# STUDIES ON THE EFFECT OF BACTERIAL GROWTH RATE ON SUBSTRATE REMOVAL IN MULTICOMPONENT SYNTHETIC WASTES

By

TA-SHON YU

Bachelor of Science
Taiwan Provincial Cheng Kung University
Tainan, Taiwan, Republic of China
1963

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1966

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
May, 1969

# STUDIES ON THE EFFECT OF BACTERIAL GROWTH RATE ON SUBSTRATE REMOVAL IN MULTICOMPONENT SYNTHETIC WASTES

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

## ACKNOWLEDGEMENTS

The author wishes to acknowledge the help rendered by the following persons and organizations, and expresses his sincere appreciation and deep gratitude to them.

To Dr. A. F. Gaudy, Jr., academic and thesis adviser, for his invaluable advice, precious friendship, and constant encouragement from the very inception to the completion of the thesis research, which made this work possible.

To Dr. E. T. Gaudy who patiently perused the manuscript of the dissertation and offered valuable suggestions.

To Professor Q. B. Graves, Dr. R. E. Koeppe, and Dr. D. F. Kincannon for their service as members of the advisory committee.

To Mrs. Grayce Wynd for her sincere friendship and excellent skills in typing this thesis.

To his wife, Yung-Tzu, who sacrificed so much, yet always endured with extended love and patience; his parents and other members of his family for their encouragement and sacrifices.

To the Federal Water Pollution Control Administration, U. S. Department of the Interior for financial support of the Research Project WP-00-786, "Metabolic Control Mechanisms in Biological Treatment."

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# CHAPTER I

# INTRODUCTION

## A. Utilization of Multicomponent Carbon Sources by Activated Sludge

Various types of waste waters differ in their degree of heterogeneity with respect to the number and types of carbon sources they contain. Domestic sewage may be expected to contain a great variety of carbon sources, whereas many industrial wastes contain a smaller number of carbon sources but these are usually present in higher concentrations. For many years the prevalent thinking in the field was that due to the heterogeneity of the microbial population, the various carbon sources in any given waste were removed concurrently, i.e., each of the various microbial species attacked the various types of compounds it could metabolize, simultaneously, and the action of all segments of the microbial population therefore caused the carbon sources to be removed concurrently. However, it was shown by Gaudy some time ago that a heterogeneous microbial population acclimated to sorbitol removed glucose and sorbitol sequentially. Subsequent work has shown that the phenomenon is not restricted to such compounds as glucose and sorbitol. Past work has provided some indication that the age of the heterogeneous population may play a role in determining whether sequential or concurrent removal of carbon sources occurs.

B. Purpose and Scope of the Present Investigation

It can readily be seen that sequential removal of the carbon sources in a waste or the partial blockage of the removal of one compound by the presence of another can cause changes in the kinetic mode and rate of overall waste water purification in biological treatment processes. Since such considerations affect the economic design and effectiveness of biological treatment processes, the study of substrate removal in multicomponent systems provides a worthwhile channel for investigation in the pollution control field.

The work undertaken in the present study was designed to gain a further insight into the general mode of substrate removal in multicomponent substrate systems, i.e., sequential removal, concurrent removal, or partial blockage of removal of one compound by the presence of another. To accomplish this purpose, various combinations of carbohydrates and carbohydrates with glycerol were studied in batch systems.

Studies were also designed to gain an insight into the possible effects of the past growth history of the organisms on the occurrence of sequential or concurrent substrate removal. A major portion of the research effort was devoted to assessing the effect of the cell age, as reflected in the past growth rate history of the cells, upon the mode of carbon source removal. For these studies the multicomponent carbon source employed was one which consisted of glucose and glycerol. Cell age was controlled in a modified chemostat operated at detention times of 4, 12, 24, 36, 48, 72, 96, and 192 hours. Cells harvested from the chemostat at various detention times or growth rates were used in batch studies to examine the mode of substrate removal. Effects on induction and repression of enzyme synthesis were examined in systems

employing low initial solids concentrations, whereas blockage of the function of existing enzyme(s) was assessed using systems with fairly high initial biological solids concentration. In some cases the blockage effect was examined under nonproliferating conditions.

Since the inflowing substrate concentration employed in the chemostat remained constant and growth rate was changed by either increasing or decreasing the hydraulic rate of flow, the studies provided an excellent opportunity to gain further insight into the response to the hydraulic shock load. Also, the studies offered an opportunity to determine whether a steady state condition could exist in the chemostat at extremely low growth rates, and to examine the effect of growth rate on such "steady state" parameters as substrate removal efficiency and cell yield.

# CHAPTER II

## LITERATURE REVIEW

The first experimental data indicating preferential bacterial growth on one carbon source over another when both were present in a medium was obtained by Monod. In experiments with Escherichia coli and Bacillus subtilis he observed diphasic optical density (growth) curves when the medium consisted of glucose and a polyalcohol (1). This data left little doubt that the diphasic growth curves were caused by sequential utilization of glucose and the polyalcohol. His interpretation of these early experiments suggested the existence in bacteria of regulatory mechanisms which controlled metabolism (growth). Springing from these early experiments and Monod's interpretative powers, a new and vital area of microbiological research has arisen. Today this area, which embodies microbial genetics and metabolic control mechanisms, comprises one of the most active and useful segments in the microbiological field. Also, much of the present knowledge concerning basic molecular genetics of all living matter has arisen from studies employing the bacteria or bacterial extracts. Today this field offers mankind one of its greatest challenges and hopes for fruitful rewards as well as one of its greatest responsibilities. When more complete understanding of molecular genetics is obtained, the next logical step would be attempts to exert possible external (or artificial) controls over the natural process. In

so doing, it may be possible to alter the natural hereditary process. Herein lies the awesome responsibility which mankind must assume. While man has long been involved in altering the "traits" of various plants and food animals by essentially "cut and try" methods for the purpose of producing traits deemed desirable, he will, with more knowledge of genetics, eventually be in a position to attempt to alter the traits of his own species. When this crossroad is reached, the philosophical considerations will dwarf those recently aired concerning the use of atomic energy. The statements above may seem somewhat afield of matters pertinent to the subject of the present thesis research; they are intended to delineate the importance of the underlying mechanisms which govern wholly or in part the biological response patterns observed during the course of the present research investigations. It must certainly be reasoned that if the basic genetics of all living matter is similar at the molecular level, the control mechanism must also function and play a role in determining the responses observed in natural ecosystems (e.g., receiving streams) and in ecosystems engineered (or supposedly so) to accomplish specific functions, e.g., the ecosystems developed in biological treatment processes such as activated sludge and trickling filtration plants. The extent to which such control mechanisms contribute or are manifested in the functional behavior of such systems has, until fairly recently, received no investigational attention.

Various metabolic mechanisms operative at both the genetic level and the enzyme level are known. While these mechanisms have not been delineated in complete detail, the great amount of work which has been done in this area has delineated the general mode of action of these

metabolic control processes and has permitted them to be characterized. In general, five distinct mechanisms are known to exist; two are operative in biosynthetic (anabolic) pathways, and three in degradative (catabolic) pathways. Gaudy has discussed and diagrammed these mechanisms in seminar presentations at Notre Dame University and the University of North Carolina, and the diagrams and portions of the text have been quoted by Su (2). Their delineation here would therefore seem to be a needless repetition, and the interested reader is referred to the thesis of Su (2). However, a brief description of the mechanisms, notation of the ones believed to play a prominent role in the responses observed in the present work, and the means of detecting them is useful, since they will be referred to in the discussion of the results.

## A. Biosynthetic Pathways

### 1. Repression of Enzyme Synthesis

The synthesis of enzymes needed by the cells to produce various cellular constituents is not generally subject to an induction (or "turning on") process. These are "constitutive" enzymes; the mechanism for their manufacture is always "turned on." These enzymes will be made so long as the raw materials (e.g., carbon, nitrogen source, etc.) for their manufacture are present in the environment. However, there is a mechanism to stop (repress) the manufacture of these enzymes, when the cell has produced enough of the particular product(s), component(s), or cell constituent(s) that are made through reactions which these enzymes catalyze. This mechanism which functions at the genetic level may be looked upon as means of permitting the cell to shut down the factory which makes the tools (enzymes) which, in turn, are used to make various

products the cell needs to grow. The genetic basis and diagrammic representation of the mechanism are given elsewhere (2).

2. Feedback Inhibition of Enzyme Function

The mechanism (repression) described above provides control over the making (synthesis) of enzymes employed in biosynthetic pathways, but the cell also possesses a means of shutting down the "secondary factory" in which these "tools" are employed. This mechanism, termed "feedback inhibition," prevents the use of enzymes which have already been synthesized, thus providing the cell with a means for rapid cessation of production of a particular compound or cellular constituent. Both repression and feedback inhibition accomplish the same end, but they function at different metabolic levels- repression at the genetic level, and feedback inhibition at the level of enzyme function. Together they provide the cell with positive control over the "turning off" process.

B. Degradative Pathways

1. Induction and Repression of Enzyme Synthesis

Enzymes which are required for metabolism (energy-yielding) of specific organic carbon sources, with the possible exception of glucose, are "inducible enzymes," i.e., they are not normally manufactured. The mechanism for their synthesis is not in a "turned on" state, as is the case for enzymes in a biosynthetic pathway. Enzymes common to the general degradative pathways found in many organisms may be inducible or constitutive. For example, the enzymes of the Emden-Meyerhoff-Parnas pathway, the hexose monophosphate pathway, and the tricarboxylic acid cycle may be considered as constitutive, whereas the key enzymes of the glyoxylate pathway are inducible. In any case, the enzymes needed to

catalyze reactions which permit the substrate (carbon source) to enter the common degradative pathways are inducible enzymes. They are not normally manufactured; the genetic mechanism for their manufacture exists in a repressed state. Induction (or acclimation) of a microbial cell to a particular carbon source comes about by release of the natural state of repression. Such release of repression is brought about by specific compounds termed "inducers." In nearly every case thus far examined, the substrate or carbon source is an inducer for the synthesis of the enzyme needed to act upon it. It can be seen that this mechanism (like the ones previously described) allows the cell to practice economy of metabolic effort. These enzymes are produced only when their inducer is present; production is stopped when the inducer is removed from the environment or is depleted in the environmental resource, i.e., when the substrate has been metabolized. Thus it may be seen that, concerning enzyme synthesis, the difference between biosynthetic and degradative pathways is that in the former, induction is not needed, and the control is exercised by repression, whereas in the latter, induction is needed and the control is exercised by release of repression.

2. Metabolite Repression

The mechanism given immediately above provides control of metabolism of a specific carbon source, but does not explain metabolic behavior often observed when two or more carbon sources are present. For example, it offers no insight into the diphasic growth curves for Escherichia coli reported by Monod (1) when glucose and a polyalcohol were used as joint carbon sources, since the cells had been previously grown on the polyalcohol. Their ability to synthesize the required enzymes for its metabolism had already been turned on. Also, the rates

of growth on glucose and the polyalcohol when used as sole carbon sources were not so great that all of the glucose would have been removed before growth on the polyalcohol would have started. In cases where the above two conditions obtain and sequential substrate removal (a sequential growth) occurs, it is necessary to seek another explanation obviously one which involves some kind of interaction between the two substrates. Such a control mechanism has recently been hypothesized; it has been termed "metabolite repression" by McFall and Mandelstam (3). If two compounds, A and B, are present, it may be expected that they will act as inducers which inactivate the natural state of repression and the genetic mechanism for synthesis of the degradative enzyme systems needed to bring both compounds into a common energy yielding pathway is released. If the breakdown of either compound leads to a common metabolic intermediate product, P, and if the rate of production of this intermediate compound is faster for compound A, then P can activate a repression of synthesis of enzymes needed to degrade carbon source B. The repression of B enzymes should last as long as the level of P produced from A is high enough to form effective repression. When the concentration of the repressor falls below some critical concentration, the repression is lifted, induction of B enzymes proceeds, and compound B is metabolized.

This proposed mechanism may be subjected to criticism, since P is also produced from compound B; thus, it might be expected that metabolism of compound B could prevent synthesis of enzymes needed for its own metabolism. Indeed, such a situation may be responsible for controlling the rate of utilization of compound B when it is used as a sole carbon source. The implication is that for compound A to shut off

synthesis of B enzymes, the intermediate compound P must be produced at a much more rapid rate from A than from B and that it must be present at a high concentration in order to effect complete shutdown of the synthesis of B enzyme(s). Such a surmise could provide a convenient explanation for cases of partial rather than complete sequential substrate removal.

Another criticism of the hypothesized mechanism of metabolite repression is that the sequential removal may not involve the action of a common metabolic intermediate but may be, instead, competition for a common permease. Thus compound A may combine so rapidly with the permease that it ties up all of the transport protein so that compound B cannot enter the cell until compound A has been used. However, the work of Zwaig and Lin (4) would seem to indicate that at least in some cases, e.g., those for which entry of the compounds into the cell is not mediated by permeases, or in any event by a common permease, metabolite repression does occur.

3. Feedback Inhibition of Enzyme Function

The mechanism of metabolite repression provides an explanation for sequential growth (and consequent sequential substrate removal) but it does not explain sequential substrate removal in some of the systems recently studied by Gaudy and his co-workers. As early as 1963, Gaudy, Komolrit, and Bhatla (5) suggested that sequential substrate removal could come about not only by repression of the synthesis of the enzyme(s) required to metabolize one of the substrates but also by a mechanism which involved the blockage of the function of the enzyme(s) already synthesized. Their conclusion was based upon the finding that sequential substrate removal of glucose and sorbitol was observed using a

rather high initial concentration of cells which had been previously acclimated to sorbitol. The existence of such a mechanism was later supported by the work of Gaudy, Gaudy, and Komolrit (6), who observed sequential substrate removal (glucose and sorbitol) for a prototrophic strain of Escherichia coli under nonproliferating conditions. They suggested that the mechanism of the blockage was similar to feedback inhibition in biosynthetic pathways. In such a mechanism a common intermediate in the degradative pathways of glucose and of sorbitol is produced faster from glucose and blocks the site of action of the first enzyme in the degradation of sorbitol.

It might be argued that the sequential substrate removal is brought about by more successful competition of glucose for a possible common permease thus preventing sorbitol from entering the cell. Indeed, such may be the case in many systems, but the work reported on glucose and glycerol in 1966 by Zwaig and Lin (4), who used a mutant strain of Escherichia coli which could take up glycerol while growing on glucose (i.e., passage of glycerol into the cell was by diffusion rather than by an active-permease transport) indicated that the blockage of glycerol metabolism was brought about by a feedback mechanism. Glycerol kinase, the first enzyme in the pathway of glycerol degradation, was inhibited and in the system they studied, fructose-1, 6-diphosphate was found to inhibit the kinase. This work was significant, since it substantiated the conclusions of Gaudy and his co-workers concerning the existence of a feedback inhibition mechanism in degradative pathways, and demonstrated that the inhibition could be brought about by a metabolic intermediate.

It can be reasoned that since the above mechanisms underlie

determination of the control of metabolism and the rate at which it progresses, they must play a determinative role in biological treatment of waste waters. However, the work in the basic microbiological fields on metabolic control mechanisms (except for that of Gaudy and his co-workers) has, for the most part, been accomplished using a limited number of pure cultures of bacteria. In the treatment of waste waters and in the case of most natural microbial systems, the population exhibits varying degrees of heterogeneity with respect to the species it contains; microorganisms other than bacteria often exist in the ecosystem. It could be argued that the heterogeneity of the population might tend to mask manifestation of the effects of the control mechanisms on the rate and mode of removal of the various substrates in a given waste water, e.g., a given industrial waste. Indeed, it was the manifestation of the effects of the control mechanism which interested Gaudy in his early studies on sequential substrate removal (7). He reasoned that if diphasic growth could be shown for two bacterial species (as done by Monod [1]), it might be a phenomenon common to a great many species and therefore should be demonstrable using heterogeneous populations. Using equal concentrations of glucose and sorbitol as mixed carbon sources, and an inoculum of cells which had been previously acclimated to sorbitol (grown from an initial seed of municipal sewage), he was able to show that all of the glucose (as measured by the anthrone test) was removed from the system while half of the total organic substrate (attributable to sorbitol) as measured by the COD test remained in solution. The point of glucose removal corresponded to a break or discontinuity in the growth curve (measured by either optical density or membrane solids determination). This work

provided the first evidence for the occurrence of sequential substrate removal in heterogeneous or mixed populations of microorganisms. A small initial cell inoculum was employed; thus this finding demonstrated repression of the synthesis of sorbitol degrading enzyme(s) while glucose remained in the medium.

This initial result precipitated an extensive continuing research effort in Gaudy's laboratory; indeed, the present report forms a portion of the overall investigational effort. Gaudy's initial results were later substantiated and extended by the work of Gaudy, Komolrit, and Bhatla (5). It was this work which led to the conclusion (tentative at the time) that another mechanism besides enzyme repression existed as a cause for sequential substrate removal.

It was also found that operational conditions which determined the "past history" of cells seemed to play a role in determining whether sequential or concurrent substrate removal occurred. Batch operation on sorbitol over a prolonged period of time leading to highly flocculated systems resulted in concurrent removal of sorbitol and glucose, whereas young populations (grown through one or two transplants and harvested for study in or at the end of the logarithmic growth phase) yielded sequential removal. This finding was later substantiated using substrate systems consisting of glucose-sorbitol and glucose-mannitol (8). The observation of sequential substrate removal on glucose and sorbitol was also substantiated by the work of Prakasam and Dondero (9) who concluded from their data that "in agreement with Gaudy's observations, it can be seen that there is a definite indication that metabolism of sorbitol is upset due to the presence of glucose." However, they criticized the work on two points. First, in Gaudy's original

work and their own, direct analysis for the polyalcohol was not performed, and there was, therefore, some doubt as to the validity of the subtraction method which was used; secondly, they felt that acclimation to sorbitol selected a narrow range of species which was not representative of a municipal sewage. The first criticism was overcome by use of a modification of the periodate test as reported in later work by Gaudy, Komolrit, and Gaudy (8). The latter statement is probably correct, since it is obvious that growth on a particular carbon source should be expected to select a narrower range of microbial species than that found in municipal sewage. However, it can also be argued that the procedure of selection provides a more severe test of the existence of sequential substrate removal in mixed populations because it tends to ensure that all of the cells in the system can metabolize the substrate. Also, it should be recalled that many industrial wastes contain only a few carbon sources and would be expected to exert a selective pressure.

Komolrit and Gaudy (10) have conducted studies using a variety of substrate systems under severe shock loading conditions in which one compound was injected into the medium during the period of active removal of another compound. A system rapidly metabolizing ducitol was blocked in the same manner by glucose as had been previously found in the case of sorbitol and mannitol. Sorbitol removal was also partially blocked by injection of galactose; injection of lactose had no effect on sorbitol removal. A shock loading of glycerol had no effect on the removal of ribose, and when this finding was analyzed in light of other experiments it was felt that the results indicated that, if a metabolic product produced from one compound was responsible for blocking removal

of another, it would be expected to lie above the triose level.

Recently, Prakasam and Dondero (11) published results of further experiments using the glucose-sorbitol system. In these studies they used a sorbitol-acclimated sludge as well as sludge taken from an activated sludge plant. They employed $^{14}C$-labeled sorbitol, and again concluded in accord with the original results of Gaudy concerning sequential removal of glucose and sorbitol by a sorbitol-acclimated population. It is also interesting to note that they were able to show with simple isolation procedures at least six different types of organisms in the sorbitol-acclimated population. In experiments with biological solids from an activated sludge plant in which labeled $CO_2$ evolved in the Warburg apparatus was measured, these authors concluded that concurrent removal of glucose and sorbitol occurred. They felt that this type of removal ensued because the activated sludge had a greater variety of microbial species. However, they presented no data showing the number of types of bacteria present in their sample of activated sludge. It might also be reasoned that the municipal activated sludge could be rightly characterized by the term "old cells."

Other than the research by Gaudy and his co-workers, and the two investigations of Prakasam and Dondero cited above, the only other published effort in this line of research has been undertaken by Stumm-Zollinger. Her initial study (12) was designed in an attempt "to confirm the relevance of Gaudy's findings to the processess occurring in natural habitats by extending the range of experimental conditions." She employed various substrate systems, and her results confirmed that sequential substrate utilization is observed in heterogeneous bacterial populations. In later work (13) she, like Prakasam and Dondero before

her, was much concerned with the heterogeneity of the microbial population. Her findings were in general accord with those of Gaudy and his co-workers, and of Prakasam and Dondero, in that old cells or cells taken directly from a field activated sludge installation yielded concurrent rather than sequential removal (glucose-galactose as carbon source). However, she also observed concurrent removal with young cell populations and felt that for her cultures the mode of removal (sequential or concurrent) seemed "to be related to the kinds and relative numbers of bacteria present rather than the physiological state of the cells." She also found that sequential substrate removal was observed in cultures in which coliforms predominated.

Komolrit and Gaudy have also observed the effects of sequential substrate removal in completely mixed continuous flow reactors under shock loading conditions (14). These findings attest to the significance of the phenomenon in waste water treatment reactors. Detailed critique of this work and other publications of Gaudy and his co-workers concerning sequential substrate removal (15)(16)(17)(18)(19)(20)(21)(22)(23)(24) is not needed here to demonstrate the importance of this line of research. The above brief history of past work delineates points of agreement and possible disagreement between the investigations accomplished in this laboratory and in other laboratories. The present investigation was designed to broaden the scope of substrate combinations investigated, and to gain further insight into the effect of the past growth history on the mode of substrate removal.

# CHAPTER III

# MATERIALS AND METHODS

## A. Analytical Techniques

### 1. Biological Solids Determination

The biological solids concentration was determined using the membrane filter technique as described in Standard Methods for the Examination of Water and Waste Water (25), and/or by the optical density of the cell suspension using the Bausch & Lomb Spectronic 2D (540 mμ). The relation between optical density and biological solids concentration is given in the calibration curve shown in Figure 1. It is seen that a fairly constant relationship exists up to biological solids concentrations of 300 mg/l.

### 2. Chemical Oxygen Demand (COD)

The principle of the COD test is well known, and it can be said with considerable certainty that the great majority of organic compounds can be totally oxidized to $CO_2$ and $H_2O$ by the action of a strong oxidizing agent, potassium dichromate, under the acidic conditions of the test. The procedure was run in accordance with Standard Methods for the Examination of Water and Waste Water (25).

### 3. Anthrone Test

The carbohydrate content of the filtrate or of the cell suspension

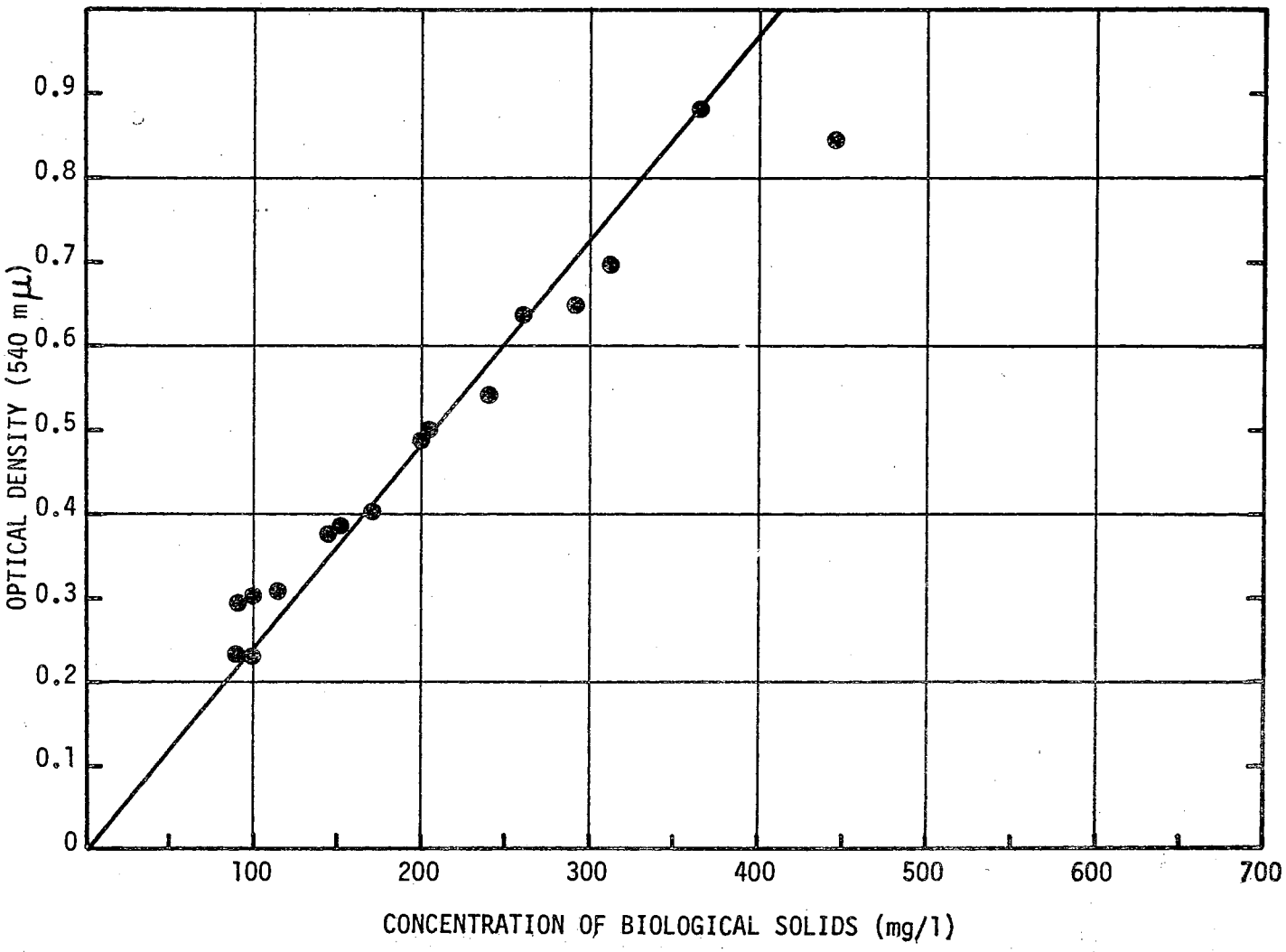



Figure 1 - Biological Solids Concentration versus Optical Density for Heterogeneous Populations.

was determined using the anthrone test. In this test the strong sulfuric acid solution catalyzes the hydrolysis of carbohydrates and dehydration to furfural or hydroxymethyl furfural, which then condenses with anthrone to give a colored product (26). The experimental procedure was run in accordance with the recommendations of Gaudy (27).

4. Glucostat Test

The glucose concentration was determined using the glucostat method, which is an enzymatic determination for β-D-glucose and 2-deoxy-D-glucose. The latter compound is oxidized at a much slower rate than is glucose, approximately 12 percent the rate of glucose oxidation. The procedure used was in accordance with the manufacturer's specifications (Method I-A)(28).

5. Galactostat Test

Galactose concentrations were measured using the galactostat test which, like the Glucostat test, is an enzymatic analysis but is specific for galactose. The test was performed in accordance with the procedure outlined by the Worthington Biochemical Corporation (29).

6. Orcinol Test

Under carefully controlled conditions of temperature, time, and HCl concentration, pentoses are rapidly converted to furfural. In the presence of ferric ion and orcinol (5-methyl resorcinol), furfural condenses and yields a colored product (26). The most widely used modification for pentose determination is that of Mejbaum (30). The procedure employed in the present studies was in accordance with that given in Experimental Biochemistry (26).

7. Resorcinol Test

The resorcinol test of Roe is a modification of the Seliwanoff reaction and provides a specific chemical analysis for ketoses. In concentrated HCl solutions, ketoses undergo dehydration to yield furfural derivatives much more rapidly than do aldoses. The furfural derivatives form a complex with resorcinol, and develop the specific color of the resorcinol test (26). The method employed in the present investigation was that given in Methods in Enzymology (30); it is noted that the color reaction is specific not only for ketoses, but also for disaccharides containing a ketose component, e.g., sucrose.

8. Nelson Test

This analysis is commonly used for quantitative determination of reducing sugar. In this test the $Cu^{++}$ is reduced to $Cu^{+}$ in alkaline solution. Cuprous oxide is precipitated quantitatively and its amount is determined by addition of arsenomolybdic acid, which is quantitatively reduced to arsenomolybdous acid by $Cu^{+}$ ion. The rust-blue color of arsenomolybdous acid is then measured colorimetrically. The procedure used in the present research was that given in Experimental Biochemistry (26).

9. Biuret Test

In the biuret test for determination of cellular protein, color development is due to the chemical reaction between copper sulfate and peptide bonds in alkaline solution (26). The procedure used in the present investigation was that outlined by Gaudy (27).

10. Measurement of Oxygen Uptake (Warburg Technique)

The Warburg respirometer has been employed for direct measurement of biochemical oxygen demand (31). More generally it is used to measure the oxygen uptake (respiration) of biological materials. The detailed techniques for use of the apparatus are given in Standard Methods for the Examination of Water and Waste Water (25) and in Manometric Techniques (32).

11. Periodate Test

In the present investigation the periodate test was used for quantitative assessment of sugar alcohols, e.g., sorbitol and glycerol. Periodate oxidation of such compounds yields formaldehyde which forms a stable violet-red color with chromotropic acid. The excess periodate is reduced to iodide by excess arsenite before the color is developed. The procedure employed in the present investigation was that outlined by Komolrit (33).

12. Dissolved Oxygen

Dissolved oxygen concentration in the mixed liquor of the continuous flow reactor was measured using a galvanic cell oxygen analyzer. The instrument used in the present investigation was manufactured by Precision Scientific Company, Chicago, Illinois.

B. Apparatus and Experimental Design

Phase A

a. Selection of Substrates

The compounds selected were carbohydrates and sugar alcohols known to compose a part of various waste waters. Also, the compounds selected

are those for which reasonably accurate quantitative analyses were available. Compounds used in this study include the following: glucose, fructose, galactose, mannose, ribose, arabinose, lactose, sucrose, and glycerol.

b. Synthetic Waste

For all batch studies the growth medium was standardized and contained the following inorganic constituents (made up in distilled water): potassium phosphate buffer (1.0 M) pH 7.0, 10 ml/l; $(NH_4)_2 \cdot SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; $MnSO_4 \cdot H_2O$, 20 mg/l; $CaCl_2 \cdot 2H_2O$, 15 mg/l; $Fecl_3 \cdot 6H_2O$, 1.0 mg/l; tap water, 67 ml/l. The various organic compounds were added to this minimal medium in desired concentrations for individual experiments.

c. Development of Microbial Populations

In these studies two different types of populations were employed. For one set of experiments the initial population consisted of 100 ml of settled sewage taken from the primary clarifier effluent of the sewage treatment plant at Stillwater, Oklahoma. All other experiments were conducted using an initial inoculum of cells (originally obtained from the same source) which had been previously acclimated to a specific carbon source.

For experiments employing an acclimated population, the microbial seed was developed as follows: 50 ml of standard growth medium containing 1000 mg/l of the specific carbon source to which the cells were to be acclimated were inoculated with 5 ml of fresh sewage from the Stillwater, Oklahoma, municipal treatment plant. This growth mixture was placed in a 250 ml Erlenmeyer flask and aerated at room temperature

on a reciprocal shaker at 100 osc/min. After 36 hours, 25 ml of this suspension were transferred to 475 ml of fresh growth medium, and the mixture was distributed in 50 ml portions to a series of 250 ml Erlenmeyer flasks. These systems were then aerated on the shaking apparatus, as described above. After 18 hours of aeration, during which time the carbon source was exhausted, the cells were harvested from the Erlenmeyer flasks and used for experimentation.

d. Experimental Protocol

In all studies the experimental systems consisted of two control reactors, each containing one of the specific carbon sources to be studied in combination, and one reactor containing both carbon sources. The specific carbon source under question was added to its control system at a concentration of 500 mg/l, whereas the combined system received 500 mg/l of each carbon source. One hundred ml of seeding population was added (either sewage or acclimated seed); the total volume of the mixed liquor in each reactor was 1.5 liters. The batch reactors (4-inch diameter test tubes) were aerated vigorously by compressed air admitted to the reactor through porous diffuser stones. During these studies temperature of the reaction liquor was maintained at 22°C. The course of biological growth was assessed by measurement of optical density. The optical density versus biological solids concentration curve was used to determine the frequency of obtaining samples for substrate analysis during a particular experiment. Twenty-five ml samples were taken periodically from the reactor, placed in a Sorvall centrifuge type SS-1, and spun at 12,000 rpm for fifteen minutes. After centrifugation, the supernatants were carefully removed and, as a safeguard, passed through a Millipore filter (HA 0.45 μ) into collection tubes.

Either 10 or 20 ml of the collected supernatant (filtrates) were used for measurement of total COD, and the remaining volume was placed in a freezer for later analysis for specific substrates.

Phase B

a. Apparatus

The apparatus used for continuous culture of the heterogeneous population was a completely mixed once-through chemostat of the type shown in Figure 2. The carbon source was the growth-limiting substrate, and under such conditions the growth rate is equal to the reciprocal of the reactor detention time. The aeration liquor volume in the reactor was 2.4 liters. The medium was delivered to the reactor through a pump calibrated to deliver various rates of flow, at a rate depending upon the desired detention time. Complete mixing of the reactor was checked by measuring the optical density of the cell suspension in the reactor and in the effluent from the reactor. Compressed air was admitted through porous diffuser stones. Air was supplied at a rate of either 4000 ml/min per 2.4 liters of aeration liquor, or 8000 ml/min per 2.0 liters. The higher airflow rate was employed at dilution rates lower than 1/24 $hr^{-1}$. The increase in aeration rates was necessary, since at this and lower dilution rates the development of filamentous organisms and side growth prevented complete mixing at the lower airflow rate. The continuous flow unit was run at a temperature of 22°C.

b. Selection of Substrate and Synthetic Waste

Glycerol was selected as the sole carbon source fed to the continuous flow reactor. This compound was chosen since it is one on which a considerable amount of research had already been accomplished in the

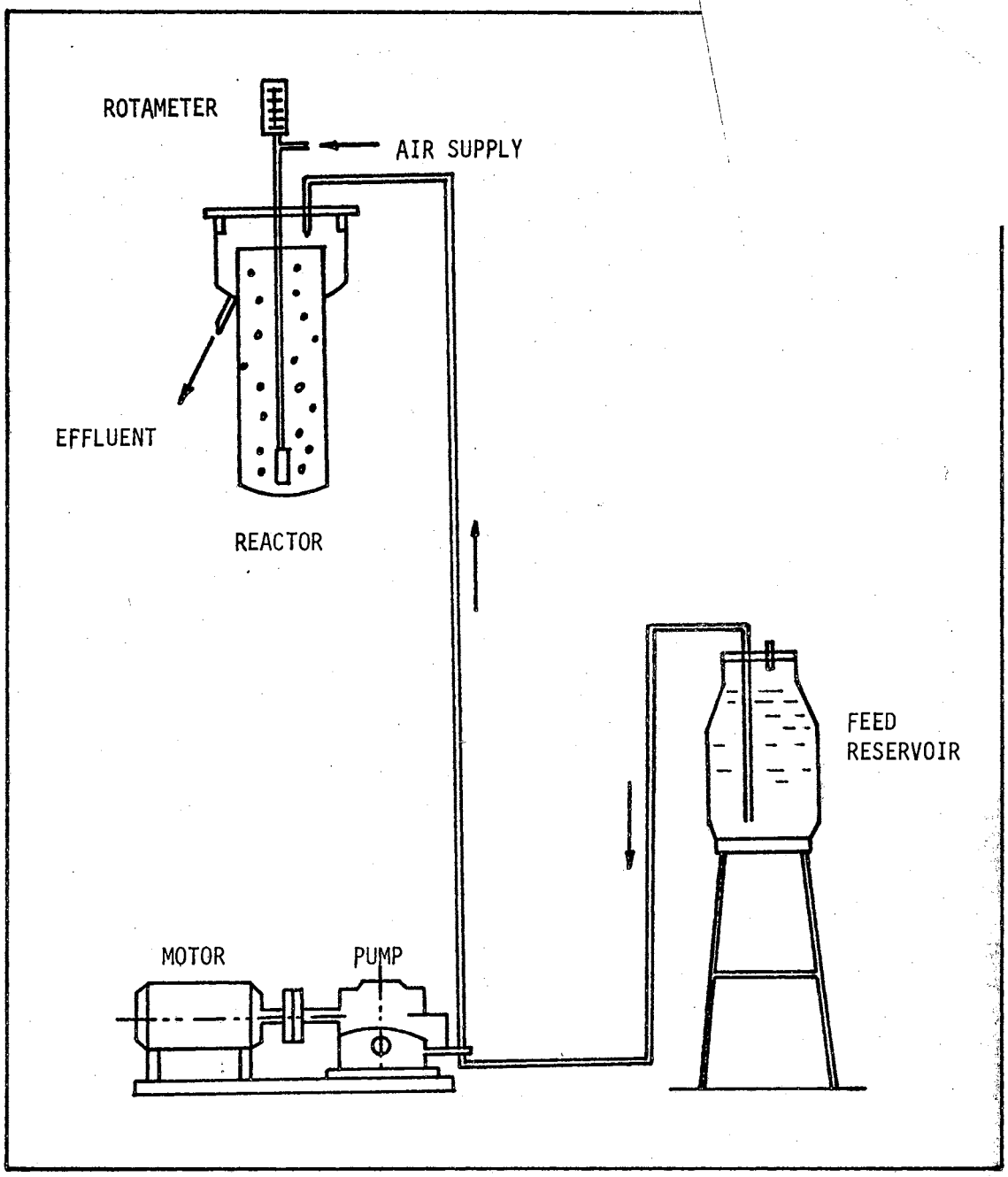



Figure 2 - Completely Mixed Continuous Flow Apparatus.

bioengineering laboratories, and it had been shown that glycerol metabolism was blocked in the presence of glucose. It therefore was an ideal carbon source for use in this stage of the investigation. Glycerol was fed to the continuous flow unit at a concentration of 2000 mg/l; the inorganic constituents of the synthetic waste were the same as those used in Phase A except that the concentrations were doubled to accommodate the increased concentration of carbon source. For batch studies using cells acclimated to glycerol harvested from the chemostat as in studies, glucose is in combination with glycerol. As in previous studies using other biological populations, the glucose control contained 500 mg/l glucose; the glycerol control contained 500 mg/l glycerol, and the combined unit contained 500 mg/l of each substrate. The inorganic constituents of the medium were the same as those given previously. In batch studies run under nonproliferating conditions, all constituents of the synthetic waste were the same as those used under growth conditions, with the exception that the nitrogen source was withheld.

c. Development of Microbial Populations

The chemostat was seeded initially with 100 ml of settled sewage obtained from the primary clarifier of the municipal sewage treatment plant at Stillwater, Oklahoma. Throughout subsequent months of study on the behavior of the continuous flow unit in transient and steady states, and batch studies to test for the mode of substrate removal when the cells were subjected to glucose and glycerol, the microbial populations employed were those which were developed in continuous culture from this initial sewage seed.

d. Experimental Protocol

The chemostat was run initially at a dilution rate of 1/4 $hr^{-1}$. It was allowed to come into steady state operation and checked for steady state by measuring the optical density of biological solids concentration in the reactor and in the effluent from the reactor. When these values were the same or nearly so, the unit was adjudged to be completely mixed, and when the biological solids concentration remained steady the unit was adjudged to be in the steady state. Samples were then taken to assess the steady state parameters. For each sampling period, two 25-ml samples of mixed liquor or effluent were removed, centrifuged and filtered as described earlier. One of the 25-ml samples was used to measure biological solids concentration, and the biological solids from the second sample were re-suspended in 10 ml of distilled water in a 25 ml vial and frozen for further analysis for cellular carbohydrates and proteins. Twenty ml of the supernatant were used for COD determination, and a 10 ml portion of the remaining liquor was stored for glycerol analysis.

After a sufficient number of samples was obtained to describe the behavior of the unit in a steady state, the dilution rate was changed. During the resultant transient state, samples were collected and processed to determine the behavior of the system in response to the hydraulic shock loading produced by the change in dilution rate. The number of samples collected in the transient state was determined by the severity of the change in detention time (or dilution rate). Later, as the unit came into the new steady state, samples were in general less closely spaced but sufficient sampling was conducted to enable assessment of the behavior of the unit at the new steady state.

For operation in the steady state at all dilution rates examined in the present investigation, cells were harvested for batch studies to determine the mode of substrate removal when glucose and glycerol comprised the joint carbon source. A glucose control system and a glycerol control system were also run as previously described. In these batch studies using cells harvested from the chemostat, both high and low initial biological solids concentrations were employed. In determining the growth of biological solids in the three batch units, the membrane filter technique rather than optical density measurement was used.

# CHAPTER IV

# RESULTS

## Phase A

### Studies on Substrate Removal in Systems Containing Two Carbon Sources

This phase of the work is presented in six subsections. The results are grouped in accordance with the previous growth history of the heterogeneous populations which were used. In general, the substrates consisted of various combinations of carbohydrates, and combinations of carbohydrates with a sugar alcohol. For all studies made using a multicomponent medium, control systems using each compound as sole carbon source were also run. In some studies the systems were also subjected to shock loading conditions. Following a graphical presentation of the results, summary tables, which include pertinent kinetic and operational parameters, are presented.

#### 1. Combinations of Glucose with Various Carbohydrates (Direct Inoculum of Domestic Sewage as Source of Heterogeneous Populations)

For all of the experiments in this section of the results, small initial inocula of sewage seed were employed. This procedure ensured obtaining the most heterogeneous population possible. These experiments were designed to assess the order and rapidity of acclimation to the various compounds, and to seek evidence for induction and/or

repression of one compound by the presence of another.

a. Combined Substrate - Glucose and Galactose

The control systems for glucose and for galactose are shown in Figure 3. The data indicate that more rapid growth was attained using glucose as substrate. On both substrates there is evidence for the production and accumulation in the medium of a small amount of metabolic intermediates and/or endproducts (compare total COD curves with curves for the specific substrates).

The substrate removal and growth pattern when these two compounds were used as a combined carbon source is shown in Figure 4. The curve labeled "galactose + intermediates" was obtained by subtracting the COD due to glucose from the anthrone COD. This was done since no specific test for galactose was used in this study. It can be seen that initiation of galactose removal did not occur until approximately half of the glucose had been metabolized. Although the removal was essentially sequential in nature, this was not reflected in a diphasic growth curve.

b. Combined Substrate - Glucose and Mannose

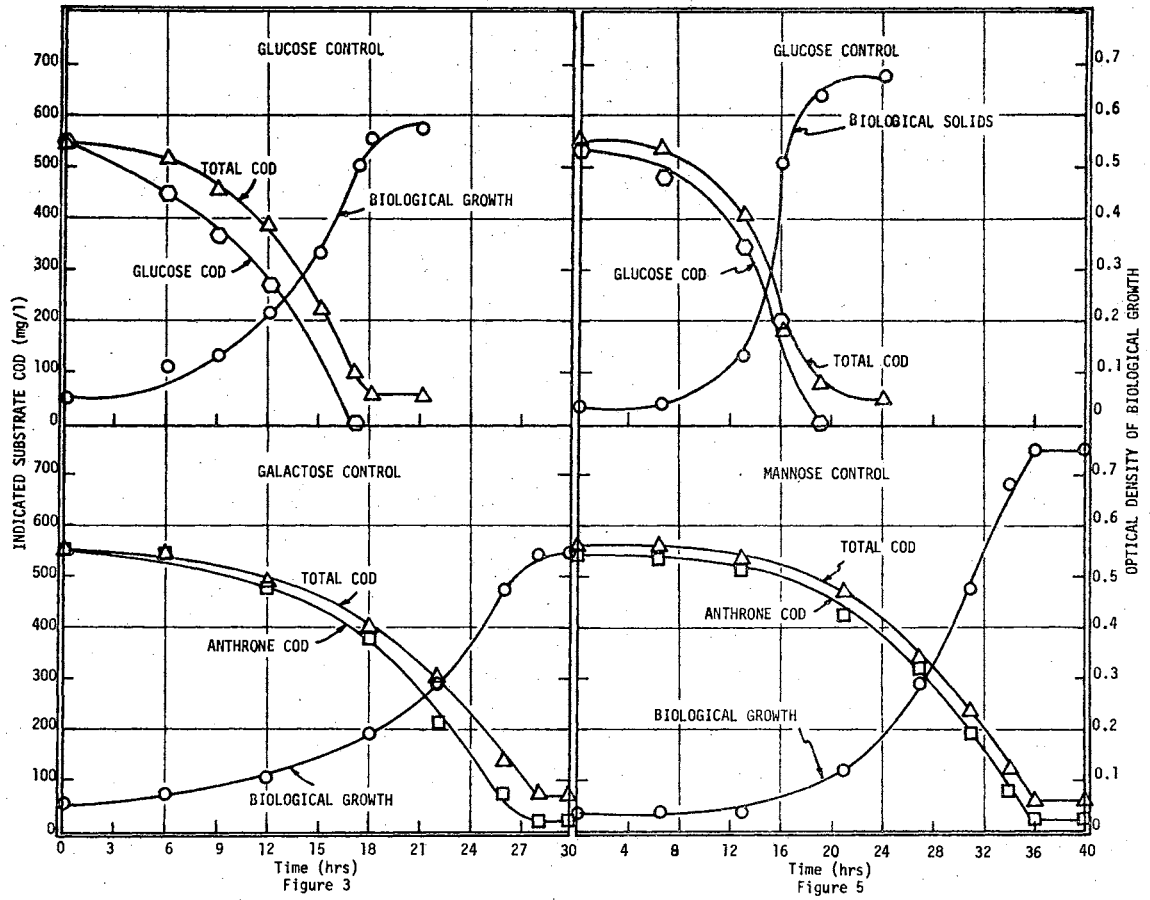
In Figure 5 it is seen that a considerably longer period of time was required to initiate growth on mannose than on glucose. It is interesting to note the significantly higher biological solids concentration which was obtained using mannose as the carbon source. Results for the combined system are shown in Figure 6. Glucose and mannose were removed sequentially. The curve labeled "mannose + intermediates" was obtained by subtracting glucose COD from the anthrone COD. Since the anthrone COD and the total COD follow essentially along the same line, the rise in the curve labeled "mannose + intermediates" indicates that,

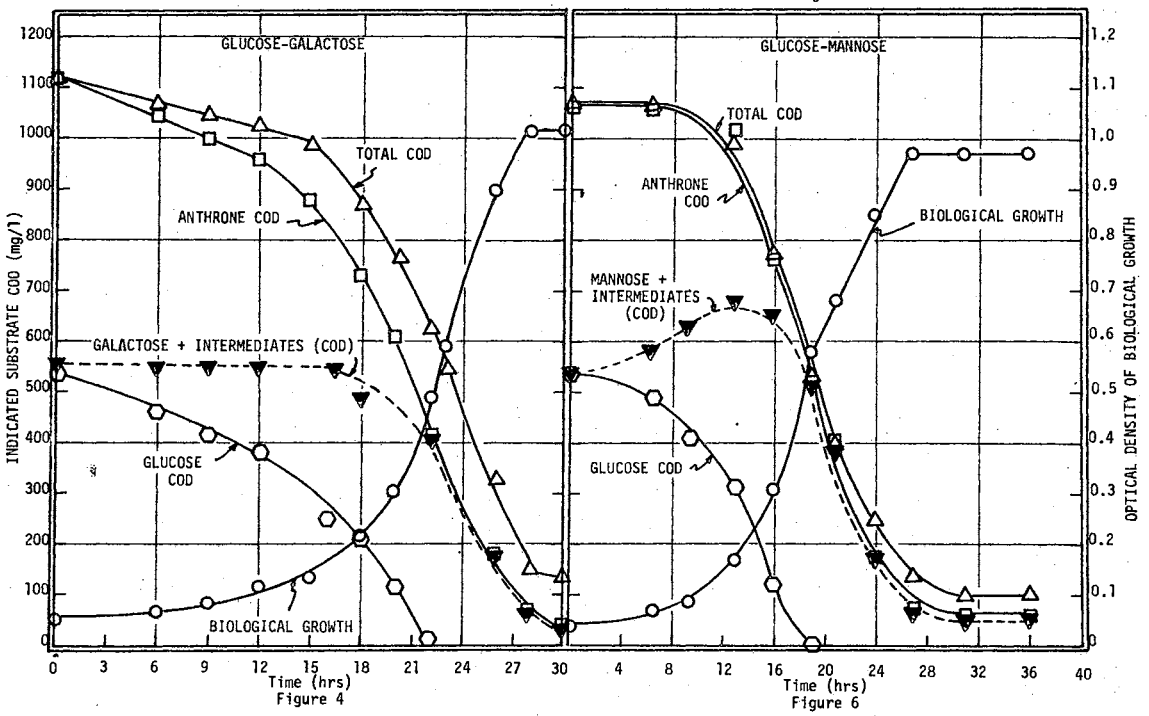
Figure 3 - Metabolic Responses of Glucose and Galactose Control Units Using Fresh Sewage as Inoculum.

Figure 4 - Metabolic Responses of Glucose-Galactose Combined Unit Using Fresh Sewage as Inoculum.

Figure 5 - Metabolic Responses of Glucose and Mannose Control Units Using Fresh Sewage as Inoculum.

Figure 6 - Metabolic Responses of Glucose-Mannose Combined Unit Using Fresh Sewage as Inoculum.


GLUCOSE CONTROL
TOTAL COD
BIOLOGICAL GROWTH
GLUCOSE COD
GALACTOSE CONTROL
TOTAL COD
ANTHRONE COD
BIOLOGICAL GROWTH
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 3
GLUCOSE CONTROL
BIOLOGICAL SOLIDS
GLUCOSE COD
TOTAL COD
MANNOSE CONTROL
TOTAL COD
ANTHRONE COD
BIOLOGICAL GROWTH
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 5


GLUCOSE-GALACTOSE
TOTAL COD
ANTHRONE COD
GALACTOSE + INTERMEDIATES (COD)
GLUCOSE COD
BIOLOGICAL GROWTH
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 4
GLUCOSE-MANNOSE
TOTAL COD
ANTHRONE COD
BIOLOGICAL GROWTH
MANNOSE + INTERMEDIATES (COD)
GLUCOSE COD
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 6

during the period of removal of glucose, intermediates of a non-carbohydrate nature were produced. It is seen that these were later metabolized. It is interesting to note that after the period of glucose removal the growth curve for biological solids can be fitted to a straight line.

c. Combined Substrate - Glucose and Ribose

In this experiment there was a particularly long lag period for growth on either glucose or ribose (Figure 7). The lag period was much longer when ribose was used as substrate. In the combined substrate system (see Figure 8) it is clearly shown that all glucose was removed before initiation of ribose removal. It is unfortunate that this experiment was not carried out longer than forty-six hours. It would appear from comparison of the total COD and ribose COD curves, and the growth curves, that a considerable amount of metabolic intermediates was produced as a result of ribose utilization, and that this phenomenon could have led to production of a diphasic growth curve.

d. Combined Substrate - Glucose and Lactose

Lactose was chosen for combination with glucose since it is a compound found in various waste waters, and also because it was interesting to determine the biochemical behavior of the system when a disaccharide was combined with glucose. The control systems are shown in Figure 9. It is seen that the responses were in general similar with respect to the growth pattern and the pattern of removal of the total COD. However, in the glucose control there was evidence for the production of metabolic intermediates or endproducts, since glucose was removed with considerably more speed than was the total COD. When the substrates

Figure 7 - Metabolic Responses of Glucose and Ribose Control Units Using Fresh Sewage as Inoculum.

