was there a concurrent release of glucose and sorbitol in the effluent, even though both substrates were applied equally to the systems, unless the sorbitol concentration had already reached its maximum limit; then glucose was also released. It cannot be argued that this occurrence is due solely to the differential in the rates at which each substrate can be metabolized by the activated sludge but not due to the substrate interaction. If such were the case, a total replacement of sorbitol metabolism by glucose, as shown in Figure 29, should not be possible, i. e., sorbitol concentration in the effluent should be reduced to some extent during the first 12 hours after shock loading and sorbitol removal should not be delayed until glucose concentration was reduced to a low level (also cf. Figure 27). Furthermore, the results with the control system, which was fed with only one component at the same concentration as that in the combined system, also indicated that if only one component was present in the influent, the system was able to respond quite readily. It is true that glucose can be metabolized at a faster rate than sorbitol (as was also observed previously in the batch studies) which may indirectly play an important part in regulating this phenomenon. These results suggest that, regardless of the length of detention period employed, the level of residual glucose concentration remaining in the system is an important factor in determining the manifestation of substrate interaction and is controlled by the rate at which glucose is supplied as well as the rate at which it can be utilized.

c. Effects of Nitrogen Deficiency: The inhibitory effect of glucose on sorbitol metabolism is more apparent under a nitrogen-deficient condition. When the system was supplied with an amount of nitrogen sufficient to utilize for growth only half of the substrate supplied, the system selectively metabolized only glucose and used no sorbitol (cf. Figure 36). There was no tendency for more sorbitol to be removed even after 50 hours of such loading. Had the nitrogen source been sufficiently supplied, both glucose and sorbitol would have been removed readily within that length of time (cf. Figure 19-B). The limiting of sludge growth by the nitrogen source had greatly reduced the ability of the system to successfully respond to shock loading and under such conditions the system would utilize the glucose prior to sorbitol. It should be noted that in this case the ratio of organic loading (as BOD) to nitrogen was approximately 40 to 1, and only about 50 per cent of the substrate was removed. Thus it is apparent that the maximum limit of BOD to nitrogen would be approximately 20 to 1 for a complete removal, which is also in accord with that recommended by several investigators in the field (20, 21, 22). The results obtained in this study also have confirmed that an activated sludge system can be operated quite successfully at the minimum nitrogen limit, i. e., BOD to nitrogen ratio of about 20 to 1 (cf. Figure 33-A). However, the modes of response of such activated sludge systems appeared to be quite different from those of systems with an excess supply of nitrogen. The system under such a limited supply of nitrogen responded by a greater increase in its

carbohydrate content and not in its protein content, which is not the characteristic of a system operated under optimum growth conditions. The portion of substrate channeled into synthesis of the sludge mass also appeared to be greater for such nitrogen-limiting conditions. This observation is actually in agreement with previous reports by Gaudy and Engelbrecht (24) and by Symons and McKinney (23) that under a nitrogen-limited condition, the system would favor synthesis of high carbohydrate or polysaccharide content. The supply of nitrogen at this minimum ratio, therefore, could enhance this type of response.

When a system being operated at the minimum nitrogen concentration was shock-loaded by rapid injection of glucose (cf. Figure 34), it was seen that the system's ability to metabolize sorbitol was also inhibited but was recovered quite rapidly after exhaustion of glucose. It can be noted that the control mechanisms involved in these substrate interactions act in a relatively rapid manner. Apparently, there was no delay in shifting from one substrate to another, and the action seems to be controlled primarily by the concentration of glucose. Due to such rapidity in occurrence, it is very unlikely that such response could be governed by a shift in predominance of the system. It is quite surprising to observe that only glucose was selectively metabolized, in preference to sorbitol, especially under a severe nitrogen deficiency (cf. Figure 35, 36). The control mechanisms must be quite delicately balanced to provide that no sorbitol would be assimilated as long as glucose is present above a certain level. Such inhibition obviously cannot be due to simple

competition between substrates for a common enzyme sequence, since, if this were the case it would be expected that more sorbitol would be removed in the presence of glucose or a greater portion of glucose should appear in the effluent. The effect of glucose is therefore, a total blockage of sorbitol utilization. It cannot be ruled out that those mechanisms which have already been proposed by various investigators in the area of basic biological science were not involved in this glucose-sorbitol system. However, it is felt that these already well-known mechanisms cannot adequately explain the interaction which occurred in this case, since "repression" is known primarily as the mechanism which involves the inhibition of enzyme synthesis and acts relatively slowly whereas "feed-back inhibition" acts more rapidly but involves only in the inhibition of enzyme function in the biosynthetic pathway (77). It is quite logical to postulate that the substrate interaction as observed in this case would involve the inhibition of substrate assimilation by another substrate such as glucose or by some intermediary metabolite(s) derived from glucose in a manner analogous to metabolite repression as proposed by Mandelstam (55). In addition it would appear that the concentration of the inhibitory substrate or the metabolic product(s) that can be derived from it, would play an important role in regulating such mechanism. One interesting engineering application which may be gained from this study is the possibility of separation of two organic carbon sources by biological means such as displayed in Figure 36.

2. Glucose Shock Loadings to Glycerol-Acclimated Systems.

It is readily seen that glucose also has an inhibitory effect on glycerol metabolism; the addition of glucose as shock loading to a glycerol system caused the system to release a small portion of glycerol in the effluent. The glucose interference, however, is more apparent under severe nitrogen-deficient conditions (cf. Figure 40). In most cases of the study herein presented, the system appeared to be able to respond quite successfully to glucose shock loading. The systems could accept a shock loading of at least twice the previous steady state concentration (cf. Figure 38) without either glucose or glycerol being released in the effluent. This observation seems to be in agreement with McKinney's (12) statement that the completely mixed activated sludge process was quite efficient in coping with an organic shock loading. In all cases of glycerol-acclimated systems with excess nitrogen, there was no significant increase in the glycerol level as compared with that found in the sorbitol systems, and the interactions were relatively less immediate. It is possible that the control mechanism for glycerol metabolism is not so sensitive to glucose as that of the sorbitol system and the critical concentration of glucose for glycerol-acclimated systems may be higher than that for the sorbitol-acclimated system. Thus it would require a longer period to set the control mechanism into full operation in a gradual shock loading situation, but at the same time glucose could also be rapidly metabolized by the activated sludge which would help to avert the effect of glucose inhibition.

However, under severe shock loading conditions, glucose inhibition of glycerol metabolism became apparent (cf. Figure 39).