Figure 8 - Metabolic Responses of Glucose-Ribose Combined Unit Using Fresh Sewage as Inoculum.

Figure 9 - Metabolic Responses of Glucose and Lactose Control Units Using Fresh Sewage as Inoculum.

Figure 10 - Metabolic Responses of Glucose-Lactose Combined Unit Using Fresh Sewage as Inoculum.

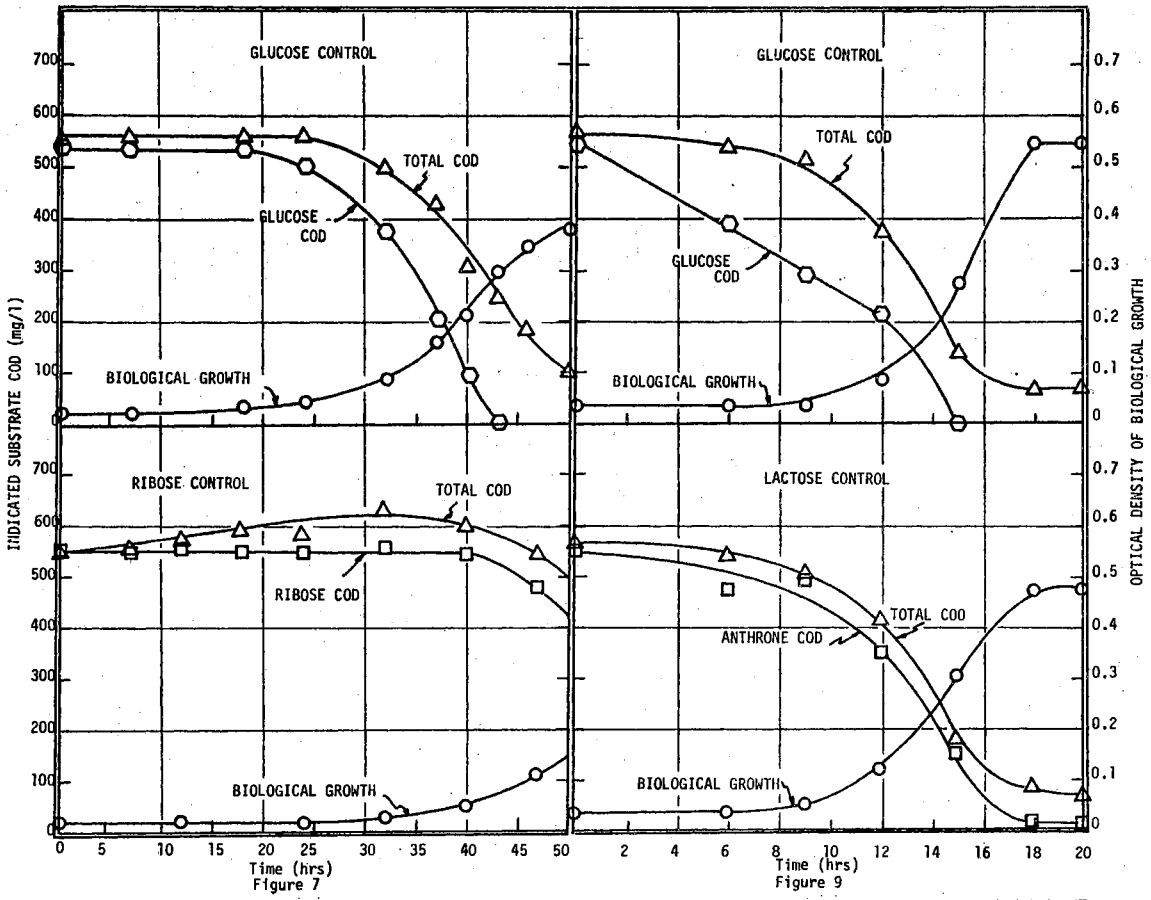



Figure 7

Figure 9

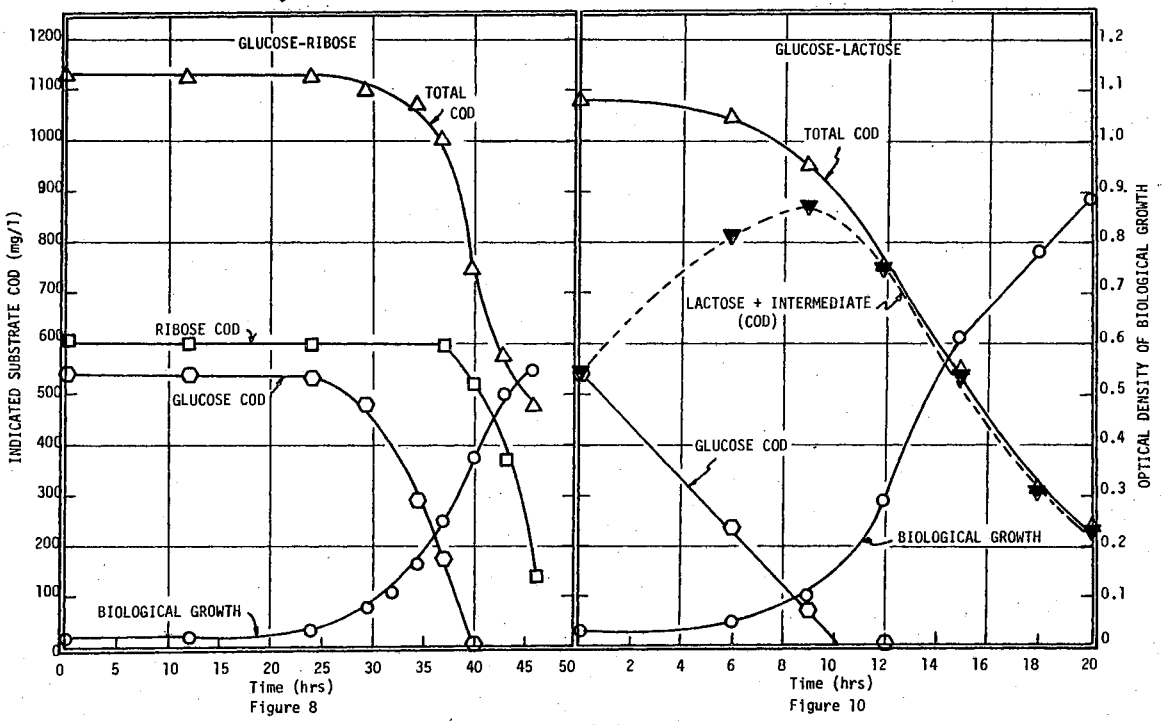



Figure 8

Figure 10

were combined (see Figure 10), glucose was removed more rapidly than was lactose, and it is seen that a considerable amount of intermediates was produced from either the metabolism of glucose or lactose. It would seem, however, from examination of the control systems and the rapidity with which glucose was removed in the combined system that most of the intermediates which did accumulate in this system arose from metabolism of glucose. Also, it is noted that the biological growth pattern was diphasic in nature.

e. Combined Substrate - Glucose and Sucrose

The control systems shown in Figure 11 indicate that rates of substrate removal and growth were slower for the sucrose system than for glucose. Intermediates accumulated in both systems. When the substrates were combined, the results shown in Figure 12 indicate that these compounds were metabolized sequentially with glucose utilization preceding sucrose utilization.

f. Summary of Data for Phase A

The results of this phase of the study using sewage seed inocula are summarized in Table I, which gives pertinent data on cell yields and rates of growth and substrate removal. Following the table, explanation of each column in this and similar tables are given.

2. Mixed Substrate Systems Consisting of Glucose in Combination with Various Compounds (Heterogeneous Microbial Seed Acclimated to the Various Compounds)

In this study the aim was to determine whether heterogeneous populations which were previously acclimated to various keto hexoses, aldopentoses, disaccharides, and various sugar alcohols would exhibit

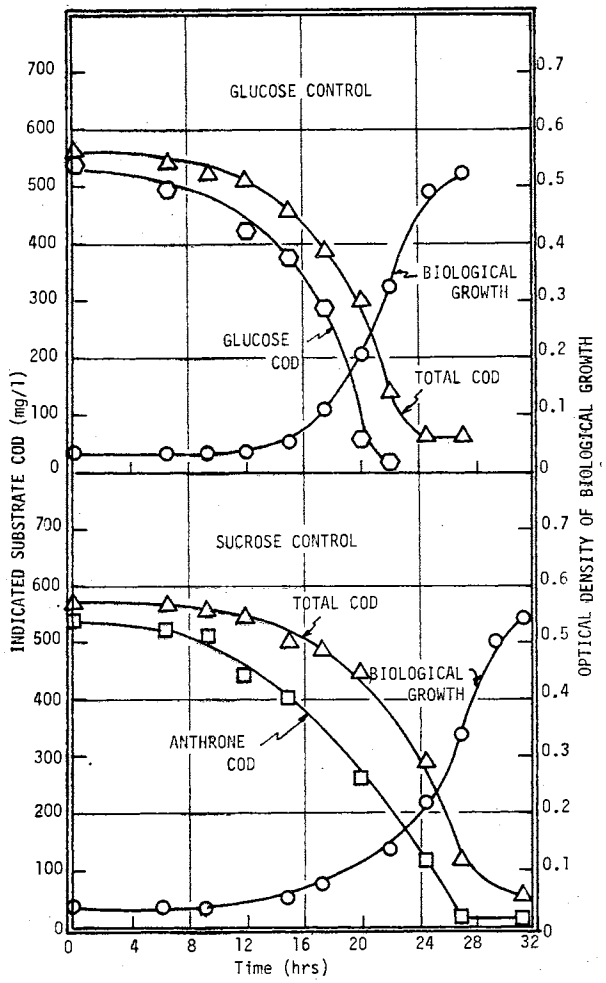



Figure 11 - Metabolic Responses of Glucose and Sucrose Control Units Using Fresh Sewage as Inoculum.

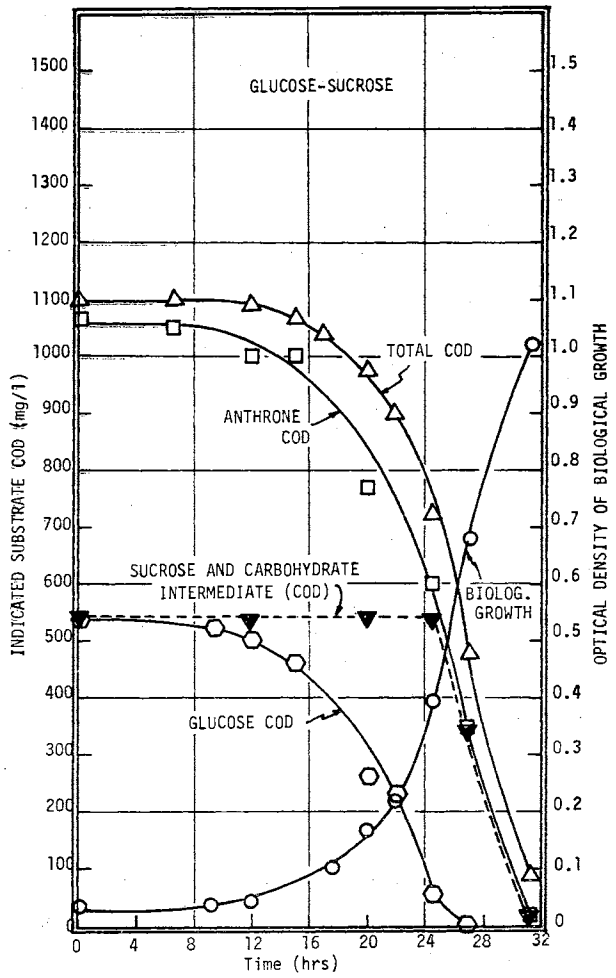



Figure 12 - Metabolic Responses of Glucose-Sucrose Combined Unit Using Fresh Sewage as Inoculum.

TABLE I

BIOLOGICAL RESPONSES OF MICROBIAL POPULATIONS FROM SEWAGE ON MULTI-SUBSTRATE SYSTEM

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | Glucose $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 3 | | glucose | 25 | 290 | 265 | 490 | 54 | 0 | 17 | $\mu_{11}=0.150$ | $K_{11}=0.211$ | $K_{11}=0.214$ | | |
| 3 | sewage | galactose | 25 | 270 | 245 | 480 | 51 | 6 | 28 | $\mu_{11}=0.077$ | $K_{11}=0153$ | | $K_{11}=0.176$ | Sequential |
| 4 | | combined | 25 | 510 | 485 | 985 | 49.3 | 0<br>16 | 22<br>28 | $\mu_{11}=0.132$ | $K_{01}=8.67$<br>$K_{12}=0.285$ | $K_{01}=12.9$<br>$K_{12}=0.222$ | $K_{12}=0.392$ | |
| 5 | | glucose | 15 | 340 | 325 | 500 | 65 | 0 | 19 | $\mu_{11}=0.203$ | $K_{11}=0.305$ | $K_{11}=0.305$ | | |
| 5 | sewage | mannose | 15 | 375 | 360 | 500 | 72 | 13 | 36 | $\mu_{11}=0.167$ | $K_{11}=0.165$ | | $K_{11}=0.165$ | Sequential |
| 6 | | combined | 15 | 480 | 465 | 965 | 48.2 | 0<br>19 | 19<br>27 | $\mu_{11}=0.161$ | $K_{11}=0.463$ | $K_{11}=0.217$ | $K_{12}=0.180$ | |
| 7 | | glucose | 10 | 185 | 175 | 460 | 38 | 18 | 43 | $\mu_{11}=0.102$ | $K_{11}=0.148$ | $K_{11}=0.175$ | | |
| 7 | sewage | ribose | 10 | 77 | 67 | 120 | 55.7 | 40 | | $\mu_{11}=0.086$ | $K_{11}=0.277$ | | $K_{11}=0.277$ | Sequential |
| 8 | | combined | 10 | 275 | 265 | 650 | 40.2 | 24<br>37 | 40 | $\mu_{11}=0.139$ | $K_{11}=0.312$ | $K_{11}=0.24$ | $K_{12}=0.283$ | |
| 9 | | glucose | 15 | 265 | 250 | 500 | 50 | 0 | 15 | $\mu_{11}=0.328$ | $K_{11}=0.506$ | $K_{01}=27$ | | |
| 9 | sewage | lactose | 15 | 240 | 225 | 490 | 45.9 | 6 | 18 | $\mu_{11}=0.299$ | $K_{11}=0.335$ | | $K_{11}=0.335$ | Sequential |
| 10 | | combined | 15 | 440 | 425 | 840 | 50.6 | 0<br>11 | 11<br>20 | $\mu_{11}=0.355$<br>$\mu_{02}=27$ | $K_{11}=0.335$ | $K_{01}=51$ | | |
| 11 | | glucose | 15 | 260 | 245 | 510 | 48 | 0 | 20 | $\mu_{11}=0.220$ | $K_{11}=0.211$ | $K_{11}=0.211$ | | |
| 11 | sewage | sucrose | 15 | 277 | 262 | 510 | 51.4 | 6 | 27 | $\mu_{11}=0.143$ | $K_{11}=0.211$ | | $K_{11}=0.235$ | Sequential |
| 12 | | combined | 15 | 510 | 495 | 1010 | 49 | 4<br>25 | 27<br>31 | $\mu_{11}=0.145$ | $K_{11}=0.230$ | $K_{11}=0.230$ | $K_{02}=86.5$ | |

## EXPLANATION OF COLUMNS FROM TABLE I THROUGH TABLE VII

1. Column 1 shows the figure number.
2. Column 2 shows the substrate to which the seed population was acclimated.
3. Column 3 shows the carbon source in the batch reactors, i.e., control systems for each carbon source and the combined system.
4. Column 4 shows initial biological solids concentrations calculated from the calibration curve for solids concentrations versus optical density at 540 mμ.
5. Column 5 shows the peak solids concentration calculated from the calibration curve for solids concentration versus optical density at 540 mμ.
6. Column 6 shows the increase in biological solids concentration calculated by subtraction of column 4 from column 5.
7. Column 7 shows total COD removed at the time of peak solids concentration.
8. Column 8 shows cell yield in percent calculated as 100 x column 6 divided by column 7.
9. Column 9 shows the time required before initiation of COD removal. In the combined systems, values are given for each substrate (first value refers to first substrate listed for the set).
10. Column 10 shows the time required to complete the removal of COD. As in column 9, values are given for each substrate.
11. Column 11 shows the rate of growth. When the biological growth followed first order increasing or decreasing kinetics, the unit of expression is $hour^{-1}$. When the biological growth followed zero order kinetics, the unit of expression is mg/l/hr (see note below for identification of kinetic order).
12. Column 12 shows the rate of removal of total COD. When the rate of total COD removal followed first order increasing or decreasing kinetics, the unit of expression is $hour^{-1}$. When the rate of total COD removal followed zero order kinetics, the unit of expression is mg/l/hr (see note below for identification of kinetic order).
13. Column 13 shows the rate of removal, based upon specific substrate analysis, expressed as COD, for one of the substrates.
14. Column 14 shows the rate of removal based upon specific substrate analysis, expressed as COD, for the other compound in the set.
15. Column 15 shows the mode of substrate removal, i.e., concurrent removal or sequential removal.

Note: Explanation of the subscripts for rate symbols, column 11 through column 14:

The first number of the subscript indicates the type of kinetics: 1 and -1 represent first order increasing kinetics and first order decreasing kinetics, respectively. 0 represents zero order kinetics. The second number of the subscript indicates the sequences of kinetic phases: 1 denotes either the first phase or that only a single phase occurred, whereas 2 refers to the second phase.

sequential or concurrent removal when these compounds were mixed with glucose, a compound to which no acclimation is (usually) required. Heterogeneous populations of sewage origin were pre-acclimated to the various compounds in order to ensure that the various species present contained the necessary enzyme complement for utilization of the carbon source.

a. Combination of Glucose and Fructose with Cells Acclimated to Fructose

The control systems shown in Figure 13 indicate that glucose was metabolized slightly more rapidly than fructose, even though the microbial population was acclimated to fructose. There was a significant amount of intermediates produced during glucose metabolism, as evidenced by the difference between the total COD and glucose COD curves. There was little or no evidence for the production of metabolic intermediates from fructose metabolism. Figure 14 shows that when these two carbon sources were combined, glucose blocked the removal of fructose; fructose was not utilized until the glucose had been removed completely or reduced to a low level.

b. Combination of Glucose and Galactose with Cells Acclimated to Galactose

In this experiment the control systems were initiated using 1000 mg/l of each substrate and, in order to facilitate analyses of the curves without reducing the scale, both control systems are plotted on the same graph (see Figure 15). Even though the cells were previously acclimated to galactose, glucose was removed more rapidly. It is interesting to note that for the galactose system there was an unusually high residual COD after cessation of growth. The sequential removal of

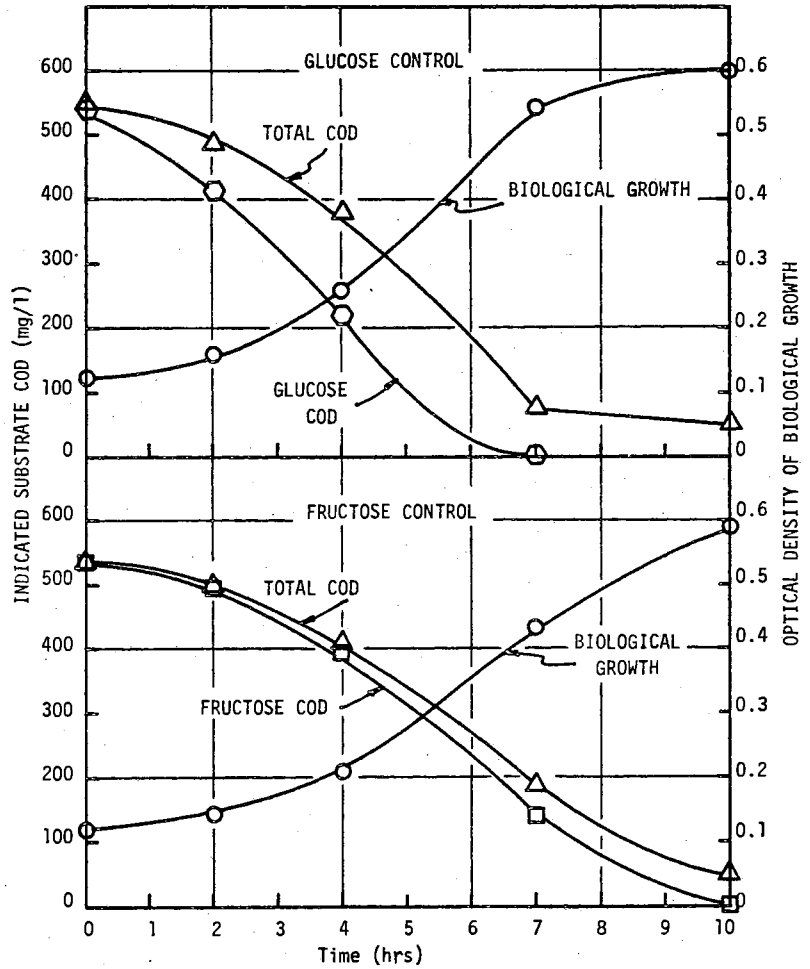



Figure 13 - Metabolic Responses of Fructose-acclimated Heterogeneous Populations in the Glucose and Fructose Control Units.

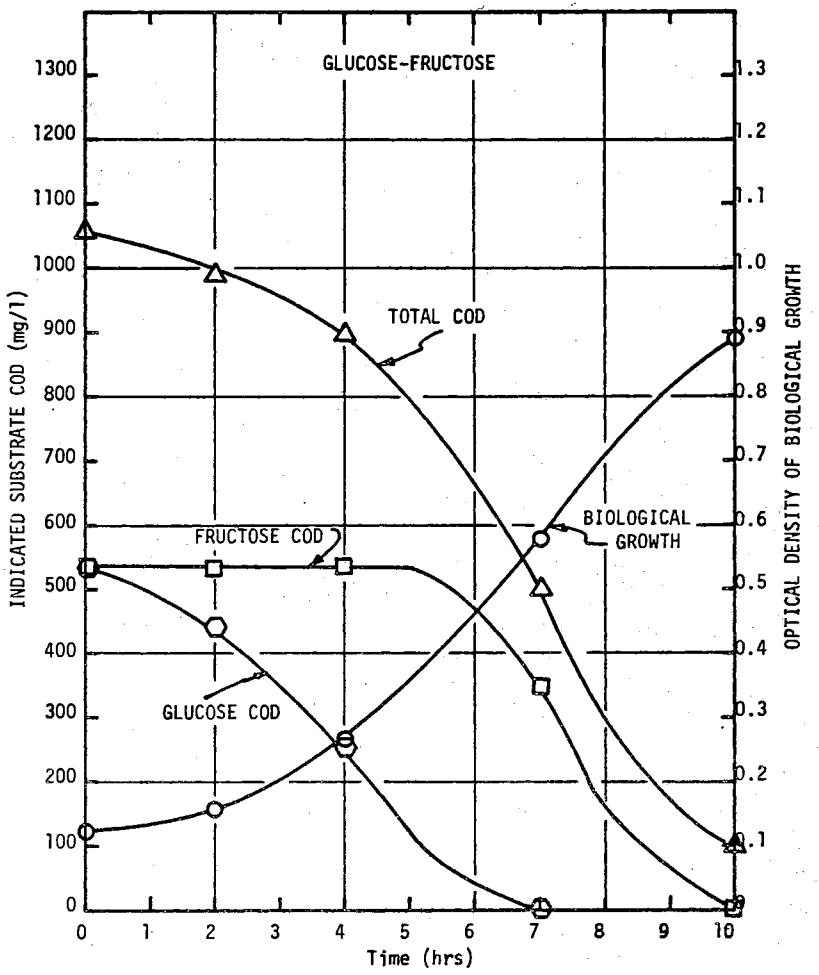



Figure 14 - Metabolic Responses of Fructose-acclimated Heterogeneous Populations in the Glucose-Fructose Combined Unit.

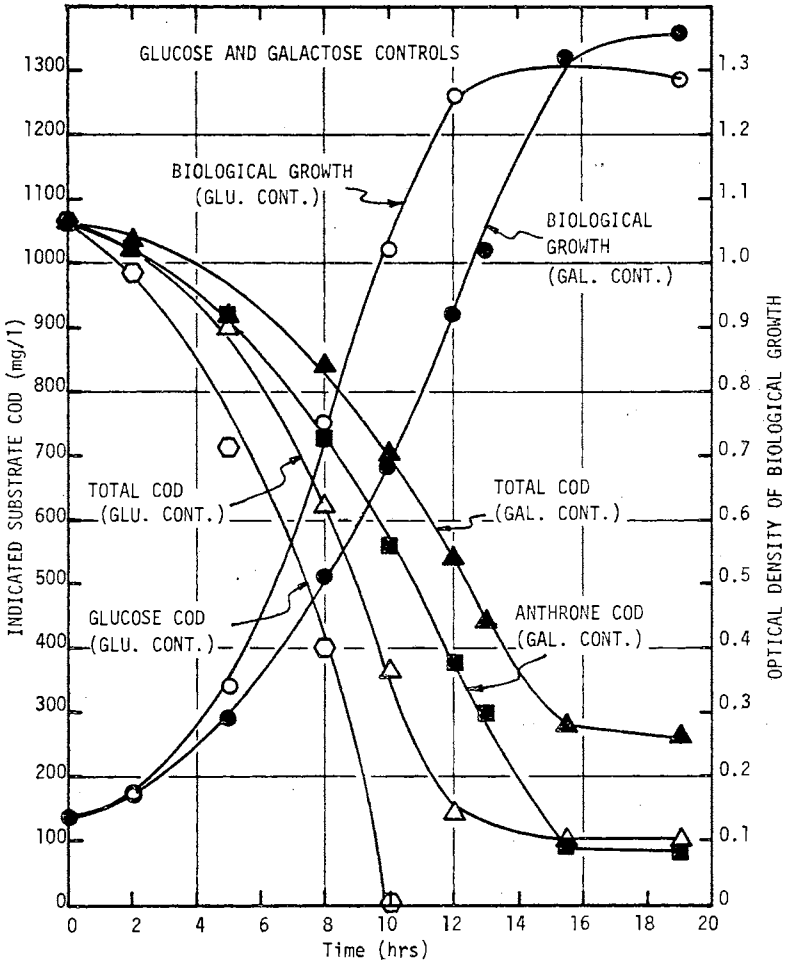



Figure 15 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Glucose and Galactose Control Units.

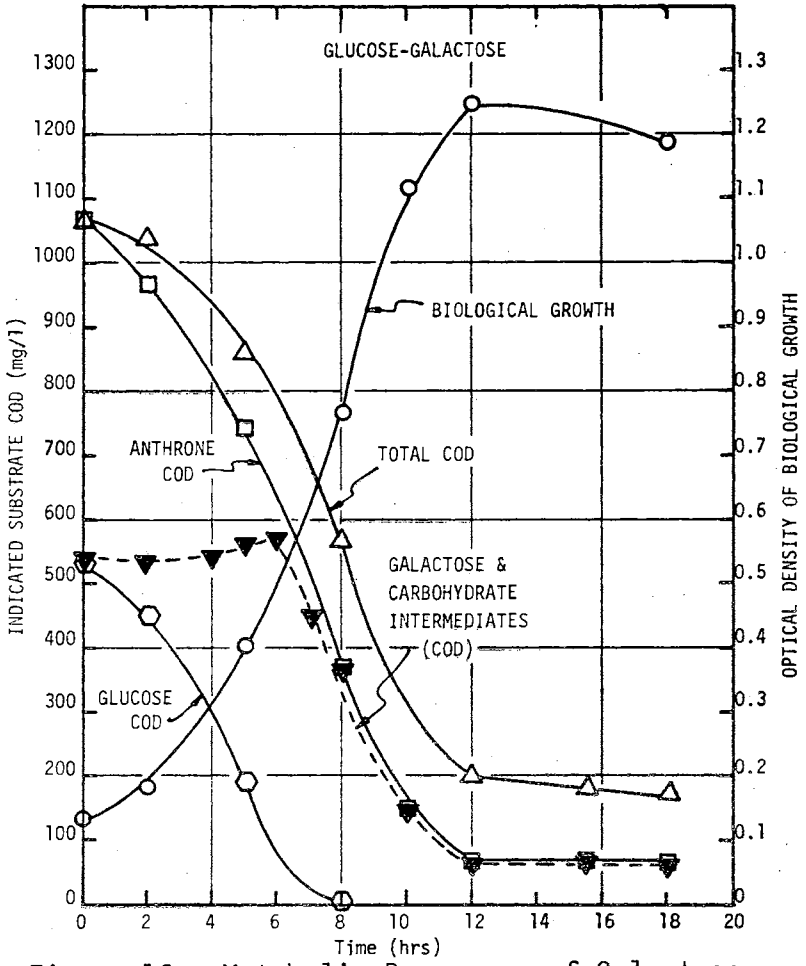



Figure 16 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Glucose-Galactose Combined Unit.

glucose and galactose is clearly demonstrated in Figure 16.

c. Combination of Glucose and Mannose with Cells Acclimated to Mannose

The control systems are shown in Figure 17, where it is seen that growth on glucose was initiated somewhat more rapidly than was growth on mannose. Also, in the glucose control system there is evidence for the production of metabolic intermediates. It would appear from the total COD curve that there was some need to acclimate to these metabolic products. In Figure 18 it is seen that mannose and glucose were removed sequentially, with glucose being metabolized first. The sequential nature of substrate removal was reflected in the anthrone COD curve, but not in the total COD curve.

d. Combination of Glucose and Ribose with Cells Acclimated to Ribose

In Figure 19 it is seen that even though the cells were previously acclimated to ribose, growth and substrate removal using glucose as carbon source proceeded considerably more rapidly than on ribose. Also, there was some evidence for the production of metabolic intermediates as the rapidity of ribose removal increased. In Figure 20 it is seen that when these substrates were used as a combined carbon source, the presence of glucose did exhibit a blocking effect on ribose utilization, and the sequential removal of these substrates was reflected in both the total COD curve and the growth curve. Initiation of the second phase of COD removal and biological growth coincided with the time of glucose removal.

e. Combination of Glucose and Arabinose with Cells Acclimated to Arabinose

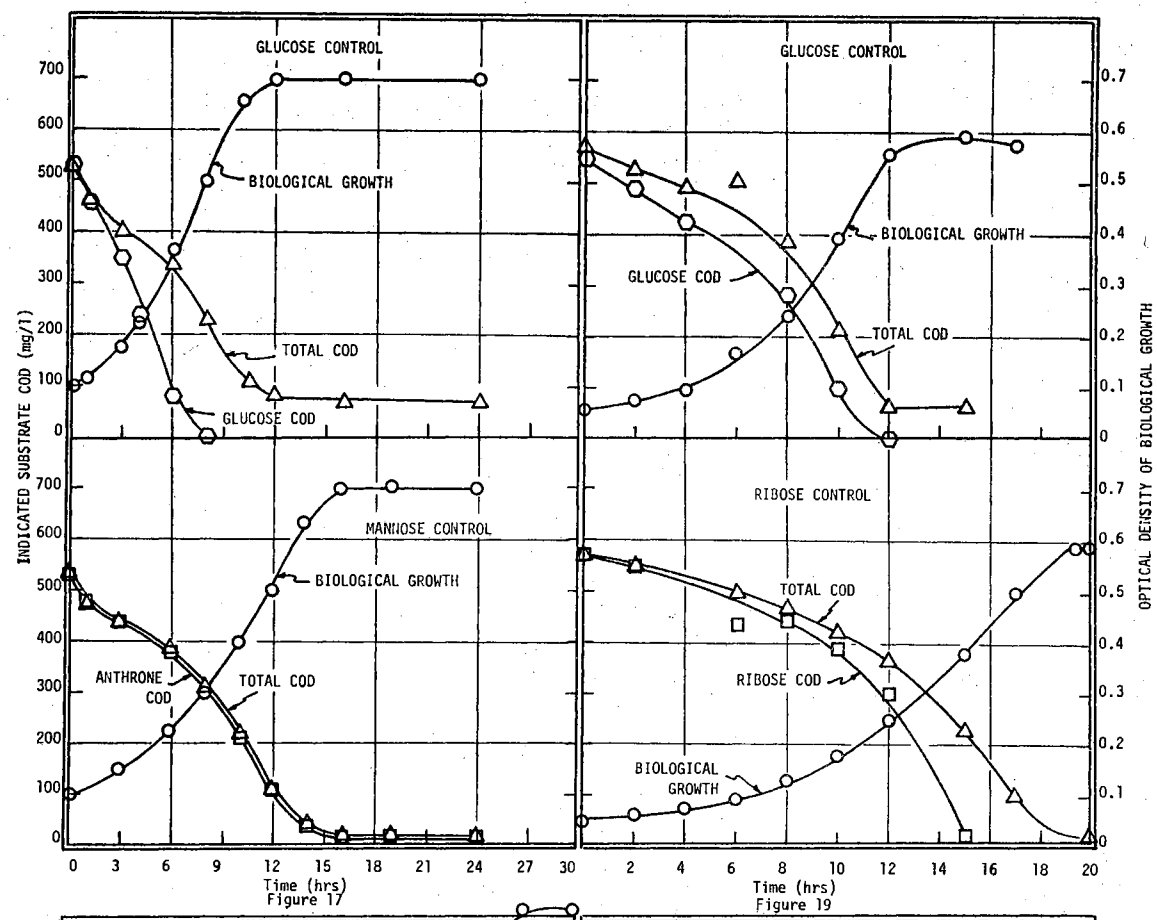
The course of substrate removal and growth in the control systems is shown in Figure 21. It is seen that the arabinose-acclimated cells

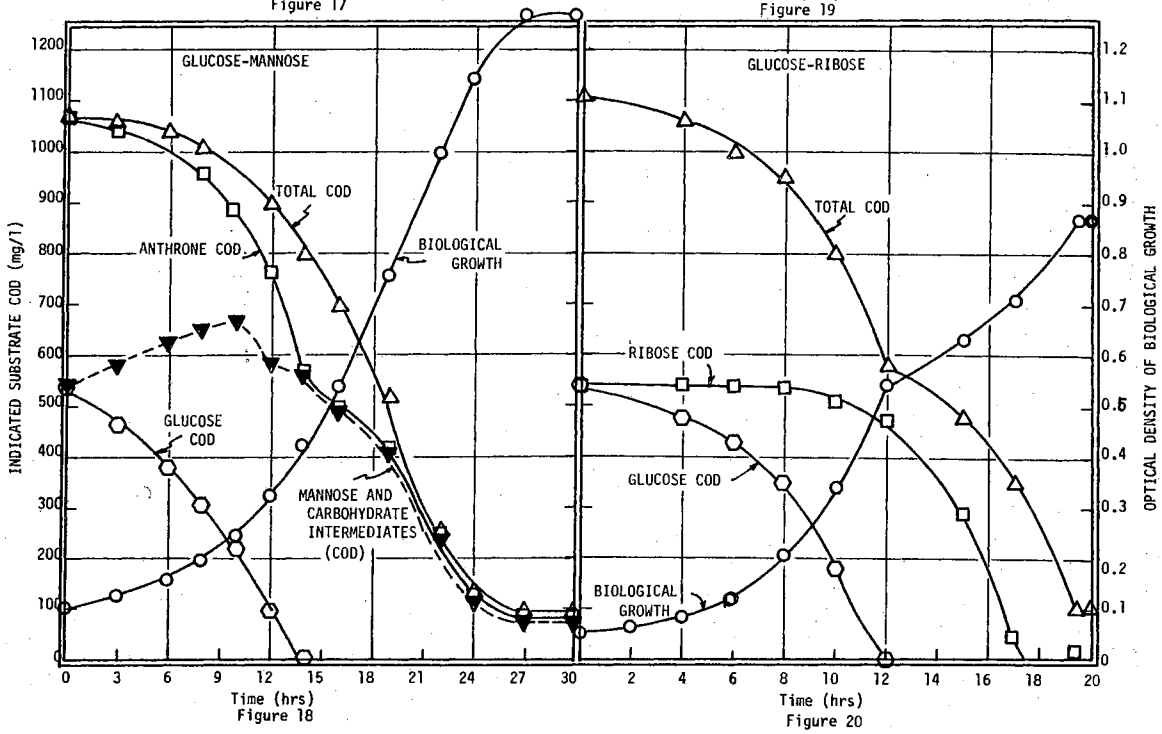
Figure 17 - Metabolic Responses of Mannose-acclimated Heterogeneous Populations in the Glucose and Mannose Control Units.

Figure 18 - Metabolic Responses of Mannose-acclimated Heterogeneous Populations in the Glucose-Mannose Combined Unit.

Figure 19 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Glucose and Ribose Control Units.

Figure 20 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Glucose-Ribose Combined Unit.


GLUCOSE CONTROL
BIOLOGICAL GROWTH
TOTAL COD
GLUCOSE COD
MANNOSE CONTROL
BIOLOGICAL GROWTH
ANTHRONE COD
TOTAL COD
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 17
GLUCOSE CONTROL
BIOLOGICAL GROWTH
GLUCOSE COD
TOTAL COD
RIBOSE CONTROL
TOTAL COD
RIBOSE COD
BIOLOGICAL GROWTH
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 19


GLUCOSE-MANNOSE
TOTAL COD
ANTHRONE COD
BIOLOGICAL GROWTH
GLUCOSE COD
MANNOSE AND CARBOHYDRATE INTERMEDIATES (COD)
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 18
GLUCOSE-RIBOSE
TOTAL COD
RIBOSE COD
GLUCOSE COD
BIOLOGICAL GROWTH
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 20

required no acclimation to glucose. It is interesting to note that glucose was removed in accordance with zero order kinetics, however. A considerable amount of metabolic intermediates accumulated in the medium during glucose removal.

Results for the combined system are shown in Figure 22. Glucose was removed at a faster rate, but the presence of glucose did not block the removal of arabinose. In the combined system, glucose was not removed in accordance with zero order kinetics. Based upon analysis of the control systems, it would appear that most of the intermediates which were produced were due to glucose metabolism.

f. Combination of Glucose and Lactose with Cells Acclimated to Lactose

In Figure 23 it is seen that lactose and glucose were used with approximately the same efficiency; however, when these substrates were used as the combined carbon source, the presence of glucose retarded lactose utilization rather severely. It is interesting to note that in all of these experiments the initial concentration of biological solids was rather high, and the metabolic blockage that was effected can be attributed to inhibition as well as repression of enzyme synthesis.

g. Combination of Glucose and Sucrose with Cells Acclimated to Sucrose

The control systems shown in Figure 25 indicate that both compounds were metabolized with approximately equal facility, even though the cells had been previouly acclimated to sucrose. The removal of sucrose was plotted in accordance with zero order kinetics; however, it can not be said with certainty that zero order elimination of sucrose really took place in the system. Analysis for glucose in the sucrose system indicated that there was no free glucose present. In Figure 26 it is

Figure 21 - Metabolic Responses of Arabinose-acclimated Heterogeneous Populations in the Glucose and Arabinose Control Units.

Figure 22 - Metabolic Responses of Arabinose-acclimated Heterogeneous Populations in the Glucose-Arabinose Combined Unit.

Figure 23 - Metabolic Responses of Lactose-acclimated Heterogeneous Populations in the Glucose and Lactose Control Units.

Figure 24 - Metabolic Responses of Lactose-acclimated Heterogeneous Populations in the Glucose-Lactose Combined Unit.

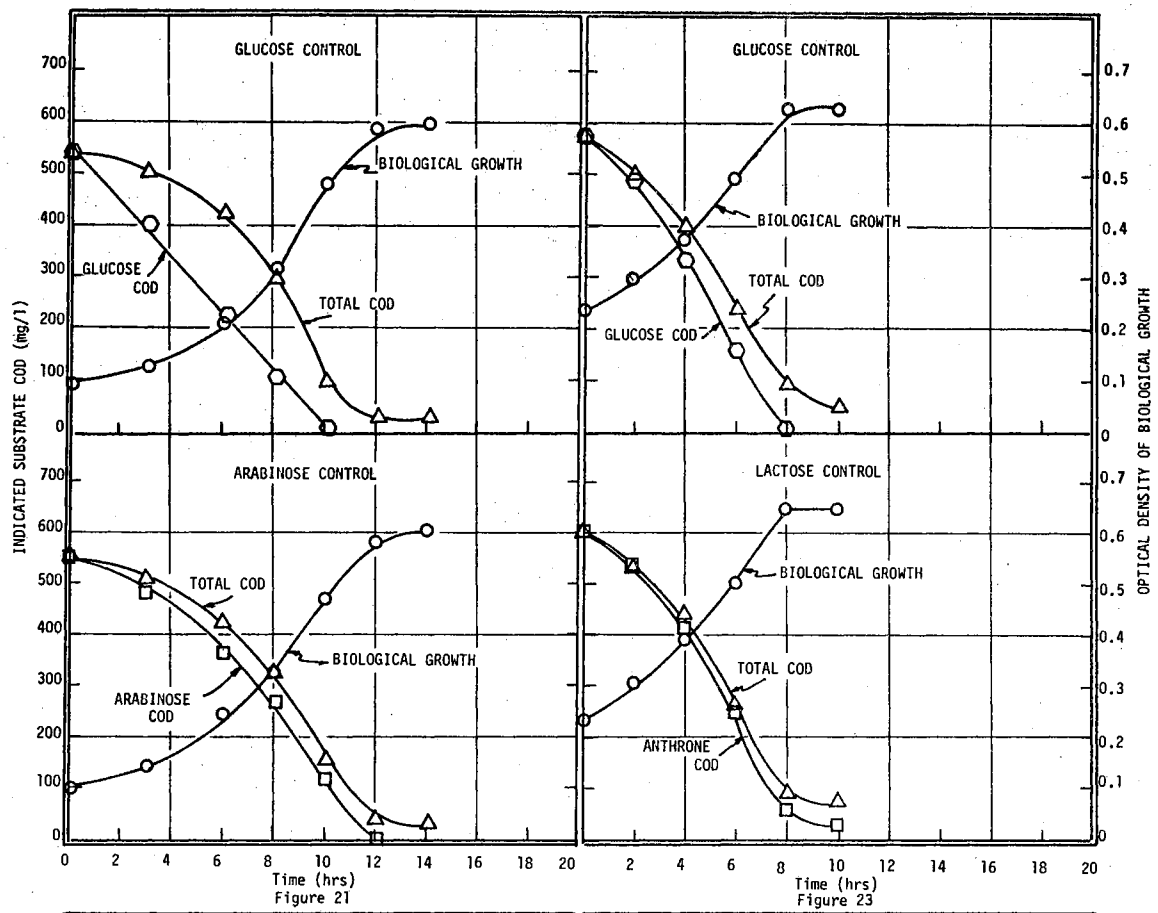



Figure 21




Figure 23

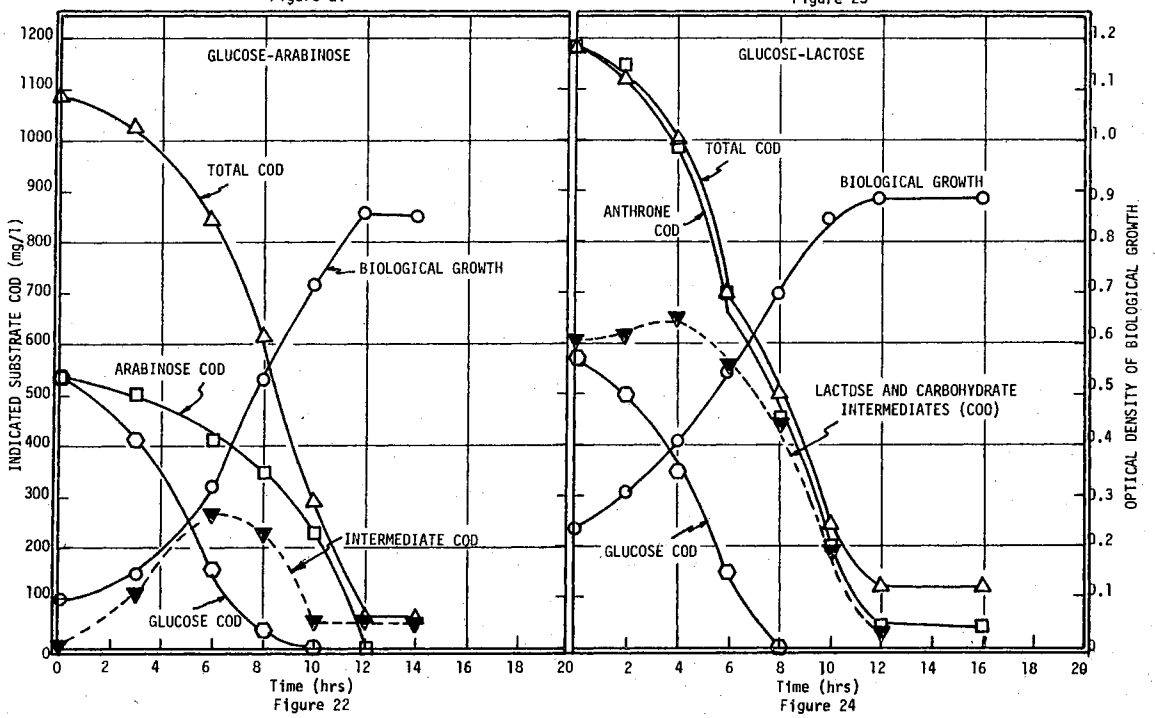



Figure 22




Figure 24

Figure 25 - Metabolic Responses of Sucrose-acclimated Heterogeneous Populations in the Glucose and Sucrose Control Units.

Figure 26 - Metabolic Responses of Sucrose-acclimated Heterogeneous Populations in the Glucose-Sucrose Combined Unit.

Figure 27 - Metabolic Responses of Glycerol-acclimated Heterogeneous Populations in the Glucose and Glycerol Control Units.

Figure 28 - Metabolic Responses of Glycerol-acclimated Heterogeneous Populations in the Glucose-Glycerol Combined Unit.

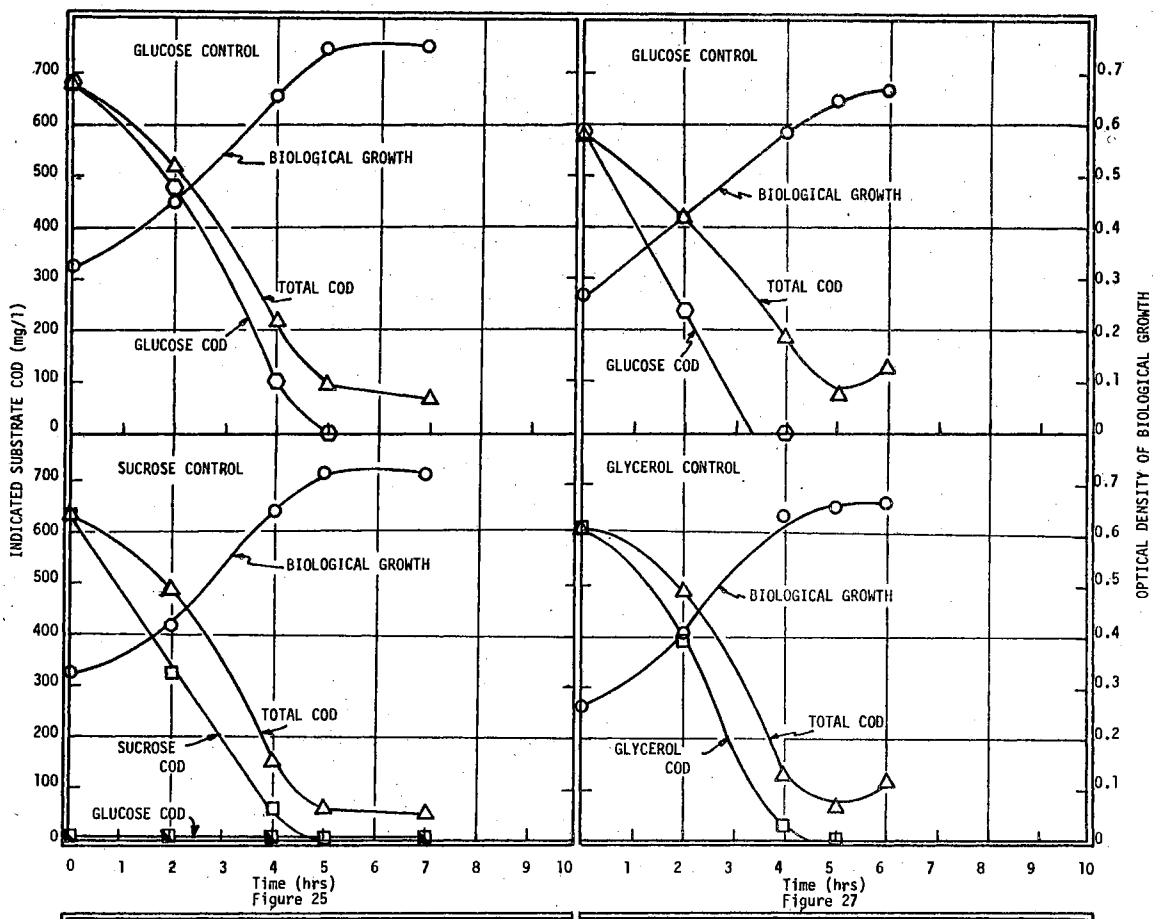



Figure 25

Figure 27

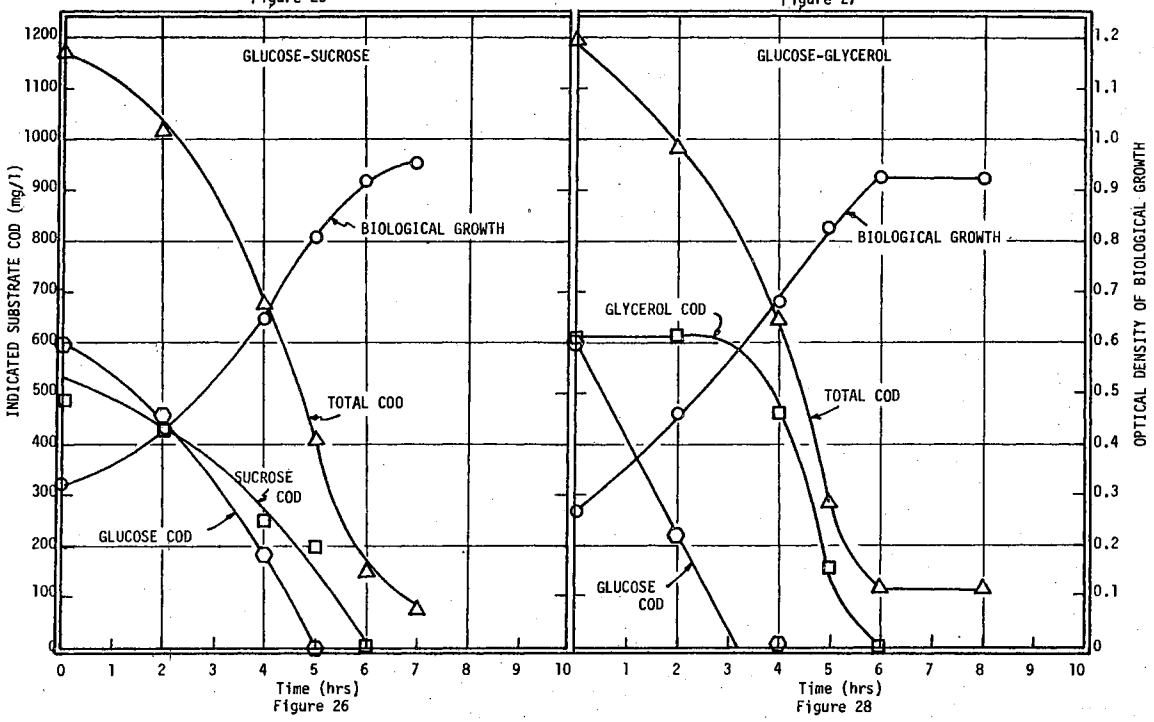



Figure 26

Figure 28

seen that the presence of glucose did not block the removal of sucrose, i.e., both carbon sources were removed concurrently.

h. Combination of Glucose and Glycerol with Cells Acclimated to Glycerol

Figure 27 shows that acclimation to glycerol also conferred acclimation to glucose, and in general the biological growth and total COD responses were the same on both substrates. The biological growth curve for the glucose system was fitted to a straight line, and it therefore seemed appropriate to show the glucose COD removal as zero order. It is interesting to note that in both systems there was a release of total COD at the 6-hour sampling time.