Under a severe nitrogen deficiency (cf. Figure 40), the growth of the system was limited by the nitrogen scurce; thus the sludge growth could not rapidly respond to the increase in glucose concentration and as a result glucose concentration in the system reached its critical level quite rapidly and its inhibitory effect was set into full operation. Recent studies in batch-operated systems by Krishnan (68) also indicated that glucose could exert its inhibitory effect on glycerol metabolism in a pattern very similar to that found in the sorbitol system; glucose also could be metabolized by the glycerol-acclimated sludge at an even faster rate than the glycerol itself. These findings, therefore, help to suggest that the control mechanisms in both sorbitol-and glycerol-acclimated systems which are affected by glucose may be operated on the same basis, being regulated by the glucose concentration present in the system.

The modes of response of the glycerol-acclimated system under a nitrogen-limited condition when it was shock-loaded with glucose, as shown in Figure 41, indicated that the response of the system was also offset by a significant increase in biological solids synthesis as well as by the increase in its carbohydrate content, whereas its biological solids protein remained constant. It is evident that the composition of the activated sludge mass does not remain constant but can vary with the growth conditions. It would be quite interesting to determine whether the metabolic activity of such a sludge population, which has been previously subjected to the

nitrogen-limiting condition, would be impaired. This aspect could play an important role in regulating the recycling of sludge in a waste treatment process especially when the system is operated with a minimum supply of nitrogen.

3. Sorbitol Shock Loading to Glucose-Acclimated Systems

The aim of this portion of the study was to detemine whether there would be any deleterious effects as the result of a situation the reverse of that previously studied. It was noted that with a complete change of influent feed from 1000 mg/l glucose to 1000 mg/l sorbitol a considerable amount of sorbitol was released in the effluent but the system recovered readily within 8 hours (cf. Figure 42). With an increase in the influent concentration by 500 mg/l sorbitol, there was no significant increase in either glucose or sorbitol in the effluent (cf. Figure 43). It is unlikely that the enzyme(s) required for utilizing sorbitol would be "constitutive" in the glucose-acclimated system. However, with the manifestation of such rapid response, it is evident that a rapid induction of enzyme(s) could be involved. It should be noted that when the system influent was changed completely from 1000 mg/l glucose to 1000 mg/l sorbitol, which was strictly a qualitative shock loading condition, the system could not respond readily. But when a similar system was shock-loaded with 1000 mg/l glucose plus 500 mg/l sorbitol influent, thus subjecting the system to both qualitative and quantitative shock loads, the system could respond successfully. It is possible that in the latter case, the presence of glucose might enable the system to

induce the necessary enzyme(s) within a shorter period of time by supplying the metabolites to carry out the essential induction process. It should be noted, however, that in all of these glucose-acclimated systems, the residual glucose remained at a very low level; therefore, it could not exert its inhibitory effects. On the contrary the presence of glucose might help to promote acclimation in a manner analogous to that observed by McQuillan and Halvorson (58) in yeasts, in which they found that glucose at a low concentration showed a stimulatory effect on induction of enzyme synthesis whereas at high concentrations it caused repression of enzyme synthesis. It should be noted that the sludge population such as employed in this portion of the study, which can be operationally defined as a "young cell" sludge, can achieve acclimation more readily than can an "old cell" sludge. From the standpoint of application, this observation would play a decisive role in determining whether a waste engineer should choose to operate a system under conditions fostering a "young cell" or an "old cell" population. A "young cell" sludge would be able to acclimate more readily to a shock load component and would possess greater metabolic activity per unit weight of sludge mass, but would be highly subjected to repressive and suppressive phenomena such as those observed in these studies. An "old cell" sludge, however, would be less subjected to such control mechanisms but would acclimate relatively slowly to a shock component and possess lesser metabolic activity.

CHAPTER VII

SUMMARY AND CONCLUSIONS

1. The suppressive or repressive effects of one waste component on another in an activated sludge population have been observed in steady state continuous flow and in discontinuous flow systems. Such phenomena appear from these studies to be of fairly general occurrence. Some of these substrate interactions have been explained on the basis of known biochemical principles and the suppressive mechanisms proposed on the basis of work previously reported from this laboratory.

2. From the results of this study and the studies previously reported, a new cellular control mechanism involving substrate interaction phenomena has been postulated. This new mechanism would involve the suppression of existing enzyme function(s) by another substrate, preventing assimilation of the substrate which the cell had previously been metabolizing.

3. The physiological condition of the cells, which is operationally defined as cell age, plays an important role in controlling the effects of substrate interactions.

4. The young cell sludge appears to respond more readily to qualitative as well as quantitative shock loads than the old cell

sludge, but is more subject to the effects of substrate interaction.

5. An adequate supply of nitrogen is essential for enhancing the successful acclimation of an activated sludge system to qualitative shock loading especially when the system consists of old cell sludge.

6. The completely mixed continuous flow activated sludge system under steady state conditions as employed in this study is not free from deleterious effects of shock load; however, it possess an ability to accept shock loading to a certain extent. Such ability is dependent on the system dilution rate, the biological solids concentration maintained in the system, the sludge activity, and the quantity as well as the rate at which the shock substrate is applied.

7. A release of metabolic intermediate(s) has been noted and appears to be more prevalent in the young cell population. The concentrations released seem to be dependent on the types of substrate and increase with the concentration of organic loading applied.

8. The occurrence of substrate interaction is not apparent in the steady state continuous flow activated sludge system when shock loading is applied within the limits of the ability of the system to accept shock load.

9. Under severe shock loading conditions, substrate interaction becomes apparent. Its occurrence is dependent on the ratio of the concentrations of an inhibitory substrate and of the

biological solids present in the system or the rate at which the inhibitory substrate is eliminated.

10. The hydraulic loading rate indirectly controls the manifestation of substrate inhibition by controlling the growth of biological solids in the system, the rate at which the substrate can be metabolized and the concentration of the residual substrate.

11. The preferential substrate utilization or substrate interaction phenomenon is more apparent under a nitrogen-limited condition which controls the biological solids concentration as well as limiting the substrate removal rates.

12. The modes of response of an activated sludge system under a nitrogen-limited condition seem to be in accordance with previous findings by Gaudy (37), i. e., a greater proportion of the substrate removed is channeled into synthesis of the sludge mass and this corresponds with a significant increase in cabohydrate content.

CHAPTER VIII

SUGGESTIONS FOR FUTURE WORK

In view of the present study herein reported, it is felt that that following research aspects would be valuable:

1. The study should be extended further to include other types of substrates such as proteins, amino acids, fatty acids, other pure organic compounds and industrial wastes, using the methods employed in this study in order to establish general patterns of response of activated sludge as well as possible measures of the effect of substrate interaction due to various types of qualitative shock load.

2. Another similar aspect which also should warrant further research is the possibility of preventing or delaying acclimation of the activated sludge to a newly introduced compound by the compound already being metabolized.

3. The nature of the metabolic intermediates, which are released into the system, should be investigated with regard to their identity and the quantities which arise from metabolism of various types of compounds. Techniques which may be employed are the use of radioactive carbon sources, various types of chromatography and electrophoresis as well as specific analytical methods for identification of such metabolic products.

4. The study of kinetics as well as mechanisms of the response of an activated sludge system to qualitative as well as quantitative shock loads, when the system is provided with a final clarifier and a controlling device to return various sludge quantities, would be of great interest and applicability from a practical viewpoint.

5. The metabolic responses and the activities of the activated sludge maintained under a nitrogen-limited condition should be compared with those under a normal growth condition, since from the results of the present study, it was found that the system still could remove the supplied organic carbon sources quite successfully under a nitrogen deficient condition to a certain extent. From a practical standpoint, especially when a return sludge is contemplated, it would be desirable to determine whether the sludge, maintained under such a nitrogen deficient condition, would still maintain a high metabolic activity.

A SELECTED BIBLIOGRAPHY

1. Nemerow, N. L., Theories and Practies of Industrial Waste Treatment. Addison-Wesley Publishing Co., Reading, Mass. (1963).

2. Anon., "Financial Guideline," Water and Sewage Works Jour., 111, 7A (1964).

3. Subcommittee on Combined Treatment of Municipal and Industrial Wastes of the Chemical Industry Advisory Committee of the Ohio River Water Sanitary Commission," Current Practices in Municipal Treatment of Industrial Wastes," Sewage and Industrial Wastes, 29, 672 (1957).

4. Byrd, J. F. and Gex, V. E., "Combined Treatment of Industrial and Municipal Wastes - An Industrial Viewpoint," Sewage and Industrial Wastes, 29, 414 (1957).

5. Gaudy, A. F., Jr. and Engelbrecht, R. S., "Quantitative and Qualitative Shock Loading of Activated Sludge Systems," Jour. Water Pollution Control Fed., 33, 800 (1961).

6. Gaudy,A. F., Jr., "Studies on Induction and Repression in Activated Sludge Systems," Applied Microbiology, 10, 264, (1962).

7. Gaudy, A. F., Jr., Komolrit, K., Gaudy, E. T. and Bhatla, M. N., "Multicomponent Substrate Removal by Activated Sludge and by Pure Culture Systems," Symp. Microbiol. Aspects of Waste Disposal, Bact. Proc. p. 17 (1963).

8. Sawyer, C. N., "Activated Sludge Modifications," Jour. Water Pollution Control Fed., 32, 233 (1960).

9. Haseltine, T. R., "A Rational Approach to the Design of Activated Sludge Plants," -- Biological Treatment of Sewage and Industrial Wastes Vol. 1 Edited by J., McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Co., New York (1956).

10. Kraus, L. S., "Combined Treatment of Industrial and Domestic Wastes," Sewage and Industrial Wastes, 30, 199 (1958).

11. Busch, A. W. and Kalinske, A. A., "The Utilization of the Kinetics of Activated Sludge in Process and Equipment Design," -- Biological Treatment of Sewage and Industrial Wastes Vol. 1 Edited by J. McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Co., New York (1956).

12. McKinney, R. E., "Mathematics of Complete-Mixing Activated Sludge Jour. Sanitary Engr. Div., American Soc. Civil Engr., 88, 3133 (1962).

13. McKinney,R. E., Symons, J. M., Shifrin, W. G. and Maurice, V., "Design and Operation of a Completely-Mixed Activated Sludge System," Sewage and Industrial Wastes, 30, 287 (1958).

14. Stack, V. T. and Conway, R. A., "Design Data for Completely Mixed Activated Sludge Treatment," Sewage and Industrial Wastes, 31, 1181 (1959).

15. Dryden, F. E., Barrett, P. A., Kissinger, J. C. and Eckenfelder, W. W., Jr., "High Rate Activated Sludge Treatment of Fine Chemical Wastes," Sewage and Industrial Wastes, 28, 183 (1956).

16. Kalinske, A. A., "The Completely Mixed Activated Sludge Process," Public Works, 91, 146 (1960).

17. Zablatzsky, H. R., Cornish, M. S. and Adams, J. K., "An Application of the Principles of Biological Engineering to Activated Sludge Treatment," Sewage and Industrial Wastes, 31, 1281 (1959).

18. Spohr,G., Hershey, W., and Brenner, T. E., "From Old Conventional Activated Sludge to Contact Stabilization," Wastes Engineering, 33, 70 (1962).

19. Anon., "Rx for Industrial Shock Loadings: Ohio Tries Contact Stabilization," Wastes Engineering, 33, 453 (1962).

20. Sawyer, C. N., "Bacterial Nutrition and Synthesis," -- Biological Treatment of Sewage and Industrial Wastes Vol. 1 Edited by J. McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Co., New York (1956).

21. Eckenfelder, W. W. and O'Connor, D. J., Biological Waste Treatment, McMillan Co., New York (1961).

22. Helmer, E. N., Frame, J. D., Greenbern, A. E. and Sawyer, C. N., "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, III Treatment with Supplementary Nutrients," Sewage and Industrial Wastes, 24, 496 (1952).

23. Symons, J. M. and McKinney, R. E., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge," Sewage and Industrial Wastes, 30, 874 (1958).

24. Gaudy, A. F., Jr., and Engelbrecht, R. S., "Basic Biochemical Consideration during Metabolism in Growing vs. Respiring Systems," Proceedings 3rd Conference on Biological Waste Treatment, Manhattan College, New York (1960).

25. Kountz, R. R., and Forney, C. Jr., "Metabolic Energy Balances in a Total Oxidation Activated Sludge System," Sewage and Industrial Wastes, 31, 819 (1959).

26. Washington, D. R. and Symons, J. M., "Volatile Sludge Accumulation in Activated Sludge Systems," Jour. Water Pollution Control Fed., 34, 767 (1962).

27. Morgan, G., "Utilization of Nitrogen Containing Compounds for the Bio-synthesis of Protein in Secondary Treatment," Sewage and Industrial Wastes, 31, 1275 (1959).