In Figure 28 it is seen that in the combined substrate system glucose blocked the removal of glycerol. As in the previous experiment, the initial solids concentration was somewhat high, and the blockage of glycerol can be attributed largely to inhibition of enzyme function.

i. Summary of Data for Phase A, 2

The data for all experiments in this segment of the study, in which cells acclimated to other substrates were tested with glucose in combination with the previous substrate, are summarized in Table II. (See Section IV, A, I, f for explanation of calculations and symbols.)

3. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Galactose in Combination with Various Compounds (Heterogeneous Populations Acclimated to Galactose)

a. Combination of Glucose and Galactose

Experiments using heterogeneous populations acclimated to galactose and subjected to aeration in the presence of glucose and galactose were

TABLE II

BIOLOGICAL RESPONSES IN THE MIXED SUBSTRATE SYSTEMS CONSISTING OF GLUCOSE WITH VARIOUS COMPOUNDS

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | Glucose $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 13 | | glucose | 60 | 300 | 240 | 500 | 48 | 0 | 7 | $\mu_{11}$=0.241 | $K_{11}$=0.447 | $K_{11}$=0.462 | | |
| 13 | fructose | fructose | 60 | 300 | 240 | 485 | 49.5 | 0 | 10 | $\mu_{11}$=0.203 | $K_{11}$=0.452 | | $K_{11}$=0.625 | Sequential |
| 14 | | combined | 60 | 445 | 385 | 960 | 40 | 0<br>5 | 7<br>10 | $\mu_{11}$=0.268 | $K_{11}$=0.490 | $K_{11}$=0.55 | $K_{12}$=1.11 | |
| 15 | | glucose | 65 | 660 | 595 | 965 | 61.7 | 0 | 10 | $\mu_{11}$=0.238 | $K_{11}$=0.374 | $K_{11}$=0.440 | | |
| 15 | galactose | galactose | 65 | 680 | 615 | 980 | 63.0 | 0 | 15.5 | $\mu_{11}$=0.184 | $K_{11}$=0.275 | | $K_{11}$=0.402 | Sequential |
| 16 | | combined | 65 | 625 | 560 | 900 | 62.3 | 0<br>7 | 7<br>12 | $\mu_{11}$=0.270 | $K_{11}$=0.305 | $K_{11}$=0.416 | $K_{02}$=100 | |
| 17 | | glucose | 50 | 350 | 300 | 465 | 64.5 | 0 | 8 | $\mu_{11}$=0.231 | $K_{11}$=0.548 | $K_{11}$=0.537 | | |
| 17 | mannose | mannose | 50 | 350 | 300 | 515 | 58.2 | 0 | 15 | $\mu_{11}$=0.135 | $K_{11}$=0.515 | | $K_{11}$=0.515 | Sequential |
| 18 | | combined | 50 | 638 | 588 | 965 | 60.7 | 0<br>14 | 14<br>25 | $\mu_{11}$=0.133 | $K_{11}$=0.197 | $K_{11}$=0.22 | $K_{12}$=0.693 | |
| 19 | | glucose | 25 | 290 | 265 | 500 | 53 | 0 | 12 | $\mu_{11}$=0.231 | $K_{11}$=0.263 | $K_{11}$=0.263 | | |
| 19 | ribose | ribose | 25 | 290 | 265 | 550 | 48.3 | 0 | 15 | $\mu_{11}$=0.168 | $K_{11}$=0.191 | | $K_{11}$=0.192 | Sequential |
| 20 | | combined | 25 | 435 | 410 | 1010 | 40.5 | 0<br>8 | 12<br>17 | $\mu_{11}$=0.231<br>$\mu_{12}$=0.054 | $K_{11}$=0.300<br>$K_{12}$=0.456 | $K_{11}$=0.308 | $K_{12}$=0.456 | |
| 21 | | glucose | 50 | 300 | 250 | 520 | 48.2 | 0 | 10 | $\mu_{11}$=0.173 | $K_{11}$=0.389 | $K_{01}$=53.5 | | |
| 21 | arabinose | arabinose | 50 | 300 | 250 | 520 | 48.2 | 0 | 12 | $\mu_{11}$=0.164 | $K_{11}$=0.271 | | $K_{11}$=0.271 | Concurrent |
| 22 | | combined | 50 | 430 | 380 | 1020 | 37.3 | 0<br>0 | 9<br>12 | $\mu_{11}$=0.252 | $K_{11}$=0.412 | $K_{11}$=0.375 | $K_{11}$=0.218 | |
| 23 | | glucose | 115 | 315 | 200 | 520 | 38.4 | 0 | 8 | $\mu_{11}$=0.122 | $K_{11}$=0.354 | $K_{11}$=0.507 | | |
| 23 | lactose | lactose | 115 | 325 | 210 | 530 | 39.6 | 0 | 8 | $\mu_{11}$=0.126 | $K_{11}$=0.433 | | $K_{11}$=0.433 | Sequential |
| 24 | | combined | 115 | 443 | 326 | 1060 | 30.8 | 0<br>6 | 8<br>12 | $\mu_{11}$=0.145 | $K_{11}$=0.51<br>$K_{12}$=0.8 | $K_{11}$=0.573 | $K_{12}$=0.547 | |
| 25 | | glucose | 160 | 380 | 220 | 610 | 36 | 0 | 5 | $\mu_{11}$=0.196 | $K_{11}$=0.526 | $K_{11}$=0.532 | | |
| 25 | sucrose | sucrose | 160 | 365 | 205 | 580 | 35.4 | 0 | 5 | $\mu_{11}$=0.214 | $K_{11}$=0.582 | | $K_{01}$=142 | Concurrent |
| 26 | | combined | 160 | 480 | 320 | 1100 | 29.1 | 0<br>0 | 5<br>6 | $\mu_{11}$=0.206 | $K_{11}$=0.600 | $K_{11}$=0.473 | $K_{11}$=0.453 | |
| 27 | | glucose | 135 | 335 | 200 | 500 | 40 | 0 | 3.5 | $\mu_{01}$=40 | $K_{01}$=120 | $K_{01}$=178 | | |
| 27 | glycerol | glycerol | 135 | 335 | 200 | 540 | 37 | 0 | 4 | $\mu_{11}$=0.221 | $K_{11}$=0.694 | | $K_{11}$=0.694 | Sequential |
| 28 | | combined | 135 | 465 | 330 | 1080 | 30.5 | 0<br>3 | 3<br>6 | $\mu_{01}$=70 | $K_{11}$=0.493 | $K_{01}$=190 | $K_{12}$=1.12 | |

presented in Figures 15 and 16.

b. Combination of Fructose and Galactose

In Figure 29 it is seen that acclimation to galactose conferred acclimation to fructose. Indeed, these galactose-acclimated cells utilized fructose more readily than galactose. In Figure 30 the response of the galactose-acclimated population to the mixed carbon source system of galactose and fructose is one which indicates that neither compound interfered with the metabolism of the other. As could be surmised from the behavior of the control systems, fructose was eliminated more rapidly than was galactose.

c. Combination of Ribose and Galactose

Cells which were previously grown on galactose could readily metabolize ribose (see Figure 31). However, growth on galactose was somewhat more rapid than on ribose. When these compounds were used as a joint carbon source, they were removed concurrently (see Figure 32). The results indicate that neither compound influenced the metabolism of the other.

d. Combination of Arabinose and Galactose

Figure 33 shows that the heterogeneous population acclimated to galactose was completely unable to metabolize arabinose. In any event it can be stated that the galactose-acclimated cells did not acclimate to arabinose during the 20-hour period of the experiment. The same conclusion may be drawn from the results shown in Figure 34, wherein both substrates were present in the medium. It is interesting to note that galactose, which could have been used as a source of energy to produce enzymes required for arabinose metabolism, was not used for this purpose.

Figure 29 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Fructose and Galactose Control Units.

Figure 30 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Fructose-Galactose Combined Unit.

Figure 31 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Ribose and Galactose Control Units.

Figure 32 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Ribose-Galactose Combined Unit.

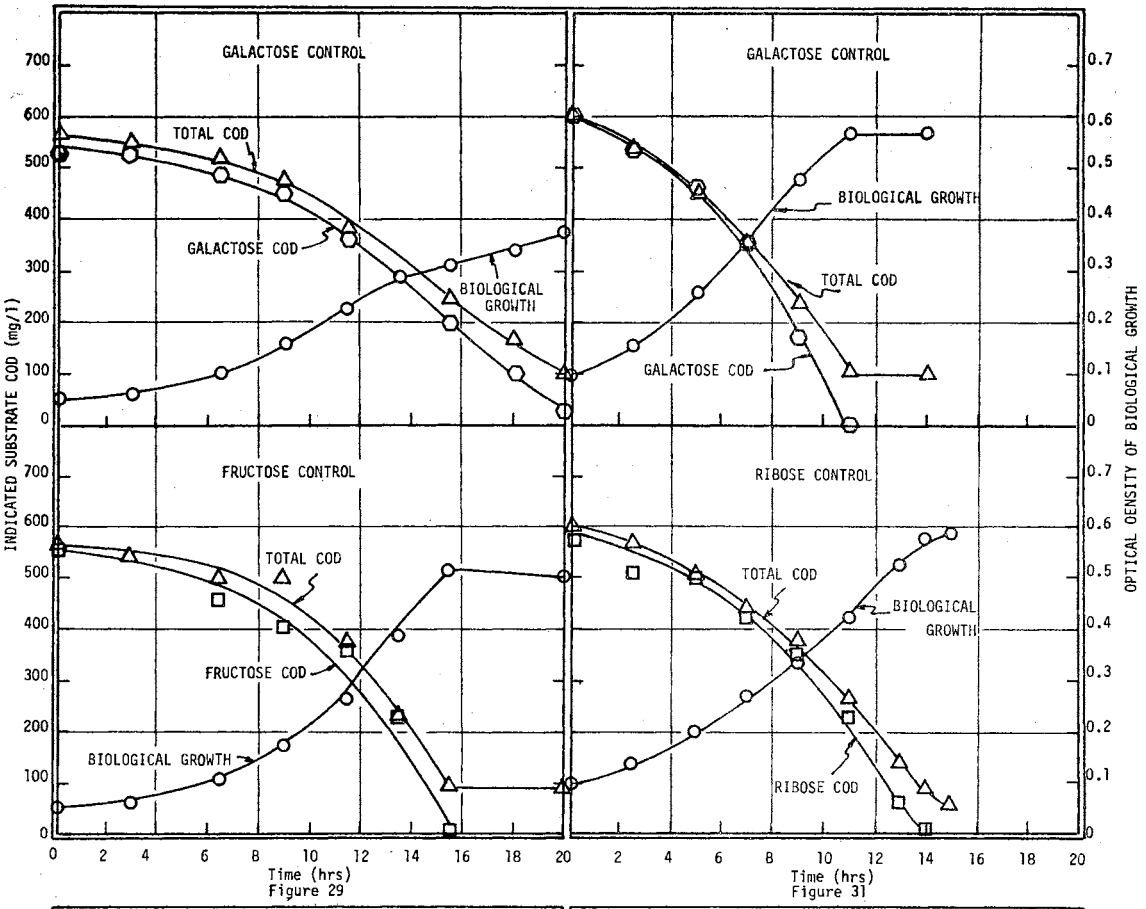



Figure 29




Figure 31

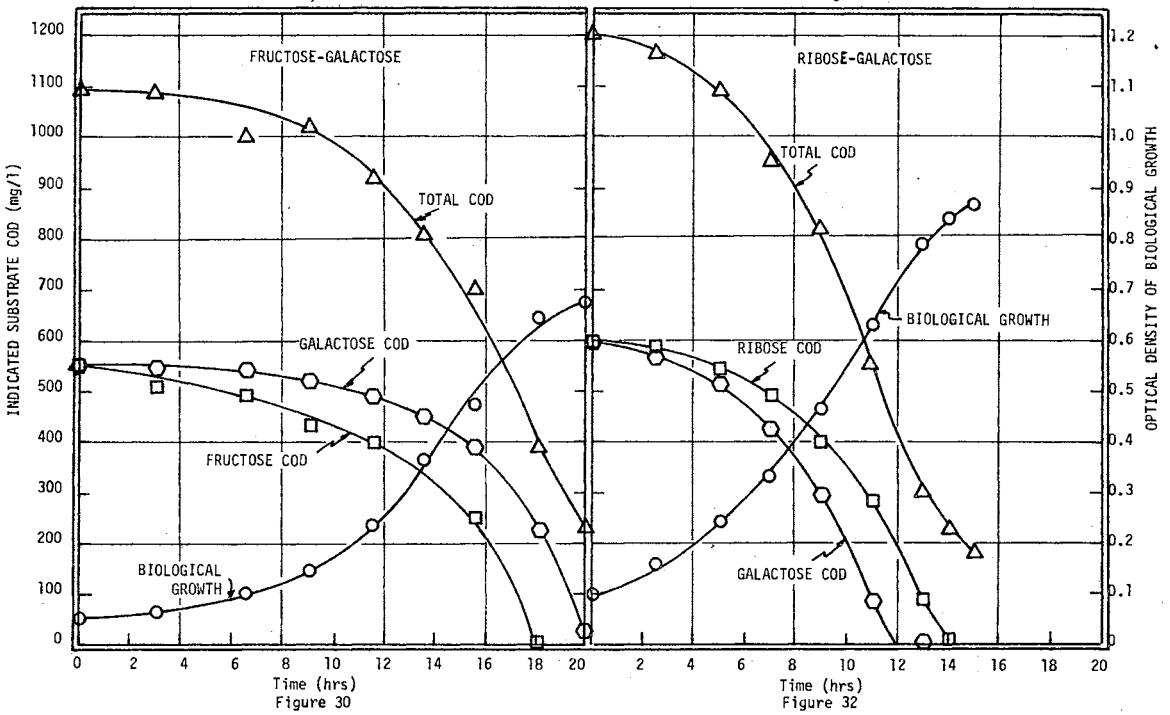



Figure 30




Figure 32

Figure 33 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Arabinose and Galactose Control Units.

Figure 34 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Arabinose-Galactose Combined Unit.

Figure 35 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Sucrose and Galactose Control Units.

Figure 36 - Metabolic Responses of Galactose-acclimated Heterogeneous Populations in the Sucrose-Galactose Combined Unit.

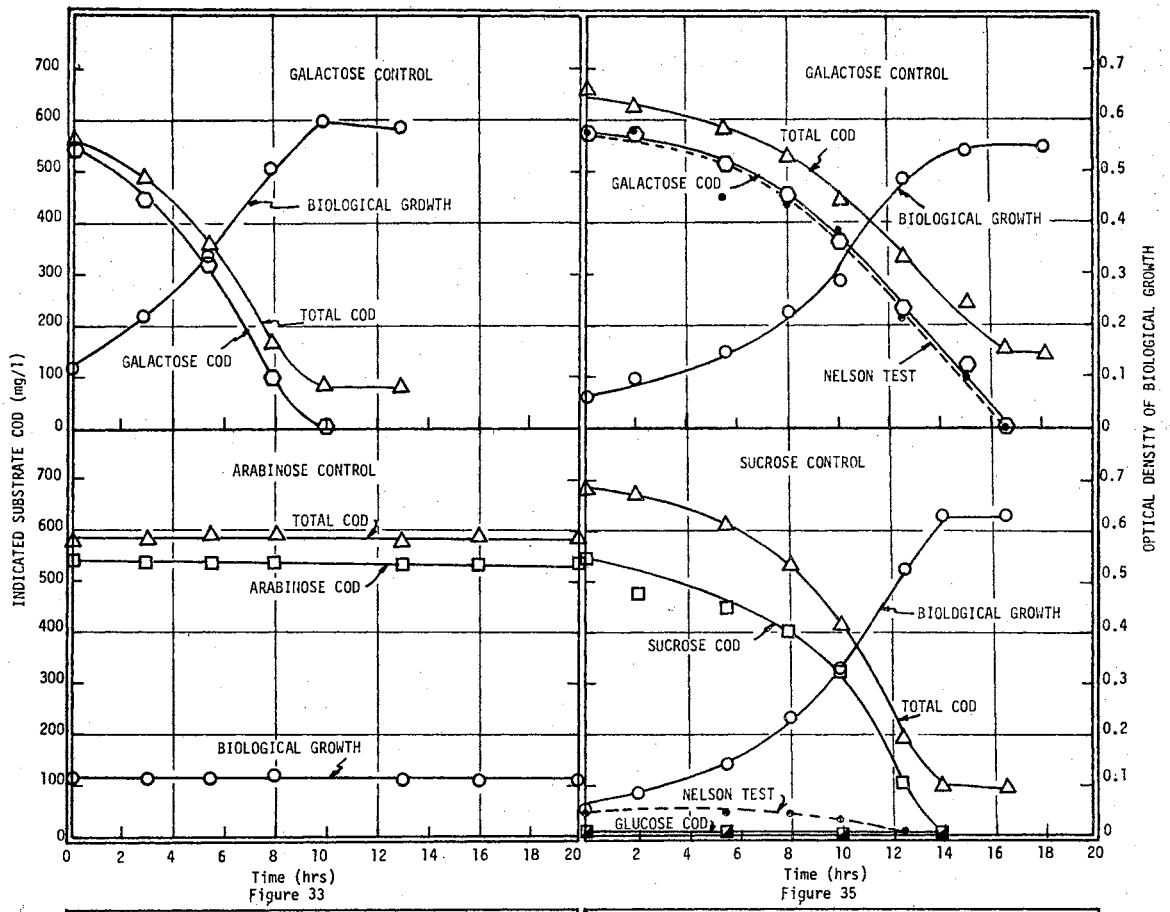



Figure 33

Figure 35

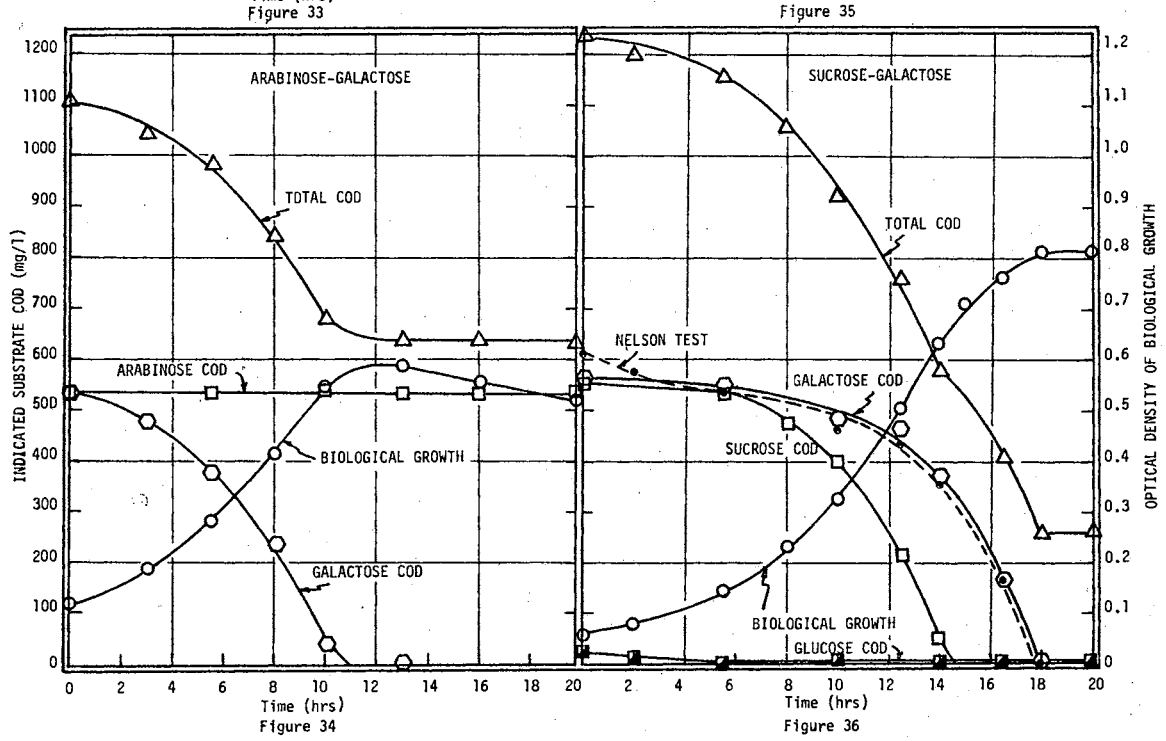



Figure 34

Figure 36

e. Combination of Sucrose and Galactose

As may be seen in Figure 35, galactose-acclimated cells utilized sucrose more rapidly than galactose. Sucrose is a non-reducing sugar, and therefore should not react to the Nelson test; however, approximately 50 mg/l of reducing sugar were detected during the period of sucrose removal. Since no glucose was found in the medium, it would appear that the reducing-sugar test registered a small amount of free fructose in the medium.

The results obtained when these sugars were used in combination are shown in Figure 36. Metabolism of galactose was partially blocked by sucrose. Sucrose was, for all practical purposes, eliminated from the medium in fourteen hours, at which time the galactose concentration was approximately 375 mg/l. The overall kinetics of removal of total COD was not seriously affected by the partially sequential nature of removal of the carbon sources, but the diphasic nature of removal is reflected in the total COD curve after fourteen hours.

f. Summary of Data for Phase A, 3

The results for all experiments using galactose-acclimated populations are summarized in Table III.

4. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Ribose in Combination with Various Compounds (Heterogeneous Populations Acclimated to Ribose)

a. Combination of Glucose and Ribose

The blockage of ribose metabolism in the presence of glucose has been previously shown in Figure 20. The control systems for these experiments were shown in Figure 19.

TABLE III

BIOLOGICAL RESPONSES IN THE MIXED SUBSTRATE SYSTEMS CONSISTING OF GALACTOSE WITH VARIOUS COMPOUNDS

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | galactose $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 15 | | glucose | 65 | 660 | 595 | 965 | 61.7 | 0 | 10 | $\mu_{11}$=0.238 | $K_{11}$=0.374 | | $K_{11}$=0.44 | |
| 15 | galactose | galactose | 65 | 680 | 615 | 980 | 63.0 | 0 | 15.5 | $\mu_{11}$=0.184 | $K_{11}$=0.275 | $K_{11}$=0.402 | | Sequential |
| 16 | | combined | 65 | 625 | 560 | 900 | 62.3 | 0<br>7 | 7<br>12 | $\mu_{11}$=0.270 | $K_{11}$=0.305 | $K_{02}$=100 | $K_{11}$=0.416 | |
| 29 | | fructose | 25 | 255 | 230 | 475 | 48.5 | 0 | 15 | $\mu_{11}$=0.186 | $K_{11}$=0.265 | | $K_{11}$=0.265 | |
| 29 | galactose | galactose | 25 | 190 | 165 | 460 | 35.9 | 0 | 20 | $\mu_{11}$=0.158<br>$\mu_{02}$=7.7 | $K_{11}$=0.272<br>$K_{02}$=33.5 | $K_{11}$=0.272<br>$K_{02}$=41 | | Concurrent |
| 30 | | combined | 25 | 338 | 313 | 860 | 36.4 | 0<br>3 | 18<br>20 | $\mu_{11}$=0.183 | $K_{11}$=0.322 | $K_{11}$=0.267 | $K_{11}$=0.231 | |
| 31 | | ribose | 48 | 295 | 247 | 540 | 45.8 | 0 | 14 | $\mu_{11}$=0.154 | $K_{11}$=0.272 | | $K_{11}$=0.272 | |
| 31 | galactose | galactose | 48 | 285 | 237 | 500 | 47.4 | 0 | 11 | $\mu_{11}$=0.202 | $K_{11}$=0.308 | $K_{11}$=0.308 | | Concurrent |
| 32 | | combined | 48 | 433 | 385 | 1020 | 37.8 | 0<br>0 | 14<br>13 | $\mu_{11}$=0.178 | $K_{11}$=0.408 | $K_{11}$=0.331 | $K_{11}$=0.334 | |
| 33 | | arabinose | 60 | 60 | | | | | | | | | | Arabinose |
| 33 | galactose | galactose | 60 | 300 | 240 | 480 | 50 | 0 | 10 | $\mu_{11}$=0.198 | $K_{11}$=0.418 | $K_{11}$=0.445 | | cannot |
| 34 | | combined | 60 | 295 | 235 | 460 | 51 | -<br>0 | -<br>10 | $\mu_{11}$=0.16 | $K_{11}$=0.374 | $K_{11}$=0.36 | | be used |
| 35 | | sucrose | 27 | 315 | 288 | 580 | 49.6 | 0 | 14 | $\mu_{11}$=0.182 | $K_{11}$=0.3 | | $K_{11}$=0.2 | |
| 35 | galactose | galactose | 27 | 285 | 258 | 520 | 49.5 | 0 | 16.5 | $\mu_{11}$=0.177 | $K_{11}$=0.332 | $K_{11}$=0.332 | | Sequential |
| 36 | | combined | 27 | 408 | 381 | 980 | 38.8 | 0<br>0 | 14<br>18 | $\mu_{11}$=0.182 | $K_{11}$=0.308 | $K_{12}$=0.235 | $K_{11}$=0.319 | |

b. Combination of Fructose and Ribose

It is seen in Figure 37 that ribose-acclimated cells grew on fructose at a more rapid rate than they did on ribose. Biological growth was slightly greater when these cells were grown on ribose. When these substrates were used as the combined carbon source (see Figure 38) they were removed concurrently, and the biological solids production was notably lower than the aggregate sludge accumulation in the controls.

c. Combination of Galactose and Ribose

As seen in Figure 39, acclimation to ribose conferred acclimation to galactose, and both substrates were removed rather rapidly. When employed as a joint carbon source, these compounds were removed concurrently (see Figure 40) and the overall rate of COD removal was significantly higher in the combined system.

d. Combination of Lactose and Ribose

The results shown in Figure 41 indicate that acclimation to ribose did not confer acclimation to lactose, as evidenced by a lag period of approximately twelve hours in the lactose control. The lag period probably represents the time required for the population to synthesize a galactoside-permease and the enzyme β-galactosidase required for initial biological attack of lactose. However, it is noted that once growth did begin, it proceeded more rapidly on lactose than on ribose, the compound to which the cells were previously acclimated.

The results shown in Figure 42 indicate that the joint presence of ribose and lactose did not enhance acclimation to lactose. If ribose did not exhibit a repressing effect on lactose metabolism, it might be expected that energy derived from ribose might be used to synthesize

Figure 37 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Fructose and Ribose Control Units.

Figure 38 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Fructose-Ribose Combined Unit.

Figure 39 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Galactose and Ribose Control Units.

Figure 40 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Galactose-Ribose Combined Unit.

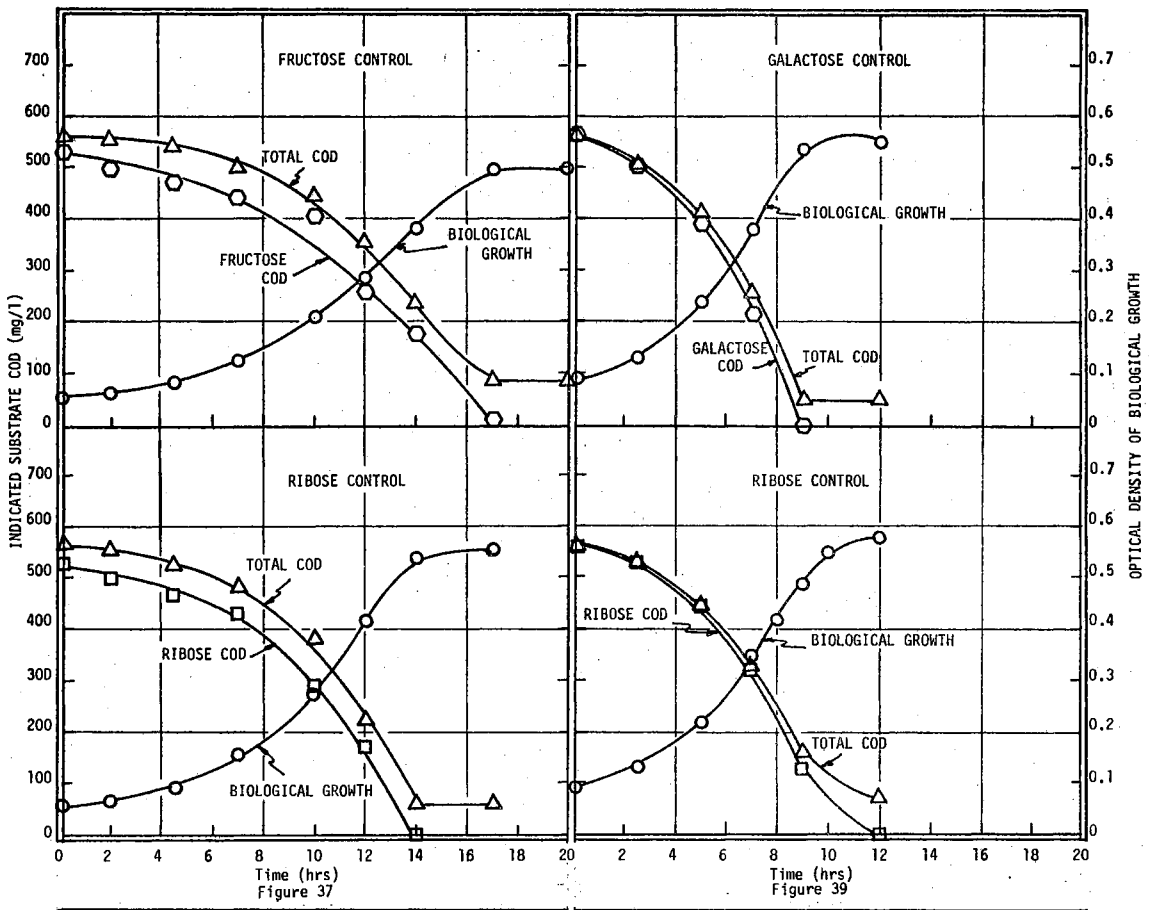



Figure 37




Figure 39

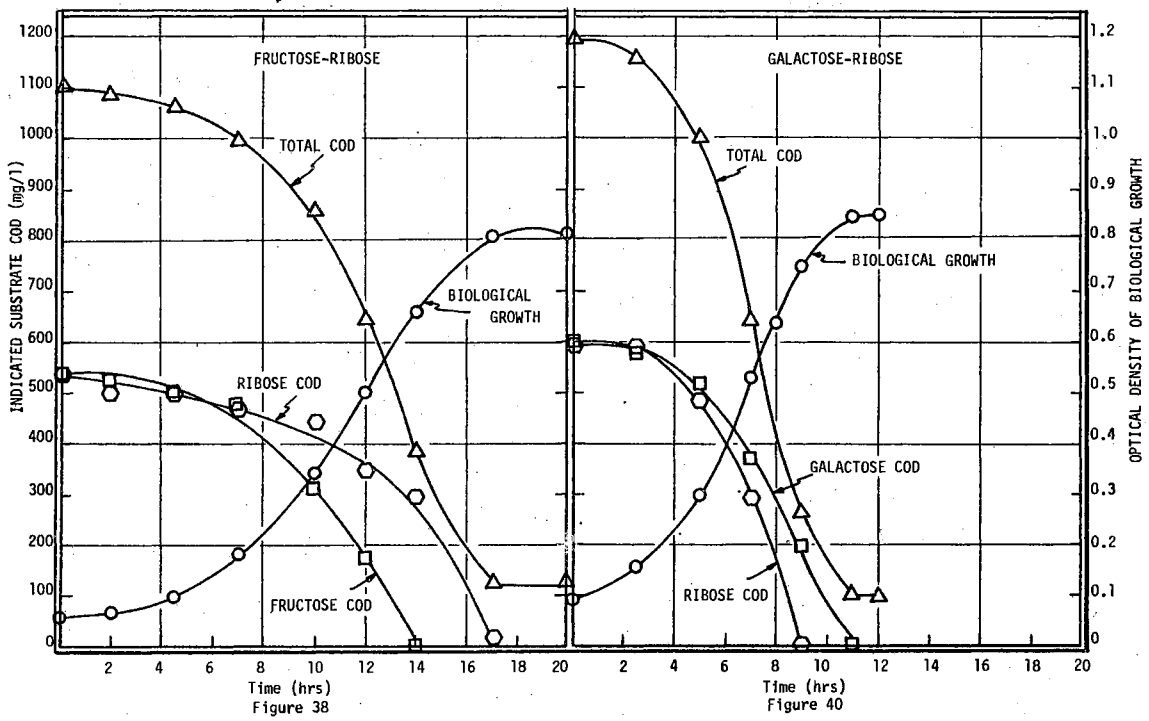



Figure 38




Figure 40

Figure 41 - Metabolic Responses of Ribose-acclimated Heterogeneous Populations in the Lactose and Ribose Control Units.

Figure 42 - Metabolic Responses of the Ribose-acclimated Heterogeneous Populations in the Lactose-Ribose Combined Unit.

Figure 43 - Metabolic Responses of the Ribose-acclimated Heterogeneous Populations in the Sucrose and Ribose Control Units.

Figure 44 - Metabolic Responses of the Ribose-acclimated Heterogeneous Populations in the Sucrose-Ribose Combined Unit.

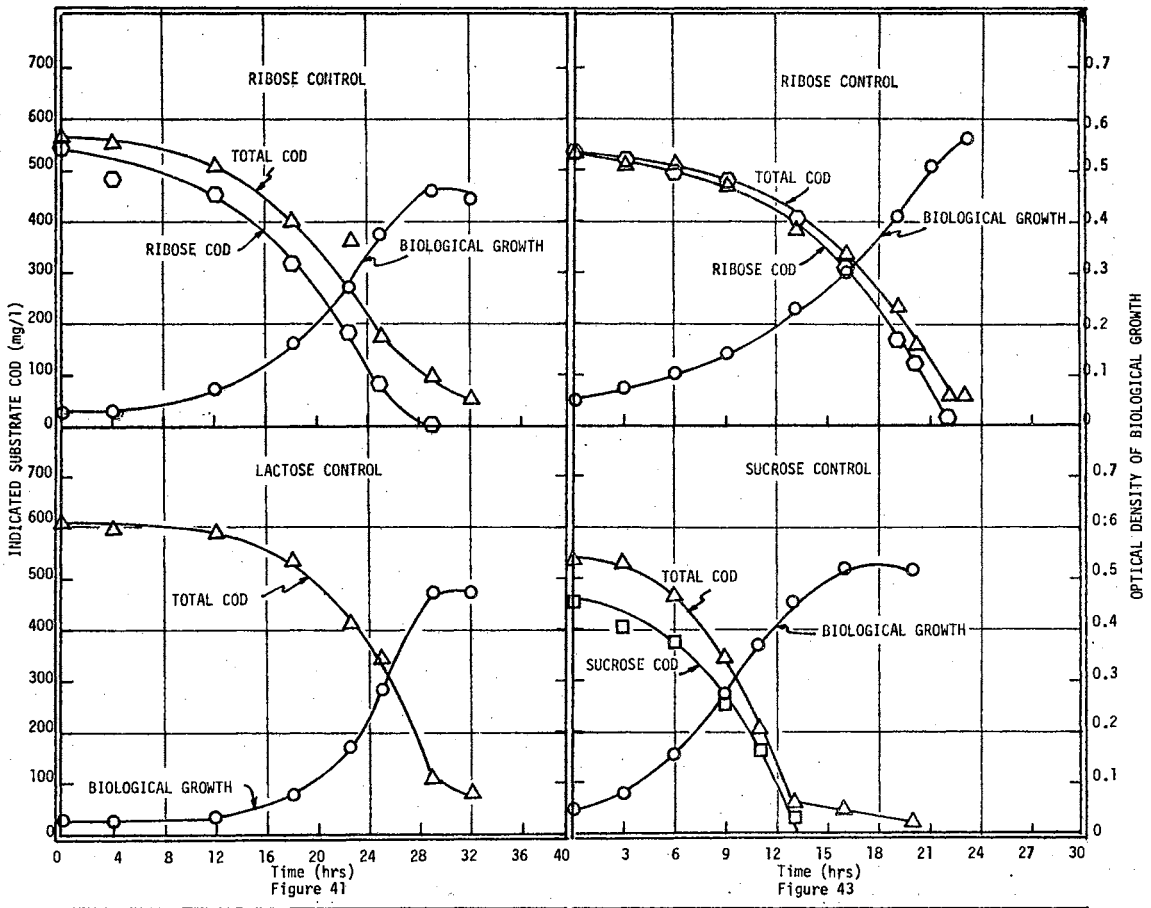



Figure 41

Figure 43

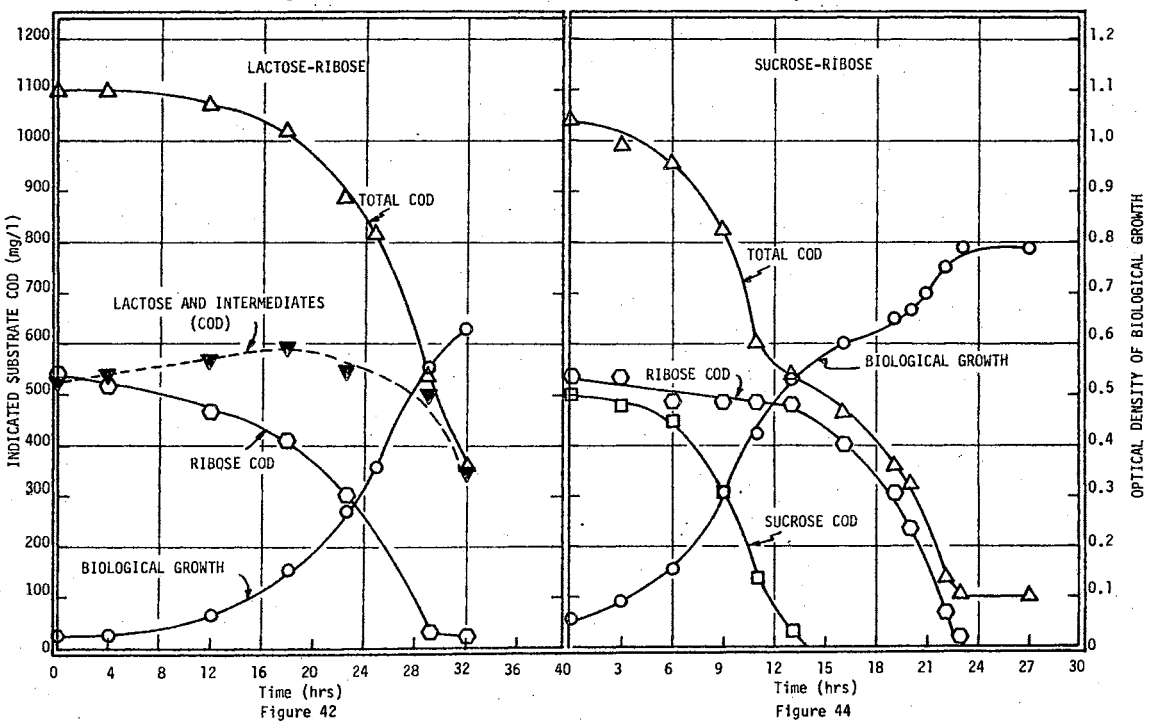



Figure 42

Figure 44

enzymes necessary for degradation of lactose. However, it is seen that this apparently did not occur, since lactose removal was not initiated until the ribose was eliminated from the medium.

e. Combination of Sucrose and Ribose

Although the cells had been previously grown on ribose, it is seen in Figure 43 that growth on sucrose proceeded considerably faster than on ribose. When these substrates were used as combined carbon sources (see Figure 44) they were without doubt utilized sequentially, and by comparing ribose removal in Figures 43 and 44, there would appear to be evidence that sucrose blocked the removal of ribose.

f. Summary of Data for Phase A, 4

The data for this segment of the study, in which ribose-acclimated cells were tested in medium containing ribose and a second substrate, are summarized in Table IV.

5. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Arabinose in Combination with Various Compounds (Heterogeneous Populations Acclimated to Arabinose)

a. Combination of Glucose and Arabinose

The results of this study have been previously given in Figures 21 and 22. Glucose did not block arabinose removal; the compounds, when used as a combined carbon source, were removed concurrently.

b. Combination of Fructose and Arabinose

Figure 45 shows that acclimation to arabinose did not confer acclimation to fructose. These results were somewhat surprising, since fructose has, for the most part, in previous work been shown to be a

TABLE IV

BIOLOGICAL RESPONSES IN THE MIXED SUBSTRATE SYSTEMS CONSISTING OF RIBOSE WITH VARIOUS COMPOUNDS

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | Ribose $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 19 | | glucose | 25 | 290 | 265 | 500 | 53 | 0 | 12 | $\mu_{11}$=0.231 | $K_{11}$=0.263 | | $K_{11}$=0.263 | |
| 19 | ribose | ribose | 25 | 290 | 265 | 550 | 48.3 | 0 | 15 | $\mu_{11}$=0.168 | $K_{11}$=0.191 | $K_{11}$=0.192 | | Sequential |
| 20 | | combined | 25 | 435 | 410 | 1010 | 40.5 | 0<br>8 | 12<br>17 | $\mu_{11}$=0.231<br>$\mu_{12}$=0.054 | $K_{11}$= 0.300<br>$K_{12}$=0.456 | $K_{12}$=0.456 | $K_{11}$=0.308 | |
| 37 | | fructose | 25 | 280 | 255 | 500 | 51 | 0 | 14 | $\mu_{11}$=0.204 | $K_{11}$=0.303 | | $K_{11}$=0.303 | |
| 37 | ribose | ribose | 25 | 250 | 225 | 475 | 47.4 | 0 | 17 | $\mu_{11}$=0.168 | $K_{11}$=0.277 | $K_{11}$=0.277 | | Concurrent |
| 38 | | combined | 25 | 405 | 380 | 975 | 39 | 0<br>0 | 14<br>17 | $\mu_{11}$=0.207 | $K_{11}$=0.305 | $K_{11}$=0.222 | $K_{11}$=0.363 | |
| 39 | | galactose | 45 | 290 | 245 | 500 | 49 | 0 | 11 | $\mu_{11}$=0.211 | $K_{11}$=0.34 | | $K_{11}$=0.34 | |
| 39 | ribose | ribose | 45 | 235 | 190 | 515 | 37 | 0 | 9 | $\mu_{11}$=0.238 | $K_{11}$=0.377 | $K_{11}$=0.395 | | Concurrent |
| 40 | | combined | 45 | 425 | 380 | 1090 | 53 | 0<br>0 | 11<br>9 | $\mu_{11}$=0.277 | $K_{11}$=0.687 | $K_{11}$=0.524 | $K_{11}$=0.529 | |
| 41 | | lactose | 13 | 235 | 222 | 520 | 42.7 | 0 | 29 | $\mu_{11}$=0.157 | $K_{11}$=0.209 | | $K_{11}$=0.209 | Sequential |
| 41 | ribose | ribose | 13 | 230 | 217 | 510 | 42.6 | 0 | 29 | $\mu_{11}$=0.128 | $K_{11}$=0.2 | $K_{11}$=0.2 | | Lactose is |
| 42 | | combined | 13 | 315 | 302 | 740 | 40.7 | 29<br>0 | 29 | $\mu_{11}$=0.135 | $K_{11}$=0.187 | $K_{11}$=0.115 | | Inhibited |
| 43 | | sucrose | 30 | 285 | 255 | 475 | 53.8 | 0 | 13 | $\mu_{11}$=0.206 | $K_{11}$=0.31 | | $K_{11}$=0.347 | |
| 43 | ribose | ribose | 30 | 260 | 230 | 500 | 46.0 | 0 | 22 | $\mu_{11}$=0.112 | $K_{11}$=0.157 | $K_{11}$=0.157 | | Sequential |
| 44 | | combined | 30 | 395 | 365 | 940 | 38.9 | 0<br>13 | 13<br>23 | $\mu_{11}$= 0.216<br>$\mu_{12}$= 0.031 | $K_{11}$=0.341<br>$K_{12}$=0.322 | $K_{01}$=4.2<br>$K_{12}$=0.322 | $K_{11}$=0.362 | |

fairly readily metabolized carbon source. From an analysis of the control systems it would be expected that these compounds when used as a joint carbon source would be removed sequentially; however, as seen in Figure 46, this was not the case. Fructose was removed more slowly than was arabinose, but the presence of arabinose apparently provided a source of energy for synthesis of the enzyme system needed to metabolize the fructose. There is, in Figure 46, a definite indication that fructose metabolism was well under way while considerable concentrations of arabinose remained in the medium. Even though the compounds were removed concurrently, there is evidence for the diphasic nature of total COD removal.

c. Combination of Galactose and Arabinose

As seen in Figure 47, cells previously acclimated to arabinose grew rather slowly when galactose was used as the carbon source. When used in combination, the substrates were metabolized concurrently (see Figure 48) although galactose was eliminated from the medium more slowly than was arabinose. Galactose utilization in the combined system was slightly more rapid than in the control, which is probably due to the larger feeding population developed because of the presence and utilization of arabinose.

d. Combination of Mannose and Arabinose

The growth of arabinose-acclimated cells on mannose was considerably slower than on arabinose (Figure 49). As a combined carbon source (Figure 50) these compounds were adjudged to be removed concurrently, since there was no evidence that arabinose blocked utilization of mannose, i.e., mannose removal did proceed, although at a slow rate,

Figure 45 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Fructose and Arabinose Control Units.

Figure 46 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Fructose-Arabinose Combined Unit.

Figure 47 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Galactose and Arabinose Control Units.

Figure 48 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Galactose-Arabinose Combined Unit.

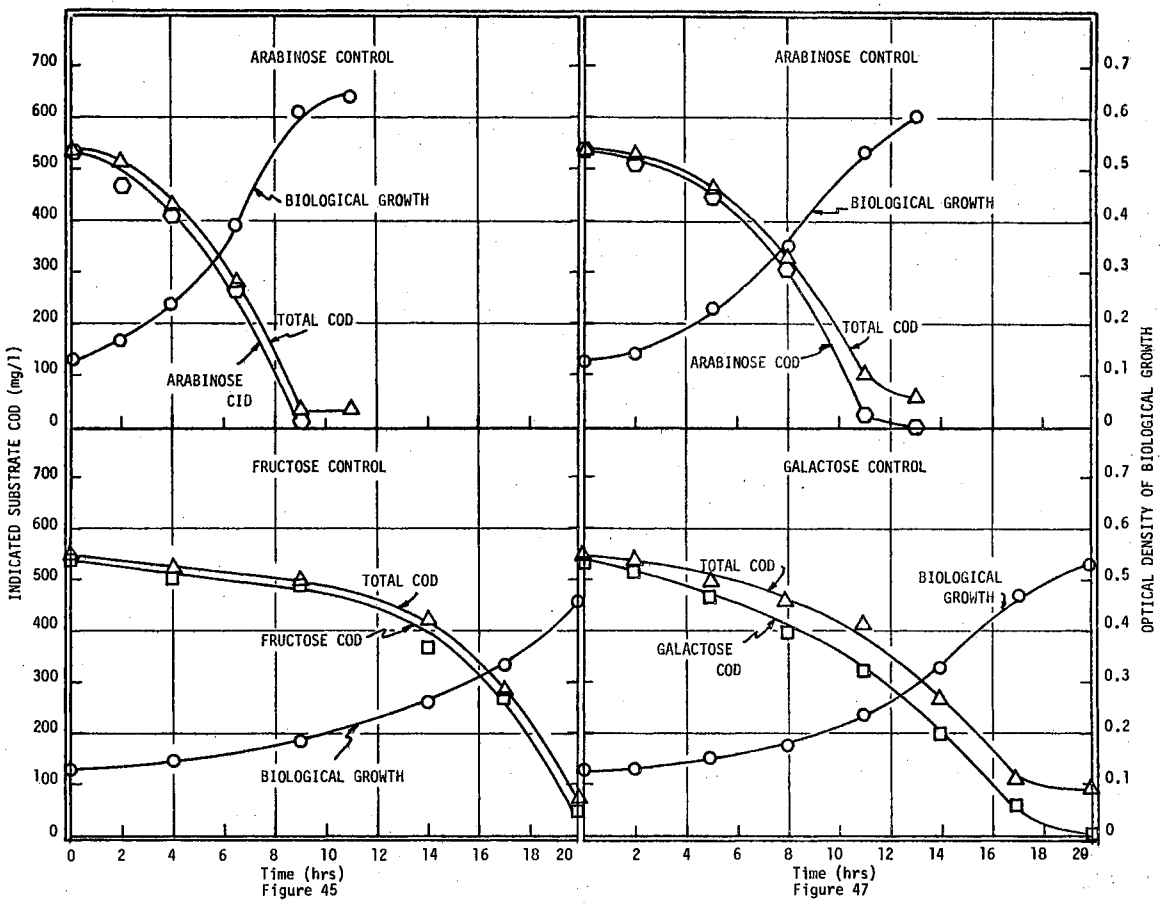



Figure 45

Figure 47

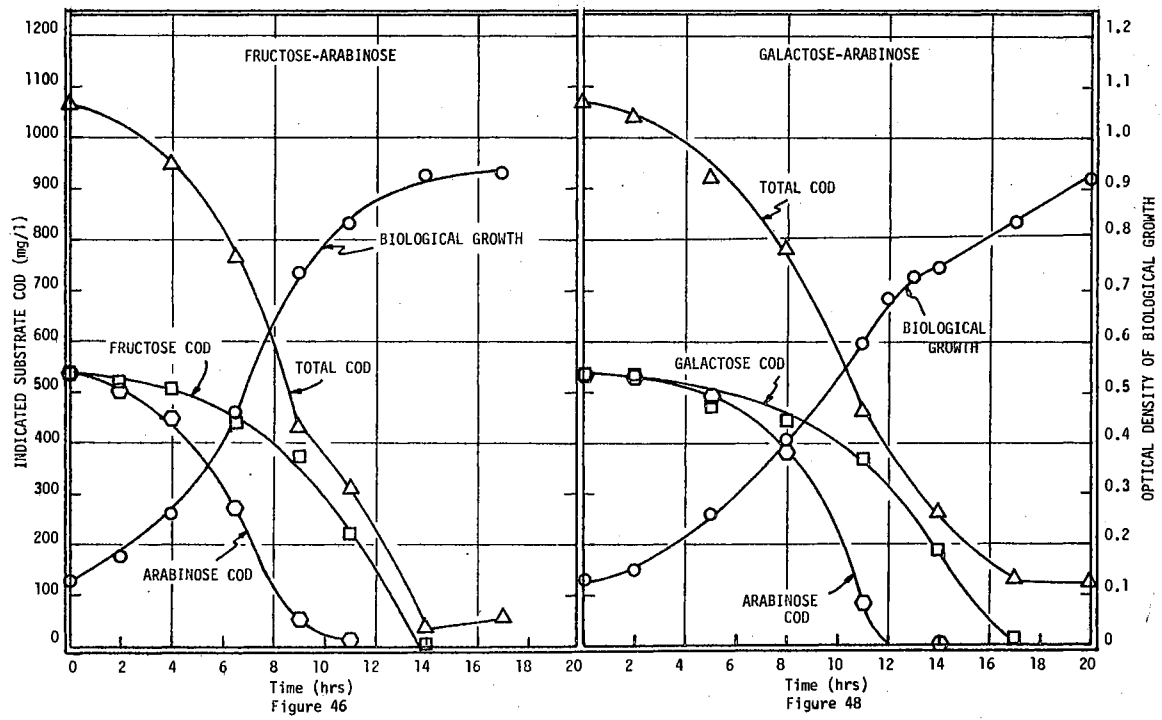



Figure 46

Figure 48

while arabinose was still present in the system. However, due to the slower rate of mannose removal, approximately eighty per cent of this carbon source remained at the time of arabinose removal, and this did give rise to decidedly diphasic growth and total COD removal curves.

e. Combination of Lactose and Arabinose

In Figure 51 it is seen that arabinose-acclimated cells metabolized lactose rather slowly. Also, in neither control system was there evidence for the accumulation of metabolic intermediates and/or endproducts. When used as a combined carbon source (see Figure 52) there was distinct evidence for diphasic removal of total COD. A small amount of lactose was taken up by the cells during the period before arabinose was essentially eliminated from the medium. It is important to note that, even after arabinose had been metabolized, removal of lactose did not proceed at a fast rate. At the 25-hour sampling time, over 300 mg/l of carbohydrate remained in the medium. This remaining carbohydrate is attributable to lactose since there was little or no evidence of accumulation of metabolic intermediates of a carbohydrate nature due to arabinose metabolism. At the same sampling time in the lactose control system essentially all of the lactose had been removed. Therefore, it seems highly probable that the presence of arabinose blocked the synthesis of the enzyme system (or a portion thereof) needed to metabolize lactose.

f. Combination of Sucrose and Arabinose

In this study, growth on arabinose (the compound to which the cells had been previously acclimated) was not initiated very rapidly. However, arabinose was removed in a shorter time than was sucrose (Figure 53).

Figure 49 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Mannose and Arabinose Control Units.

Figure 50 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Mannose-Arabinose Combined Unit.

Figure 51 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Lactose and Arabinose Control Units.

Figure 52 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Lactose-Arabinose Combined Unit.

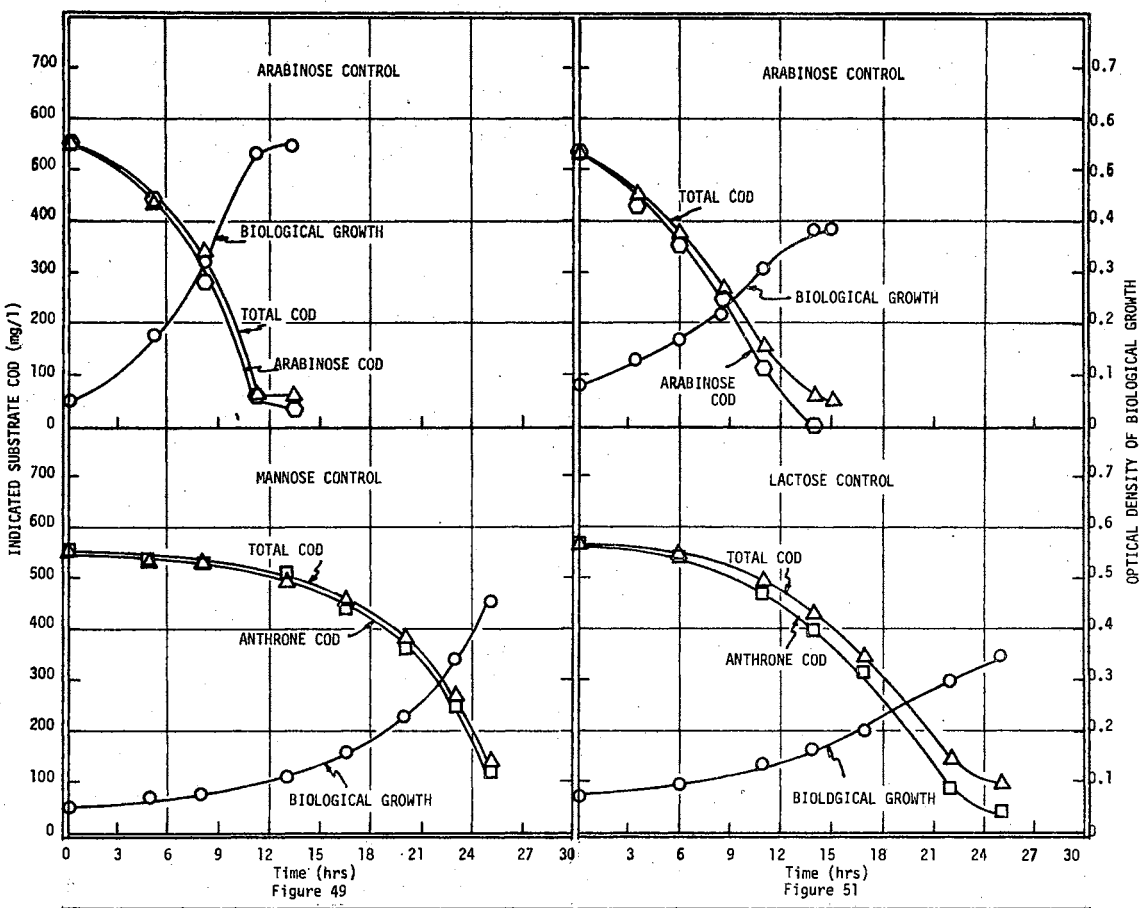



Figure 49




Figure 51

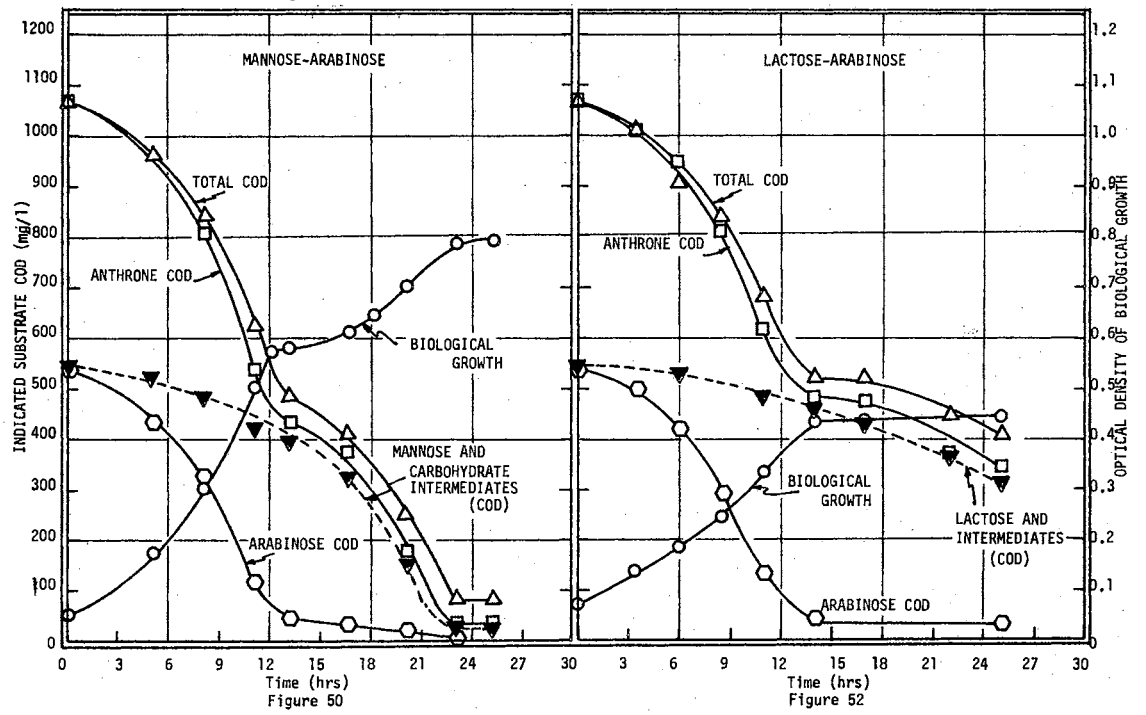



Figure 50




Figure 52

In the combined system (Figure 54) the substrates were removed concurrently and growth proceeded at a faster rate than in either control, indicating concurrent removal.

g. Summary of Data for Phase A, 5

The data for this segment of the study, using cells acclimated to arabinose, in media containing arabinose and other substrates, are summarized in Table V.

6. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Glycerol in Combination With Various Compounds (Heterogeneous Populations Acclimated to Glycerol)

a. Combination of Glucose and Glycerol

Results using these two substrates with cells acclimated to glycerol have already been presented in Figures 27 and 28, and there was ample evidence that glucose inhibited the utilization of glycerol.

b. Combination of Fructose and Glycerol

Heterogeneous populations acclimated to glycerol could readily metabolize fructose, as seen in Figure 55. However, glycerol was metabolized at a faster rate than was fructose. When the compounds were used as the combined carbon source, they were removed concurrently (Figure 56). Glycerol was removed only slightly more rapidly than fructose. The optical density (biological solids) values at five hours and seven hours suggest that growth was diphasic; however, if such really was the case, it was not reflected in the total COD removal curve.

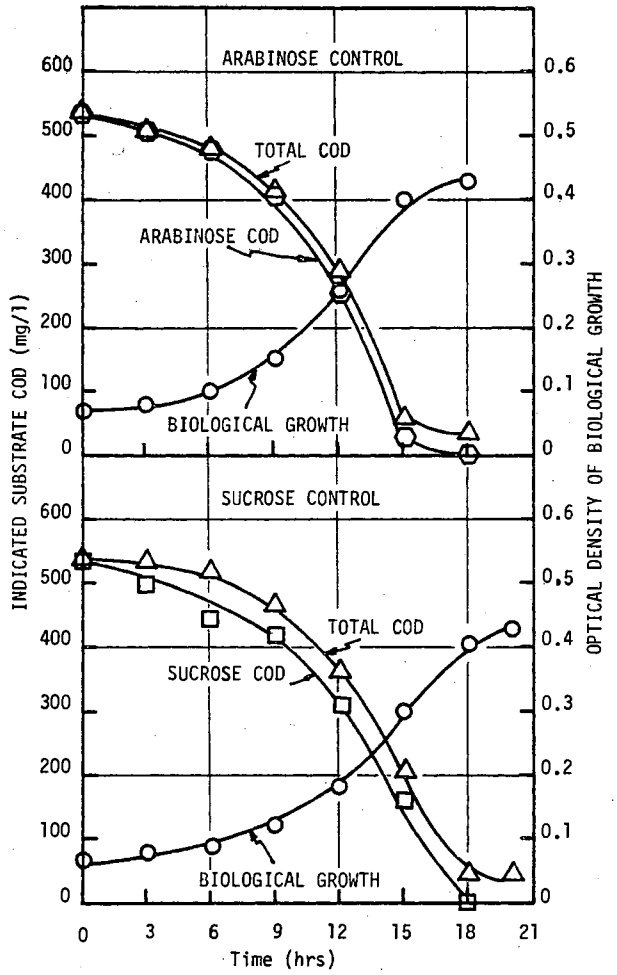



Figure 53 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Sucrose and Arabinose Control Units.

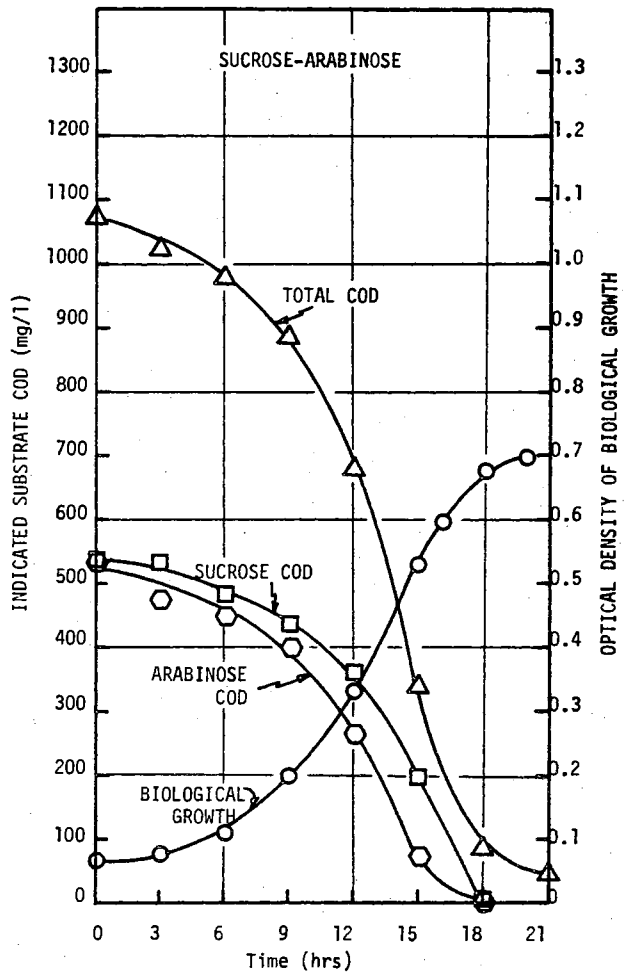



Figure 54 - Metabolic Responses of the Arabinose-acclimated Heterogeneous Populations in the Sucrose-Arabinose Combined Unit.

TABLE V

BIOLOGICAL RESPONSES IN THE MIXED SUBSTRATE SYSTEMS CONSISTING OF ARABINOSE WITH VARIOUS COMPOUNDS

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | Arabinose $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 21 | | glucose | 50 | 300 | 250 | 520 | 48.2 | 0 | 10 | $\mu_{11}=0.173$ | $K_{11}=0.389$ | | $K_{01}=53.5$ | |
| 21 | arabinose | arabinose | 50 | 300 | 250 | 520 | 48.2 | 0 | 12 | $\mu_{11}=0.164$ | $K_{11}=0.271$ | $K_{11}=0.271$ | | Concurrent |
| 22 | | combined | 50 | 430 | 380 | 1020 | 37.3 | 0<br>0 | 9<br>12 | $\mu_{11}=0.252$ | $K_{11}=0.412$ | $K_{11}=0.218$ | $K_{11}=0.375$ | |
| 45 | | fructose | 65 | 230 | 165 | 490 | 33.7 | 9 | 20 | $\mu_{11}=0.072$ | $K_{11}=0.183$ | | $K_{11}=0.183$ | |
| 45 | arabinose | arabinose | 65 | 320 | 255 | 500 | 51 | 0 | 9 | $\mu_{11}=0.178$ | $K_{11}=0.296$ | $K_{11}=0.296$ | | Concurrent |
| 46 | | combined | 65 | 465 | 400 | 1025 | 39 | 0<br>0 | 14<br>10 | $\mu_{11}=0.208$ | $K_{11}=0.34$<br>$K_{12}=0.647$ | $K_{11}=0.304$ | $K_{11}=0.213$ | |
| 47 | | galactose | 60 | 265 | 205 | 460 | 44.5 | 0 | 20 | $\mu_{11}=0.0634$ | $K_{11}=0.185$ | | $K_{11}=0.2$ | |
| 47 | arabinose | arabinose | 60 | 300 | 240 | 470 | 51 | 0 | 11 | $\mu_{11}=0.147$ | $K_{11}=0.334$ | $K_{11}=0.327$ | | Concurrent |
| 48 | | combined | 60 | 460 | 400 | 945 | 42.4 | 5<br>5 | 17<br>12 | $\mu_{11}=0.167$<br>$\mu_{02}=15$ | $K_{11}=0.242$ | $K_{11}=0.44$ | $K_{11}=0.222$ | |
| 49 | | mannose | 25 | 225 | 200 | 410 | 48.8 | 13 | 21 | $\mu_{11}=0.103$ | $K_{11}=0.149$ | | $K_{11}=0.149$ | |
| 49 | arabinose | arabinose | 25 | 275 | 250 | 490 | 51 | 0 | 11 | $\mu_{11}=0.188$ | $K_{11}=0.186$ | $K_{11}=0.186$ | | Concurrent |
| 50 | | combined | 25 | 395 | 370 | 985 | 37.6 | 0<br>0 | 23<br>13 | $\mu_{11}=0.18$<br>$\mu_{12}=0.04$ | $K_{11}=0.24$<br>$K_{12}=0.542$ | $K_{11}=0.239$ | $K_{11}=0.231$ | |
| 51 | | lactose | 35 | 175 | 140 | 460 | 30.4 | 6 | 24 | $\mu_{11}=0.069$ | $K_{11}=0.234$ | | $K_{11}=0.234$ | Sequential |
| 51 | arabinose | arabinose | 35 | 193 | 153 | 485 | 31.6 | 0 | 14 | $\mu_{11}=0.115$ | $K_{11}=0.235$ | $K_{11}=0.235$ | | Lactose is |
| 52 | | combined | 35 | 225 | 190 | 660 | 28.8 | 14<br>0 | -<br>14 | $\mu_{11}=0.109$<br>$\mu_{02}=0.625$ | $K_{11}=0.296$<br>$K_{12}=0.335$ | $K_{11}=0.310$ | $K_{11}=0.109$ | Inhibited |
| 53 | | sucrose | 35 | 215 | 180 | 495 | 36.4 | 0 | 18 | $\mu_{11}=0.131$ | $K_{11}=0.352$ | | $K_{11}=0.223$ | |
| 53 | arabinose | arabinose | 35 | 215 | 180 | 500 | 36 | 0 | 15 | $\mu_{11}=0.156$ | $K_{11}=0.231$ | $K_{11}=0.250$ | | Concurrent |
| 54 | | combined | 35 | 350 | 315 | 1020 | 30.9 | 0<br>0 | 18<br>18 | $\mu_{11}=0.188$ | $K_{11}=0.222$ | $K_{11}=0.183$ | $K_{11}=0.188$ | |

c. Combination of Galactose and Glycerol

The growth of glycerol-acclimated cells on galactose (see Figure 57) yielded a somewhat surprising kinetic response. A considerable amount of intermediates and/or endproducts accumulated in the medium as galactose was removed, and the growth curve did not exhibit a log phase. Biological solids accumulated at a decreasing rate throughout the experiment. From these results one would not expect the results shown in Figure 58 for the combined substrate system. Here it is seen that the presence of galactose interfered quite significantly with the removal of glycerol. Glycerol removal was totally blocked during the first 5.5 hours of the experiment during which nearly half of the galactose was metabolized. It is also interesting to note that there was an unusually high residual COD at the end of the experiment.

d. Combination of Mannose and Glycerol

Glycerol-acclimated cells exhibited a capability for utilization of mannose, as seen in Figure 59. However, growth and mannose utilization proceeded in an atypical fashion, i.e., approximately linear growth and substrate utilization were exhibited in this system. Neither of the controls exhibited a noticeable amount of intermediates and/or endproducts. When these substrates were used in combination (Figure 60) they were removed concurrently, and mannose appeared to decrease the rate of glycerol removal compared to the rapidity of glycerol elimination in the control.

e. Combination of Ribose and Glycerol

Figure 61 shows growth and substrate removal in the control systems, and it can be seen that the glycerol-acclimated population could

Figure 55 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Fructose and Glycerol Control Units.

Figure 56 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Fructose-Glycerol Combined Unit.

Figure 57. Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Galactose and Glycerol Control Units.

Figure 58 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Galactose-Glycerol Combined Unit.

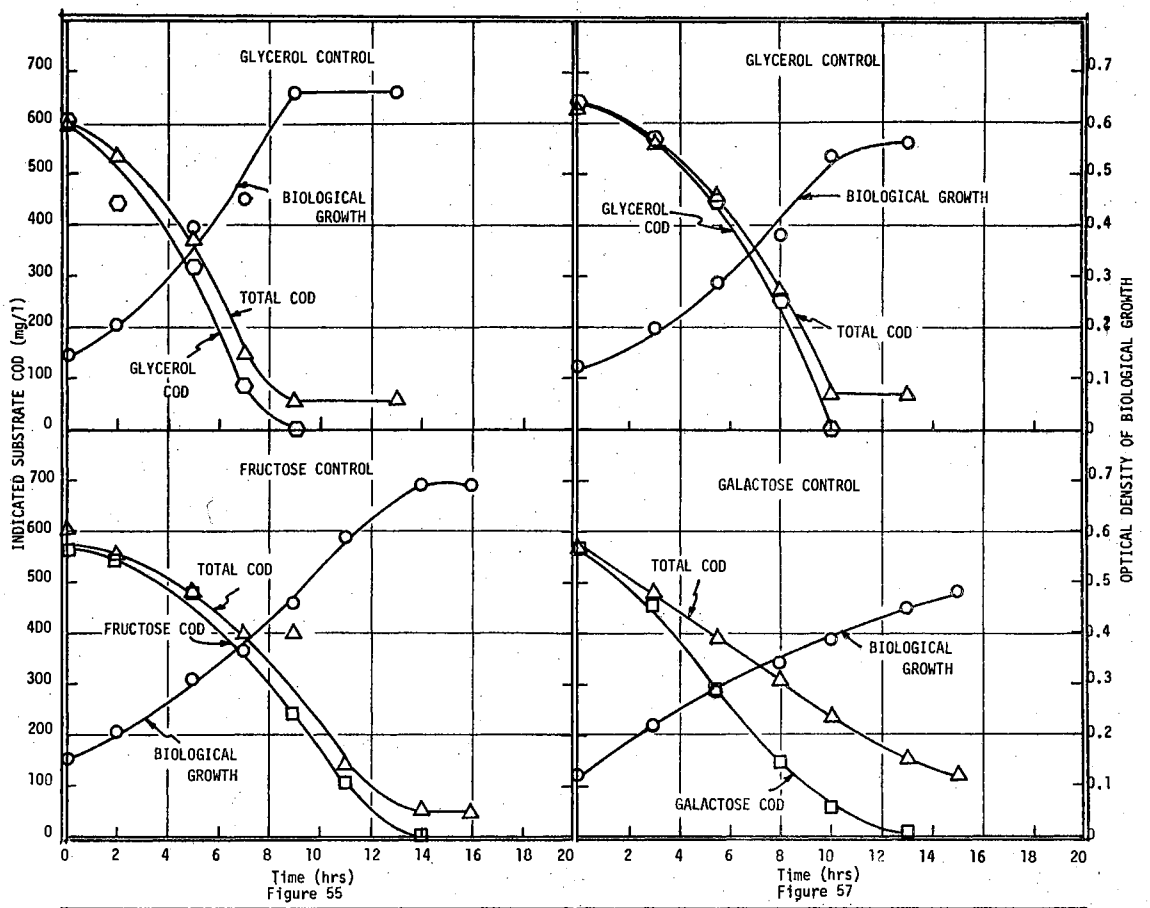



Figure 55




Figure 57

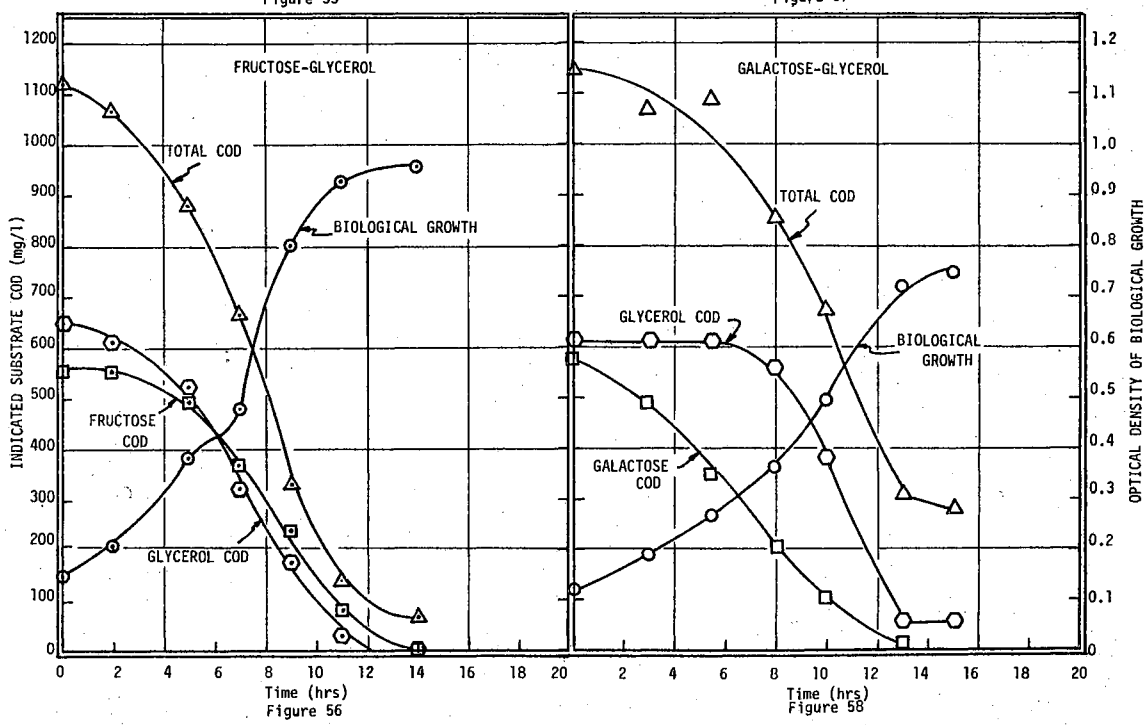



Figure 56



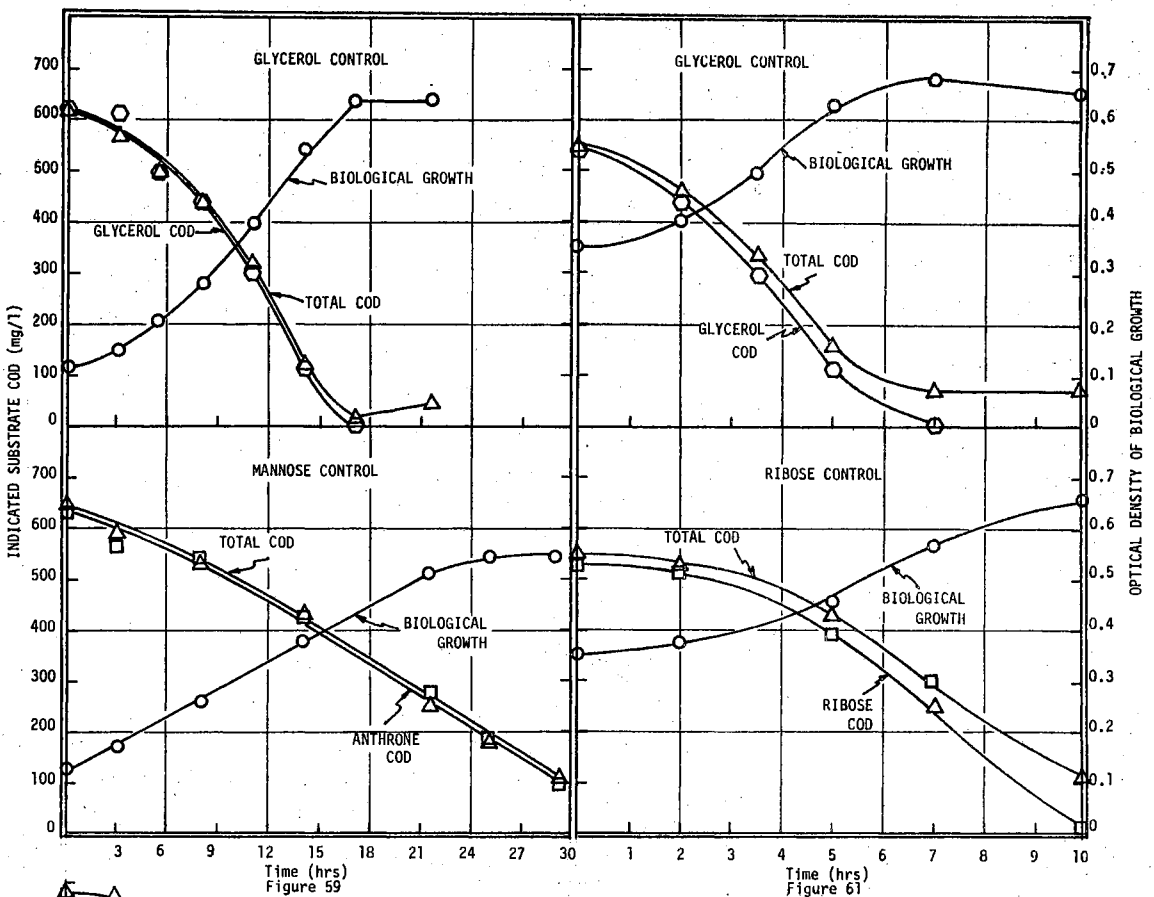

Figure 58

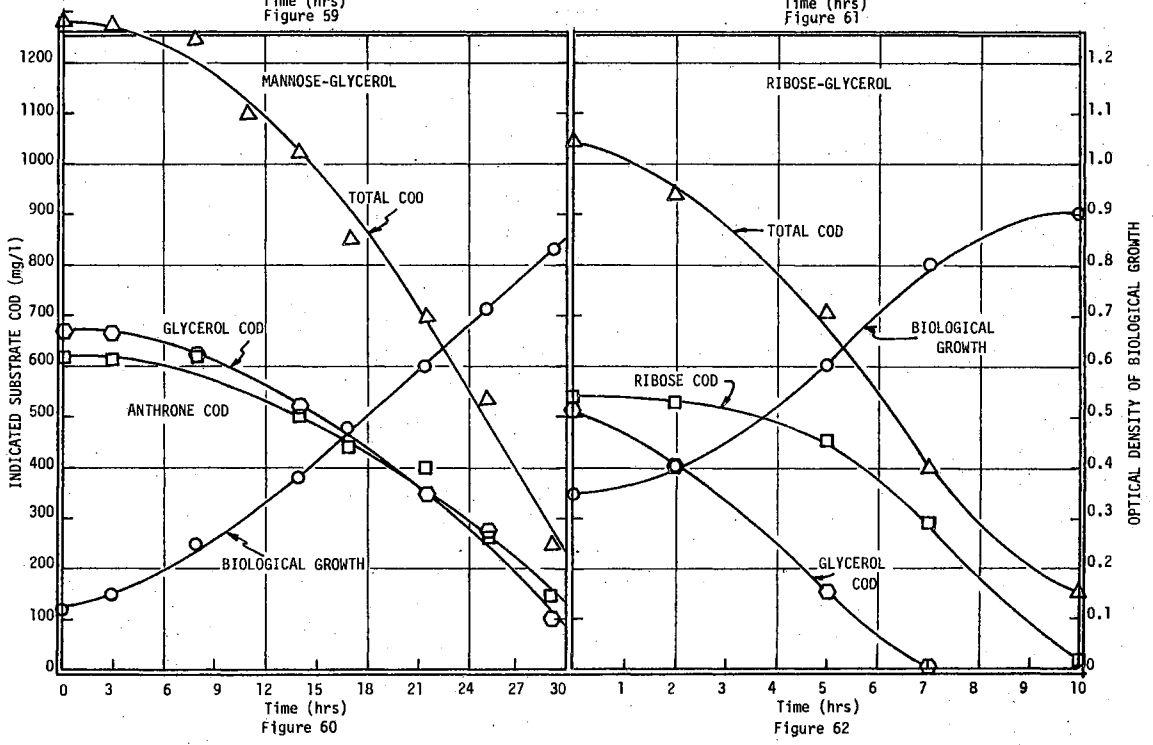
Figure 59 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Mannose and Glycerol Control Units.

Figure 60 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Mannose-Glycerol Combined Unit.

Figure 61 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Ribose and Glycerol Control Units.

Figure 62 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Ribose-Glycerol Combined Unit.


GLYCEROL CONTROL
BIOLOGICAL GROWTH
GLYCEROL COD
TOTAL COD
MANNOSE CONTROL
TOTAL COD
BIOLOGICAL GROWTH
ANTHRONE COD
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 59
GLYCEROL CONTROL
BIOLOGICAL GROWTH
TOTAL COD
GLYCEROL COD
RIBOSE CONTROL
TOTAL COD
BIOLOGICAL GROWTH
RIBOSE COD
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 61


MANNOSE-GLYCEROL
TOTAL COD
GLYCEROL COD
ANTHRONE COD
BIOLOGICAL GROWTH
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 60
RIBOSE-GLYCEROL
TOTAL COD
BIOLOGICAL GROWTH
RIBOSE COD
GLYCEROL COD
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 62

metabolize ribose although at a slower rate than glycerol. The substrate removal patterns which were exhibited when the carbon sources were used jointly (Figure 62) indicate that the metabolism of neither compound was affected by the presence of the other.

f. Combination of Arabinose and Glycerol

In this study the initial biological solids concentration was somewhat higher than that used in the previous experiments for glycerol-acclimated cells. It is seen in Figure 63 that the glycerol-acclimated population exhibited no ability to metabolize arabinose throughout the 50-hour experimental period. In the arabinose control system, biological solids concentration continually decreased. In the combined system (Figure 64) the presence of glycerol did not assist the population in acclimating to arabinose. No arabinose was removed in the 50-hour experimental period. Biological solids concentration attained a peak at the time of removal of glycerol, and thereafter the solids concentration decreased in the prolonged endogenous phase. The decrease in biological solids concentration was accompanied by a release of COD into the medium.

g. Combination of Lactose and Glycerol

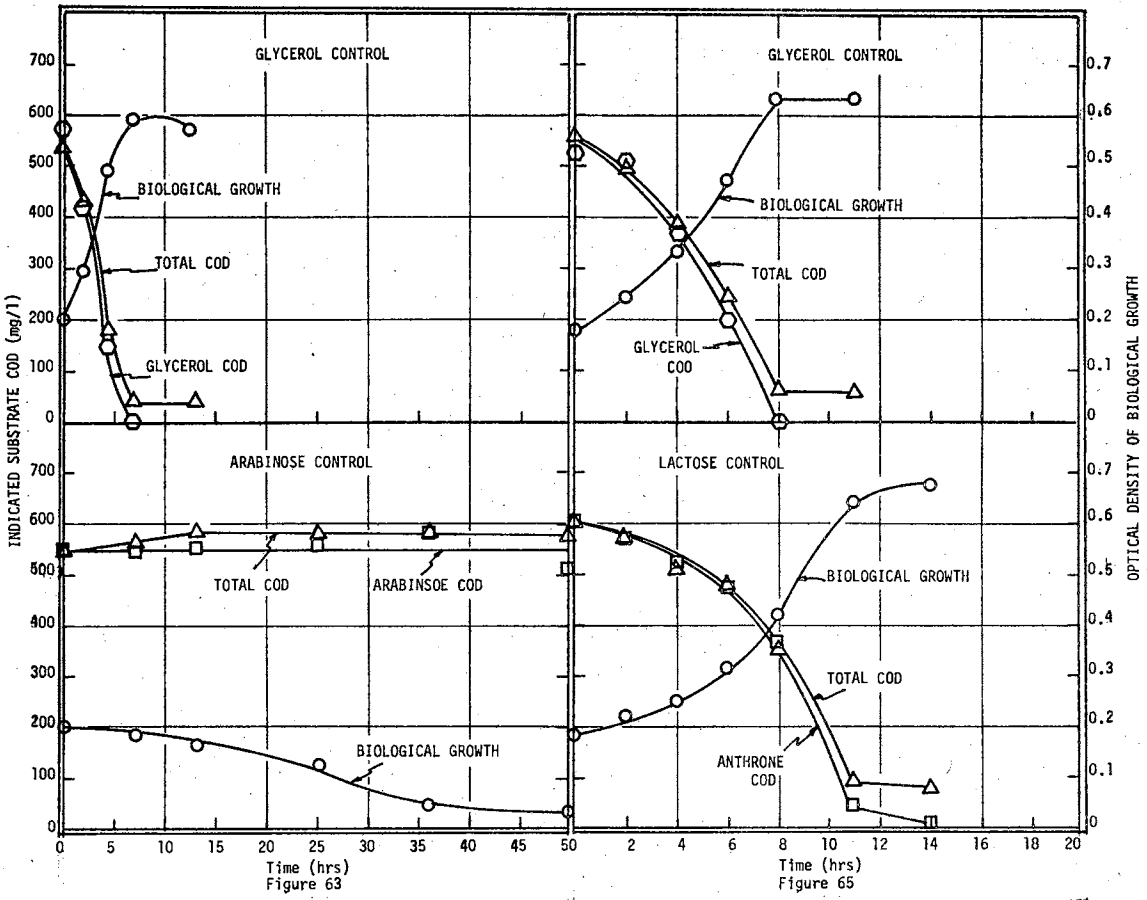
The results shown in Figure 65 would indicate that glycerol-acclimated cells possessed the ability to metabolize lactose, although growth on lactose proceeded somewhat slower than growth on glycerol. However, when lactose and glycerol were used as the combined carbon sources (Figure 66), glycerol blocked lactose utilization until approximately fifty per cent of the glycerol had been eliminated from the system. When lactose metabolism was initiated, the results indicate

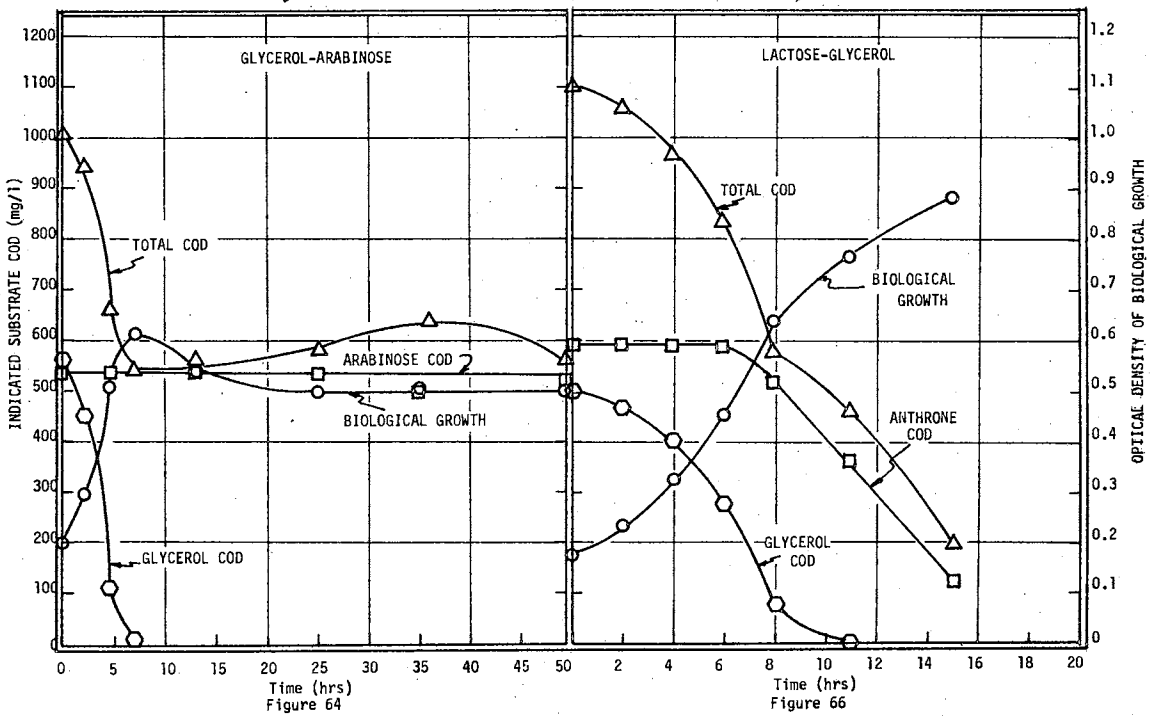
Figure 63 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Arabinose and Glycerol Control Units.

Figure 64 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Arabinose-Glycerol Combined Unit.

Figure 65 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Lactose and Glycerol Control Units.

Figure 66 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Lactose-Glycerol Combined Unit.


GLYCEROL CONTROL
BIOLOGICAL GROWTH
TOTAL COD
GLYCEROL COD
ARABINOSE CONTROL
TOTAL COD
ARABINSOE COD
BIOLOGICAL GROWTH
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 63
GLYCEROL CONTROL
BIOLOGICAL GROWTH
TOTAL COD
GLYCEROL COD
LACTOSE CONTROL
BIOLOGICAL GROWTH
TOTAL COD
ANTHRONE COD
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 65


GLYCEROL-ARABINOSE
TOTAL COD
ARABINOSE COD
BIOLOGICAL GROWTH
GLYCEROL COD
INDICATED SUBSTRATE COD (mg/l)
Time (hrs)
Figure 64
LACTOSE-GLYCEROL
TOTAL COD
BIOLOGICAL GROWTH
ANTHRONE COD
GLYCEROL COD
OPTICAL DENSITY OF BIOLOGICAL GROWTH
Time (hrs)
Figure 66

that lactose was removed in accordance with zero order kinetics. There was a definite indication of diphasic removal of total COD in the system.

h. Combination of Sucrose and Glycerol

Glycerol-acclimated cells could readily metabolize sucrose, as seen in Figure 67. When used as a combined carbon source (see Figure 68), these substrates were removed concurrently. The growth curve in the combined system indicates a slight diphasic tendency. At the point of initiation of the second phase there were in the system approximately 150 mg/l sucrose COD, 460 mg/l glycerol COD, and approximately 270 mg/l COD due to the sum of metabolic intermediates produced from either glycerol or sucrose, or both, and COD due to the inorganic salts in the medium.

i. Summary of Data for Phase A, 6

The data for this segment of the study, in which cells acclimated to glycerol were tested in media containing glycerol and other substrates, are summarized in Table VI.

7. Studies on Substrate Removal in Two-component Carbon Source Systems (Heterogeneous Populations Acclimated to One of the Compounds)

In order to gain more information concerning the generality of sequential substrate removal, a variety of systems consisting of combinations of carbohydrates not heretofore studied were used in two-component carbon source experiments.

a. Combination of Galactose and Fructose With Cells Acclimated to Fructose

In Figure 69 it is seen that fructose-acclimated cells could

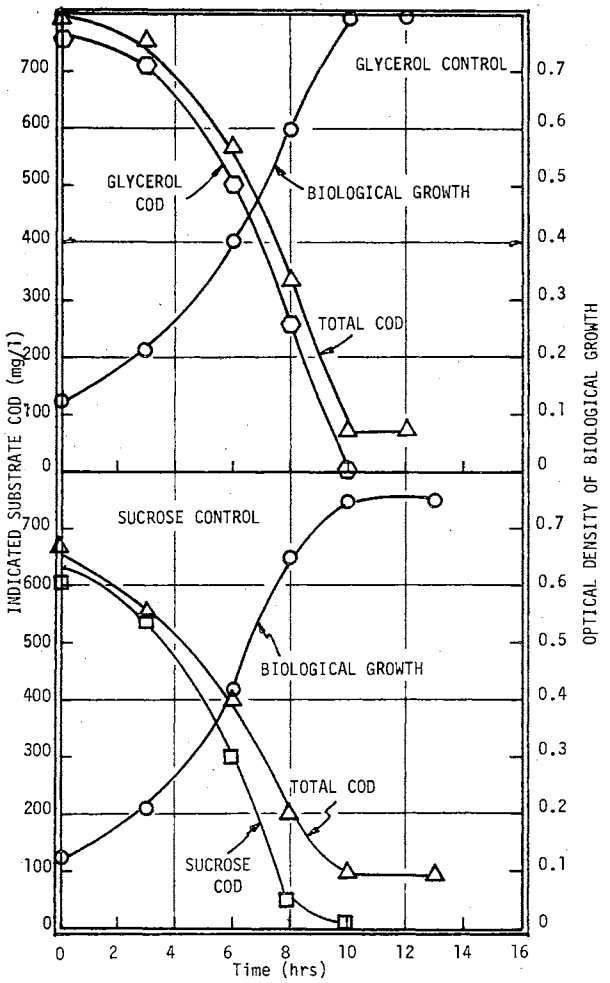



Figure 67 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Sucrose and Glycerol Control Units.

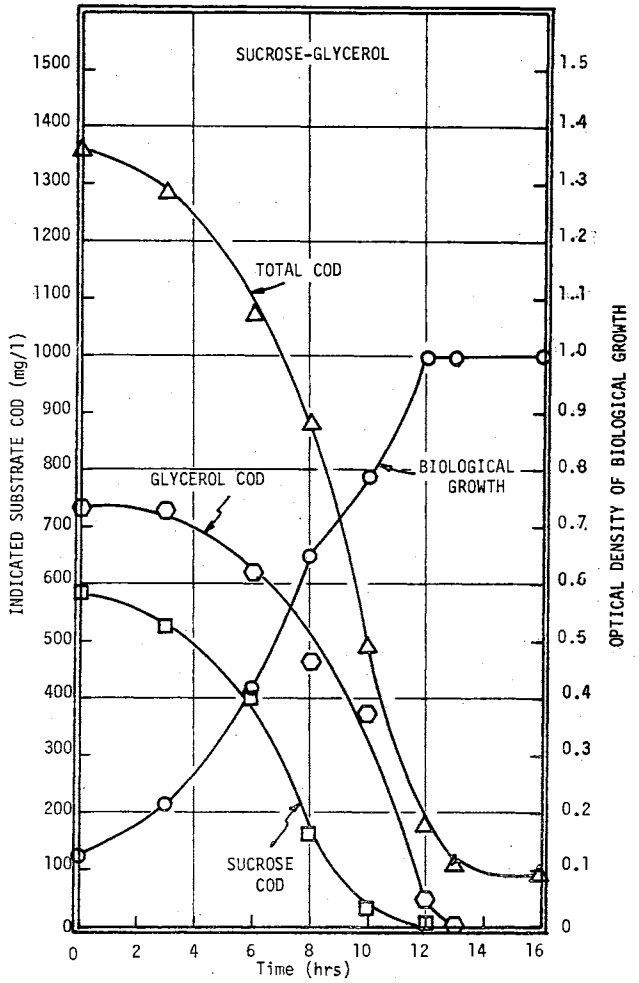



Figure 68 - Metabolic Responses of the Glycerol-acclimated Heterogeneous Populations in the Sucrose-Glycerol Combined Unit.

TABLE VI

BIOLOGICAL RESPONSES IN THE MIXED SUBSTRATE SYSTEMS CONSISTING OF GLYCEROL WITH VARIOUS COMPOUNDS

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | Glycerol $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 27 | | glucose | 135 | 335 | 200 | 500 | 40 | 0 | 3.5 | $\mu_{01}$=40 | $K_{01}$=120 | | $K_{01}$=178 | |
| 27 | glycerol | glycerol | 135 | 335 | 200 | 540 | 37 | 0 | 4 | $\mu_{11}$=0.221 | $K_{11}$=0.694 | $K_{11}$=0.694 | | Sequential |
| 28 | | combined | 135 | 465 | 330 | 1080 | 30.5 | 0<br>3 | 3<br>6 | $\mu_{01}$=70 | $K_{11}$=0.493 | $K_{12}$=1.12 | $K_{01}$=190 | |
| 55 | | fructose | 75 | 345 | 270 | 520 | 52 | 0 | 14 | $\mu_{11}$=0.115 | $K_{11}$=0.28 | | $K_{11}$=0.47 | |
| 55 | glycerol | glycerol | 75 | 330 | 255 | 520 | 49.2 | 0 | 9 | $\mu_{11}$=0.223 | $K_{11}$=0.35 | $K_{11}$=0.346 | | Concurrent |
| 56 | | combined | 75 | 480 | 405 | 1050 | 38.6 | 0<br>0 | 14<br>11 | $\mu_{11}$=0.189<br>$\mu_{12}$=0.256 | $K_{11}$=0.403 | $K_{11}$=0.422 | $K_{11}$=0.562 | |
| 57 | | galactose | 60 | 240 | 180 | 445 | 40.5 | 0 | 13 | $\mu_{11}$=0.064 | $K_{01}$=32 | | $K_{11}$=0.372 | |
| 57 | glycerol | glycerol | 60 | 280 | 220 | 550 | 40.0 | 0 | 10 | $\mu_{11}$=0.16 | $K_{11}$=0.347 | $K_{11}$=0.347 | | Sequential |
| 58 | | combined | 60 | 375 | 315 | 830 | 38 | 0<br>7 | 13<br>13 | $\mu_{11}$=0.147 | $K_{11}$=0.321 | $K_{12}$=0.764 | $K_{11}$=0.237 | |
| 59 | | mannose | 60 | 270 | 210 | 540 | 38.9 | 0 | 33 | $\mu_{01}$=9.2 | $K_{01}$=20 | | $K_{11}$=0.143 | |
| 59 | glycerol | glycerol | 60 | 320 | 260 | 600 | 43.3 | 0 | 17 | $\mu_{11}$=0.183 | $K_{11}$=0.254 | $K_{11}$=0.254 | | Concurrent |
| 60 | | combined | 60 | 415 | 355 | 1030 | 34.5 | 0<br>0 | 33<br>31 | $\mu_{11}$=0.082 | $K_{11}$=0.197 | $K_{11}$=0.101 | $K_{11}$=0.113 | |
| 61 | | ribose | 175 | 330 | 155 | 430 | 36 | 0 | 10 | $\mu_{11}$=0.065 | $K_{11}$=0.583 | | $K_{11}$=0.583 | |
| 61 | glycerol | glycerol | 175 | 340 | 165 | 470 | 35.2 | 0 | 7 | $\mu_{11}$=0.146 | $K_{11}$=0.526 | $K_{11}$=0.526 | | Concurrent |
| 62 | | combined | 175 | 450 | 275 | 890 | 31 | 0<br>0 | 10<br>7 | $\mu_{11}$=0.139 | $K_{11}$=0.358 | $K_{11}$=0.395 | $K_{11}$=0.55 | |
| 63 | | arabinose | 100 | 18 | -82 | -40 | | | | | | | | Arabinose |
| 63 | glycerol | glycerol | 100 | 300 | 200 | 500 | 38.5 | 0 | 7 | $\mu_{11}$=0.2 | $K_{11}$=0.474 | $K_{11}$=0.475 | | cannot be |
| 64 | | combined | 100 | 305 | 205 | 460 | 44.5 | -<br>0 | -<br>7 | $\mu_{11}$=0.208 | $K_{11}$=0.694 | | $K_{11}$=0.563 | used |
| 65 | | lactose | 90 | 340 | 250 | 520 | 48 | 0 | 11 | $\mu_{11}$=0.096 | $K_{11}$=0.328 | | $K_{11}$=0.328 | Sequential |
| 65 | glycerol | glycerol | 90 | 315 | 225 | 500 | 45 | 0 | 8 | $\mu_{11}$=0.152 | $K_{11}$=0.472 | $K_{11}$=0.480 | | Lactose is |
| 66 | | combined | 90 | 440 | 350 | 900 | 38.9 | 6<br>0 | 17<br>10 | $\mu_{11}$=0.155<br>$\mu_{02}$=12.5 | $K_{11}$=0.693<br>$K_{12}$=0.472 | $K_{11}$=0.875 | $K_{02}$=54.3 | Inhibited |
| 67 | | sucrose | 60 | 375 | 315 | 580 | 54.4 | 0 | 10 | $\mu_{11}$=0.209 | $K_{11}$=0.271 | | $K_{11}$=0.369 | |
| 67 | glycerol | glycerol | 60 | 400 | 340 | 730 | 46.6 | 0 | 10 | $\mu_{11}$=0.201 | $K_{11}$=0.583 | $K_{11}$=0.583 | | Concurrent |
| 68 | | combined | 60 | 500 | 440 | 1270 | 34.6 | 0<br>3 | 10<br>13 | $\mu_{11}$=0.221<br>$\mu_{12}$=0.108 | $K_{11}$=0.385 | $K_{11}$=0.430 | $K_{11}$=0.425 | |

readily utilize galactose. Little or no metabolic intermediates were produced from either substrate during the substrate removal period. When these compounds were used as combined carbon sources (Figure 70), they were each metabolized at the same rate (comparable to their rates of removal as individual carbon sources); the removal of these carbon sources was concurrent. Apparently neither compound had an effect upon the metabolism of the other.

b. Combination of Lactose and Galactose with Cells Acclimated to Lactose

In Figure 71 it is seen that lactose-acclimated cells did possess ability to metabolize galactose; however, the rates of substrate elimination and growth were lower on galactose than on lactose, and in the galactose system there was some evidence for the accumulation of metabolic intermediates and/or endproducts. When these substrates were used in combination (Figure 72) they were metabolized concurrently with production of only slight amounts of non-carbohydrate intermediates and/or endproducts. Lactose did decrease the rate of utilization of galactose. The results indicate that the non-carbohydrates present at the end of the experiment were due almost entirely to compounds produced from galactose metabolism.

c. Combination of Sucrose and Galactose with Cells Acclimated to Sucrose

The results shown in Figure 73 indicate that the sucrose-acclimated cells could metabolize galactose; however, while galactose was eliminated from the medium in the same time required to remove sucrose, the total COD curves are considerably different due to the production of a

Figure 69 - Metabolic Responses of the Fructose-acclimated Heterogeneous Populations in the Galactose and Fructose Control Units.