28. Fruton, J. S. and Simmonds, S., General Biochemistry John Wiley & Sons, Inc., New York (1963).

29. Ludzack, F. J. and Ettinger, M. B., "Controlling Operation to Minimize Activated Sludge Effluent Nitrogen," Jour. Water Pollution Control Fed., 34, 920 (1962).

30. Weston, R. F. and Eckenfelder, W. W., "Application of Biological Treatment to Industrial Wastes. I Kinetics and Equilibria of Oxidative Treatment," Sewage and Industrial Wastes, 27, 802 (1955).

31 Heukelekian, H. and Weisberg, E., "Bound Water and Activated Sludge Bulking," Sewage and Industrial Wastes, 28, 558 (1956).

32. Lackey, J. B. and Smith D. B., "Factors Influencing Development of Biological Flocs," -- Biological Treatment of Sewage and Industrial Wastes Vol. 1 Edited by J. McCabe and W. W. Eckenfelder, Jr., Reinhold Publishing Co., New York (1956).

33. Genetelli, E. J. and Heukelekian, H., "The Effects of Chemical Composition of Substrate and Loading on the Performance and Bulking of Activated Sludge," Proceedings 17th Annual Purdue Industrial Wastes Conf., Purdue Unive., Lafayette, Ind. (1962).

34. Engelbrecht, R. S. and McKinney, R. E., "Activated Sludge Cultures Developed on Pure Organic Compounds," Sewage and Industrial Wastes, 29, 1350 (1957).

35. Ludzack, F. and Ettinger, M., "Chemical Structures Resistant to Aerobic Biochemical Stabilization," Jour. Water Pollution Control Fed., 32, 1173 (1960).

36. Malaney, G. W., "Oxidative Abilities of Aniline Acclimated Activated Sludge," Jour. Water Pollution Control Fed., 32, 1300 (1960).

37. Gaudy, A. F., Jr., "Biochemical Aspects of Qualitative Shock Loading of Aerobic Waste Treatment System," Doctoral Thesis, University of Illinois, Urbana, Illinois, (1959).

38. Jasewicz, L. and Porges, N., "Biochemical Oxidation of Dairy Wastes -- Isolation and Study of Sludge Microorganisms," Sewage and Industrial Wastes, 28, 1130 (1956).

39. Prakasam, T. B. S. and Dondero, N. C., "Observations on the Behavior of a Microbial Population Adapted to A Synthetic Waste," Proceedings 19th Annual Purdue Industrial Waste Conf., Purdue Univ., Lafayette, Ind. (1964).

40. Anon., "Industrial Wastes to Sewers? Yes - If They are Amenable!" Waste Engineering, 30, 262 (1959).

41. Quirk, T. P., "Design Data for Biological Treatment of Combined Wastes," Sewage and Industrial Wastes, 31, 1288 (1959).

42. Jones, E. L., Alspaugh, T. A. and Strokes, H. B., "Aerobic Treatment of Textile Mill Wastes," Jour. Water Pollution Control Fed., 34, 495 (1962).

43. Gaudy, A.F., Jr., E. T. Gaudy, and Komolrit, K., "Multicomponent Substrate Utilization by Natural Populations and a Pure Culture of Escherichia coli," Applied Microbiology, 11, 157 (1963).

44. Gaudy, A. F., Jr., Komolrit, K. and Bhatla, M. N., "Sequential Substrate Removal in Heterogeneous Populations," Jour. Water Pollution Control Fed., 35, 903 (1963).

45. Pardee, A. B., "Response of Enzyme Synthesis and Activity to Environment - Microbial Reactions to Environment," 11th Symposium of Society for General Microbiology, London, (April, 1961).

46. Epps, H. M. R. and Gale, E. F., "The Influence of the Presence of Glucose during Growth on the Enzymic Activities of Escherichia coli," Biochem. Jour., 36, 619 (1942).

47. Monod, J., "The Phenomena of Enzymatic Adapation," Growth Jour., 11, 223 (1947).

48. Magasanik, B., "Catabolite Repression," Cold Spring Harbor Symposia on Quantitative Biology, 26, 249 (1961).

49. Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T., and Britten, R. J., "Studies of Biosynthesis in Escherichia coli," Carnegie Inst. Wash. Publ. No. 607, Washinton, D.C.

50. Moyed, H. S. and Umbarger, H. E., "Regulation of Biosynthetic Pathway," Physiological Review, 42, 444 (1962).

51. Krebs, H. A., "Control of Cellular Metabolism," - The Molecular Control of Cellular Activity Edited by J. M. Allen, McGraw-Hill Book Co., Inc., New York (1962).

52. Jacob, F. and Monod, J., "Genetic Regulatory Mechanisms in the Synthesis of Proteins," Jour. Molecular Biology, 3, 318 (1961).

53. Cohen, G. N., and Monod, J., "Bacterial Permeases," Bacteriol. Reviews, 21, 169 (1957).

54. Pardee, A. B., "The Role of Enzyme Regulation in Metabolism,"- Growth in Living Systems Edited by M. X. Zarrow, Basic Books Inc., New York (1960).

55. Mandelstam, J., "The Repression of Constitutive β-galactosidase in Escherichia coli by Glucose and Other Carbon Sources," Biochem. Jour., 82, 489 (1962).

56. McFall, E., and Mandelstam, J., "Specific Metabolic Repression of Three Induced Enzymes in Escherichia coli," Biochem. Jour., 89, 391 (1963).

57. Kornberg, H. L., Collins, J. F. and Bigley, D., "The Influence of Growth Substrates on Metabolic Pathways in Micrococcus denitrificans," Biochim. et Biophys. Acta, 39, 9 (1960).

58. MacQuillan, A. M. and Halvorson, A. O., "Metabolic Control of β- glucosidase Synthesis in Yeast," Jour. Bacteriol., 84, 23 (1962).

59. Gorini, L. and Maas, W. K., "The Potential for the Formation of Biosynthetic Enzyme in E. coli," Biochim. et. Biophs. Acta, 25, 208 (1957).

60. Lamanna, C. and Mallette, M. F., Basic Bacteriology-Its Biological and Chemical Background, The Williams & Wilkins Co., Baltimore (1959).

61. Spicer, C. C., "The Theory of Bacterial Constant Growth Apparatus," Biometrics, 11, 225 (1955).

62. Herbert, D. R., Elsworth, R., Telling, R. C., "The Continuous Culture of Bacteria - A Theoretical and Experimental Study," Jour. General Microbiology, 14, 601 (1956).