Figure 70 - Metabolic Responses of the Fructose-acclimated Heterogeneous Populations in the Galactose-Fructose Combined Unit.

Figure 71 - Metabolic Responses of the Lactose-acclimated Heterogeneous Populations in the Galactose and Lactose Control Units.

Figure 72 - Metabolic Responses of the Lactose-acclimated Heterogeneous Populations in the Galactose-Lactose Combined Unit.

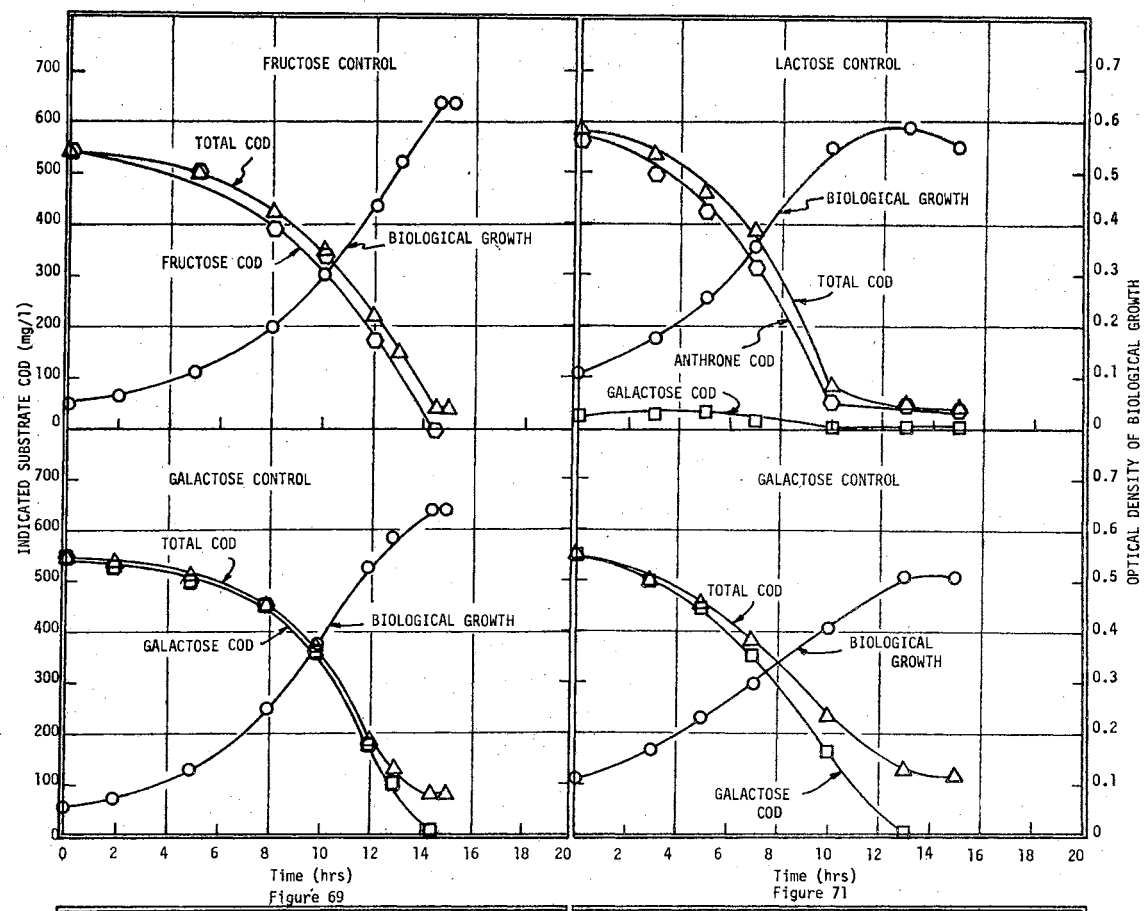



Figure 69




Figure 71

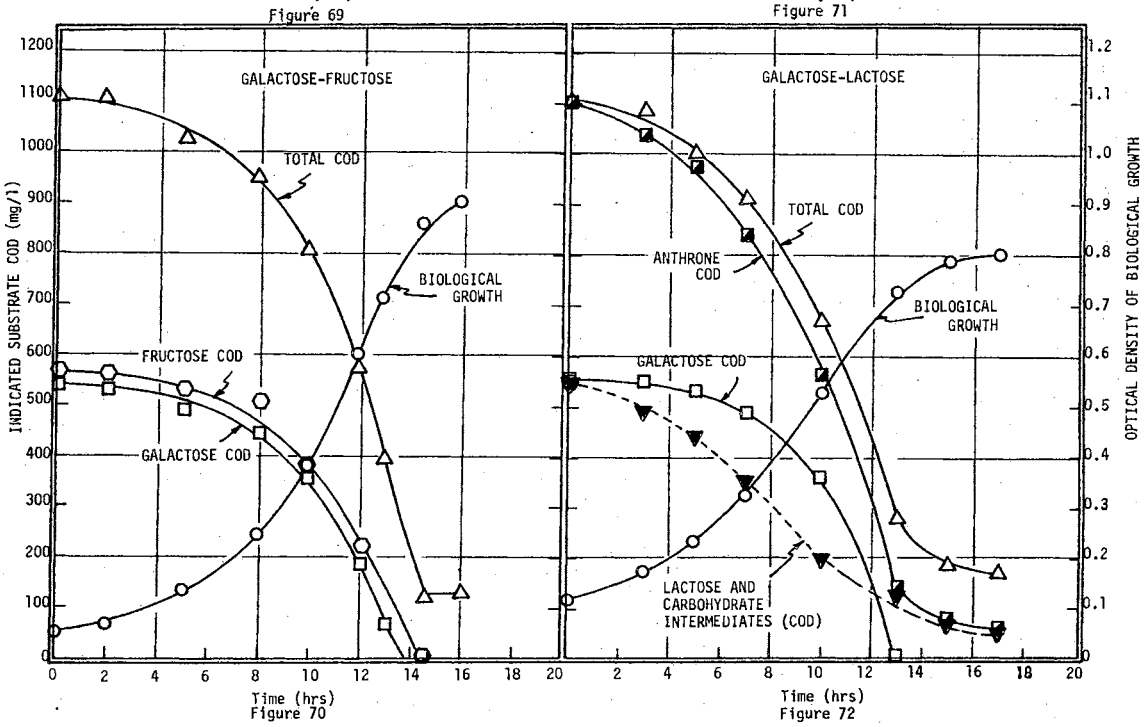



Figure 70




Figure 72

rather large amount of metabolic intermediates and/or endproducts from the metabolism of galactose. It is interesting to note that nearly the same biological solids production resulted from metabolism of galactose as from metabolism of sucrose, even though there was a considerably higher residual COD in the galactose control. In the sucrose system, glucose was not detected during the period of substrate removal; however, there was present a small amount of reducing sugar, as evidenced by a positive reaction to the Nelson test.

When these compounds were used as combined carbon source (Figure 74) the presence of galactose caused a partial blockage of sucrose metabolism. Sucrose utilization was not initiated until approximately one-third of the galactose had been removed. The results also indicate that once sucrose removal was initiated, it proceeded in accordance with zero order kinetics. The partially diphasic removal of these substrates was not reflected in either the biological solids growth or the total COD removal curve. At the end of the experiment the residual COD was quite high; approximately 320 mg/l total COD remained after both original substrates had been totally removed from the medium.

d. Combination of Lactose and Ribose with Cells Acclimated to Lactose

Control systems for these substrates are shown in Figure 75, and it is seen that cells acclimated to lactose could metabolize ribose. A long lag period was not evidenced on ribose; however, the rates of growth and substrate elimination were considerably lower than those on lactose. From the results shown in Figure 76 it is seen that when these substrates were used as a joint carbon source, the presence of lactose blocked utilization of ribose until approximately half of the lactose had been eliminated from the system. The partially sequential nature of

Figure 73 - Metabolic Responses of the Sucrose-acclimated Heterogeneous Populations in the Galactose and Sucrose Control Units.

Figure 74 - Metabolic Responses of the Sucrose-acclimated Heterogeneous Populations in the Galactose and Sucrose Combined Unit.

Figure 75 - Metabolic Responses of the Lactose-acclimated Heterogeneous Populations in the Ribose and Lactose Control Units.

Figure 76 - Metabolic Responses of the Lactose-acclimated Heterogeneous Populations in the Ribose-Lactose Combined Unit.

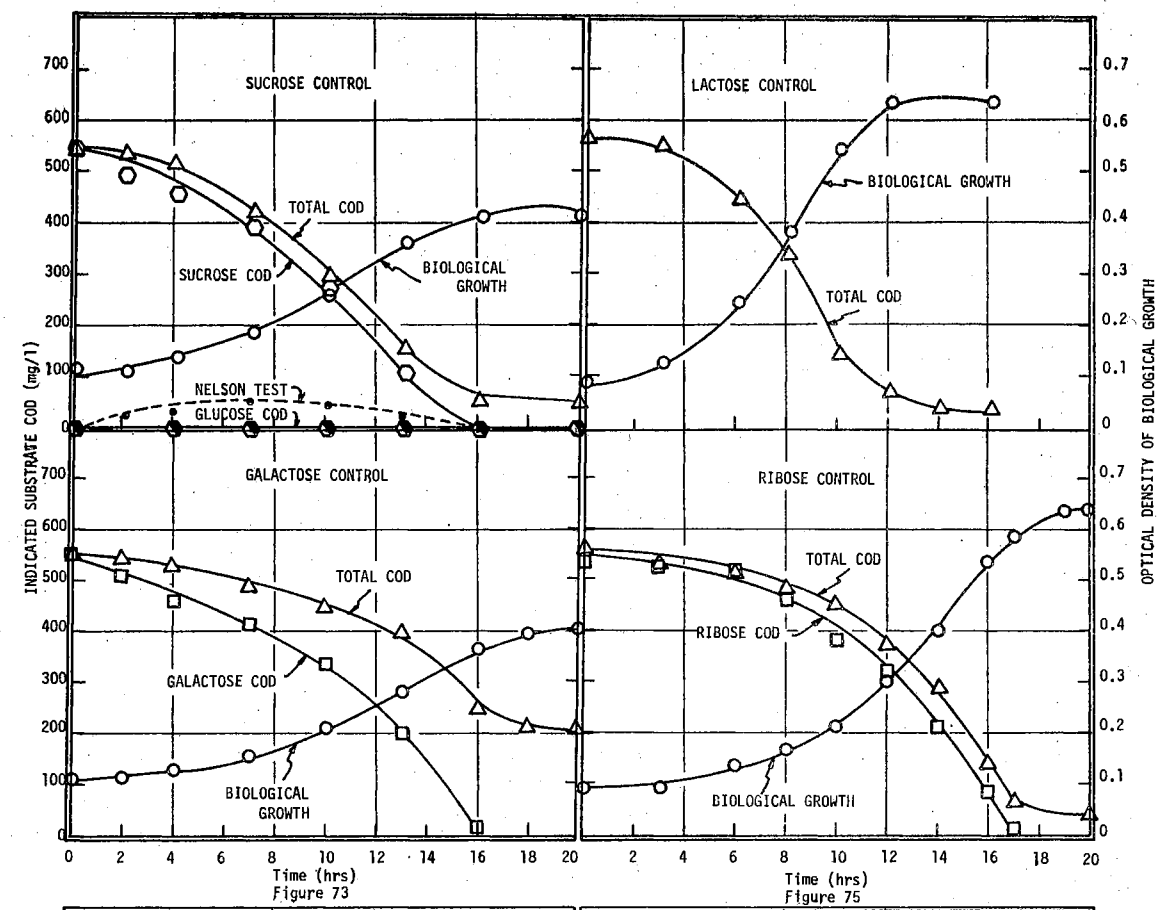



Figure 73

Figure 75

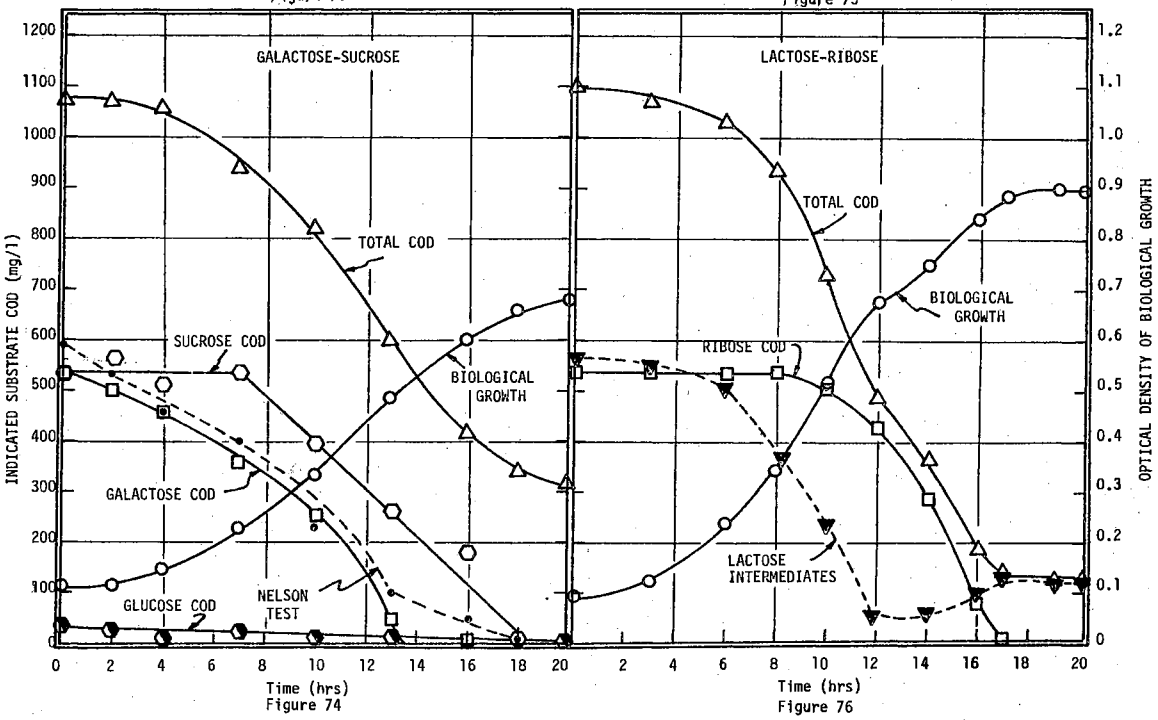



Figure 74

Figure 76

metabolism of these carbon sources led to discontinuities in the total COD and biological solids growth curves.

e. Summary of Data for Phase A, 7

The data for this segment of the study are summarized in Table VII.

Phase B

Effect of Dilution Rate on the Metabolism of Glycerol and on the Sequential Removal of Glucose and Glycerol

These experiments were designed with two major concerns in mind. First, it was desired to determine whether the dilution rate (D) or growth rate ($\mu$) exerted an effect on the mode of removal of glycerol and glucose for glycerol-acclimated cells. Stepwise decreases in growth rate (i.e., increases in reactor detention time) would yield cells of increasing age. Thus, some insight was sought into the effect of cell age on the occurrence of sequential substrate removal. Secondly, it was not known whether continuous steady state operation could be maintained at very low dilution rates, and it was envisioned that the experiments would help to answer this question. In addition, it was felt that valuable transient state data on substrate removal and biological solids production could be amassed as the system's dilution rate was progressively changed to decreasing values and eventually returned to faster growth rates, i.e., the work provided an opportunity to study various aspects of hydraulic shock loading.

For this series of experiments, the mode of presentation of the results is as follows: A figure is shown depicting behavior of the continuous flow system at the initial dilution rate, during the transient state after the change in dilution rate, and in the final steady state

TABLE VII

BIOLOGICAL RESPONSES IN THE MIXED SUBSTRATE SYSTEMS CONSISTING OF TWO COMPOUNDS

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | | |
| Figure Number | Cells Acclimated to | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Lag Time hrs | Removal Time hrs | Growth Rate $hr^{-1}$; mg/l/hr | Total COD $hr^{-1}$; mg/l/hr | Substrate COD $hr^{-1}$; mg/l/hr | Other Substrates $hr^{-1}$; mg/l/hr | Mode of Removal |
| 29 | | fructose | 25 | 255 | 230 | 475 | 48.5 | 0 | 15 | $\mu_{11}$=0.186 | $K_{11}$=0.265 | | $K_{f11}$=0.265 | |
| 29 | galactose | galactose | 25 | 190 | 165 | 460 | 35.9 | 0 | 20 | $\mu_{11}$=0.158<br>$\mu_{02}$=7.7 | $K_{11}$=0.272<br>$K_{02}$=33.5 | $K_{g11}$=0.272<br>$K_{g02}$=41 | | Concurrent |
| 30 | | combined | 25 | 338 | 313 | 860 | 36.4 | 0<br>3 | 18<br>20 | $\mu_{11}$=0.183 | $K_{11}$=0.322 | $K_{g11}$=0.267 | $K_{f11}$=0.231 | |
| 69 | | fructose | 24 | 324 | 300 | 490 | 61.2 | 0 | 14.5 | $\mu_{11}$=0.187 | $K_{11}$=0.354 | | $K_{f11}$=0.354 | |
| 69 | fructose | galactose | 24 | 324 | 300 | 455 | 65.8 | 5 | 14.5 | $\mu_{11}$=0.221 | $K_{11}$=0.354 | $K_{g11}$=0.354 | | Concurrent |
| 70 | | combined | 24 | 452 | 428 | 980 | 43.7 | 5<br>5 | 14.5<br>14.5 | $\mu_{11}$=0.221 | $K_{11}$=0.330 | $K_{g11}$=0.354 | $K_{f11}$=0.354 | |
| 23 | | glucose | 115 | 315 | 200 | 520 | 38.4 | 0 | 8 | $\mu_{11}$=0.122 | $K_{11}$=0.354 | $K_{g11}$=0.507 | | |
| 23 | lactose | lactose | 115 | 325 | 210 | 530 | 39.6 | 0 | 8 | $\mu_{11}$=0.126 | $K_{11}$=0.433 | | $K_{l11}$=0.433 | Sequential |
| 24 | | combined | 115 | 443 | 326 | 1060 | 30.8 | 0<br>6 | 8<br>12 | $\mu_{11}$=0.145 | $K_{11}$=0.51<br>$K_{12}$=0.8 | $K_{g11}$=0.573 | $K_{i12}$=0.547 | |
| 71 | | galactose | 55 | 255 | 200 | 430 | 46.5 | 0 | 13 | $\mu_{11}$=0.143 | $K_{11}$=0.205 | $K_{g11}$=0.270 | | |
| 71 | lactose | lactose | 55 | 293 | 238 | 540 | 44.2 | 0 | 10 | $\mu_{11}$=0.179 | $K_{11}$=0.283 | | $K_{l11}$=0.288 | Concurrent |
| 72 | | combined | 55 | 400 | 345 | 930 | 37.1 | 5<br>0 | 13<br>15 | $\mu_{11}$=0.162 | $K_{11}$=0.292 | $K_{g11}$=0.374 | $K_{l11}$=0.265 | |
| 75 | | ribose | 45 | 320 | 275 | 525 | 52.3 | 0 | 17 | $\mu_{11}$=0.102 | $K_{11}$=0.231 | $K_{r11}$=0.231 | | Sequential |
| 75 | lactose | lactose | 45 | 320 | 275 | 525 | 52.3 | 0 | 12 | $\mu_{11}$=0.225 | $K_{11}$=0.375 | | | Ribose is |
| 76 | | combined | 45 | 450 | 405 | 970 | 41.7 | 8<br>0 | 17<br>12 | $\mu_{11}$=0.203<br>$\mu_{12}$=0.457 | $K_{11}$=0.430<br>$K_{12}$=0.74 | $K_{r12}$=0.405 | $K_{l11}$=0.430 | Inhibited |
| 25 | | glucose | 160 | 380 | 220 | 610 | 36 | 0 | 3.5 | $\mu_{11}$=0.196 | $K_{11}$=0.526 | $K_{g11}$=0.532 | | |
| 25 | sucrose | sucrose | 160 | 365 | 205 | 580 | 35.4 | 0 | 4 | $\mu_{11}$=0.214 | $K_{11}$=0.582 | | $K_{s01}$=142 | Concurrent |
| 26 | | combined | 160 | 480 | 320 | 1100 | 29.1 | 0<br>0 | 3<br>6 | $\mu_{11}$=0.206 | $K_{11}$=0.600 | $K_{g11}$=0.473 | $K_{s11}$=0.453 | |
| 73 | | galactose | 55 | 205 | 150 | 340 | 44.2 | 0 | 16 | $\mu_{11}$=0.059 | $K_{11}$=0.202 | $K_{g11}$=0.253 | | Partial |
| 73 | sucrose | sucrose | 55 | 215 | 160 | 480 | 33.3 | 0 | 16 | $\mu_{11}$=0.103 | $K_{11}$=0.444 | | $K_{s11}$=0.444 | |
| 74 | | combined | 55 | 340 | 285 | 755 | 37.8 | 0<br>7 | 14<br>18 | $\mu_{11}$=0.14 | $K_{11}$=0.3 | $K_{g11}$=0.241 | $K_{s02}$=48.7 | Sequential |

Note: The footnotes of "g" "f" "r" "s" and "l" represent corresponding glucose, galactose, fructose, ribose, sucrose, and lactose.

at the new dilution rate. On each such figure, arrows indicate the times when cells were harvested for batch experiments to test for sequential substrate removal with glucose and glycerol as joint carbon sources. The batch experiments were run in a manner similar to those of the previous section (Phase A). The numbers shown above the arrows refer to the figure numbers in which the batch results are presented (controls and combined systems--high and/or low initial biological solids concentration) for experiments using cells taken from the continuous flow unit at that time.

1. Effect of Changing Dilution Rate from 1/4 to 1/12 $hr^{-1}$

The continuous flow unit was started using an initial seed obtained from the primary clarifier effluent of the municipal sewage treatment plant at Stillwater, Oklahoma. The unit was fed a synthetic medium containing glycerol at 2000 mg/l. The initial dilution rate was 1/4 $hr^{-1}$. After two days of continuous flow operation, the unit attained a steady state (see Figure 77) with a biological solids concentration of 920 mg/l and an effluent COD of 100 mg/l. In this first steady state, cellular carbohydrates amounted to 120 mg/l and cellular protein to 200 mg/l. Also at this time cells were harvested from the reactor effluent and used in batch experiments.

For the batch studies shown in Figures 78 and 79, a high initial concentration of cells was used under nitrogen-deficient conditions. It is evident from the control units that glycerol-acclimated cells could oxidatively assimilate glucose, but not as readily as they could utilize glycerol. A considerable amount of metabolic products remained in the glucose control after the glucose had been removed. Unfortunately, not enough samples were obtained during the substrate removal period to

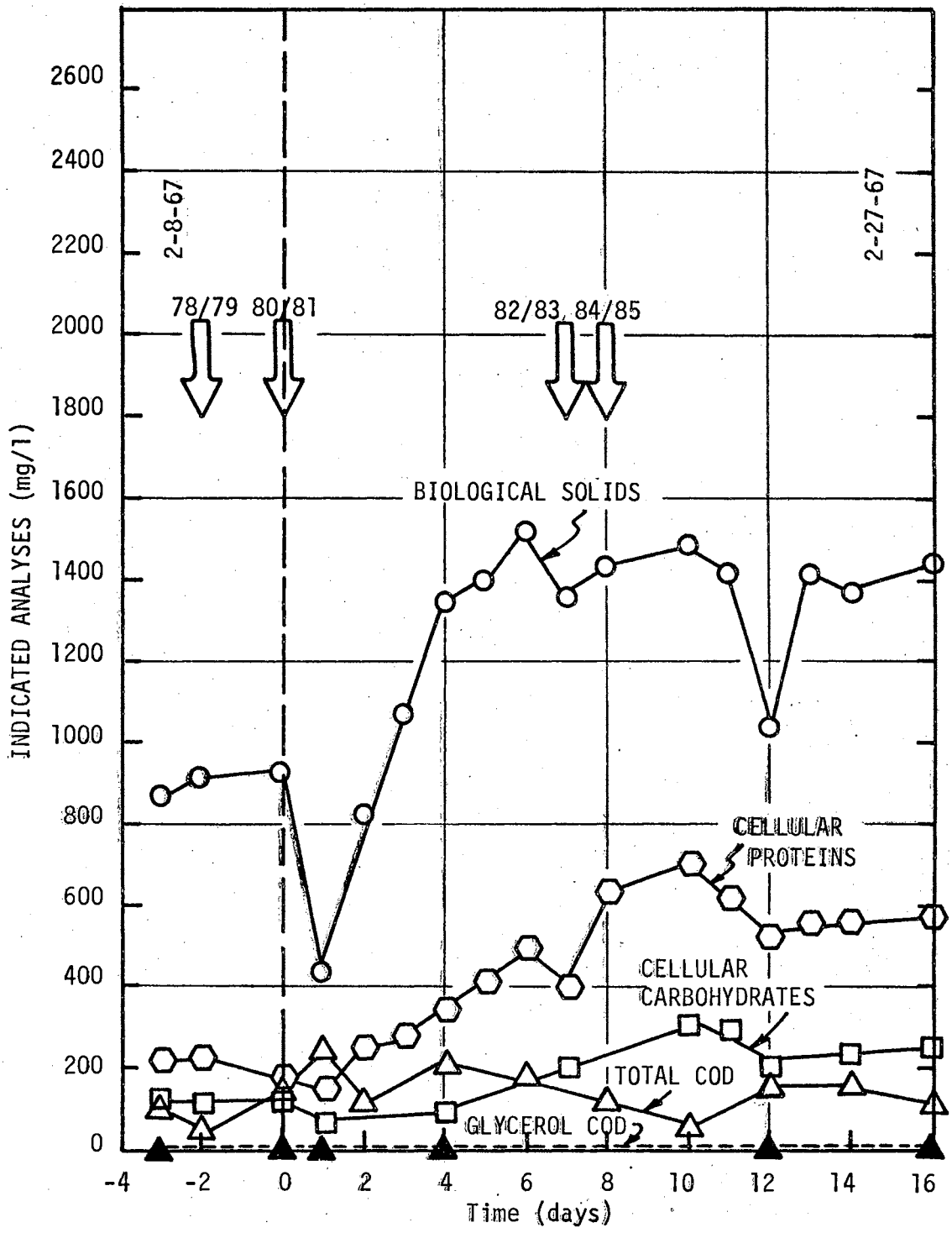



Figure 77 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/4 to 1/2 $hr^{-1}$ During Period of Operation From 2-8-67 to 2-27-67.

Figure 78 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-10-67).

Figure 79 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-10-67).

Figure 80 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-12-67).

Figure 81 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-12-67).

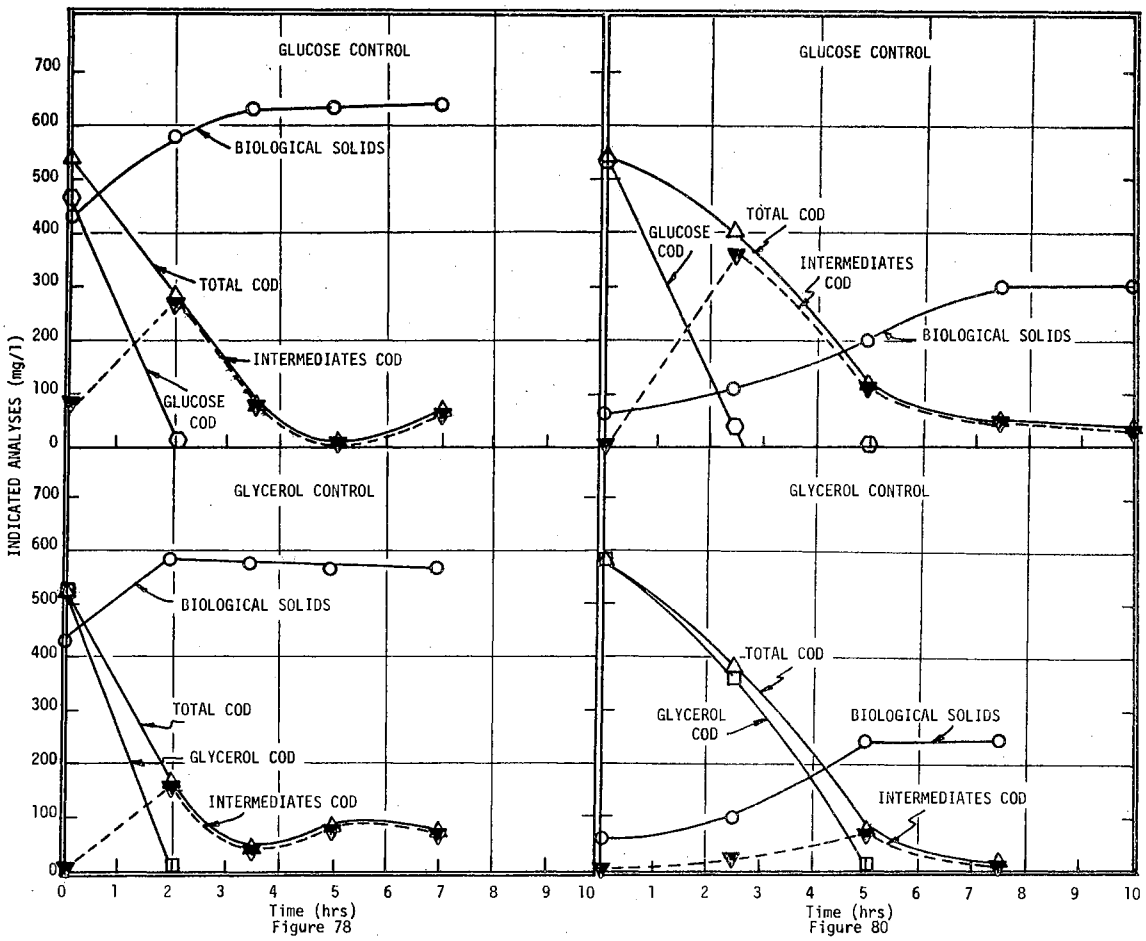



Figure 78




Figure 80

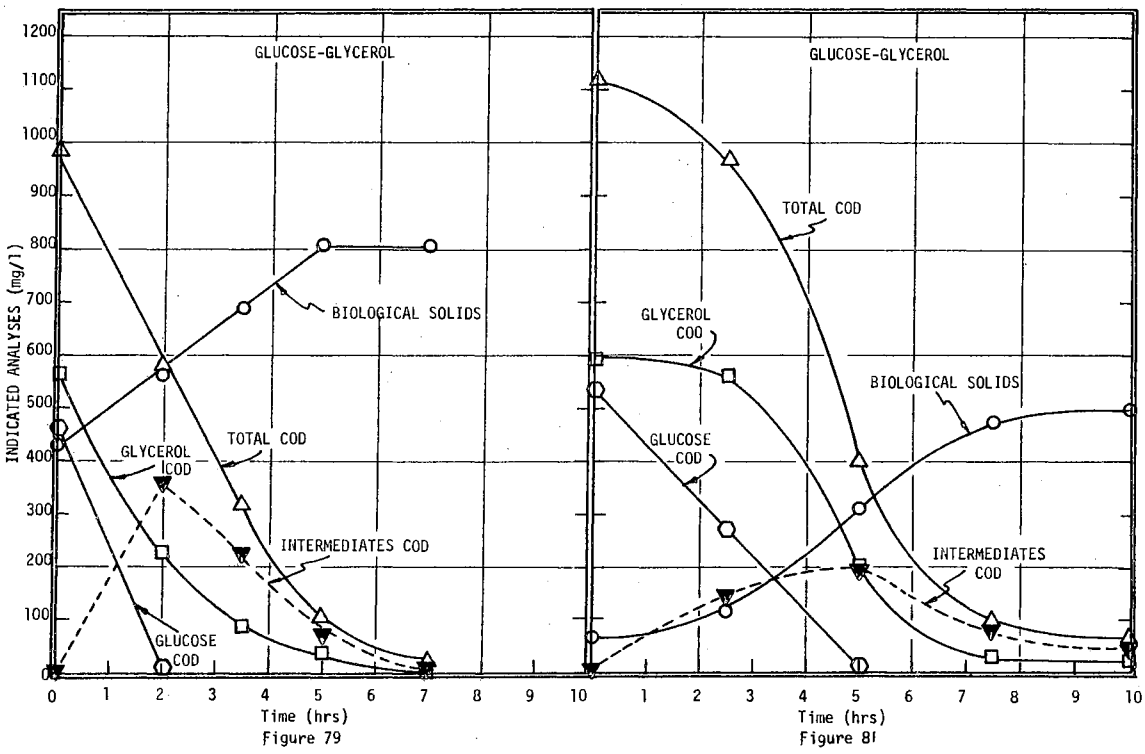



Figure 79




Figure 81

establish the kinetic mode of removal. On the basis of the data presented, the removal may be adjudged as following zero order kinetics.

In the combined system, overall COD removal does appear to follow zero order kinetics for the first 3.5 hours of the experiment. Also, biological solids accumulation appeared to follow zero order kinetics. It is difficult to determine whether removal of glycerol and glucose in the combined system was sequential or concurrent since enough samples were not obtained in the first two hours of the experiment. However, on the basis of the data presented, the removal of these carbon sources was probably concurrent during this experiment.

Forty-eight hours after initiating the batch experiment described above, cells were harvested from the continuous flow unit and batch studies were undertaken using a low initial cell inoculum under growth conditions. The most striking feature of the results for the control systems is the extremely high accumulation of metabolic products when the glycerol-acclimated cells were grown on glucose. It is also interesting to note that in the glucose control the biological solids attained a significantly higher level than in the glycerol system. In the combined system the blockage of glycerol metabolism by the presence of glucose is clearly seen by examination of the results of analyses made 2.5 hours after starting the experiment. By the time another sample was taken, the glucose had been totally removed. While it is evident that glucose did block glycerol removal, it can not be said on the basis of the data presented that the blockage existed during the total time glucose persisted in the system.

In Figure 77 it is seen that after changing the dilution rate from 1/4 to 1/12 $hr^{-1}$, there was a rather significant dilute-out of cells

followed by a rather rapid rise in cell concentration and apparent attainment of the new steady state at approximately 1400 mg/l solids. During the transition there was a slight increase in the effluent COD, but there was no leakage of glycerol in the effluent.

During operation at the new dilution rate, cells were harvested from the effluent of the continuous flow reactor and used in batch studies conducted at high biological solids concentration under nitrogen-deficient conditions. The results are shown in Figures 82 and 83. A considerable accumulation of metabolic products occurred in both control systems, and it would appear that both compounds were metabolized by the glycerol-acclimated sludge with approximately the same degree of proficiency. In the combined system there is a clear indication that glucose and glycerol were removed concurrently and that the metabolic products produced by each were removed at a rather slow linear rate after exhaustion of glycerol and glucose.

Cells were harvested on the next day of operation, and used for studies under growth conditions in systems employing a low initial inoculum of cells. The results for the control system (Figure 84) indicate that glucose was metabolized at a faster rate than was glycerol; however, the rate of total COD removal was higher in the glycerol system. In the combined system, glycerol utilization was inhibited but not totally blocked by the presence of glucose. There was evidence for the buildup and subsequent utilization of metabolic intermediates and/or endproducts.

2. Effect of Changing Dilution Rate from 1/12 to 1/24 $hr^{-1}$

After a period of operation at a dilution rate of 1/12 $hr^{-1}$, the flow was adjusted to yield a dilution rate (or growth rate) of 1/24 $hr^{-1}$.

Figure 82 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 2-19-67).

Figure 83 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 2-19-67).

Figure 84 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 2-20-67).

Figure 85 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 2-20-67).

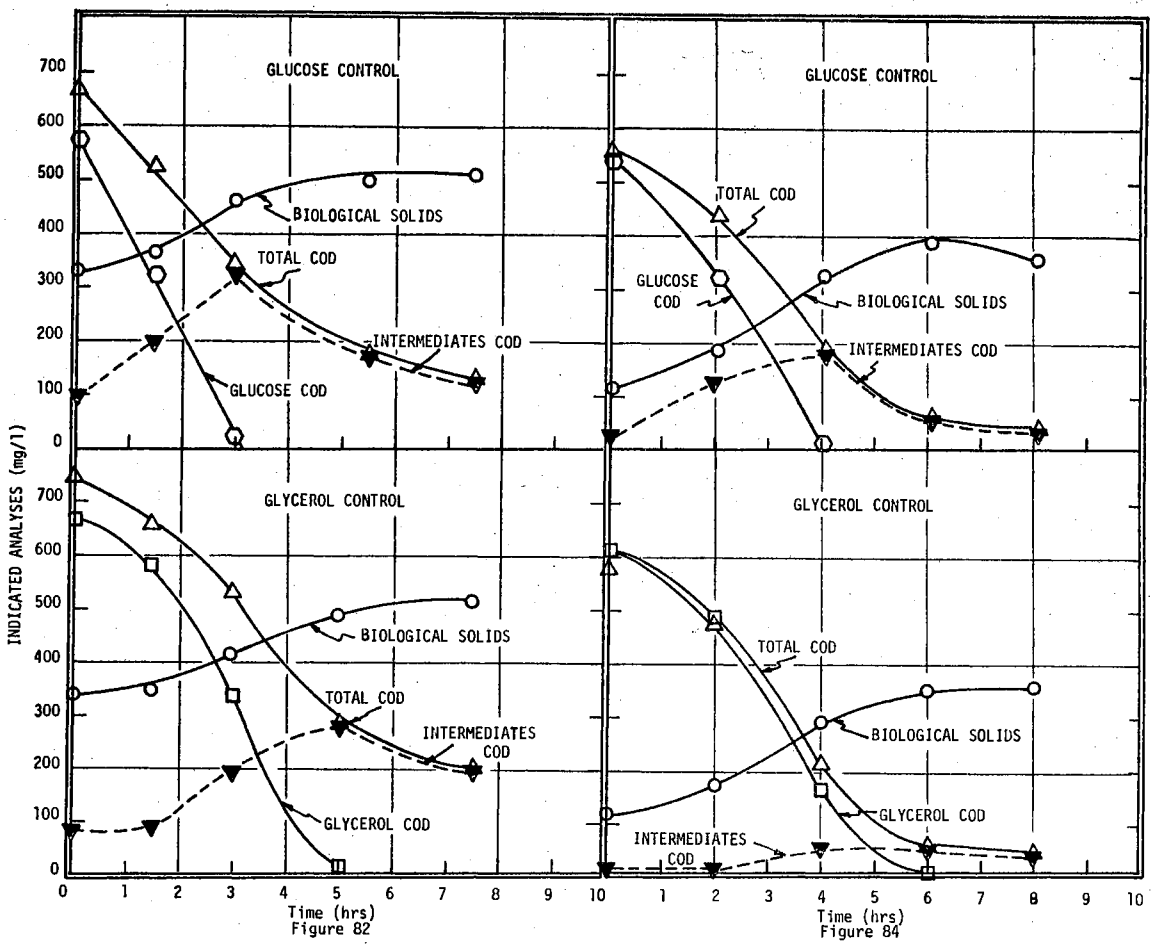



Figure 82




Figure 84

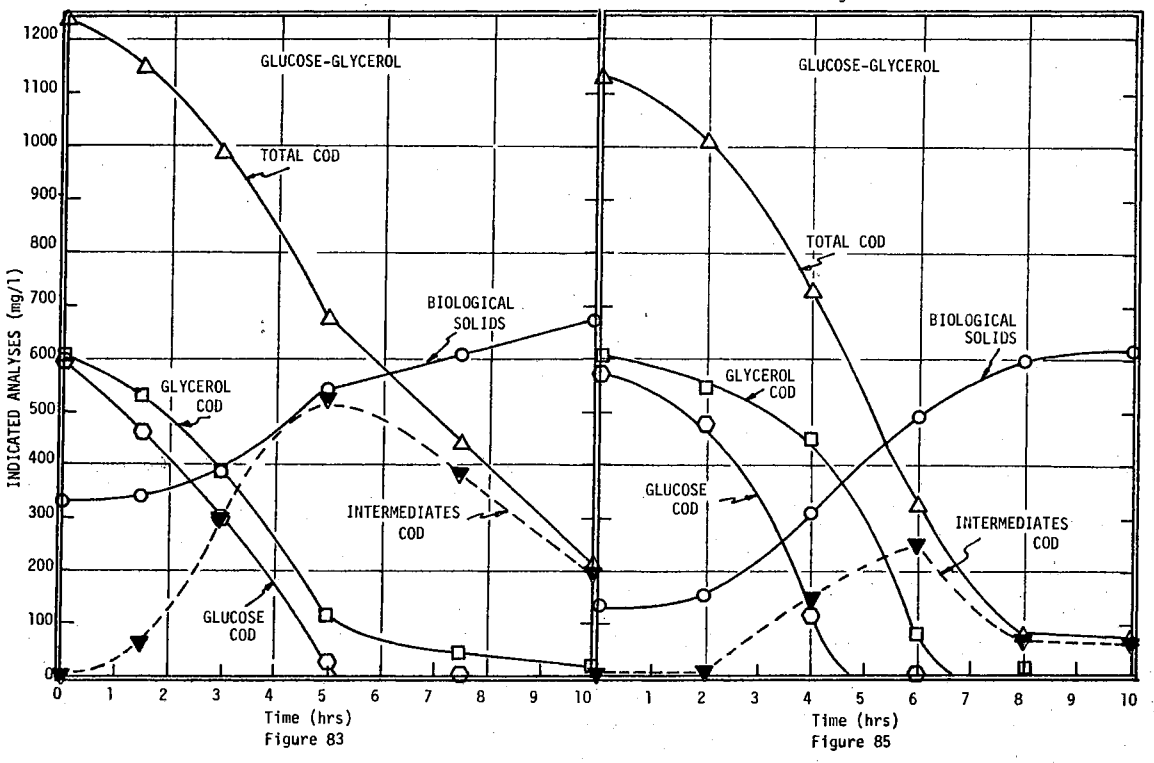



Figure 83




Figure 85

The behavior of the continuous flow unit during this period of operation is shown in Figure 86. Points plotted to the left of day 0 on the time scale (day 0 indicates the day on which the dilution rate was changed) are the same as the last three sample points shown in Figure 77. In order to facilitate clarity of presentation of results for the continuous flow reactor, some overlap in figures depicting its continuum of behavior throughout this phase of the studies is provided. Calendar dates are provided on the left and right borders of each figure. For example, Figure 77 terminates with 2-27-67, and Figure 86 begins with 2-25-67. It is seen that after slowing the growth rate to one-half the previous rate, there was a period of cell dilute-out followed by a recovery in cell concentration. During this time, glycerol did not appear in the effluent; however, there was a significant increase in total COD concentration during the transient period. After the recovery in cell concentration, the effluent COD returned to its former level. Under operation at the new dilution rate, the biological solids concentration attained higher levels than were observed before the hydraulic shock load was applied. There was a proportionately higher protein content of the cells as the solids level increased; however, cell carbohydrate remained at a fairly low level.

It is rather difficult to explain the high biological solids concentration attained after changing the dilution rate to 1/24 $hr^{-1}$. It is felt that the solids level attained does not represent an increase in cell yield, but that it is representative of operational problems sometimes encountered in dealing with heterogeneous populations. At times, the tendency of the cells to floc in heavy aggregates prevented complete mixing in the system, and the solids concentration in the

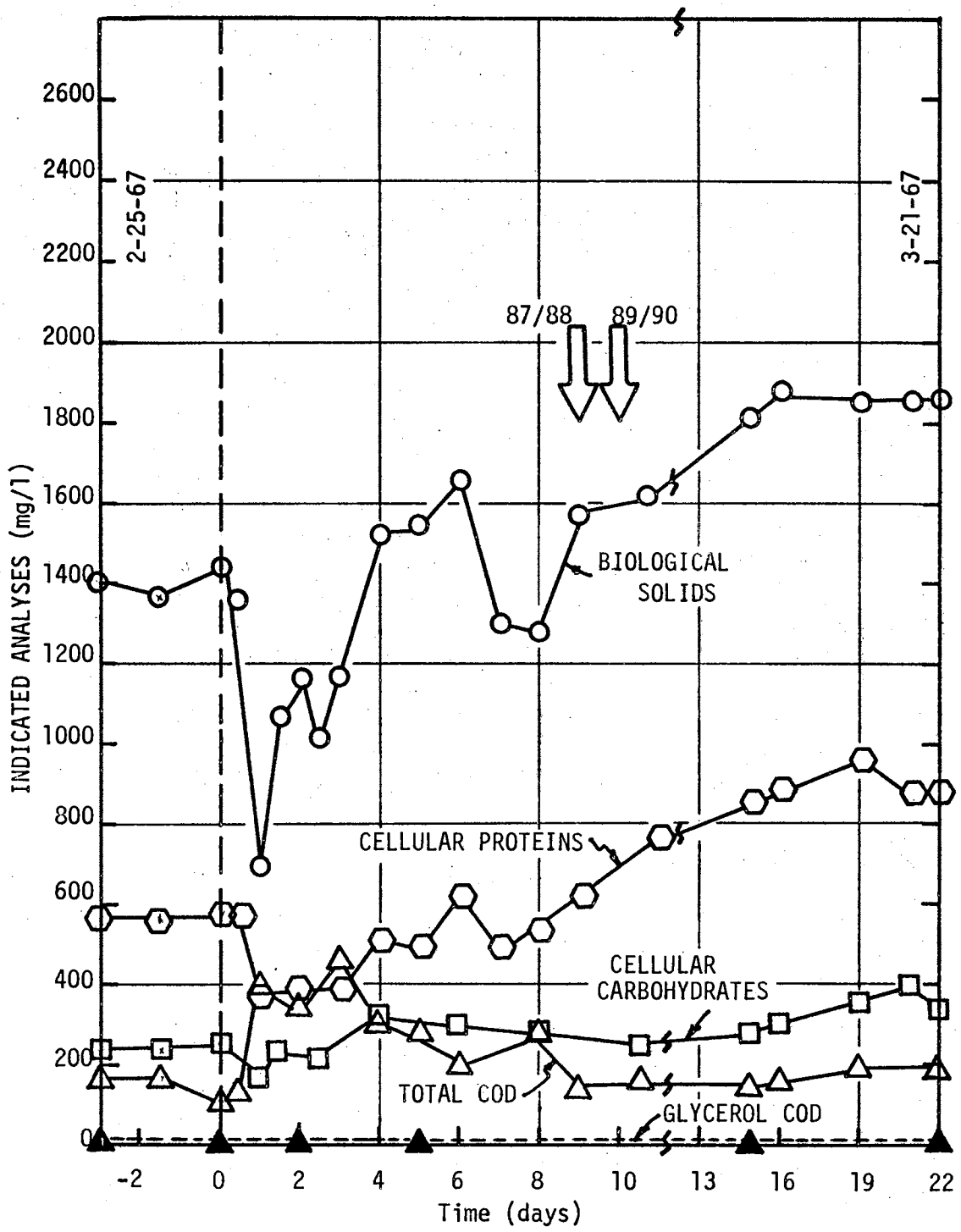



Figure 86 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/12 to 1/24 $hr^{-1}$ During Period of Operation From 2-25-67 to 3-21-67.

reactor was somewhat higher than the solids concentration in the reactor effluent.

Nine days after the change in dilution rate, cells were harvested from the reactor and used for batch experiments under nitrogen-deficient conditions. It is seen (Figure 87) that in the control systems glucose was removed more rapidly than was glycerol, and there was a considerable accumulation of metabolic intermediates and/or endproducts in the glucose system. In the combined system there is definite indication that glycerol metabolism was blocked by the presence of glucose. There was considerable production of metabolic intermediates and/or endproducts which, judging by the behavior of the control systems, may be assumed to have arisen from the metabolism of glucose.

On the following day (day 10) cells were harvested, and used at a low initial inoculum under proliferating or growth conditions; the results are shown in Figures 89 and 90. Control systems indicate that both compounds were used with approximately equal facility, although there was, as in the previous case, a considerable accumulation of metabolic products in the glucose control. In the combined system the total blockage of glycerol removal until glucose had been exhausted is clearly evident. It is also seen that after exhaustion of glucose, the removal of total COD proceeded in accordance with zero order kinetics.

3. Effect of Changing Dilution Rate from 1/24 to 1/4 $hr^{-1}$

Because of the buildup of high solids concentration at the dilution rate of 1/24 $hr^{-1}$, it was decided that a severe hudraulic shock load might tend to disperse the floc and enhance more complete mixing of the reactor contents. For this reason the dilution rate was next increased six times (from 1/24 to 1/4 $hr^{-1}$). The behavior of the system is shown

Figure 87 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 3-9-67).

Figure 88 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 3-9-67).

Figure 89 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 3-10-67).

Figure 90 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 3-10-67).

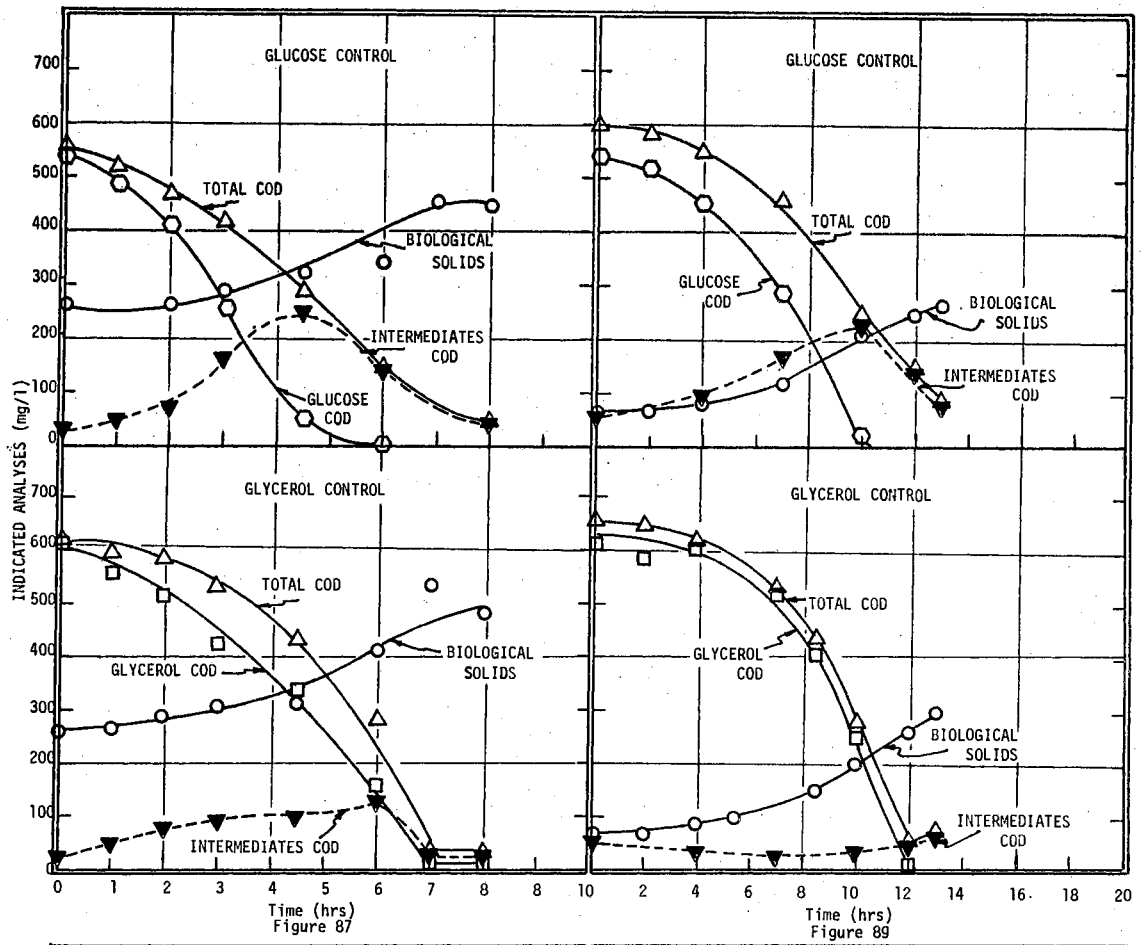



Figure 87




Figure 89

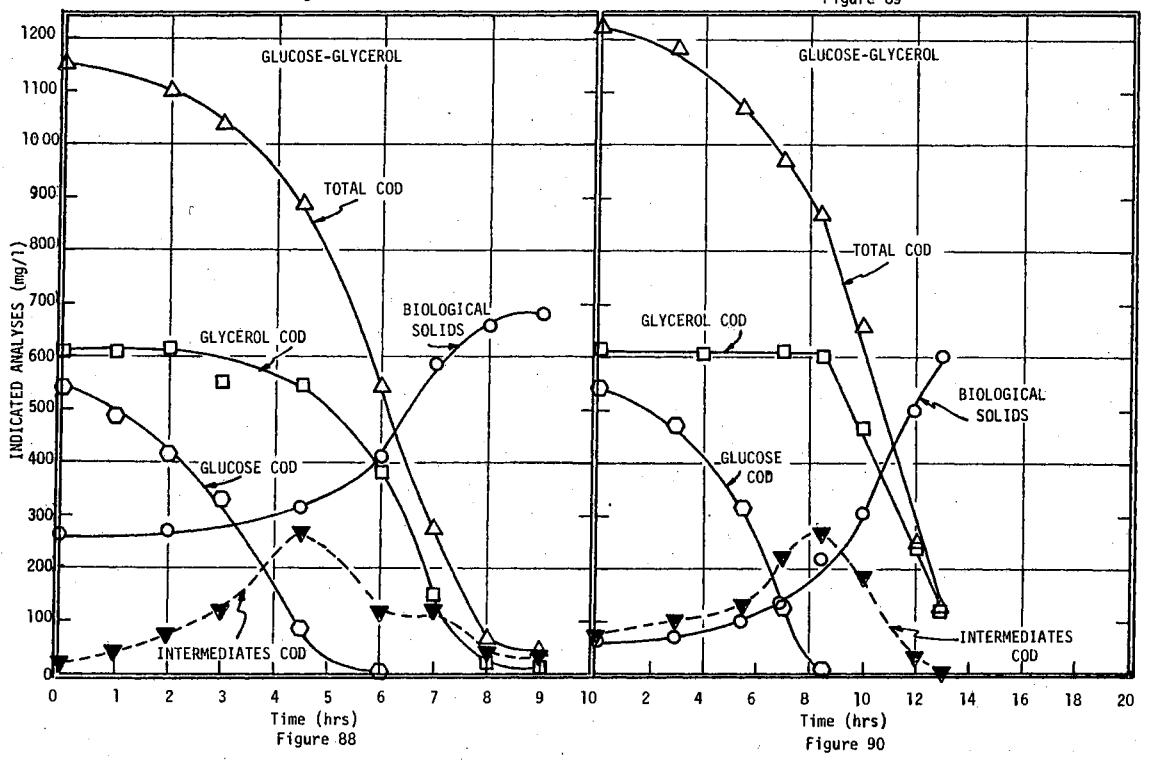



Figure 88




Figure 90

in Figure 91. The severe hydraulic shock loading was one which could not be assimilated by the system without a very severe disruption of substrate removal efficiency. Within eighteen hours after shifting the dilution rate (four and one-half detention times), the biological solids concentration dropped from 1850 to 550 mg/l, while the COD increased from 180 to 1780 mg/l. Glycerol leakage during this period amounted to 1500 mg/l. Slightly more than seven retention periods were required before the system attained a steady state. The shock did have the desired effect in providing more complete mixing, since the heavily matted filamentous organisms which had previously predominated disappeared from the system under this new mode of operation, and the cells in the reactor were observed to be quite dispersed. The unit was run for a total of twenty-four days at the 1/4 $hr^{-1}$ dilution rate (see Figure 92), and it is seen that the biological solids level remained at approximately 1400 mg/l for an extended period of time.

Shortly after the unit had attained its new steady state level (see arrow at day 2, Figure 91), cells were harvested for batch studies under nonproliferating conditions. The results are shown in Figures 93 and 94. The cells which predominated in the system at this time were very clearly different from those which had previously predominated in the unit; however, a common characteristic was the production of a considerable amount of metabolic intermediates by these glycerol-acclimated cells when grown on glucose. Also, in both control systems a very high residual COD was observed. Both glucose and glycerol were totally removed in the control systems, and the data indicate that they were eliminated in accordance with zero order kinetics. In the combined substrate system, glucose was removed much faster than was glycerol, and a

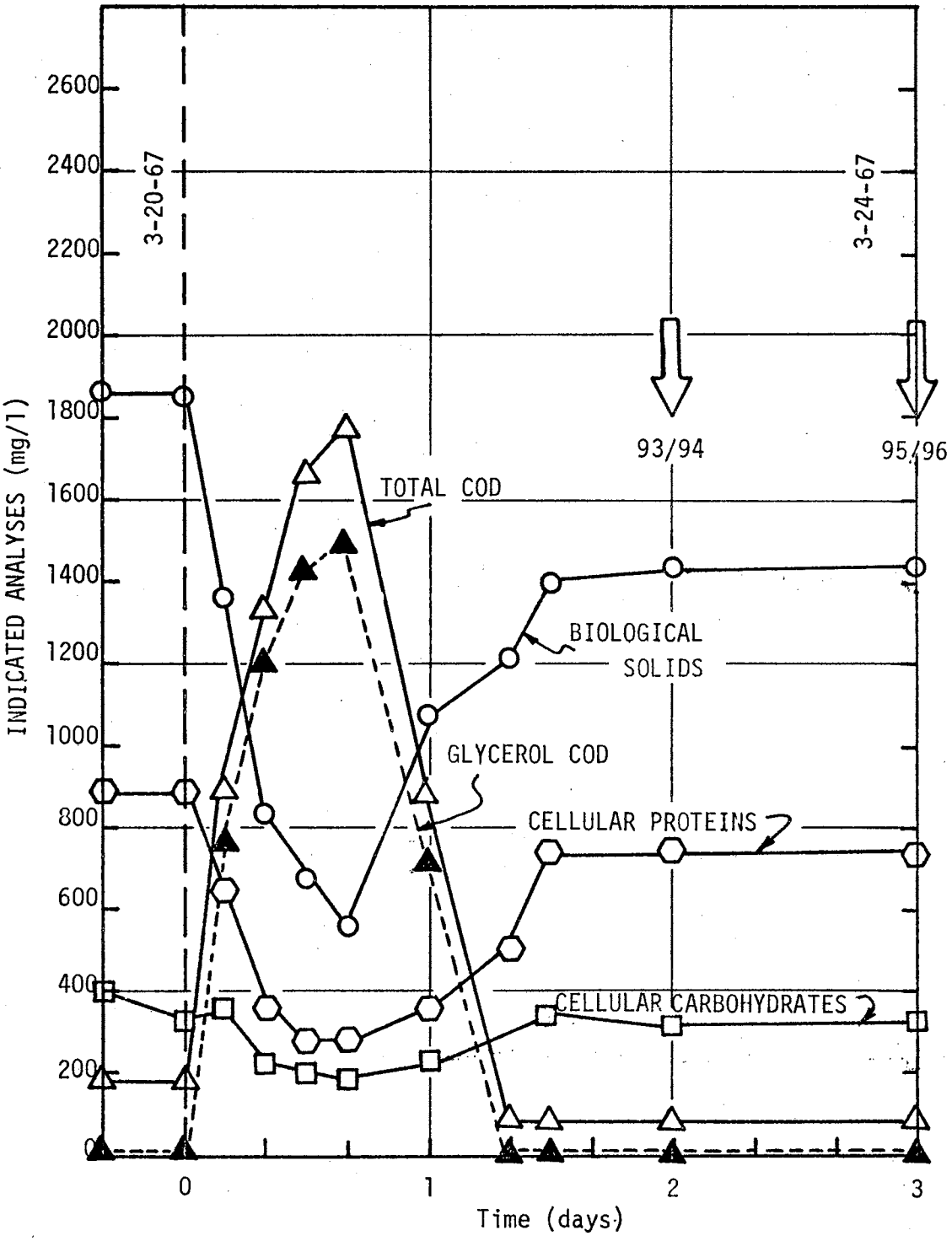



Figure 91 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/24 to 1/4 $hr^{-1}$ During Period of Operation From 3-20-67 to 3-24-67.

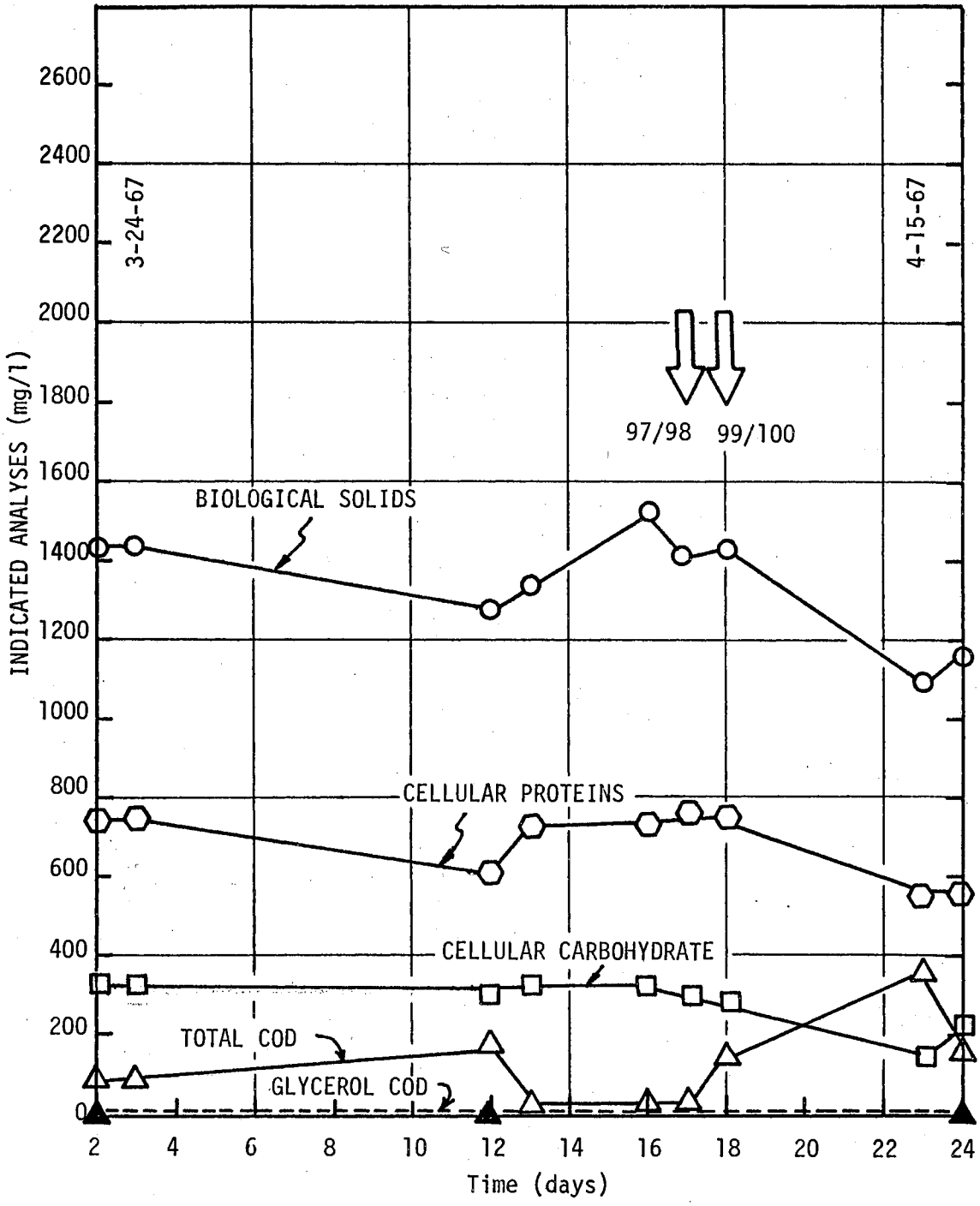



Figure 92 - Prolonged Performance of Continuous Flow Reactor Under Dilution Rate 1/4 $hr^{-1}$ During Period of Operation From 3-24-67 to 4-15-67.

Figure 93 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 3-23-67).

Figure 94 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 3-23-67).

Figure 95 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 3-24-67).

Figure 96 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 3-24-67).

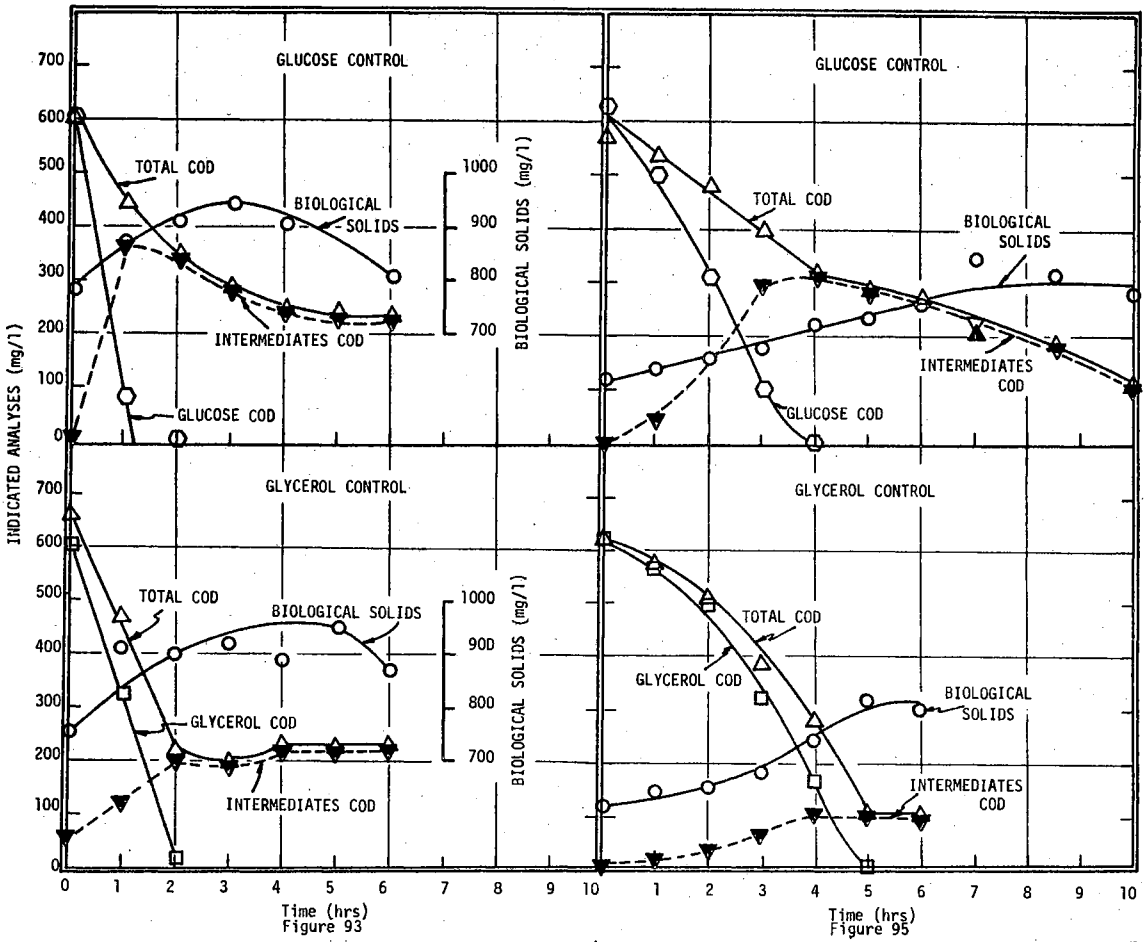



Figure 93

Figure 95

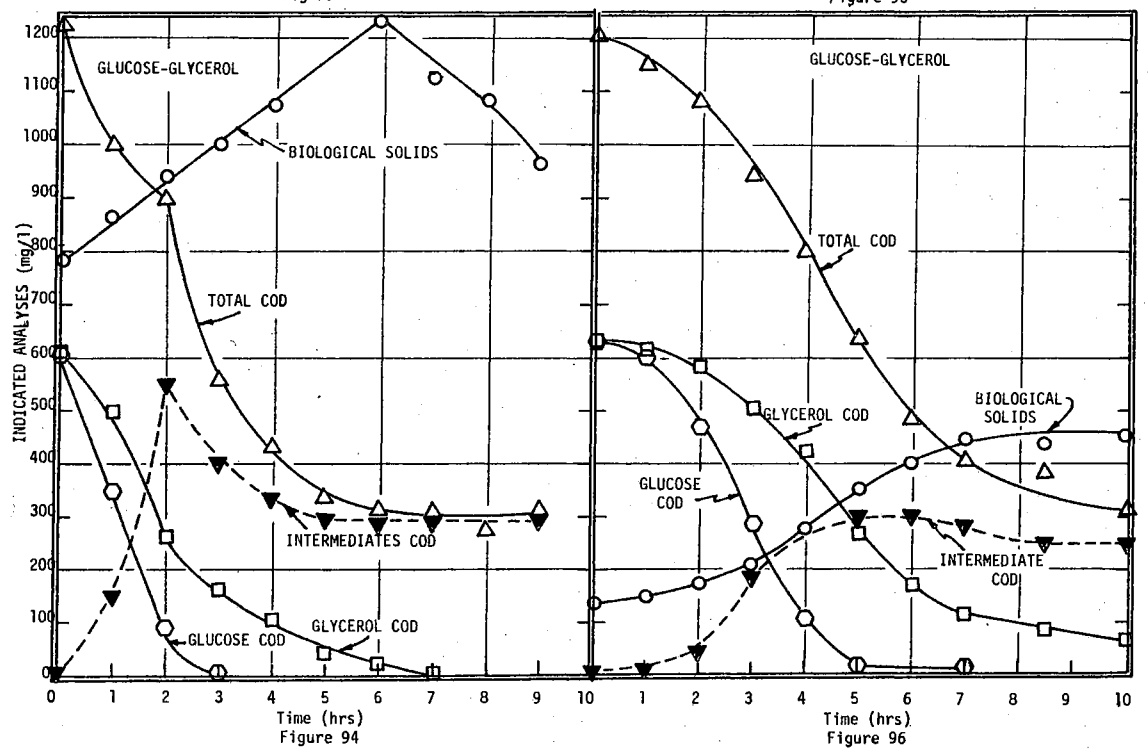



Figure 94

Figure 96

very high concentration (500 mg/l) of metabolic products accumulated in the medium; these were not completely degradable by the microbial population.

On the following day, cells were harvested from the continuous flow unit in order to perform batch experiments under growth conditions. It is seen in Figure 95 that for the glycerol control both growth and glycerol removal proceeded in accordance with kinetics which might be expected of an acclimated population; however, in the glucose control, linear growth was observed and a large amount of metabolic products was accumulated in the medium. After the elimination of glucose, these metabolic products were slowly degraded by the microbial population.

In the combined system (Figure 96), glucose and glycerol were removed concurrently. However, glycerol (periodate reactive material) persisted in the system until the end of the experiment.

After prolonged operation at this dilution rate, another series of batch experiments was conducted. The results using a high initial cell inoculum under nonproliferating conditions is shown in Figures 97 and 98. Essentially the same patterns of solids accumulation and substrate removal were observed as in the previous experimentation under nonproliferating conditions. However, it is seen that for the control systems shown in Figure 97, the residual COD was significantly lower than the residual COD observed for the previous systems (Figure 93). In the combined substrate system, glucose and glycerol were again removed concurrently and glycerol persisted in the system for a much longer time than did glucose.

Cells were also harvested for studies using a low initial inoculum under proliferating or growth conditions; these results are shown in

Figure 97 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 4-8-67).

Figure 98 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 4-8-67).

Figure 99 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 4-9-67).

Figure 100 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 4-9-67).

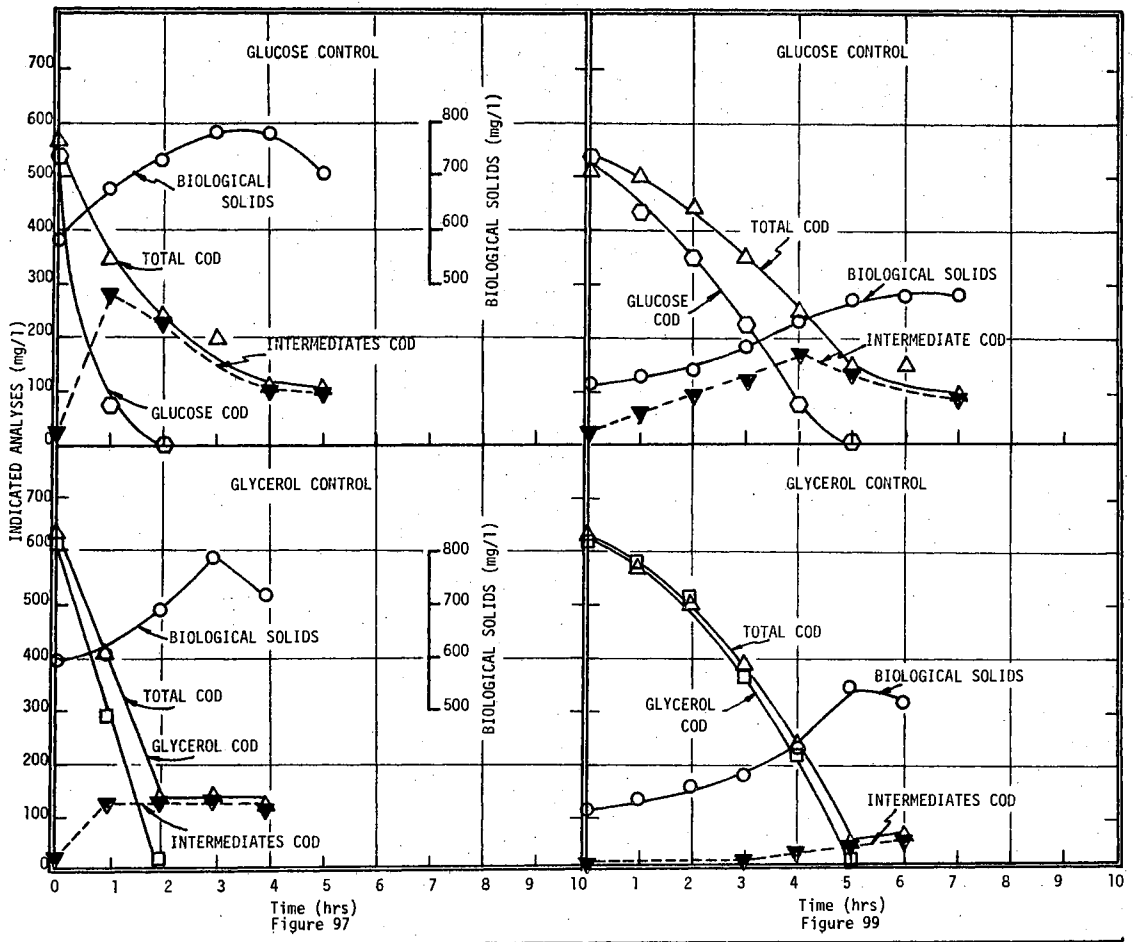



Figure 97




Figure 99

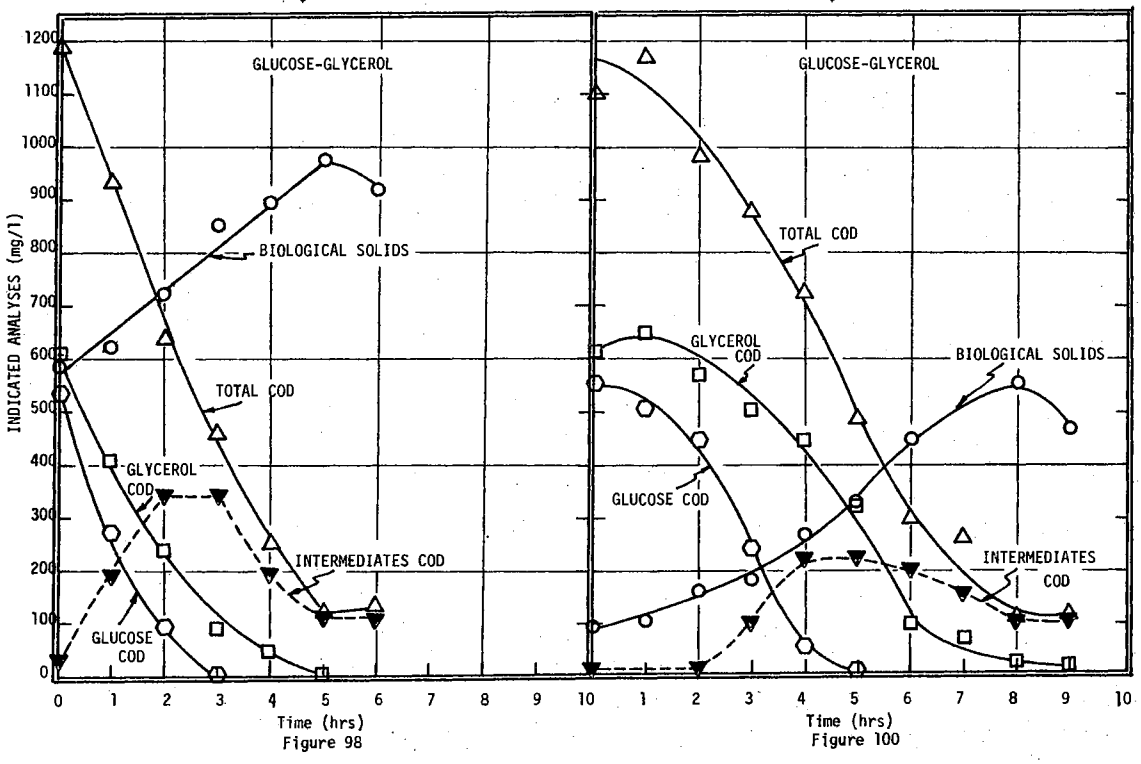



Figure 98




Figure 100

Figures 99 and 100. Typical growth and substrate removal curves were obtained with the glycerol control, and the results compare very well with those of the previous glycerol control (Figure 95). Comparison of the previous glucose control (Figure 95) with the control system shown in Figure 99 indicates that while glucose was eliminated at a slightly slower rate in Figure 99, production of intermediates was less and the removal of total COD was considerably improved. When the compounds were used as a combined carbon source, the removal must be adjudged to be concurrent; however, glucose did retard the use of glycerol. By the time over half of the glucose was eliminated from the system, only 100 mg/l glycerol had been eliminated. When all of the glucose had been metabolized, approximately fifty per cent of the glycerol remained.

4. Effect of Changing Dilution Rate from 1/4 to 1/12 $hr^{-1}$

In the next experiment the progression of the growth rate from fast to increasingly slower rates was resumed, and the continuous flow unit was subjected to a three-fold lowering of dilution rate (from 1/4 to 1/12 $hr^{-1}$). The results are shown in Figures 101 and 102. It is interesting to compare the response with that observed for a similar change in dilution rate, shown in Figure 77. In the present instance there was no rapid dilute-out of biological solids, nor was there a considerable leakage of COD in the reactor effluent.

Two days after applying the change in dilution rates, cells were harvested and used for batch studies under nonproliferating conditions; the results are shown in Figures 103 and 104. Both glucose and glycerol were metabolized with equal facility, and a considerable amount of intermediates and/or endproducts accumulated in both control systems. When used as joint carbon sources, glycerol and glucose were removed

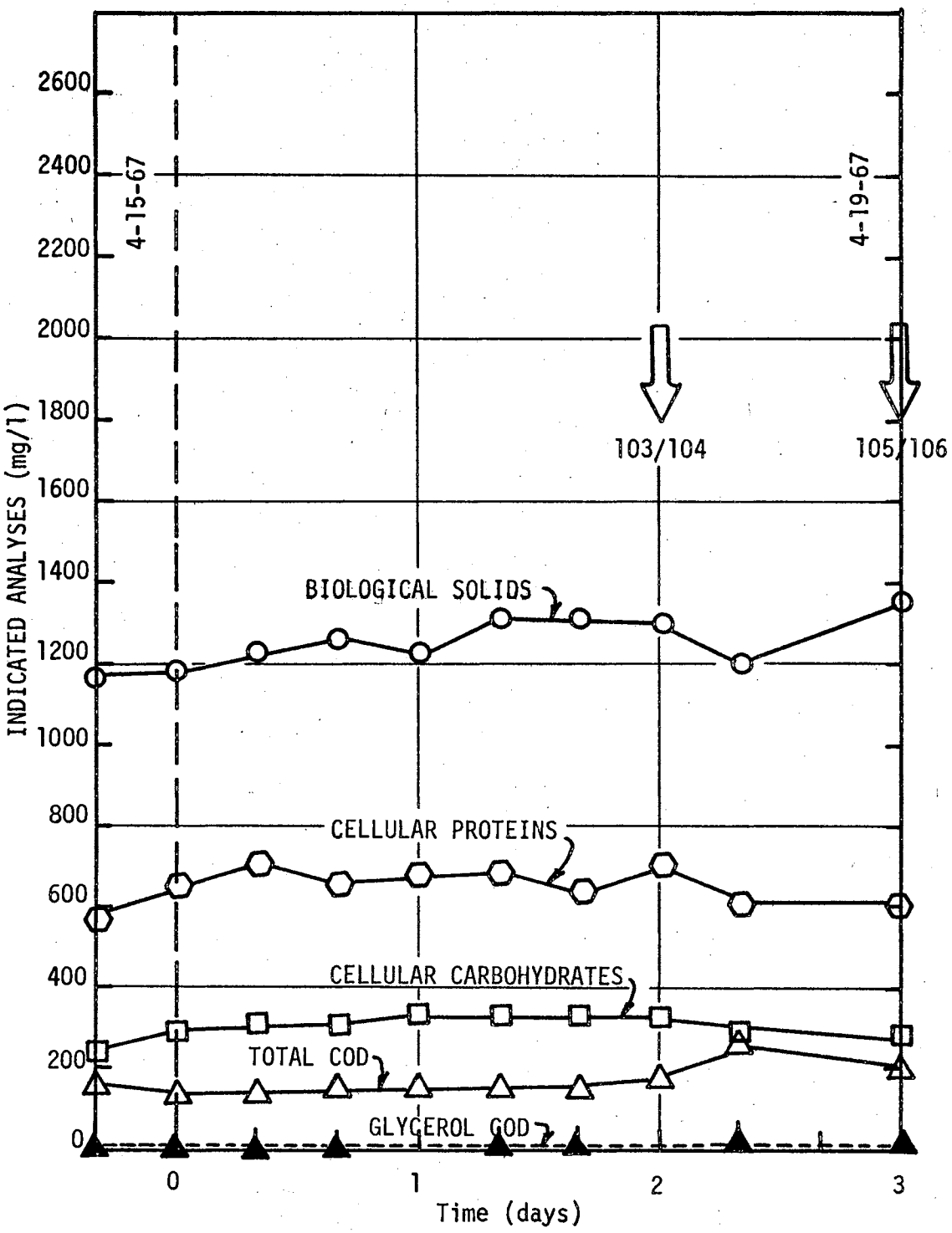



Figure 101 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/4 to 1/12 $hr^{-1}$ During Period of Operation From 4-15-67 to 4-19-67.

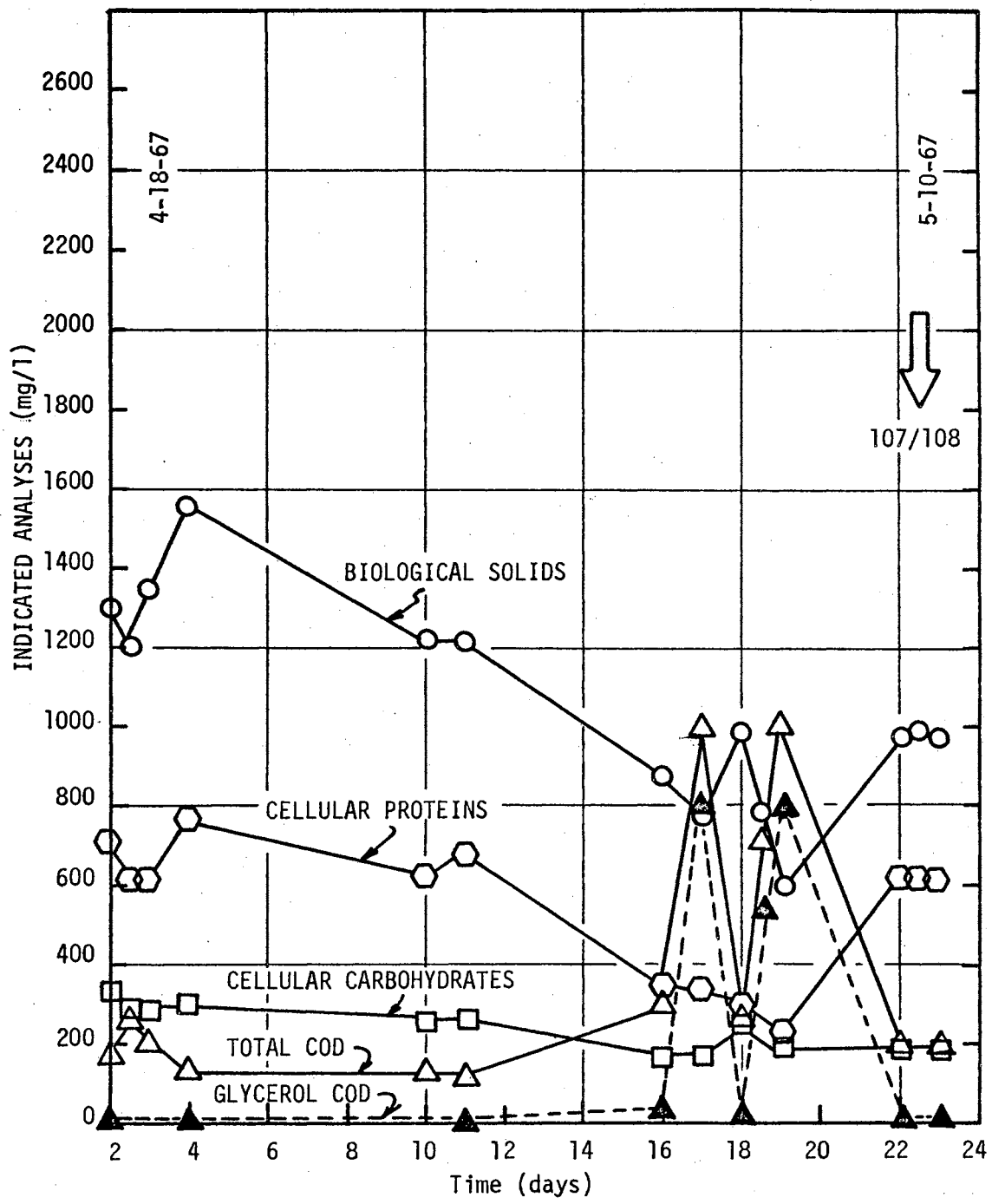



Figure 102 - Prolonged Performance of Continuous Flow Reactor Under Dilution Rate 1/12 $hr^{-1}$ During Period of Operation From 4-18-67 to 5-10-67.

Figure 103 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 4-18-67).

Figure 104 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 4-18-67).

Figure 105 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Diltuion Rate 1/12 hr-1 on 4-19-67).

Figure 106 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 4-19-67).

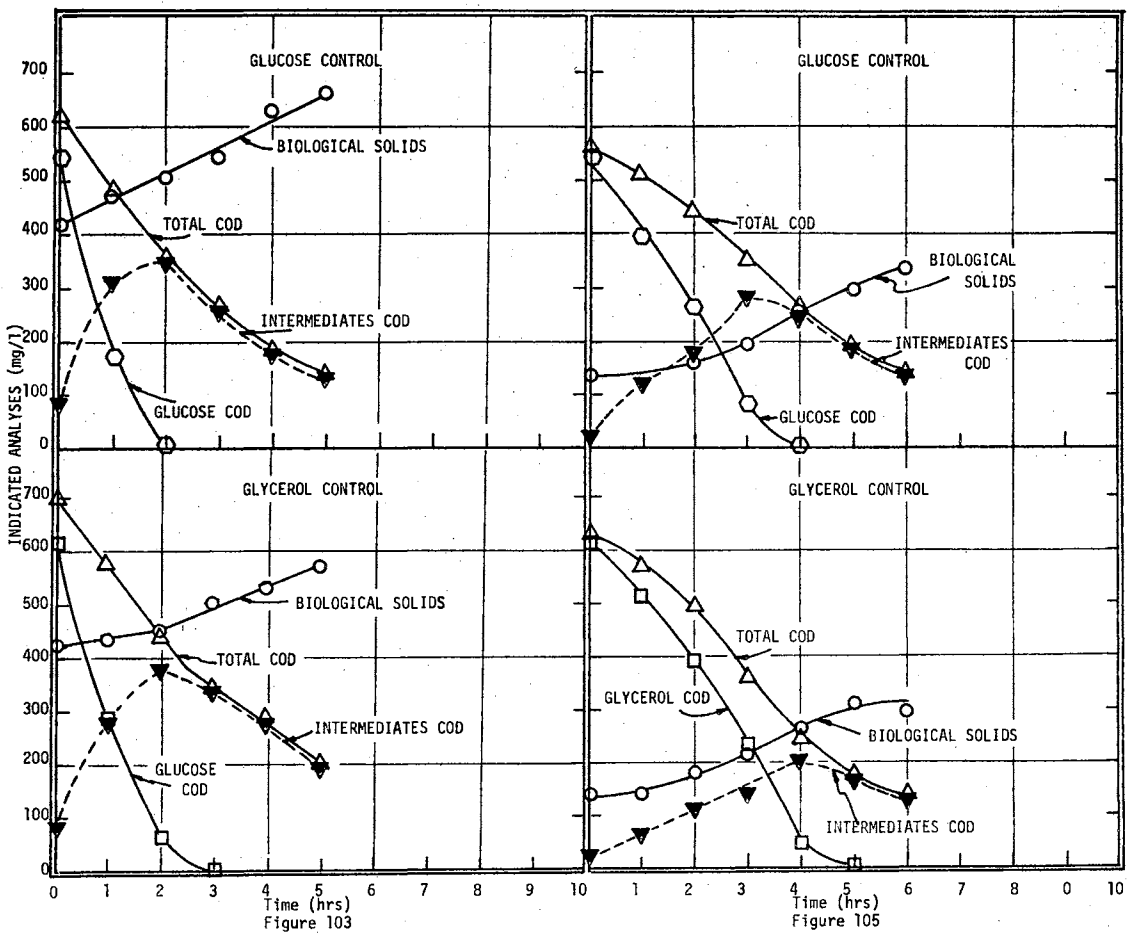



Figure 103




Figure 105

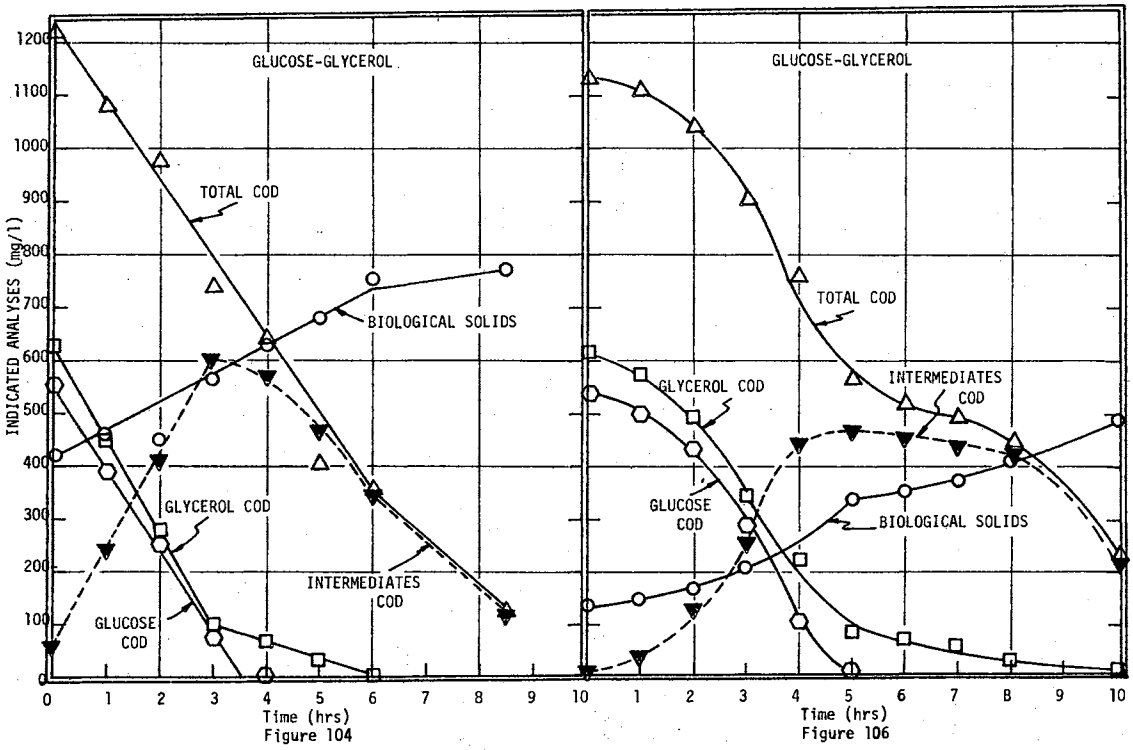



Figure 104




Figure 106

concurrently, and approximately fifty per cent of the total carbon source was converted to metabolic products which were subsequently metabolized by the nonproliferating population.

On the following day, cells were harvested from the continuous flow unit, and used for batch studies under growth conditions. The results are shown in Figures 105 and 106. Growth on glycerol was slightly more rapid than on glucose, and in both systems a considerable amount of metabolic products was released into the medium during degradation of the initial carbon sources. When used in combination, the carbon sources were removed concurrently; however, the large release of metabolic products and their subsequent utilization by the organisms imparted a distinct diphasic character to the total COD removal curve. It seems apparent that an acclimation period was required before these products could be metabolized. Also, the presence of intermediates and/or end-products would appear to be the cause for the persistence of glycerol in the medium beyond the fifth hour of the experiment.

The continuous flow unit was maintained at a dilution rate of 1/12 $hr^{-1}$ for the next three weeks, and it is seen in Figure 102 that during this time there was a gradual loss of solids, and from the 16th to the 22nd day of operation at this dilution rate there was a severe and unexplainable disruption of system efficiency. After the 22nd day of operation, it appeared that the unit had regained a condition of steady behavior, and cells were harvested at this time for another batch experiment under growth conditions. The results, as shown in Figures 107 and 108, indicate that while the cells could metabolize glucose, the removal of glycerol proceeded more rapidly. It can also be seen that growth on glucose caused a considerable accumulation of metabolic products in the

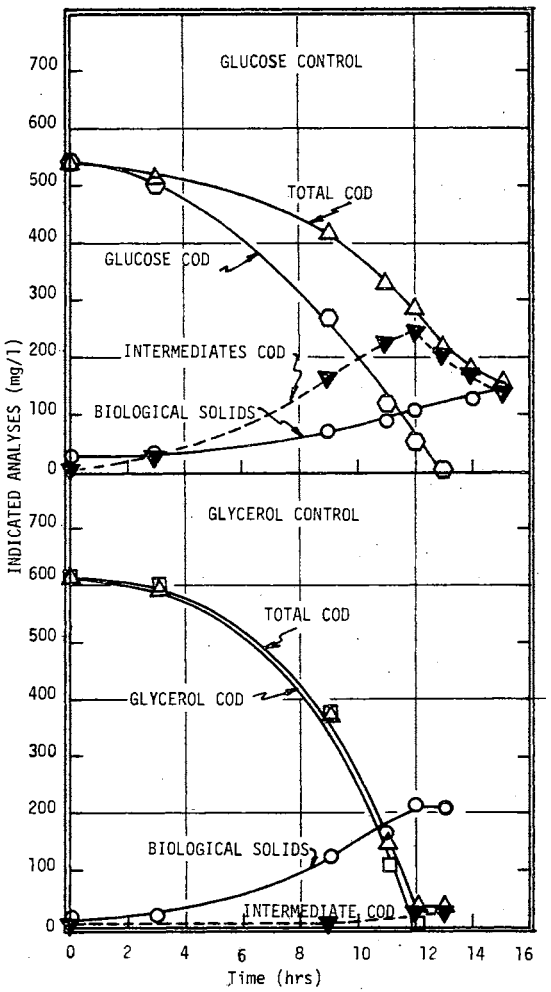



Figure 107 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 5-10-67).

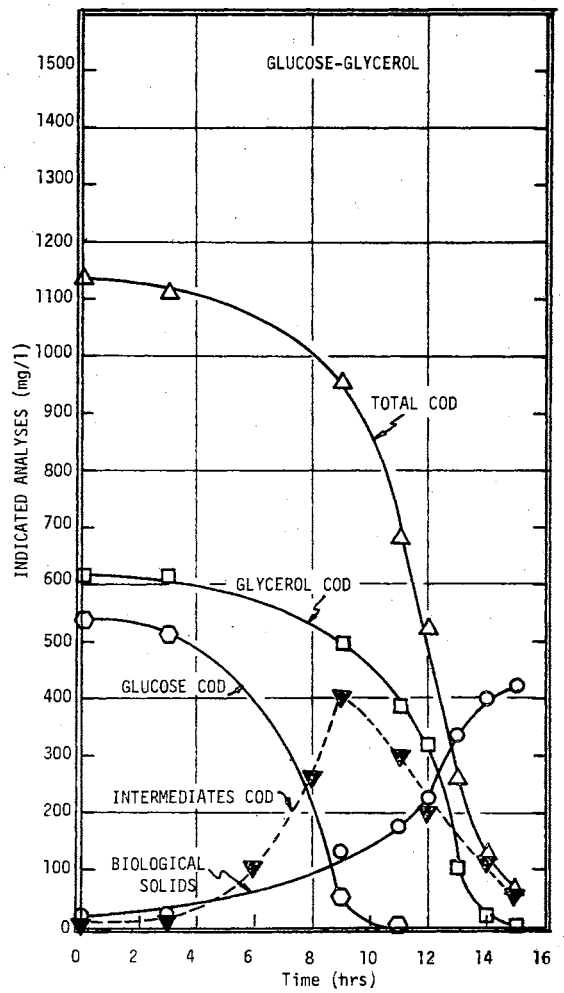



Figure 108 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 5-10-67).

medium. However, when glucose and glycerol were used as combined substrates, the presence of glucose clearly retarded the use of glycerol. Only 100 mg/l glycerol had been eliminated from the system by the time the glucose concentration reached a very low level. The extremely low initial biological solids concentration tends to ensure that the results represent a manifestation of the prevention of the synthesis of enzymes necessary for glycerol metabolism by the presence of glucose or of catabolites produced from the degradation of glucose.

5. Effect of Changing Dilution Rate from 1/12 to 1/24 $hr^{-1}$

The response of the continuous flow unit to a change in dilution rate from 1/12 to 1/24 $hr^{-1}$ is shown in Figure 109. Halving the growth rate caused biological solids to be diluted out of the system and precipitated a rise in the effluent COD from 200 mg/l to 600 mg/l. Glycerol was not detected in the reactor effluent. The drop in biological solids concentration was accompanied by a decrease in cell protein; however, the carbohydrate concentration in the cell mass remained essentially constant. The system recovered fairly rapidly, and cells were harvested for batch experiments.