63. Rao, B. S., "Unpublished Data," Bio-engineering Laboratory, Oklahoma State University (June, 1964).

64. Standard Methods for the Examination of Water and Wastewater, 11th Ed., Amer. Pub. Health Ass'n., New York (1960).

65. Neish, A. C., Analytical Methods for Bacterial Fermentations National Research Council of Canada, Report No. 46-8-3 (2nd Revision), p. 36 (1952).

66. Gaudy, A. F., Jr., "Colorimetric Methods for the Determination of Protein and Carbohydrate Content of Biological Sludges," Industrial Water and Wastes, 7, 17 (1962).

67. Fernell, W. R., and King, H. K., "The Simultaneous Determination of Pentose and Hexose in Mixtures of Sugars," Analyst, 78, 80 (1953).

68. Krishnan, P., Unpublished Data, Bio-engineering Laboratory, Oklahoma State University (June, 1964).

69. Gaudy, A. F., Engelbrecht, R. S. and De Moss, R. D., "Laboratory Scale Activated Sludge Unit," Applied Microbiology, 8, 298 (1960).

70. Touster, O. and Shaw, D. R. D., "Biochemistry of the Acyclic Polyols," Physiological Reviews, 42, 181 (1962).

71. Shaw, D. R. D., "Polyol Dehydrogenases," Biochem. Jour., 64 394 (1956).

72. Horwitz, S. B. and Kaplan, N. O., "Hexitol Dehydrogenases of Bacillus Subtilis," Jour. Biol. Chem., 239, 830 (1964).

73. Scolnich, E. M. and Edmund, C. C. Lin, "Parallel Induction of D-arabitol and D-sorbitol Dehydrogenases," Jour. Bacteriol., 84, 631 (1962).

74. Neidhart, F. C. and Magasanik, B., "Effects of Mixtures of Substrates on Biosynthesis of Inducible Enzymes in Aerobacter aerogenes," Jour. Bacteriol., 73, 260 (1957).

75. Thimann, K. V., The Life of Bacteria, Second Edition, The Macmillan Publishing Co., New York (1963).

76. Porges. N., " Radioactive Glucose Oxidation by Aerated Sludge," Sewage and Industrial Wastes, 30, 776 (1958).

77. Umbarger, H. E., "End - Product Inhibition of the Initial Enzyme in a Biosynthetic Sequence as a Mechanism of Feedback Control," - Control Mechanisms in Cellular Processes Edited by D. M. Bonner, The Ronald Press Co., New York (1961).

APPENDICES

APPENDIX A

DETERMINATION OF MINIMUM NITROGEN REQUIREMENTS

Two organic carbon sources, glucose and sorbitol, were selected for this portion of the study, since these two compounds were also the primary ones used in the continuous flow activated sludge studies. The constituents of the synthetic waste employed in this study were: the organic carbon source (either glucose or sorbitol), 500 mg/l; 1.0M potassium phosphate buffer pH 7.0, 10 ml/l; $MgSO_4.7H_2O$, 100 mg/l; $FeCl_3.6H_2O$, 0.5 mg/l; $MnSO_4.1H_2O$, 10.0 mg/l; $CaCl_2$, 7.5 mg/l; tap water, 100 ml/l; and distilled water to volume. The above synthetic waste was then divided into several portions of 100 ml each. To each portion $(NH_4)_2SO_4$ was added such that the final concentration in each portion varied as follows: 50, 100, 200, 300, 400, 500 and 600 mg/l. Each portion was then inoculated with a very small amount of an acclimated seed obtained from a continuous flow activated sludge unit which had been fed with either glucose or sorbitol at a flow rate which yielded a four-hour detention time. 50 ml of the seeded synthetic medium of each portion were then placed in 250 ml Ehrlenmeyer flasks and aerated on a reciprocal shaker (100 strokes/min). Cell growth in each flask was determined by measuring the optical density of the mixed liquor using a Coleman Model D-6 colorimeter at 540 mμ.

The results of this study are shown in Figure 45 and Figure 46. It is seen that in both cases, the results are quite similar. The growth of the systems supplied with 50 mg/l and 100 mg/l $(NH_4)_2SO_4$ initially increased at about the rate as those of the other systems with more nitrogen, but broke off sharply as the population density reached certain levels. The growth of the systems with amounts of $(NH_4)_2SO_4$ of 200 mg/l and over continued to rise and all leveled off at about the same population density. It is obvious that the growth of the systems with 50 mg/l and 100 mg/l $(NH_4)_2SO_4$ was limited by the amount of supplied nitrogen. It can be noted that the nitrogen-limited condition did not seem to affect the growth rate of the systems but only the total yield of the population.

Based on the results obtained herein, it is seen that the nitrogen requirement for 500 mg/l organic carbon source (either glucose or sorbitol) will be between 100 and 200 mg/l $(NH_4)_2SO_4$. By interpolation, it can be estimated that approximately 150 mg/l $(NH_4)_2SO_4$ would be required for metabolizing 500 mg/l of the organic carbon source.