The first batch experiments using cells harvested from the chemostat at this dilution rate were conducted under nitrogen-deficient conditions. The results are shown in Figures 110 and 111. It is seen from the controls that glucose was removed at a much faster rate than was glycerol; however, the removal of glucose was accompanied by the production of an extremely large amount of metabolic intermediates which were removed rather slowly. When used as combined substrates, glucose effected severe retardation of glycerol removal. Glycerol removal was initiated only after glucose removal was nearly complete. It is

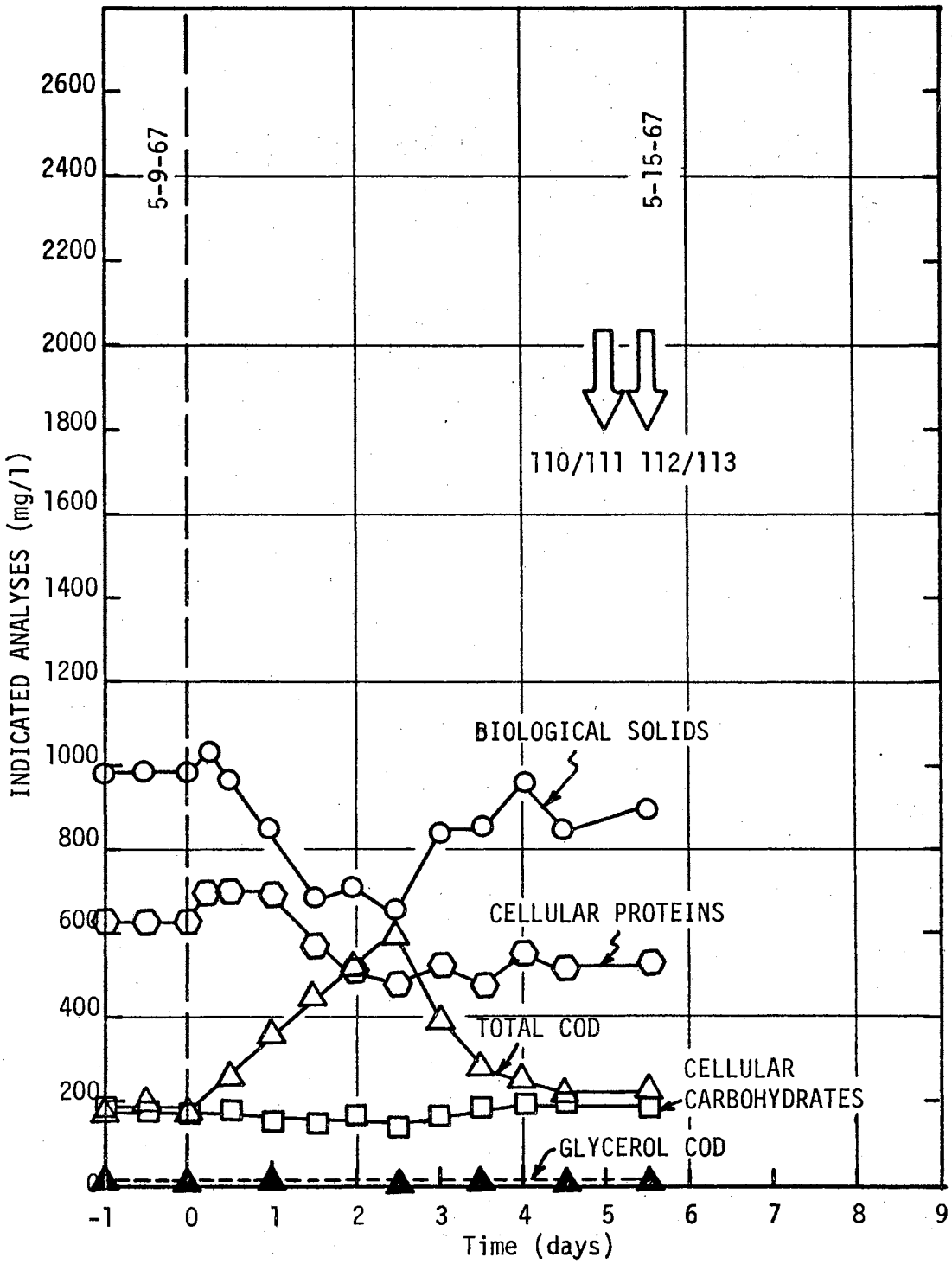



Figure 109 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/12 to 1/24 $hr^{-1}$ During Period of Operation From 5-9-67 to 5-15-67.

Figure 110 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 5-15-67).

Figure 111 - Metabolic Response Under Nitrogen-deficient Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 5-15-67).

Figure 112 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 5-15-67).

Figure 113 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 5-15-67).

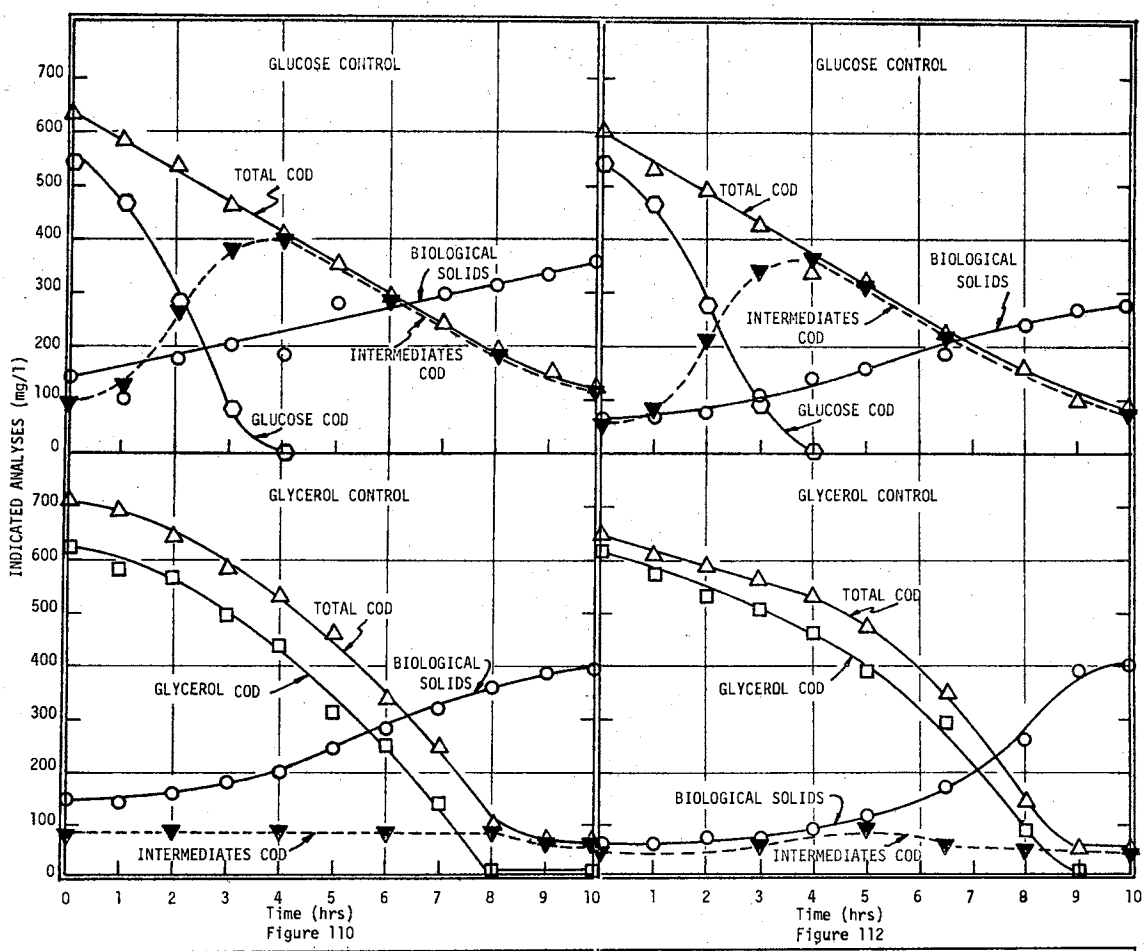



Figure 110




Figure 112

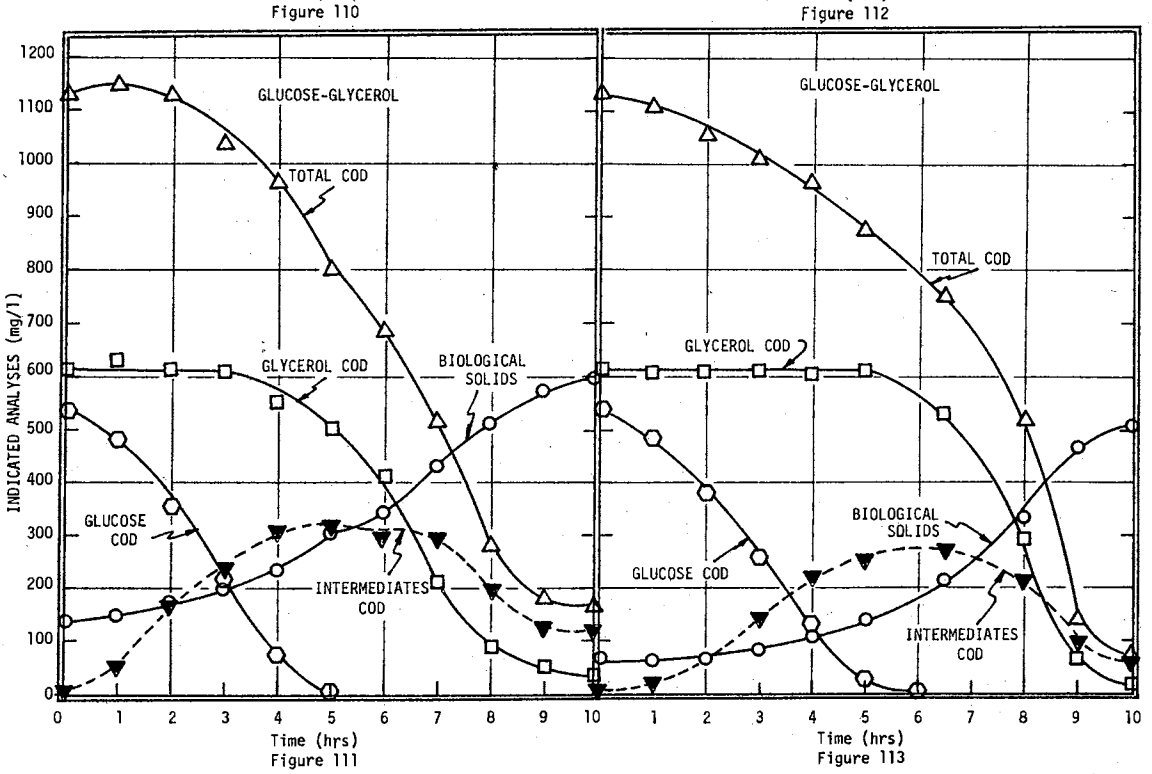



Figure 111




Figure 113

interesting to note that the intermediates which were produced (essentially all from the metabolism of glucose) remained at a fairly constant level until approximately one-half of the glycerol had been removed from the system. It would thus appear that this initially dual carbon source system produced a multi-carbon source system consisting of a least three substrates (glycerol plus glucose plus metabolic product), and that they were removed in an essentially sequential manner.

Cells were then harvested for studies under growth conditions, and the results are shown in Figures 112 and 113. A large amount of intermediates was produced in the glucose control, which behaved very similarly to the glucose control for the previous batch experiment under nitrogen-deficient conditions. When used as combined substrates under growth conditions, glucose again prevented glycerol removal until nearly all of the glucose was removed from the system. As in the previous experiment, the metabolic products accumulating in the medium due to glucose metabolism did not retard the removal of glycerol.

During operation of the chemostat at the dilution rate of 1/24 $hr^{-1}$ it was considered useful to run a separate set of experiments in which cells from the continuous flow reactor were used to seed a batch reactor operated on a 24-hour feeding cycle. Each twenty-four hours the batch reactor was fed 2000 mg/l glycerol, i.e., the same medium used in the continuous flow unit was used in the batch unit. Each day before feeding, one-third of the mixed liquor was wasted, and the remainder was settled for thirty minutes. Half of the remaining liquor (one-third of the total batch reaction liquor) was then discarded and the unit brought back to the initial volume with the feeding medium. The batch unit was operated in this manner for two weeks. This mode of operation

for batch units is consistent with standard procedures established in the bioengineering laboratories of Oklahoma State University. It had been found previously in other work in the bioengineering laboratories that batch cells grown up on this feeding cycle tended to yield concurrent removal, whereas cells freshly grown in the same medium invariably yielded sequential removal of glucose and sugar alcohol. After operating this separate batch unit for two weeks, cells were harvested for batch studies under nonproliferating and under growing conditions. The results under nonproliferating conditions are shown in Figures 114 and 115. It is seen that the cells could assimilate both carbon sources fairly rapidly and that, when the substrates were combined, removal was effected in a concurrent manner, although the removal of glycerol was retarded in the presence of glucose and the removal of total COD was diphasic.

When a slightly lower initial concentration of these batch-grown cells was employed under growth conditions (Figures 116 and 117), both substrates were again metabolized with approximately equal facility although, quite unexplainably, growth and substrate removal proceeded in accordance with apparent zero order kinetics. When used as combined carbon sources, the substrates were again utilized concurrently, with glucose again causing some retardation of glycerol removal.

During the 2-week period of batch operation using cells initially harvested from the continuous flow unit, the microbial population in the batch unit underwent a very noticeable change in predominance, whereas it did not in the continuous flow unit. Therefore, concurrent removal observed using a 24-hour detention time under batch operation as compared to sequential removal using a 24-hour detention time under

Figure 114 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Previously Grown at Dilution Rate 1/24 $hr^{-1}$ and Then Grown in Batch Unit for Two Weeks With Twenty-four Hour Feeding Cycle).

Figure 115 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Previously Grown at Dilution Rate 1/24 $hr^{-1}$ and Then Grown in Batch Unit for Two Weeks With Twenty-four Hour Feeding Cycle).

Figure 116 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cell Previously Grown at Dilution Rate 1/24 $hr^{-1}$ and Then Grown in Batch Unit for Two Weeks With Twenty-four Hour Feeding Cycle).

Figure 117 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Previously Grown at Dilution Rate 1/24 $hr^{-1}$ and Then Grown in Batch Unit for Two Weeks With Twenty-four Hour Feeding Cycle).

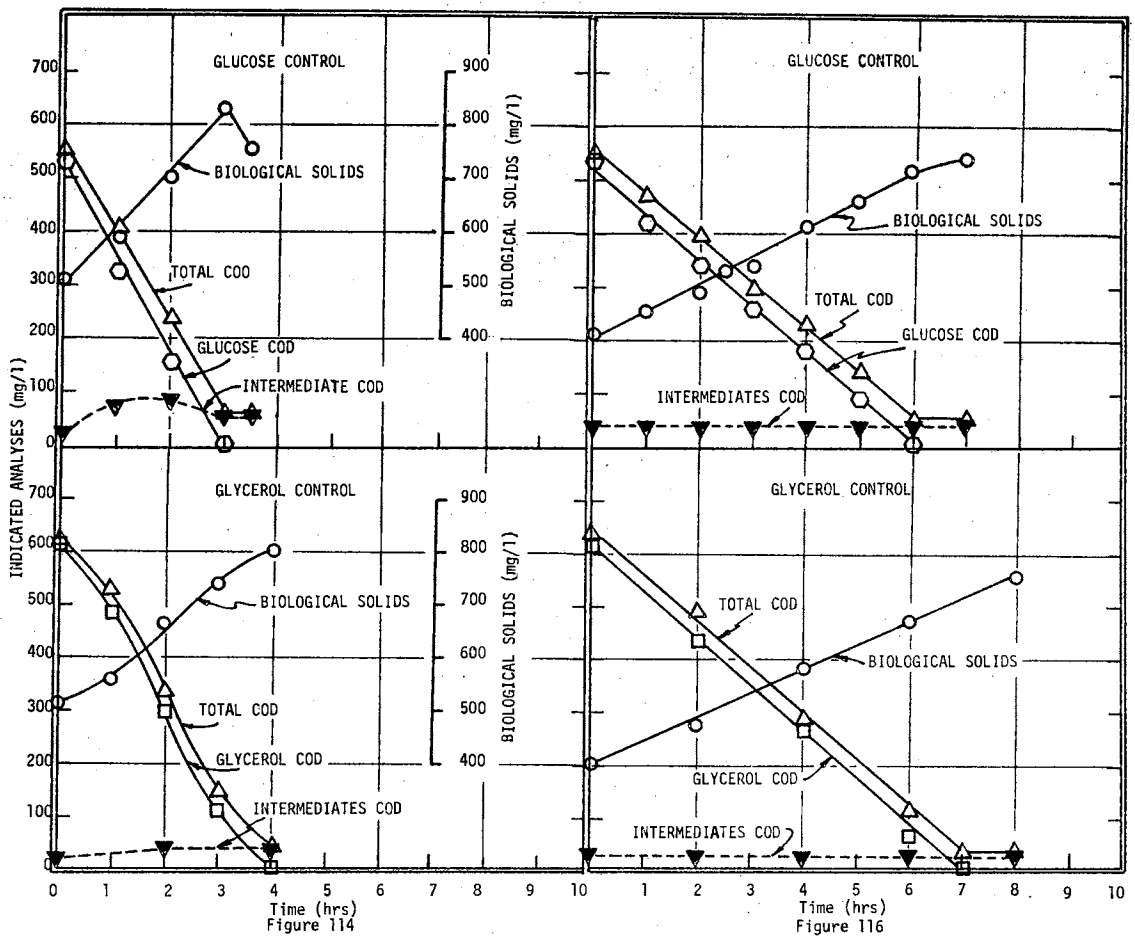



Figure 114




Figure 116

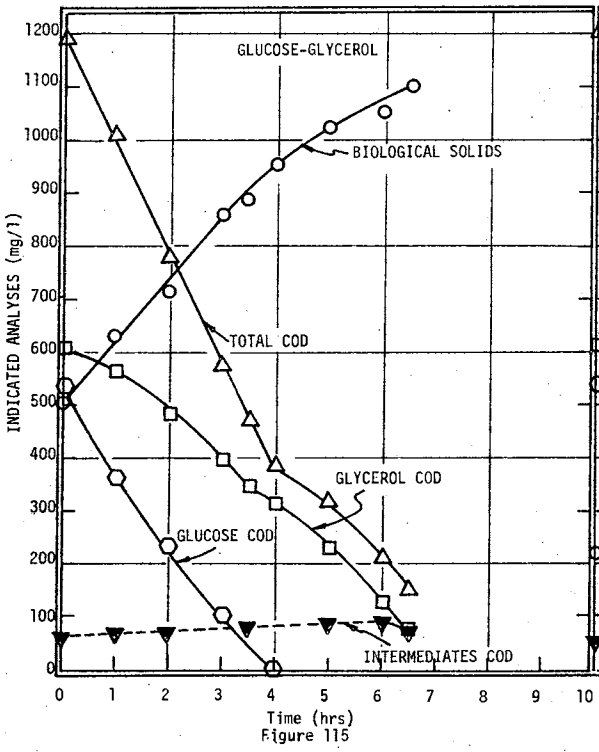



Figure 115

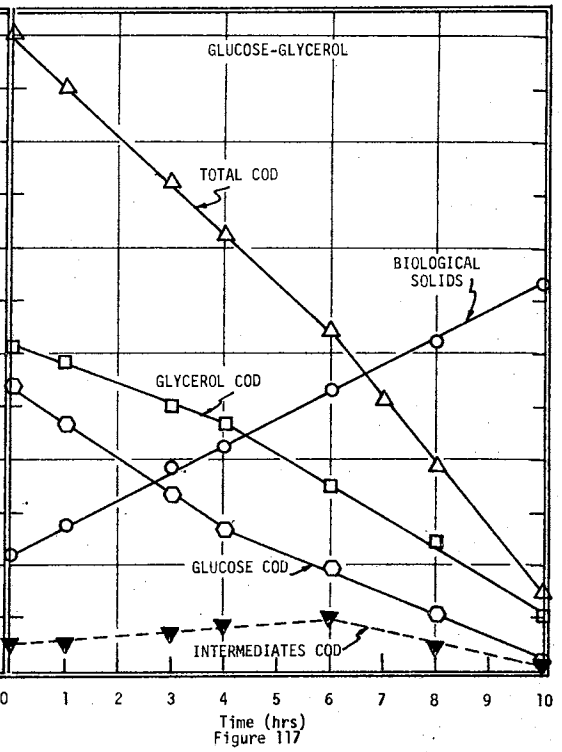



Figure 117

continuous flow operation may, in this particular case, be due in a large measure to the difference in the predominating species present. Ideally it would have been extremely valuable to grow up a fresh (young) population of the predominantly yellow pigmented organisms which existed in the batch unit and determine if these "young" cells exhibited either sequential or concurrent removal. Such an experiment may have shed some light on the perplexing problems concerning the effect of cell age on the observance of sequential removal. Unfortunately, this experiment could not be immediately scheduled.

6. Effect of Changing Dilution Rate from 1/24 to 1/36 $hr^{-1}$

The last data points shown for continuous operation of the chemostat at a dilution rate of 1/24 $hr^{-1}$ were for May 15, 1967 (Figure 109). The unit was operated at this dilution rate for approximately two more weeks; however, it was noted that during the last two weeks in May filamentous organisms began to predominate, and the condition of complete mixing could not be attained. Biological solids were being retained in the reactor, and the solids concentration began to rise.

In an effort to help alleviate this situation, the airflow rate was increased to 8000 ml/min. This helped to relieve the solids retention problem somewhat but, as is seen in Figure 118, the biological solids level was still abnormally high on June 1, 1967. Rather than increase the dilution rate in an attempt to "flush out" the retained biological solids, it was decided to proceed in accordance with the original experimental plan and the dilution rate was changed from 1/24 to 1/36 $hr^{-1}$. After nine days of such operation, the biological solids level dropped sharply, and most of the filamentous biomass disappeared from the unit. The biological solids concentration again experienced a significant

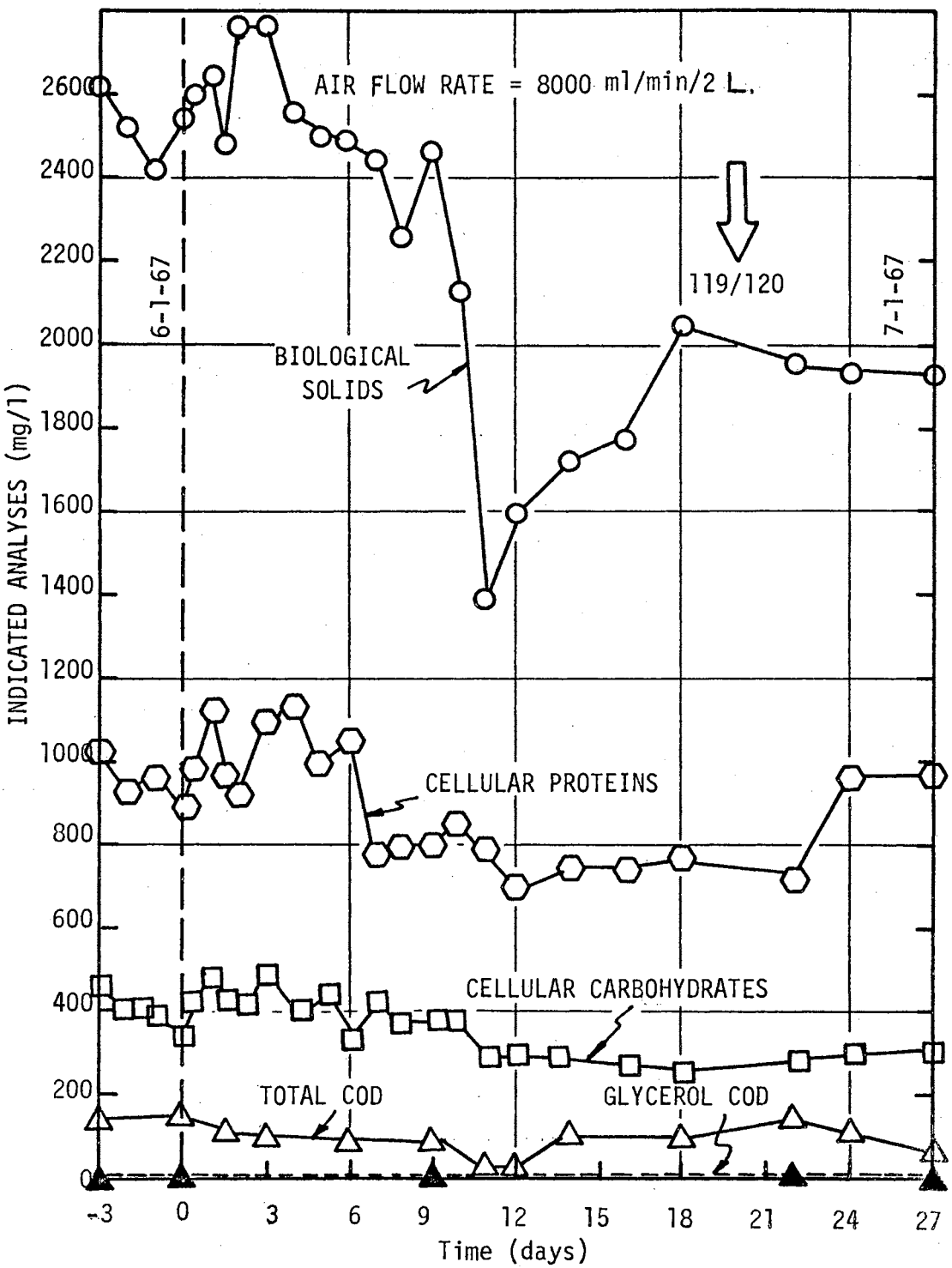



Figure 118 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/24 to 1/36 $hr^{-1}$ During Period of Operation From 6-1-67 to 7-1-67.

increase, but did not attain the levels previously observed.

During the latter part of June the unit could not be considered to be completely mixed (as evidenced by higher biological solids concentration in the reactor than in the reactor effluent); however, a decision was made to harvest cells to test for sequential substrate removal. At this relatively low dilution rate it was difficult to collect enough cells to perform experiments under nonproliferating conditions at high initial biological solids levels; therefore, experimentation was conducted under proliferating conditions only.

It is seen (Figure 119) that the cells exhibited approximately equal facility for metabolism of glucose, and for metabolism of glycerol. Glucose was removed somewhat more rapidly, but its metabolism caused the accumulation of a greater amount of metabolic intermediates and/or end-products than did the metabolism of glycerol. When used as combined carbon sources (Figure 120), glucose did not totally block glycerol metabolism; however, the utilization of glycerol was rather significantly retarded during the period when glucose was in the medium. During this time, glycerol was removed slowly and at a rate consistent with zero order kinetics. After removal of glucose, glycerol metabolism proceeded quite rapidly, but a significant glycerol concentration remained at the termination of the experiment (twelve hours).

7. Effect of Changing Dilution Rate from 1/36 to 1/48 $hr^{-1}$

On July 1, 1967, the dilution rate was changed from 1/36 to 1/48 $hr^{-1}$; the transient response and subsequent behavior at the new dilution rate are shown in Figure 121. Changing the dilution rate caused a drop in biological solids concentration, but did not cause an increase in effluent COD. Actually, at this time the effluent COD decreased

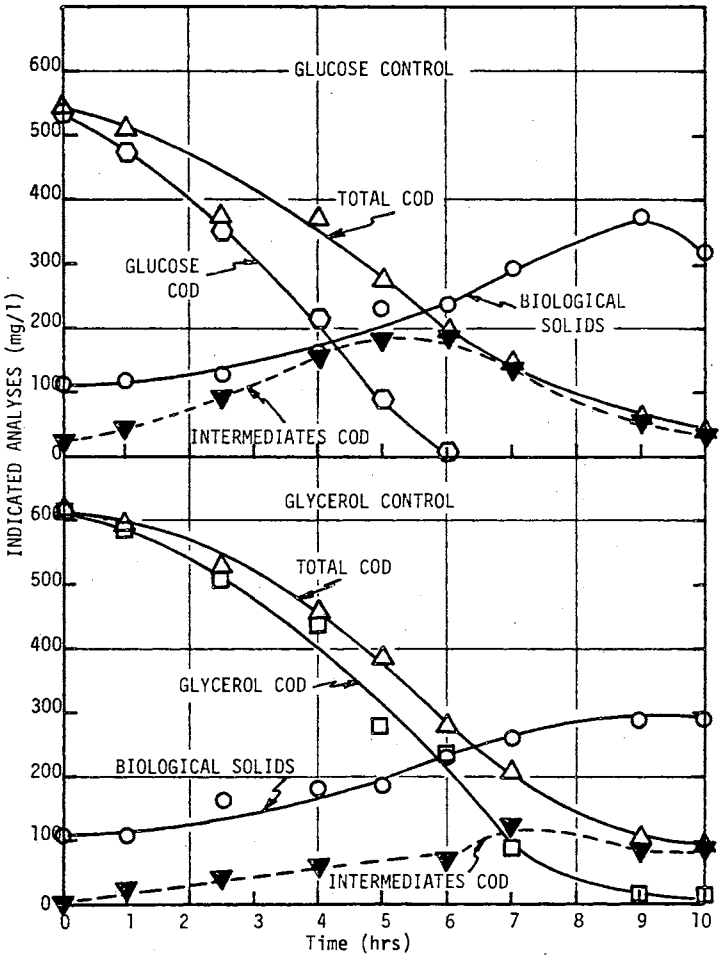



Figure 119 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/36 $hr^{-1}$ on 7-24-67).

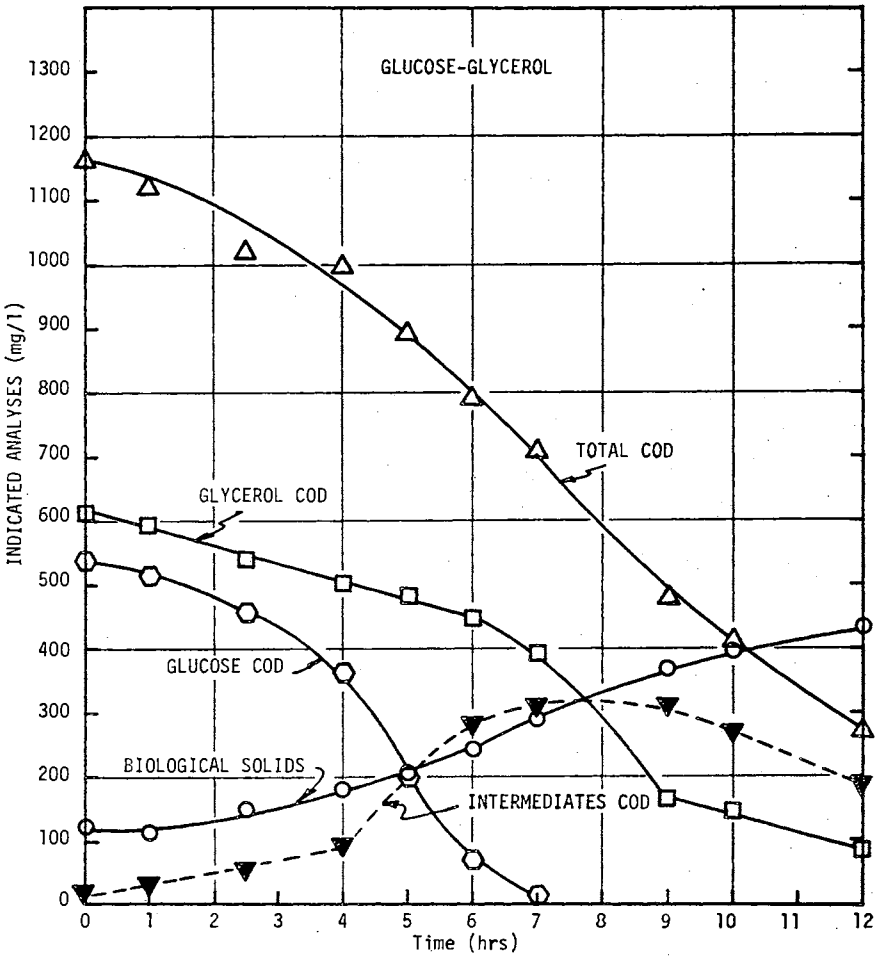



Figure 120 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/36 $hr^{-1}$ on 7-24-67).

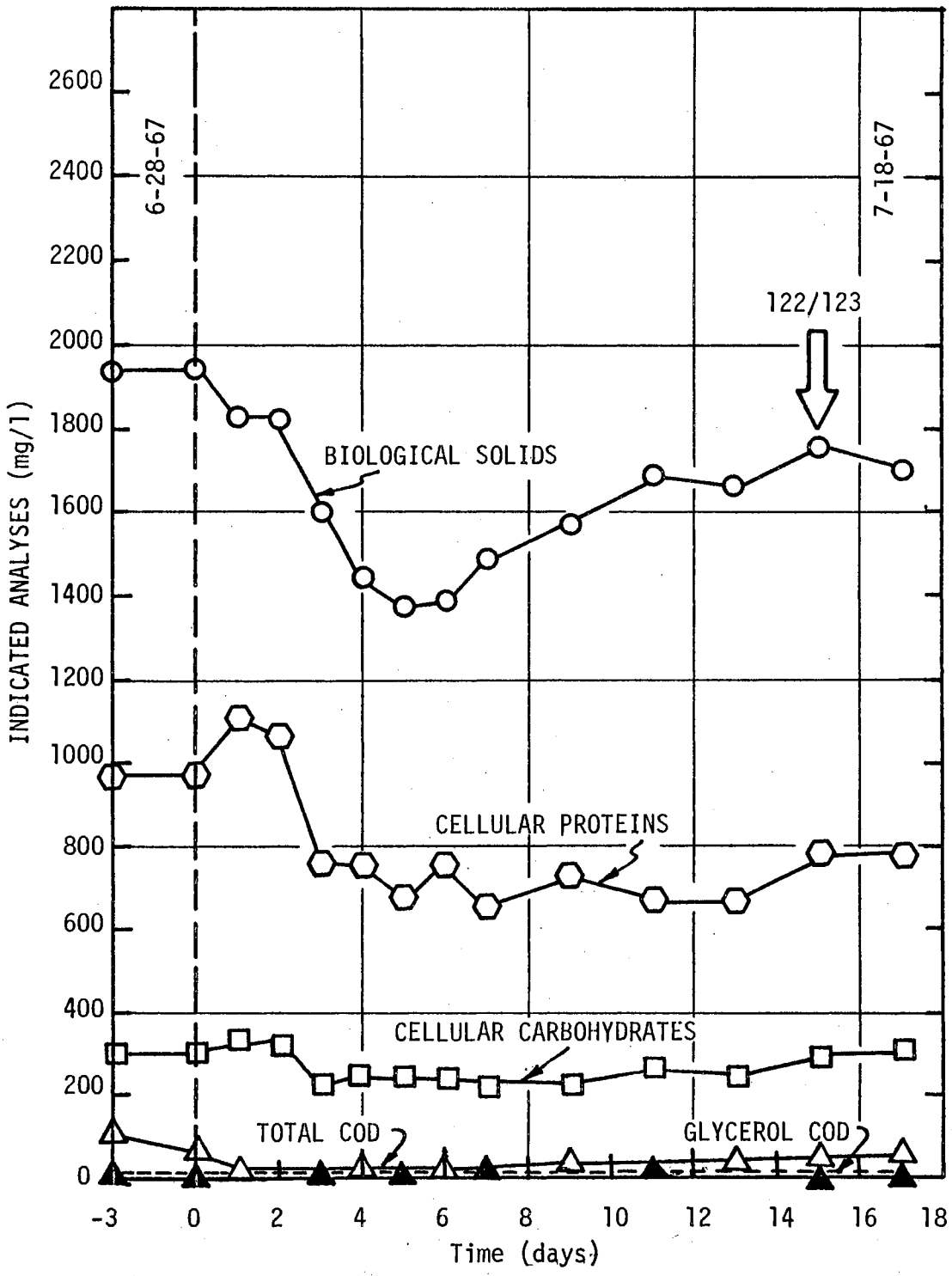



Figure 121 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/36 to 1/48 $hr^{-1}$ During Period of Operation From 6-28-67 to 7-18-67.

somewhat, and the unit was providing an extremely high percentage of COD removal. After five days of operation at the new dilution rate the biological solids gradually began to rise; however, this was not accompanied by an increase in cell protein. Again the unit was not adjudged to be completely mixed, but since it had attained a relatively stable biological solids concentration, cells were harvested for batch experiments to test for sequential substrate removal.

Results using the cells under proliferating conditions are shown in Figures 122 and 123. It is seen that unlike many of the previous control systems for glucose, these cells did not produce a large amount of metabolic intermediates. Both glucose and glycerol were used with approximately equal facility by these glycerol-acclimated cells, and when the carbon sources were used in combination, glucose and glycerol were metabolized concurrently. The presence of glucose did not appear to have any effect whatever on the metabolism of glycerol. Actually, glycerol was removed slightly before removal of glucose.

8. Effect of Changing Dilution Rate from 1/48 to 1/74 $hr^{-1}$

The continuous flow unit was operated at a dilution rate of 1/48 $hr^{-1}$ until July 25, 1967, at which time the dilution rate was decreased to 1/72 $hr^{-1}$ (See Figure 124). In response to this change in dilution rate, the biological solids concentration and cell protein decreased. The concentration of carbohydrate in the sludge mass remained essentially constant. Effluent COD did not rise in response to this shock loading.

After the transient was completed, the biological solids level remained fairly constant at approximately 1200 mg/l. During this period of operation there was a decided change in species predominance as

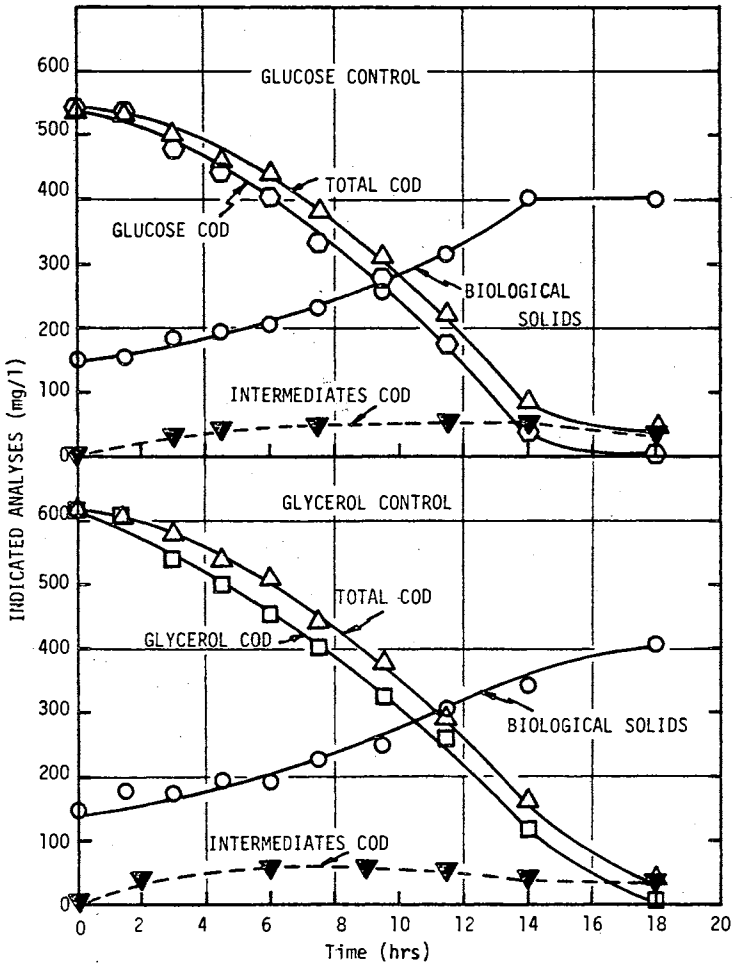



Figure 122 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/48 $hr^{-1}$ on 7-16-67).

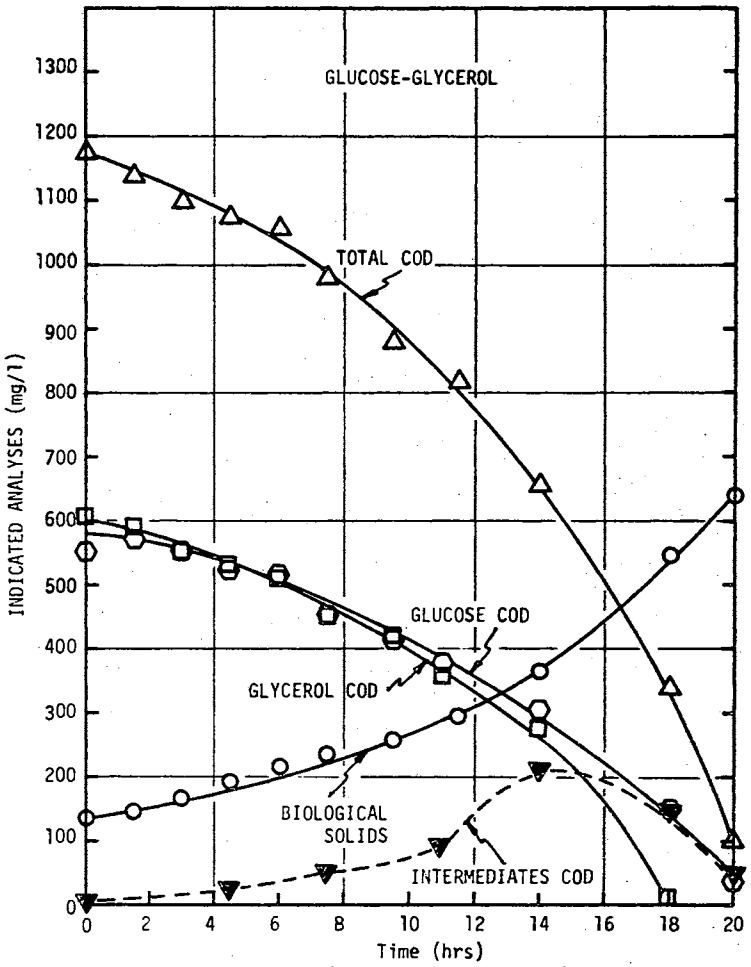



Figure 123 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/48 $hr^{-1}$ on 7-16-67).

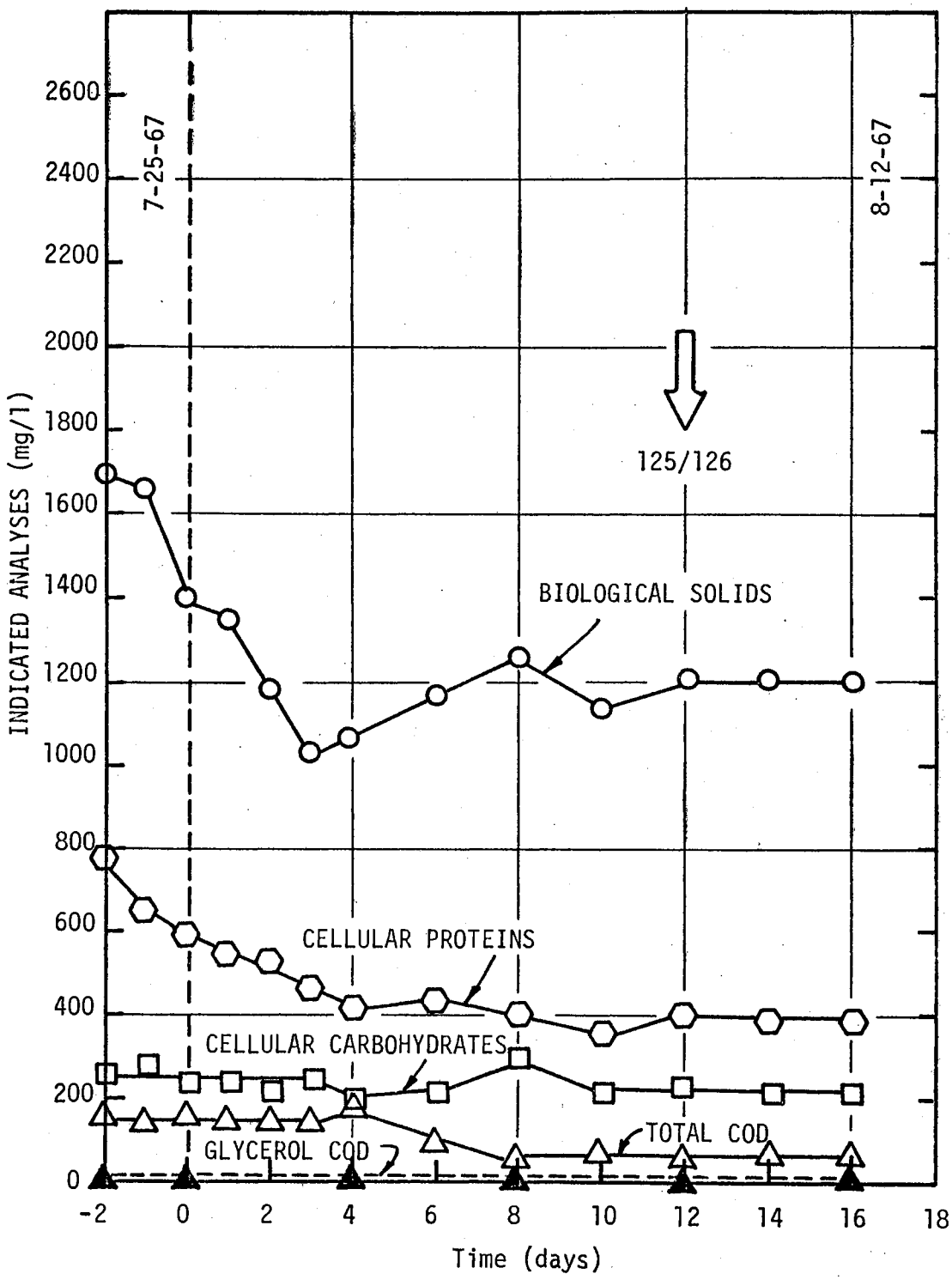



Figure 124 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/48 to 1/72 $hr^{-1}$ During Period of Operation From 7-25-67 to 8-12-67.

adjudged by the development of a distinct orange color of the reactor mixed liquor. The unit was adjudged to be completely mixed, and the cells were harvested for subsequent batch experiments to determine the mode of substrate removal. The results for the control systems, shown in Figure 125, indicate that both substrates could be used readily by the new population. Glucose was removed slightly faster than was glycerol. However, when the substrates were combined, glycerol removal was initiated before initiation of glucose metabolism (Figure 126). It can not be said that the presence of glycerol severely blocked glucose removal, since after fourteen hours of aeration a considerable amount of both compounds remained in the medium, and thereafter both were removed fairly rapidly and at approximately equal rates.

Since there was a definite indication that a change in predominance had occurred in the continuous flow reactor, a batch aerator using seed population taken from the reactor effluent was started. Operation of the batch reactor proceeded for one week in accordance with the daily feeding schedule previously described. During this one-week period of operation, color of the mixed liquor changed from orange to light yellow. Cells from the batch unit were then used for an experiment to test for sequential substrate removal. The results are shown in Figures 127 and 128. It is seen that both glucose and glycerol were used readily by this population, and that on glucose a large amount of metabolic products accumulated in the medium. When used as combined carbon sources, glycerol was not removed to any appreciable extent until the glucose had been metabolized. During glucose metabolism a significant amount of metabolic intermediates accumulated in the medium (approximately equal to the concentration which accumulated in the glucose control system),

Figure 125 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/72 $hr^{-1}$ on 8-8-67).

Figure 126 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/72 $hr^{-1}$ on 8-8-67).

Figure 127 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Previously Grown at Dilution Rate 1/72 $hr^{-1}$ and Then Grown in Batch Unit for One Week With Twenty-four Hour Feeding Cycle).

Figure 128 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Previously Grown at Dilution Rate 1/72 $hr^{-1}$ and Then Grown in Batch Unit for One Week With Twenty-four Hour Feeding Cycle).

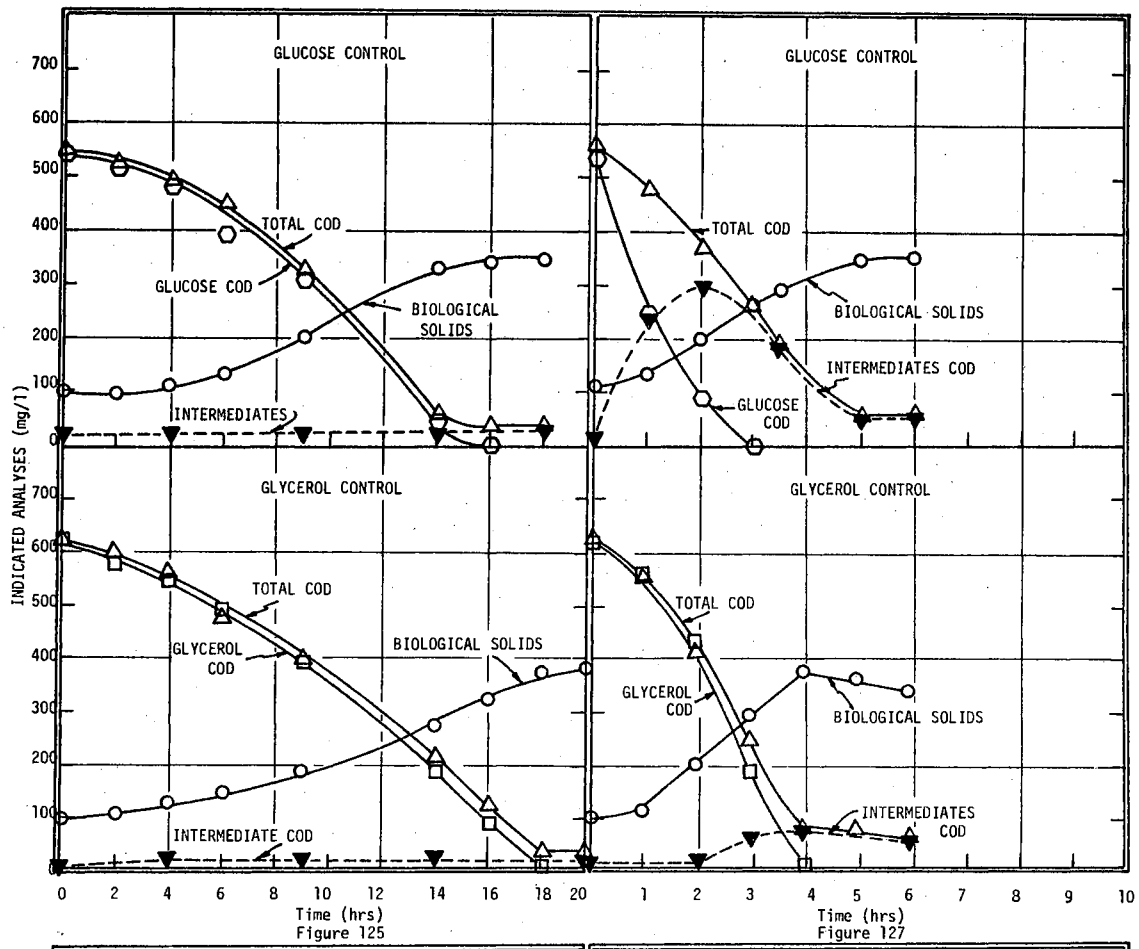



Figure 125




Figure 127

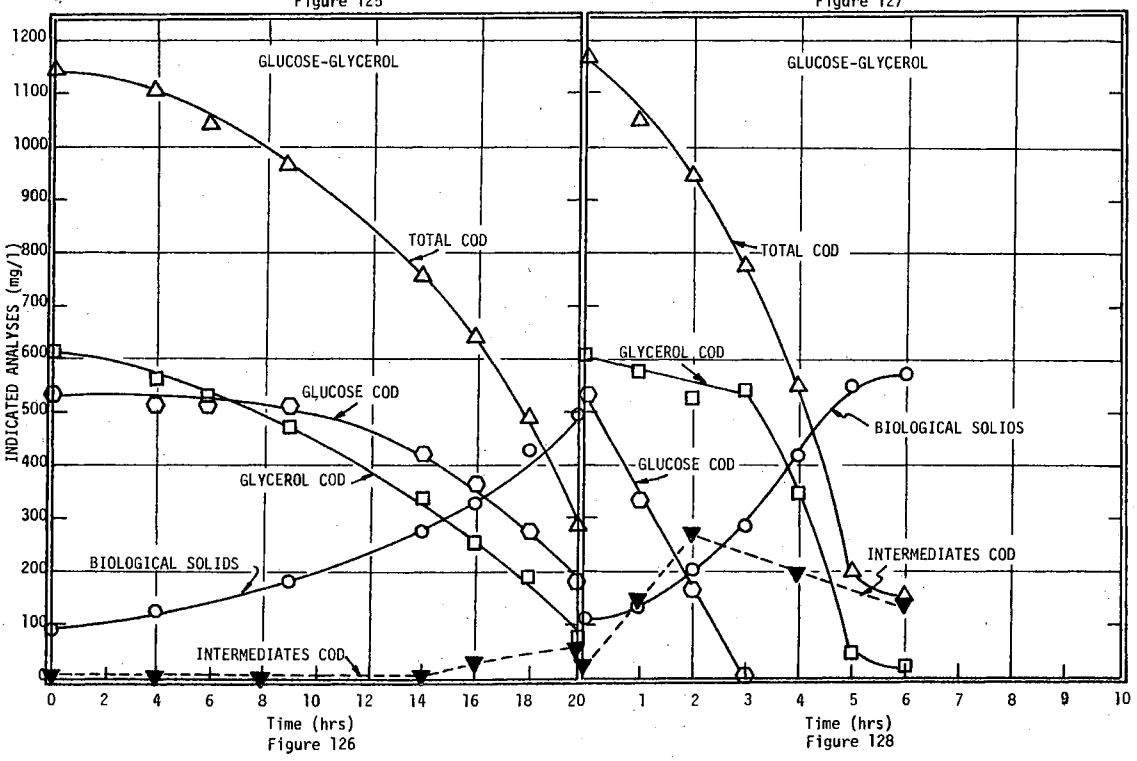



Figure 126




Figure 128

but these metabolic intermediates did not retard the removal of glycerol.