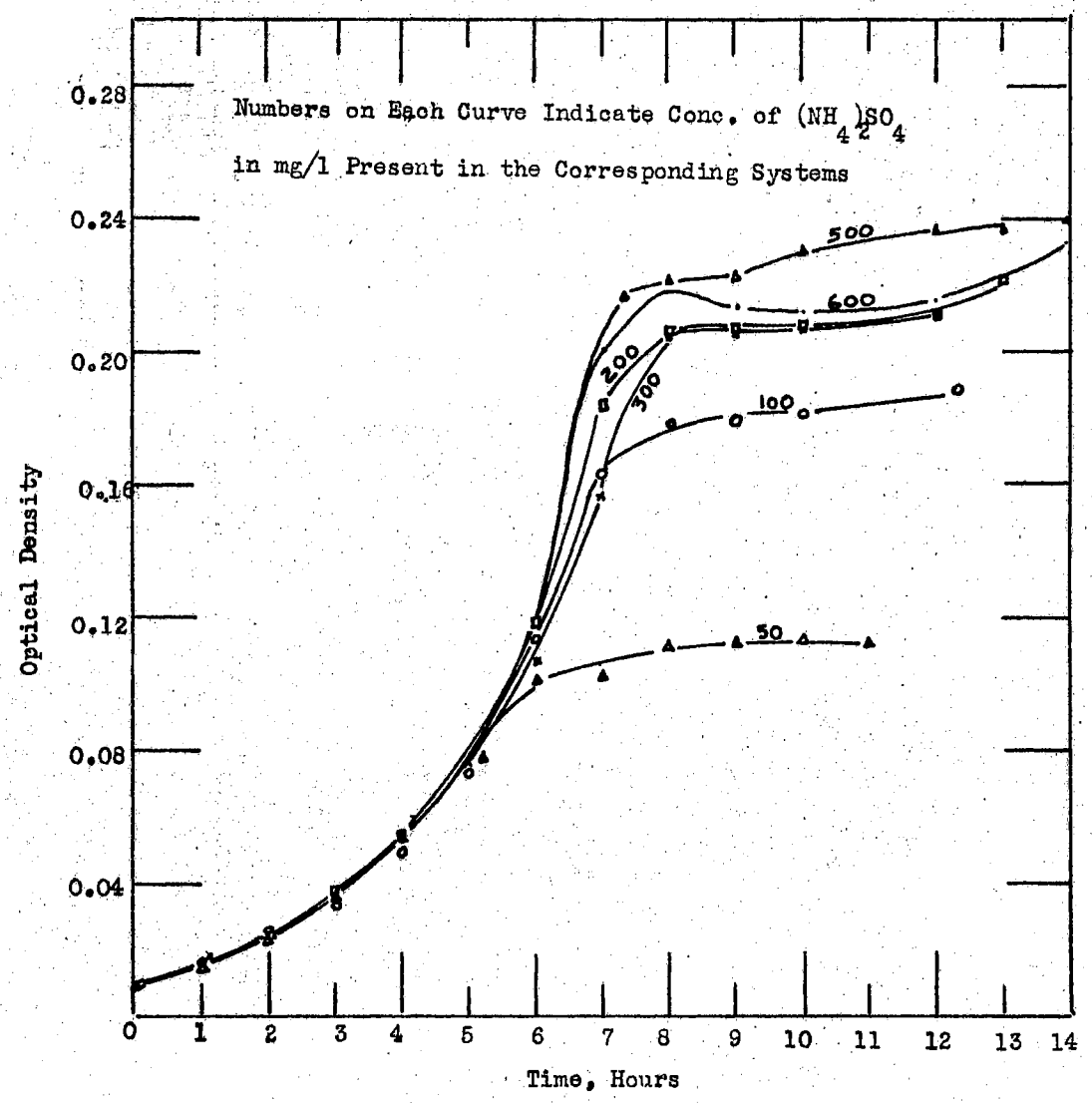

FIGURE 45 GROWTH OF SORBITOL - ACCLIMATED SLUDGE ON 500 MG/L OF SORBITOL MEDIUM WITH VARYING SUPPLY OF NITROGEN [AS MG/L $(NH_4)_2SO_4$]

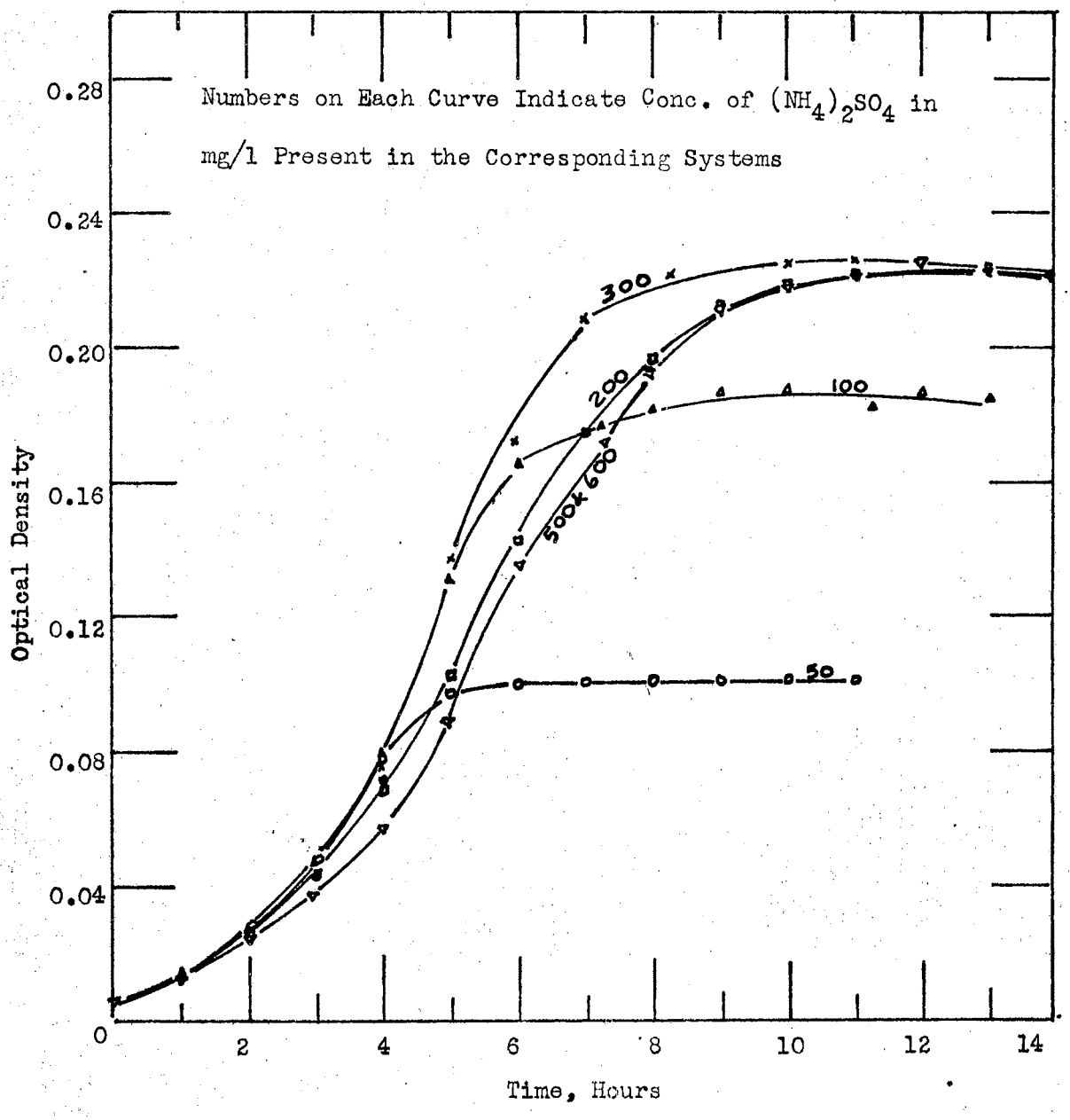

FIGURE 46 GROWTH DENSITY OF GLUCOSE - ACCLIMATED HETEROGENEOUS POPULATION IN 500 MG/L GLUCOSE MEDIUM WITH VARYING SUPPLY OF NITROGEN [AS MG/L $(NH_4)_2SO_4$]

APPENDIX B

DETERMINATION OF POLYALCOHOLS

Principle: The method of polyalcohol determination employed in this study is based on the principle that polyalcohols can be readily oxidized by periodate in an acid solution by being cleaved at each carbon-carbon bond within the compound, yielding 2 moles of formaldehyde per mole of polyalcohol. The amount of formaldehyde produced can be determined colorimetrically, since it forms a quite stable violet-red color with chromotropic acid. The excess periodate and iodate interfere in the color reaction and they are reduced to iodide by excess arsenite before the color is developed. Certain sugars or carbohydrates such as glucose or lactose can interfere with the test since they also can be oxidized to give formaldehyde but at a comparatively much slower rate. Therefore, by employing a short oxidation time, such interferences can be greatly reduced. The correction for the interferences by these sugars can be made by using a correction factor which corresponds to that sugar which is present in the medium. Obviously if these sugars are present in too high amount in comparison to the polyalcohols, the accuracy of this test would be affected. In this study, the concentration of the sugars is usually considerably less than the polyalcohol

concentration; therefore, such a problem is eliminated. The procedure herein described is primarily adapted from that suggested by Neish(65) with some modifications.

Reagents:

1. Periodic Acid (0.1 Molar) dissolve 4.6 gm of periodic acid (H_5IO_6) in 200 ml of water. This reagent should be prepared freshly before each use.

2. Sodium arsenite (1.0 Molar) Dissolve 13.0 gm of sodium arsenite ($NaAsO_2$) in water and adjust volume to 100 ml. This solution should be prepared freshly before use.

3. Sulfuric Acid (10 N) Pour 30 ml concentrated sulfuric acid (H_2SO_4) into 60 ml of water with stirring. Adjust volume to 100 ml when cool.

4. Absolute ethyl alcohol

5. Chromotropic Acid Reagent:

a. Dissolve 1.0 gm of chromotropic acid (1, 8-dihydroxy naphthalene, 3, 6-disulfonic acid) in 100 ml of distilled water.

b. Add 600 ml of concentrated H_2SO_4 to 300 ml water and cool.

c. Add the above 100 ml of chromotropic acid solution (solution a) to 900 ml of the diluted H_2SO_4 (solution b).

6. Standard solutions: the concentration of the standard solutions which are normally employed is 100 mg/l. The solution is prepared by dissolving 0.1 gm of the corresponding polyalcohols in 1000ml water. Standard sugar solutions are also prepared similarly.

Procedure:

An aliquot containing 0.05 to 0.3 mg of hexitol (e. g. mannitol, sorbitol or dulcitol) or 0.025 mg to 0.2 mg for glycerol is pipetted into a test-tube. The aliquot is then made up to 2.0 ml with distilled water. 0.1 ml of 10 N H_2SO_4 is then added to each tube. To each tube 0.5 ml of 0.1 M periodic acid reagent is then added, and exactly 10 minutes later 0.5 ml of the 1 M arsensite is added and mixed well. About twenty seconds after addition of the arsenite, iodine appears in the solution and then fades. After waiting about 10 minutes, 6.9 ml of absolute alcohol are added, which made up the total volume of the mixture to 10 ml, and the contents are well mixed. 1.0 ml of the mixture is then transferred into another test tube. 10 ml of chromotropic acid reagent are added and mixed. The tubes then are heated for 30 minutes in a boiling water bath, in diffused light. After cooling to room temperature, the percent transmittance is determined using a wavelength setting of 570 mμ. A blank is also run concurrently with each set of determination as well as several standards.

When the unknown sample also contains a sugar, the correction is made by also running the standards of the corresponding sugar present in the unknown concurrently.

Calculation:

Typical standard curves for glycerol, sorbitol and glucose as determined by the periodate-chromotropic acid test are shown in Figure 47. It was found that there were slight changes in the slope of these standard curves with each set of tests. Hence, it is recommended that a set of standards should be run concurrently with each set of determinations. It is seen that both glycerol and sorbitol give a much steeper slope than glucose. The corresponding slopes for each standard curve then can be calculated:

Glycerol Standard Curve = $4.1\ (O.D)/_{1\ mg\ glycerol}$

Sorbitol Standard Curve = $1.94\ (O.D)/_{1\ mg\ sorbitol}$

Glucose Standard Curve = $0.21\ (O.D)/_{1\ mg\ glucose}$

Hence, correction factors for glucose interference can be calculated: Correction factor for glucose in glucose-glycerol system

$$= \frac{0.21\ (O.D)}{4.1\ (O.D)}$$

$$= 0.051$$

Correction factor for glucose in glucose-sorbitol system

$$= \frac{0.21\ (O.D)}{1.94\ (O.D)}$$

$$= 0.108$$

Therefore,

glucose correction for glycerol-glucose system

= mg/l glucose (by anthrone) x 0.051

glucose correction for sorbitol-glucose system

= mg/l glucose (by anthrone) x 0.108

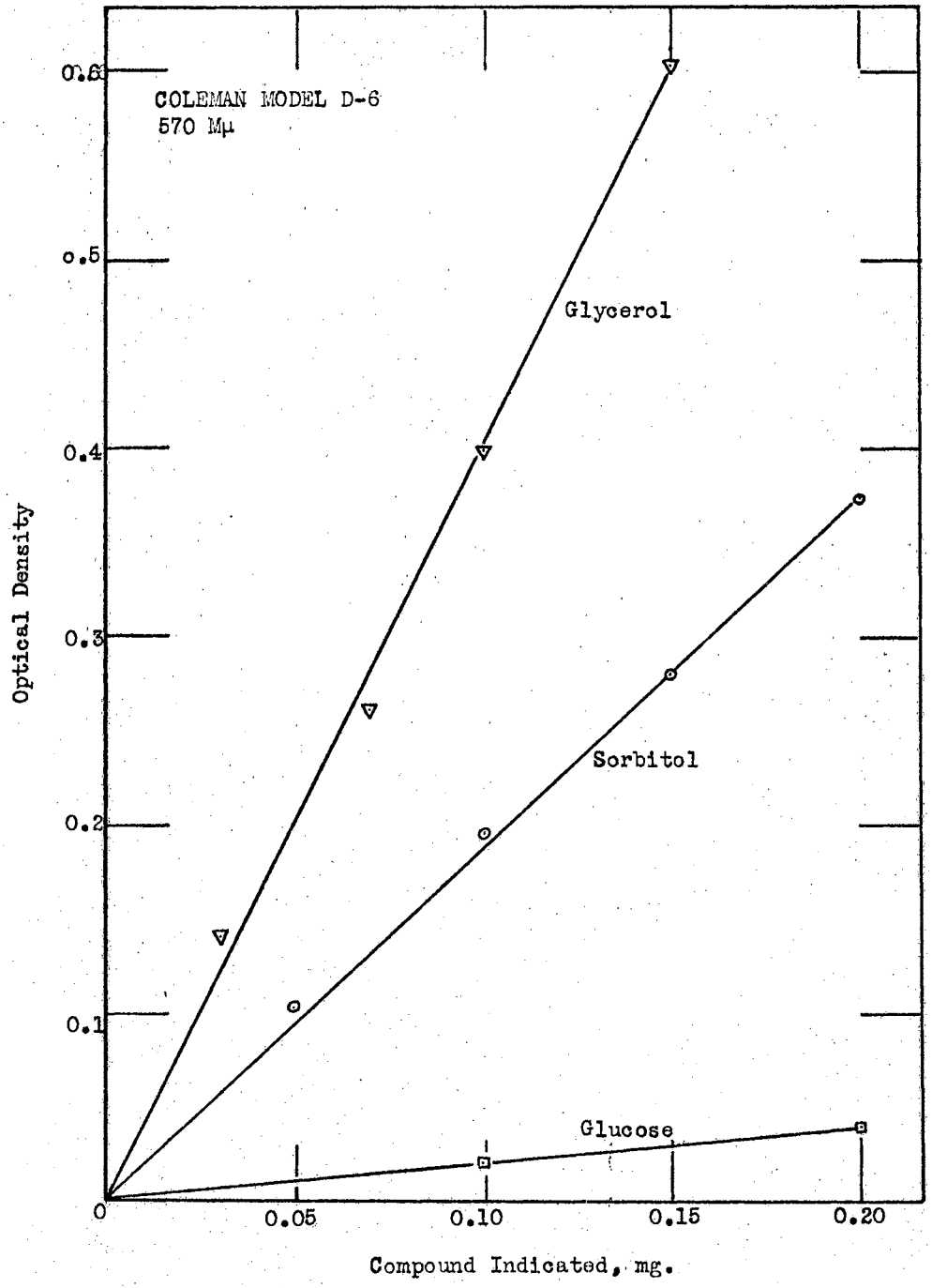

FIGURE 47 TYPICAL STANDARD CURVES FOR VARIOUS COMPOUNDS INDICATED AS DETERMINED BY PERIODATE - CHROMOTROPIC ACID TEST.

From the values of the correction factors obtained, it may be noted that approximately 20 mg/l glucose will yield an interference equivalent to 1 mg/l glycerol or 2 mg/l sorbitol. Thus the glucose interference will be relatively low when glucose is present in an aliquot in an amount equal to or less than that of glycerol or sorbitol.

VITA

KUSUG KOMOLRIT

Candidate for the Degree of

Doctor of Philosophy

Thesis: BIOCHEMICAL RESPONSE OF ACTIVATED SLUDGE PROCESSES TO ORGANIC SHOCK LOADS

Major Field: Sanitary and Public Health Engineering

Biographical:

Personal Data: Born in Bangkok, Thailand, February 24, 1937, the son of Lt. Col. King and Sa-ing Komolrit.

Education: Attended Bangkok Preparatory School in Bangkok (Thailand), 1952-54; attended the Premedical College at Chulalongkorn University, Bangkok, 1954-56; won a Thai Government Scholarship, sponsored by the Thai Public Health Service, to study in the United States in 1956; graduated from the Illinois Institute of Technology, Chicago, Illinois, in 1960, with a Bachelor's degree in Civil Engineering; while attending the Illinois Institute of Technology, received an award from the Thai Alliance in America for outstanding scholastic achievement in 1958; continued in the graduate school at the Illinois Institute of Technology, majoring in Sanitary Engineering till June, 1960; transferred to the University of Illinois, Urbana, Illinois, in September, 1960; while attending the university of Illinois, engaged in a research project on "Fundamental Factors in Treatment of Iron Bearing Waters" sponsored by the National Institute of Health, U. S. Public Health Service; was awarded the degree of Master of Science in Sanitary Engineering in January, 1962; entered Oklahoma State University, Stillwater, Oklahoma in January, 1962,

specializing in the Bio-engineering aspects of water pollution control; while attending the Oklahoma State University, engaged in the research project on "Response of Activated Sludge to Organic Shock Loads," supported by the U. S. Public Health Service; completed requirements for the degree of Doctor of Philosophy in Sanitary and Public Health Engineering at Oklahoma State University, May, 1965.

Membership in Honorary and Professional Societies: Sigma XI, Chi Epsilon, Tau Beta Pi, Oklahoma Water & Pollution Control Association.

Publications: Author and co-author of the technical publications concerning water treatment and water pollution control problems which include:

1. "Measurement of Redox Potential and Determination of Ferrous Iron in Ground Water" University of Illinois Sanitary Engineering Publication Series No. 11 (1962).

2. "Sequential Substrate Removal in Heterogeneous Populations" Jour. Water Pollution Control Fed., 35, 903 (1963).

3. "Multicomponent Substrate Utilization by Natural Populations and a Pure Culture of Escherichia coli" Applied Microbiology, 11, 157 (1963).

4. "Multicomponent Substrate Removal by Activated Sludge and by Pure Culture Systems" Symposium, Microbiological Aspects of Waste Disposal, Bacteriol. Proc. p. xvii (1963).

5. "Response of Biological Waste Treatment Process to Organic Shock Loading" Oklahoma State Engineer Jour. (Feb. 1964).

6. "Sequential Substrate Removal in Response to Qualitative Shock Loading of Activated Sludge Systems" Jour. Appl. Microbiol., 12, 280 (1964).

7. "Substrate Interactions during Shock Loadings to Biological Treatment Process" Proceedings 19th Annual Purdue Ind. Waste Conf., Purdue Univ., Lafayett, Ind. (May, 1964).

8. "Biochemical Response of Continuous Flow Activated Sludge Processes to Qualitative Shock Loadings" presented at 1964 Annual Meeting of Water Pollution Control Fed. (Oct., 1964).