9. Effect of Changing Dilution Rate from 1/72 to 1/96 $hr^{-1}$

On August 12, 1967, the dilution rate was changed from 1/72 to 1/96 $hr^{-1}$. The resulting behavior of the continuous flow reactor is shown in Figure 129. There was a slight decrease in biological solids concentration, but no definable transient state was observed. The unit attained steady state operation with respect to solids and COD removal, and was adjudged to be completely mixed.

Cells were harvested from the continuous flow unit for use in batch experiments to test for the occurrence of sequential substrate removal, and the results are shown in Figures 130 and 131. These glycerol-acclimated cells exhibited an ability to metabolize glucose, and has been observed in many previous experiments, metabolism of glucose led to the accumulation of a considerable amount of metabolic products in the medium. When used as combined substrate, glucose prevented removal of glycerol for six hours, during which time some glucose was metabolized; however, a large amount of glucose remained in the medium after six hours, and glycerol metabolism proceeded concurrently with the removal of the remaining glucose. It is interesting that the rate of glucose removal decreased at approximately the same time that glycerol removal was initiated.

At the time cells were harvested for the batch experiments described above, cells were also harvested from the continuous flow reactor and used to start a batch reactor. Cells were grown in this batch reactor for one day, and then used to test for sequential substrate removal. During the one day of batch operation there was no readily observable change in species predominance. The results of the

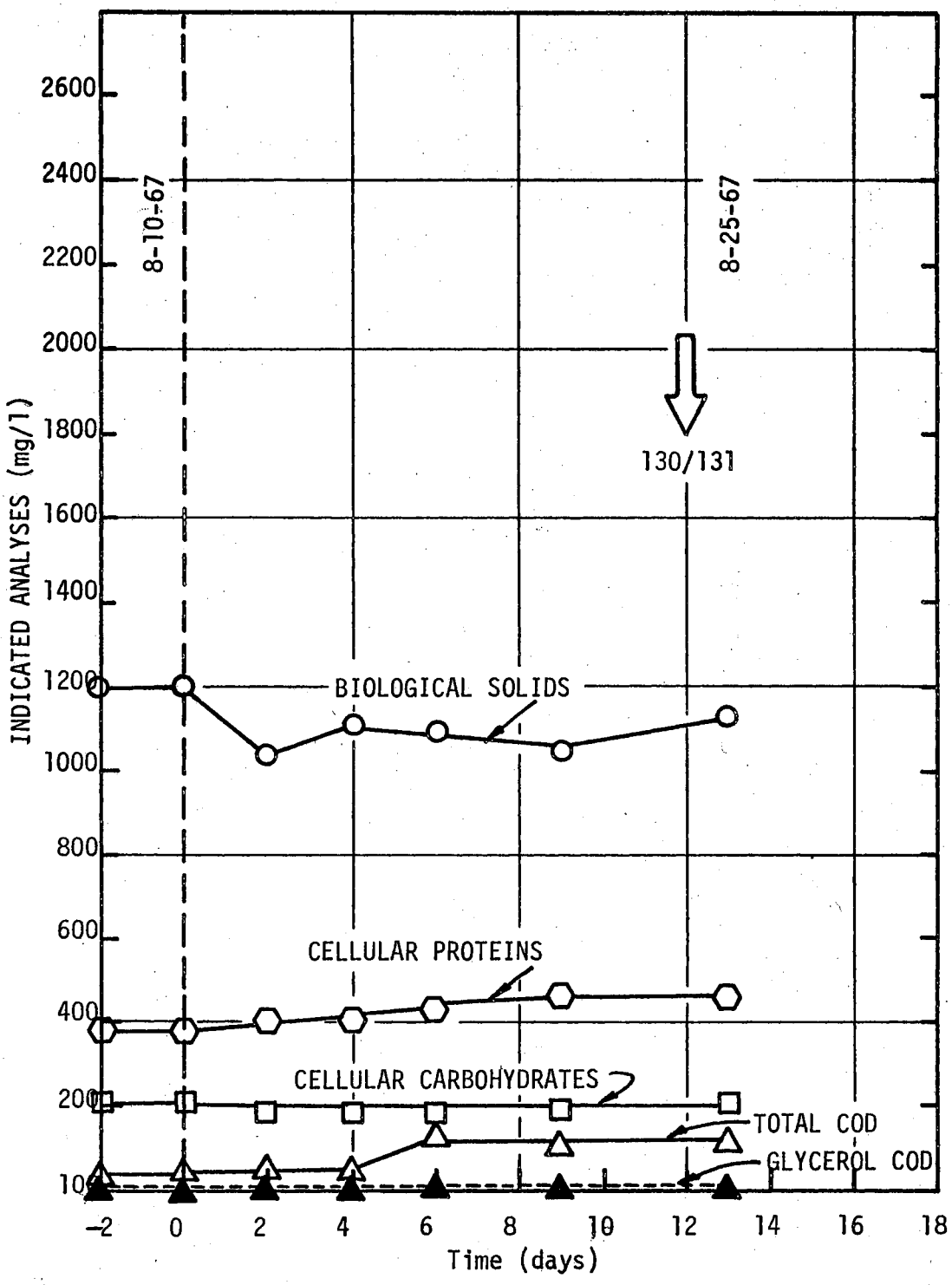



Figure 129 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/72 to 1/96 $hr^{-1}$ During Period of Operation From 8-10-67 to 8-25-67.

Figure 130 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 8-24-67).

Figure 131 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 8-24-67).

Figure 132 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Previously Grown at Dilution Rate 1/96 $hr^{-1}$ and Then Grown in Batch for Twenty-four Hours).

Figure 133 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Previously Grown at Dilution Rate 1/96 $hr^{-1}$ and Then Grown in Batch for Twenty-four Hours).

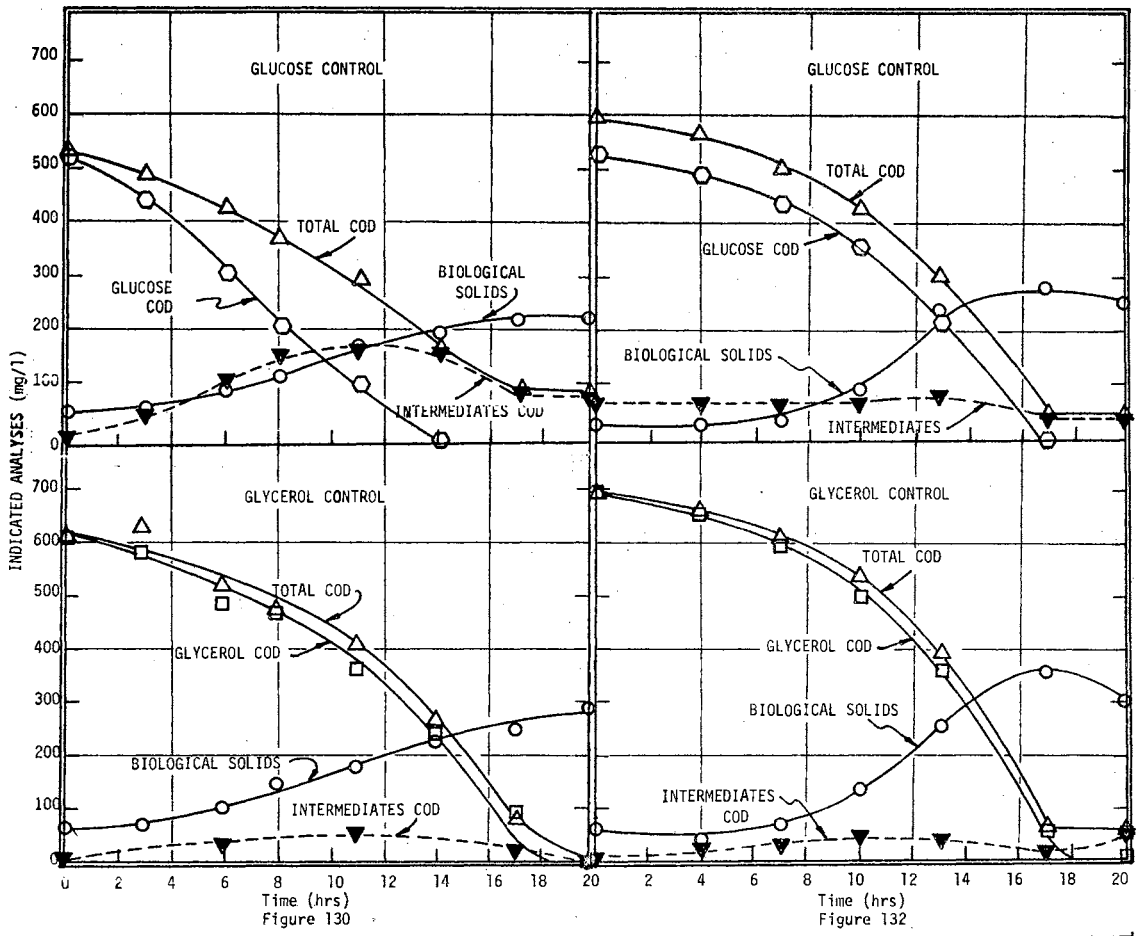



Figure 130




Figure 132

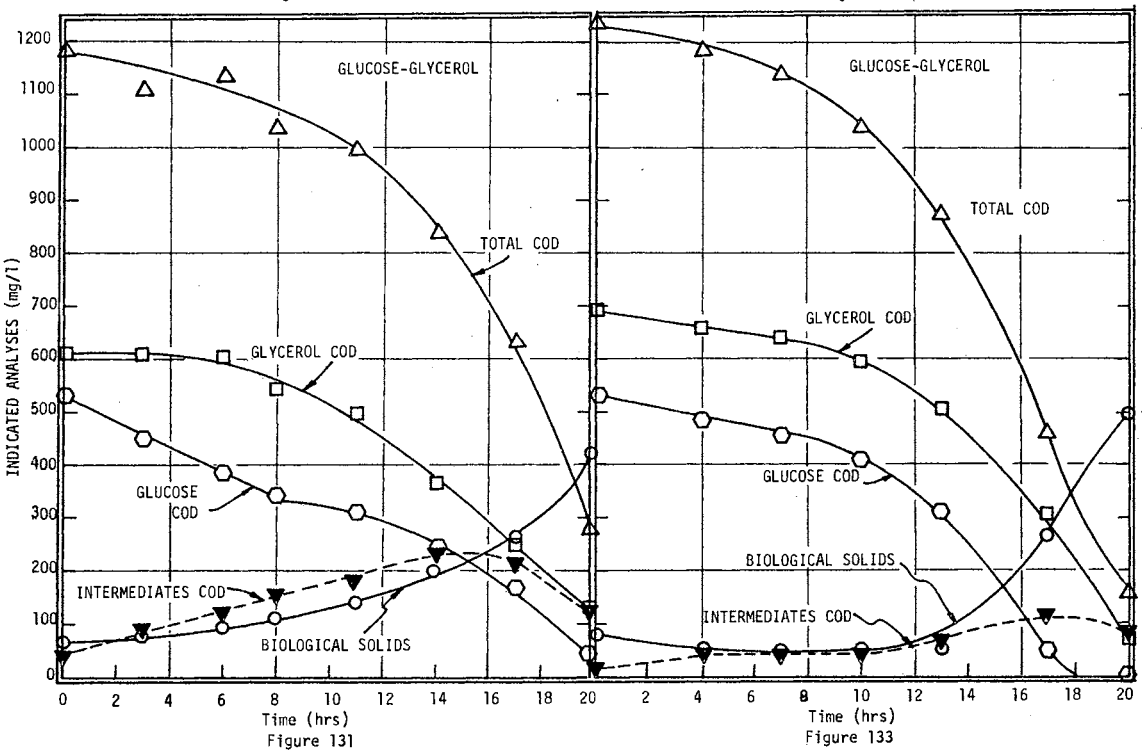



Figure 131




Figure 133

batch experimentation are shown in Figures 132 and 133. The control systems are not unlike those shown in the previous figure; however, in the glucose control there was no evidence for the accumulation of metabolic intermediates and/or endproducts in the medium. When used as combined carbon source, glycerol and glucose were removed concurrently.

10. Effect of Changing Dilution Rate from 1/96 to 1/192 $hr^{-1}$

It was seen in Figure 129 that it was possible to grow cells in a continuous flow reactor approaching a steady state condition at a growth rate as low as 1/96 $hr^{-1}$. From the viewpoint of the microbiologist, this growth rate is extremely low, and it seems somewhat surprising that it was attainable. However, in certain processes applicable to the biological treatment of polluted waters, for example, aerated lagoons, such detention times in reactors approaching conditions of complete mixing would not be uncommon, and it was of interest to learn that principles of continuous flow completely mixed reactors could possibly be applied at such slow growth rates. It was also of interest to determine whether a steady state could be approached at still lower growth rates. Accordingly, the dilution rate was changed from 1/96 to 1/192 $hr^{-1}$. The effects of such a change on the behavior of the continuous flow reactor are shown in Figure 134. It is seen that in over one month of operation at this very low dilution rate, the system maintained a relatively steady level of biological solids, and the substrate removal efficiency remained rather high.

After one month of operation at this dilution rate, cells were harvested from the reactor effluent and used in batch experimentation. While the major purpose of running the batch experimentation was to determine the mode of substrate removal (either sequential or concurrent),

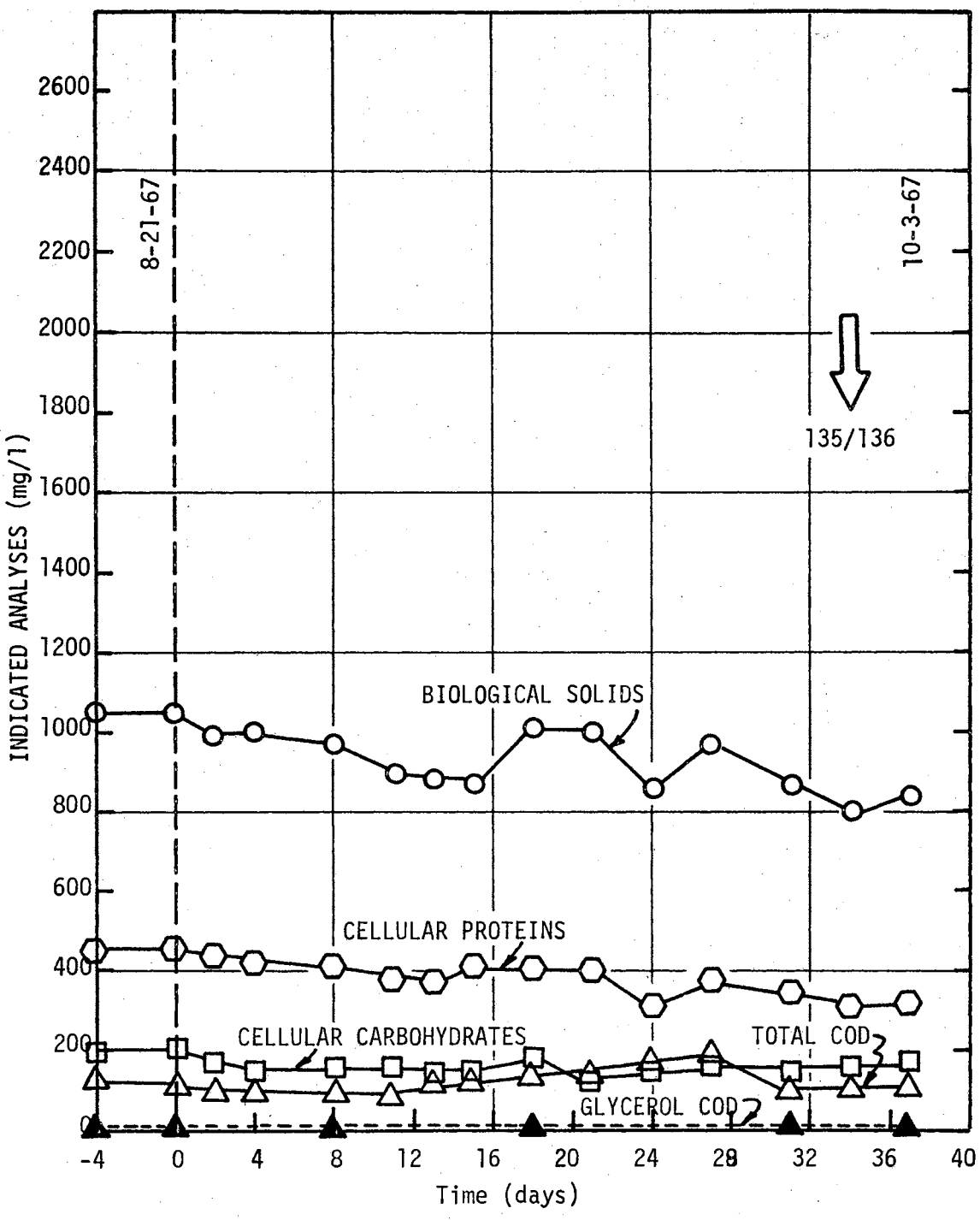



Figure 134 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/96 to 1/192 $hr^{-1}$ From 8-21-67 to 10-3-67.

the control systems are extremely interesting in themselves, since they offer an insight into the effect of the past growth history of the cells when placed in an environment containing an abundant supply of carbon source. Although the batch experiment was conducted under growth conditions, it is seen that growth on either substrate was severely retarded (Figure 135). The experiment was run until COD removal was essentially completed, and it is seen that most of the COD removal can be attributed to substrate oxidation or respiration of the cells. The biological solids production in both systems was extremely low, and the cell yield was approximately twenty per cent. When used as combined carbon sources, the substrates were removed concurrently (Figure 136).

The continuous flow unit was operated for an additional month at this dilution rate, during which time a relatively steady condition was maintained. Cells were then harvested (see arrow on left-hand side, Figure 137) for another batch experiment to test for sequential or concurrent substrate removal. The results shown in Figures 138 and 139 indicate that the control systems yielded essentially the same results as the control systems for the previous batch experiment. When used as combined carbon sources, the removal of glucose and glycerol must be adjudged as concurrent, as was the case for the previous batch experiment, although at the time of glucose exhaustion there was a considerable amount of glycerol remaining in the medium.

11. Effect of Changing Dilution Rate from 1/192 to 1/96 $hr^{-1}$

While it was interesting and surprising to learn that the completely mixed chemostat could be run in a condition approaching steady state at a detention time as high as eight days ($\mu$ = 0.0052 $hr^{-1}$), and while it would have been interesting to determine whether the logarithmic

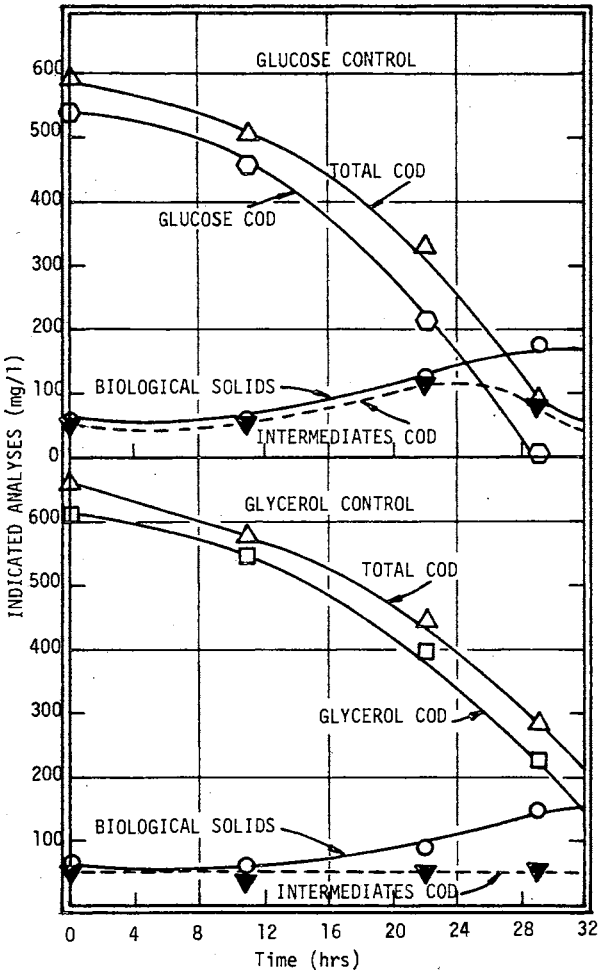



Figure 135 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/192 $hr^{-1}$ on 9-30-67).

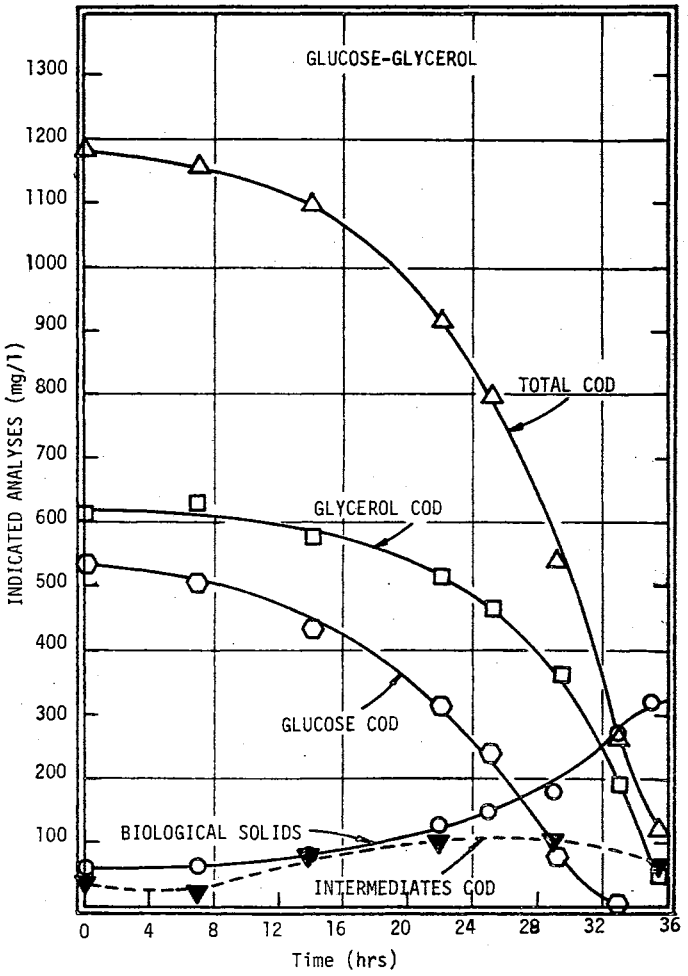



Figure 136 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate at 1/192 $hr^{-1}$ on 9-30-67).

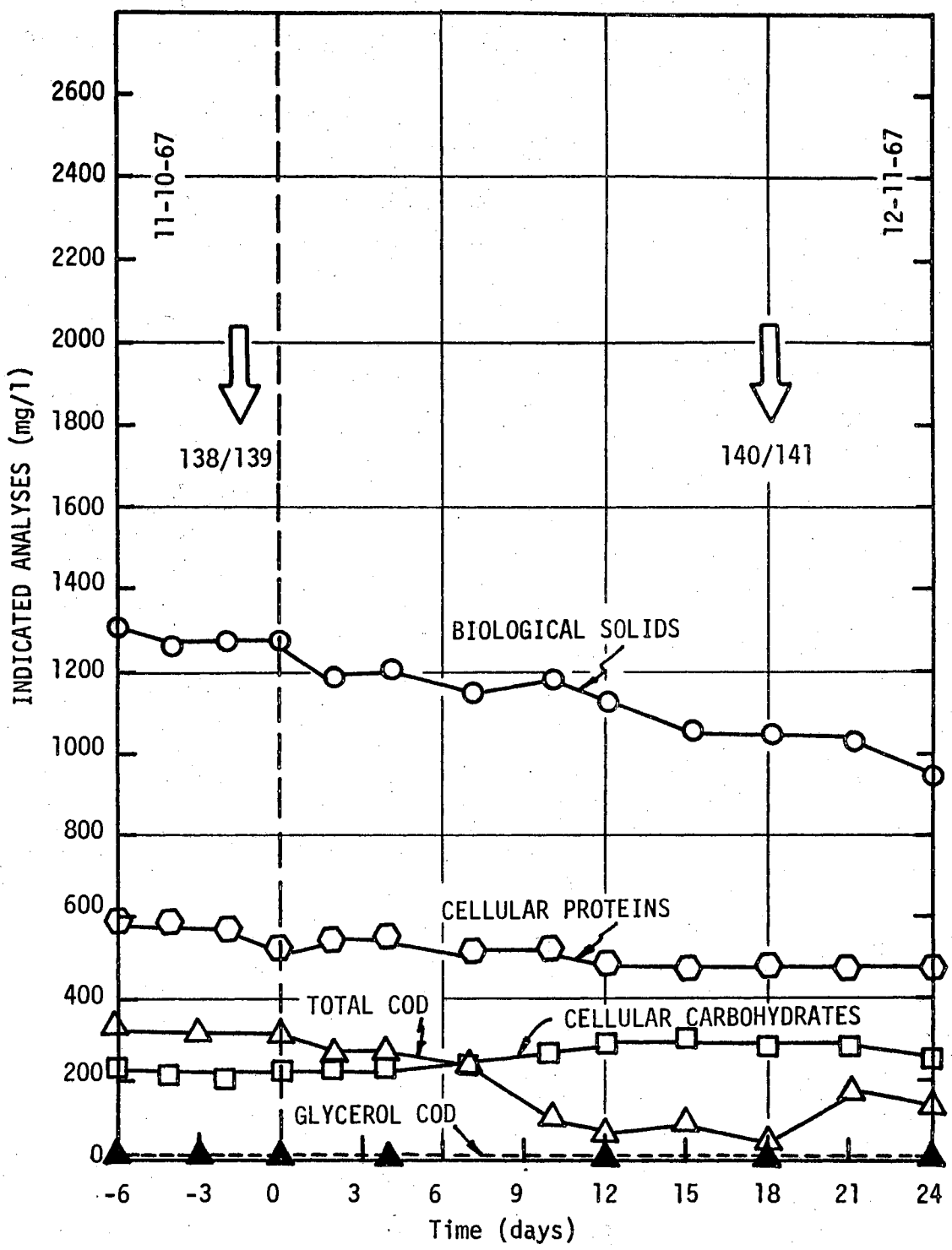



Figure 137 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/192 to 1/96 $hr^{-1}$ During Period of Operation From 11-10-67 to 12-11-67.

Figure 138 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/192 $hr^{-1}$ on 11-14-67).

Figure 139 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/192 $hr^{-1}$ on 11-14-67).

Figure 140 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 12-5-67).

Figure 141 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 12-5-67).

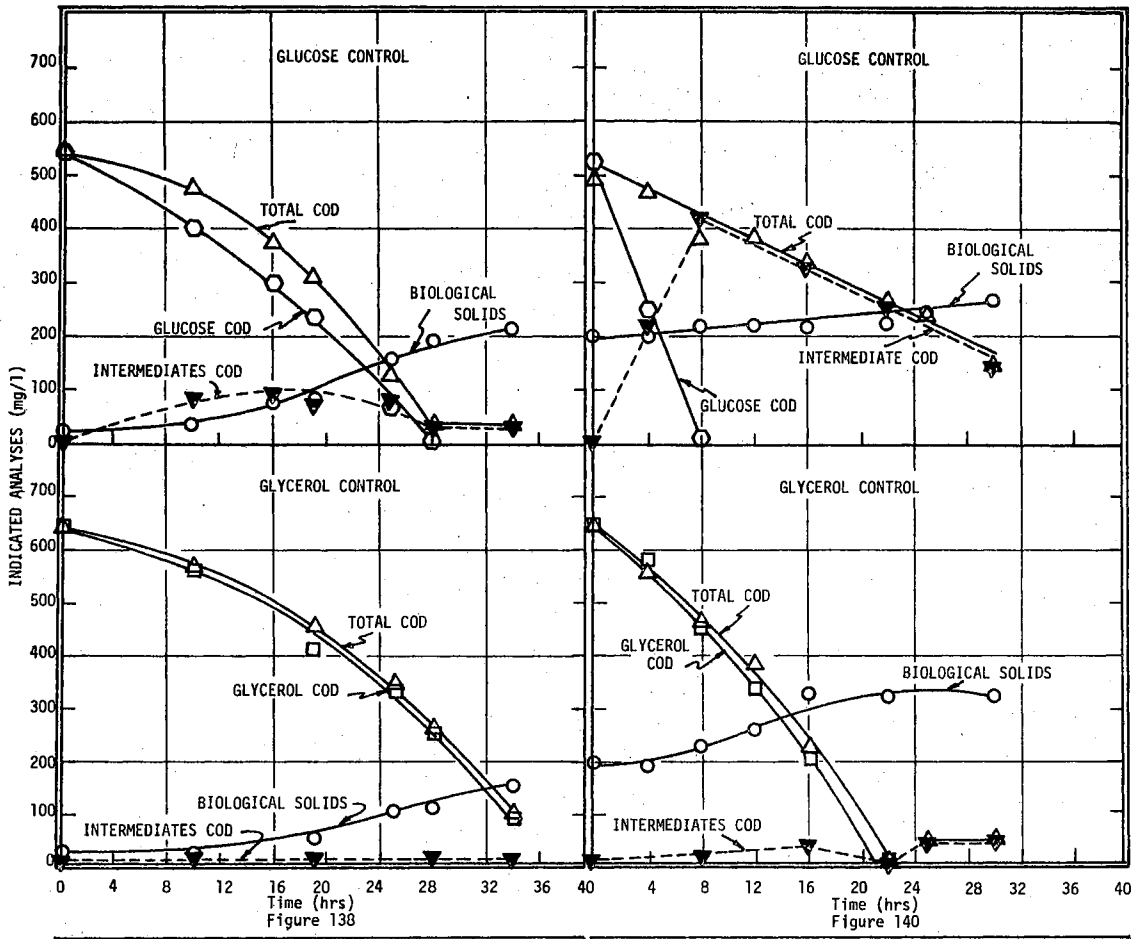



Figure 138




Figure 140

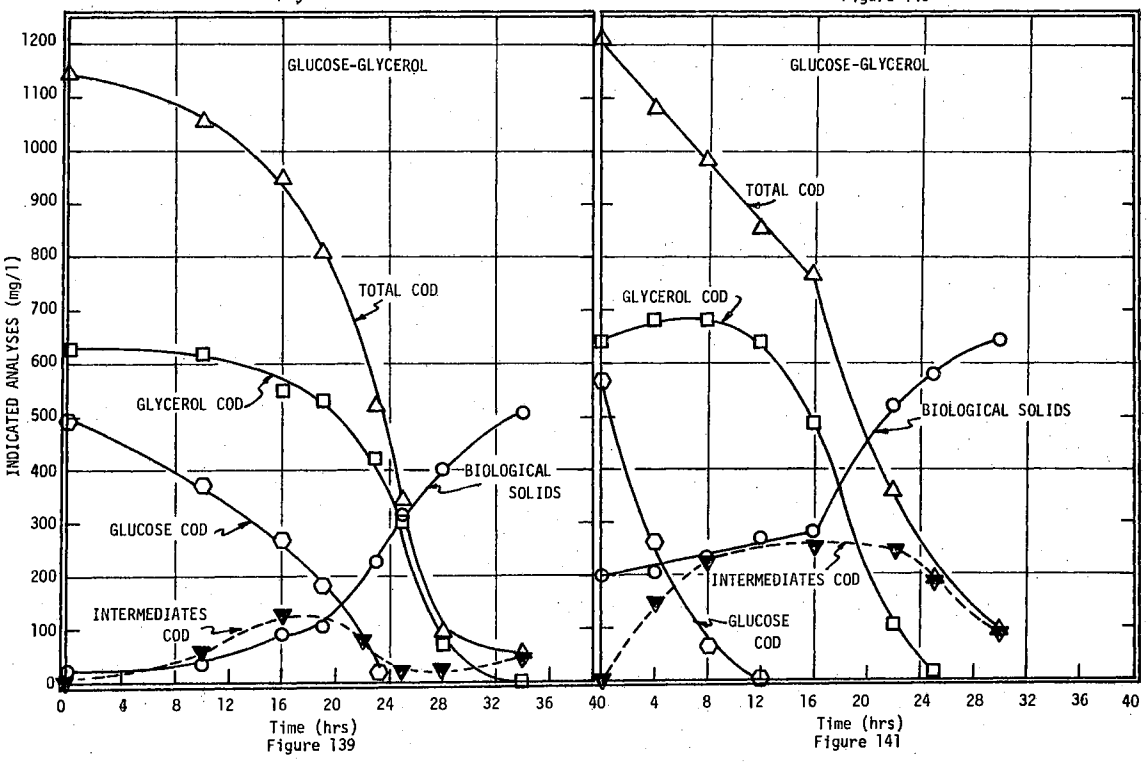



Figure 139




Figure 141

growth rate could be maintained at an even lower level, it was decided not to decrease the dilution rate further because even at the dilution rate of 1/192 $hr^{-1}$ the rate of evaporation was beginning to give operational problems which could not be solved without redesigning the continuous flow reactor. Accordingly, it was decided to allow the unit to undergo a series of successively increased dilution rates (i.e., increasingly severe shock loads leading to decreased detention times). As seen in Figure 137, the change in dilution rate from 1/192 to 1/96 $hr^{-1}$ did not cause a serious disruption in system efficiency. In fact, the effluent COD which had gradually risen from 100 to 300 during the period of operation at the lower dilution rate, gradually returned to approximately 100 mg/l. The biological solids level dropped very gradually after the change in dilution rate, but was maintained at approximately 1000 mg/l.

Eighteen days after changing the dilution rate, cells were harvested for batch experimentation, and the results are shown in Figures 140 and 141. There was a considerable difference between the glucose and the glycerol controls. On glycerol the cells behaved in a manner somewhat typical of cells acclimated to the substrate on which they were grown. The rather long period required for substrate removal and the low amount of sludge synthesis was typical of the response using cells with a similar past growth history in the continuous flow unit. When grown on glucose, an extremely large proportion of the carbon source was converted into metabolic products, and only slightly over 50 mg/l of sludge accumulated while over 350 mg/l of total COD was removed. Removal of glucose and of total COD progressed in accordance with zero order reaction kinetics. However, when these substrates were used as a

combined carbon source, the presence of glucose blocked the removal of glycerol. Glycerol removal was not initiated until the glucose was exhausted from the medium. It is also evident that the intermediates produced from glucose did not block the removal of glycerol. Removal of total COD and accumulation of biological solids exhibited a distinctly diphasic character.

12. Effect of Changing Dilution Rate from 1/96 to 1/48 $hr^{-1}$

After three weeks of operation at a dilution rate of 1/96 $hr^{-1}$, the dilution rate was changed to 1/48 $hr^{-1}$ and the response (see Figure 142) led to severe disruption of substrate removal efficiency in a well-defined transient state which was followed by recovery and maintenance of fairly steady operation four days after the shift in dilution rate. The change in volumetric feeding rate precipitated a change in microbial predominance. The mixed liquor developed a pinkish hue, and a very loose floc, which proved to be predominantly filamentous organisms when examined under the microscope, developed.

Cells were harvested from the reactor effluent and used in batch experiments. The results shown in Figures 143 and 144 indicate that glucose and glycerol could be metabolized by these cells with nearly equal facility. It is also interesting to note that sludge accumulation in both controls was greater than the sludge accumulation observed in batch studies using cells grown in the continuous flow unit at the slower growth rates. When used as a combined carbon source, glycerol and glucose were removed concurrently.

13. Effect of Changing Dilution Rate from 1/48 to 1/24 $hr^{-1}$

It is seen in Figure 145 that a change in the hydraulic loading

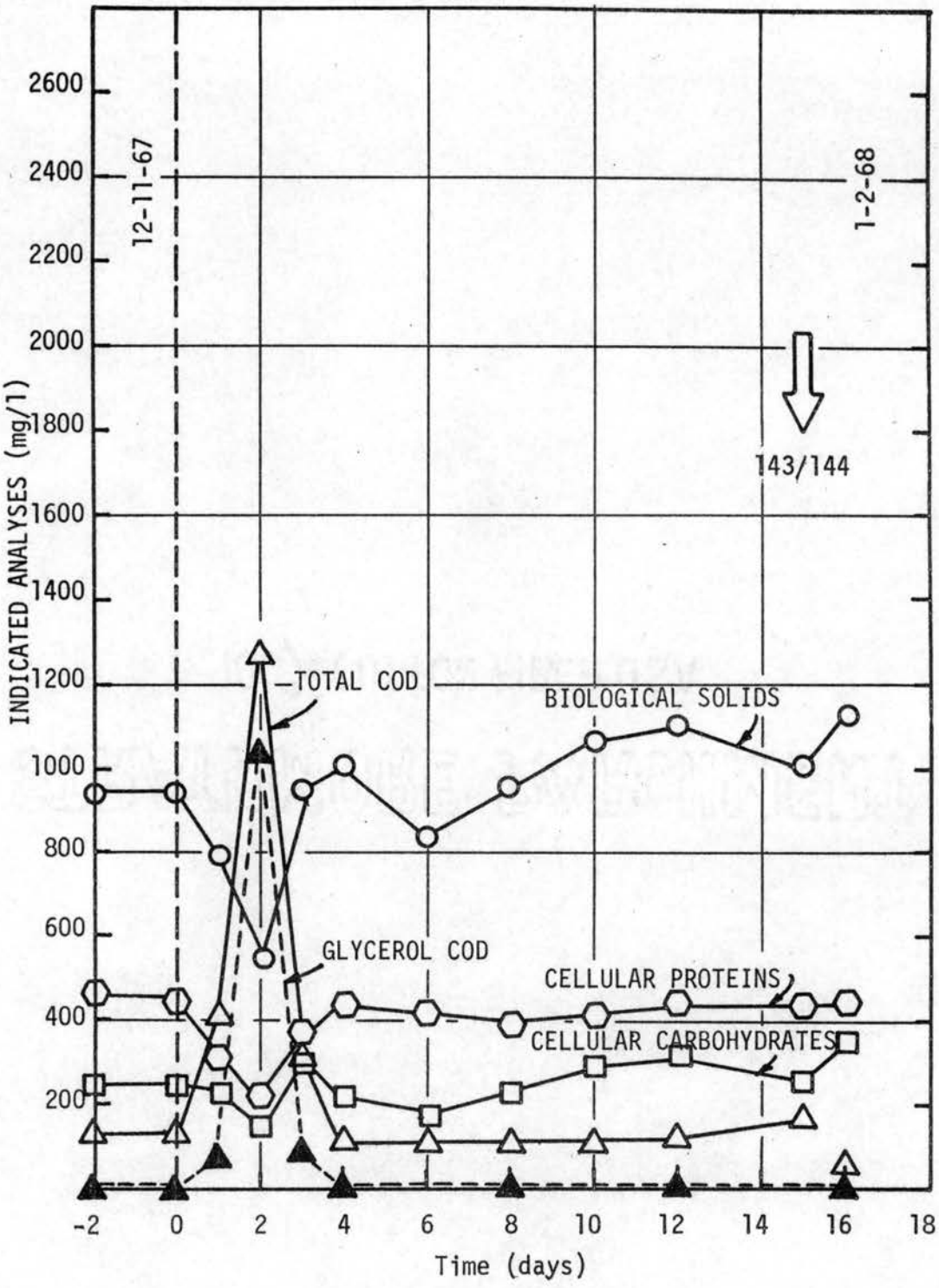



Figure 142 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/96 to 1/48 $hr^{-1}$ During Period of Operation From 12-11-67 to 1-2-68.

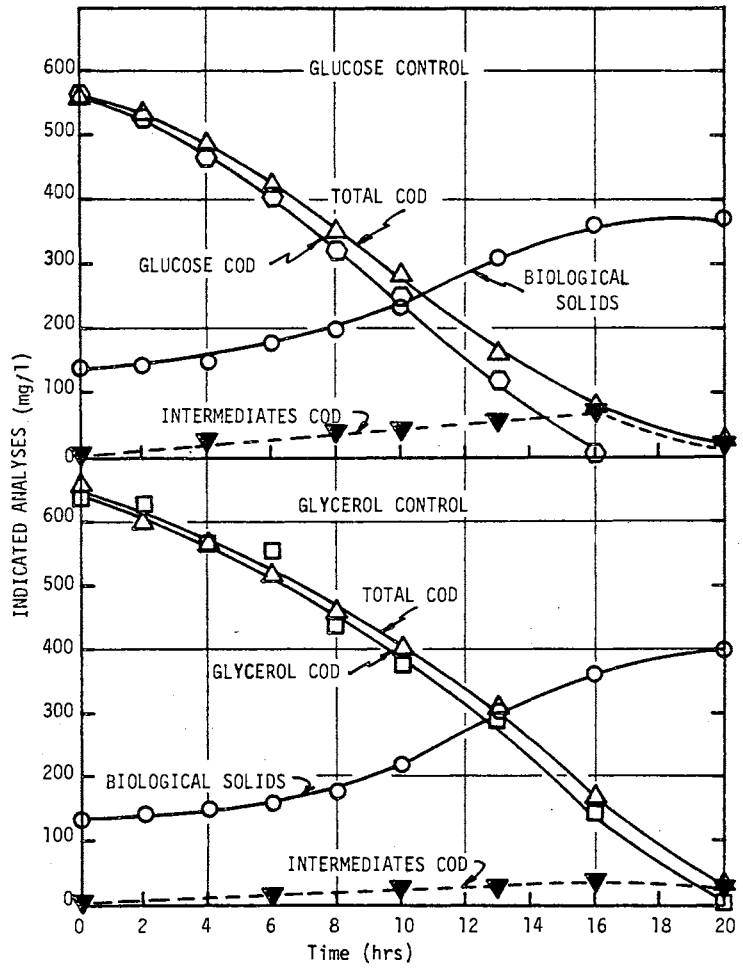



Figure 143 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/48 $hr^{-1}$ on 1-1-68).

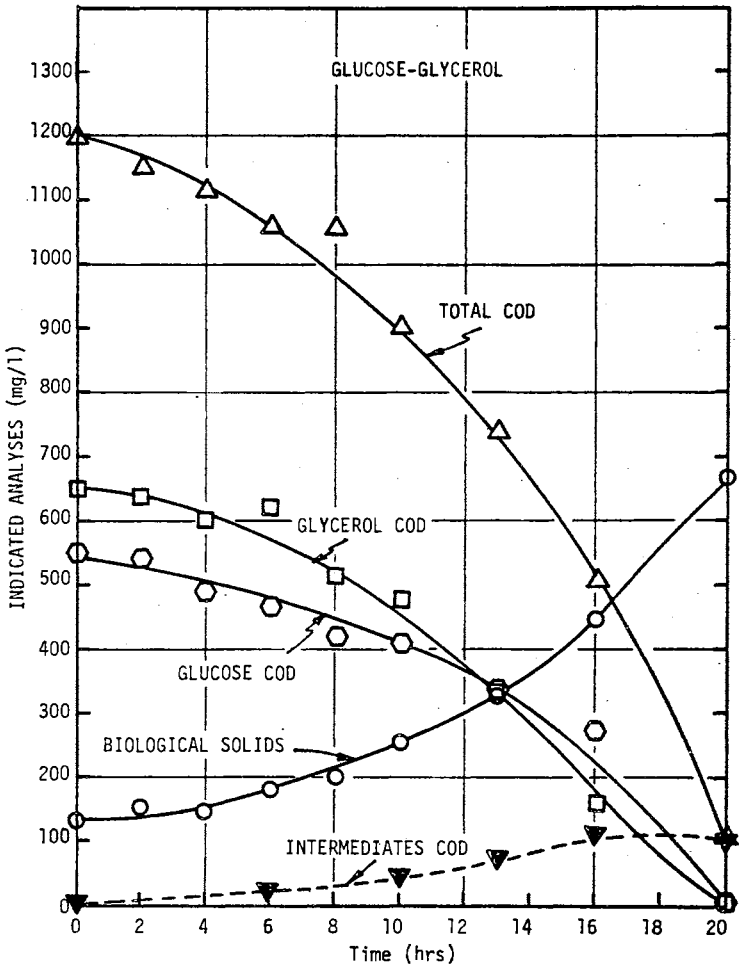



Figure 144 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/48 $hr^{-1}$ on 1-1-68).

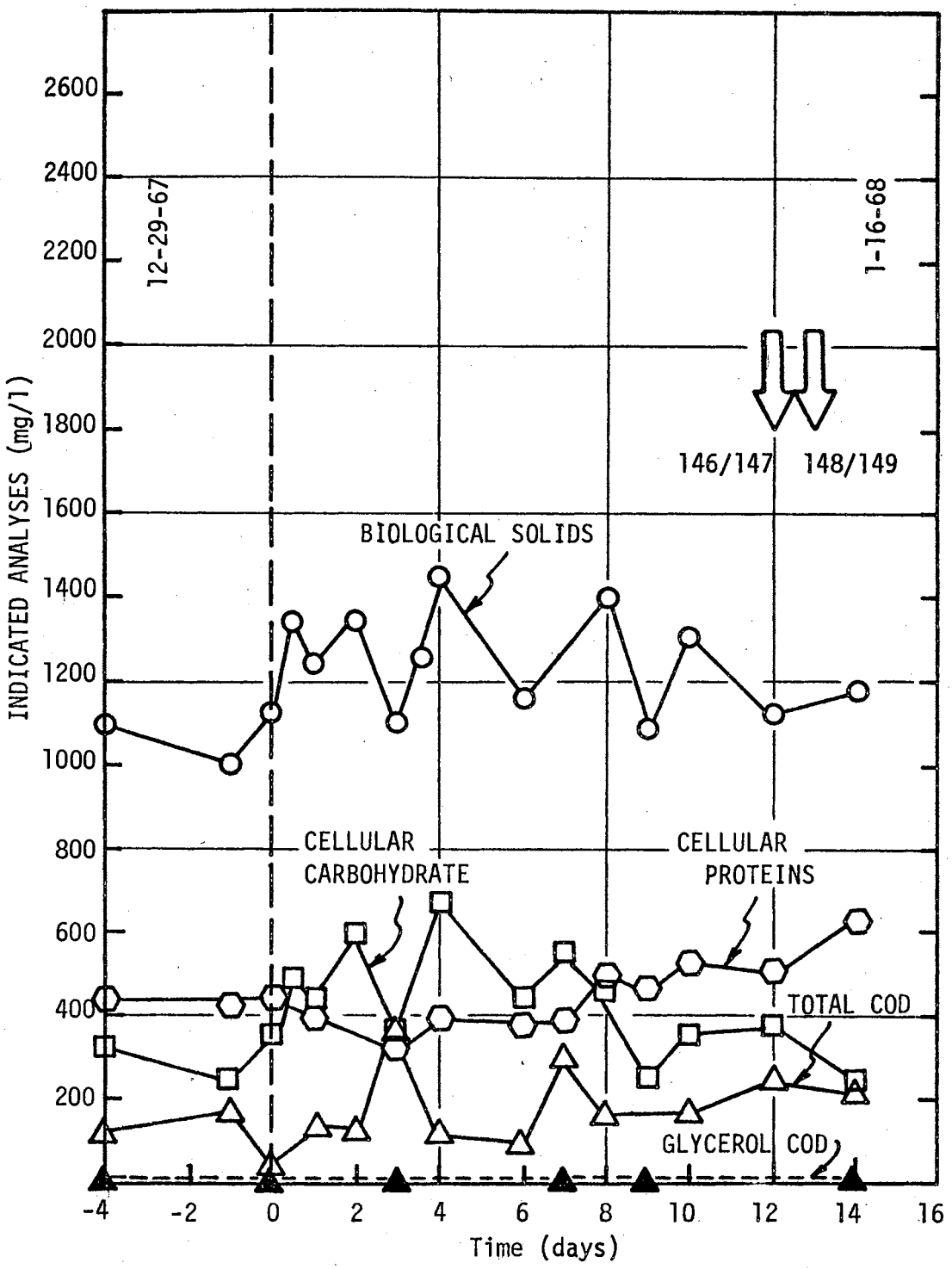



Figure 145 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/48 to 1/24 $hr^{-1}$ During Period of Operation From 12-29-67 to 1-16-68.

from a dilution rate of 1/48 to 1/14 $hr^{-1}$ did not cause a severe transient disruption of system efficiency; however, there was considerable fluctuation in biological solids concentration and effluent COD throughout the entire 14-day period of operation at the new dilution rate. It is important to note that the fluctuation which was observed is not greater than is commonly experienced for "steady state" operation of continuous flow reactors. The most notable change in system performance at this dilution rate was the general increase in carbohydrate content of the cells. During the period of operation at this dilution rate, the mixed liquor exhibited a milky appearance and filamentous organisms were predominant.

At this dilution rate the cell output was sufficiently high to permit collection of enough biological solids in a reasonable time period so that batch experiments under nonproliferating conditions using a relatively high initial biological solids concentration could be resumed. The results of such an experiment under nonproliferating conditions is shown in Figures 146 and 147. Examination of the control systems indicates that glycerol was used considerably faster than was glucose. When the substrates were used in combination, the presence of glycerol severely retarded glucose removal. Indeed, even after glycerol was essentially removed from the system, glucose metabolism proceeded at an extremely slow pace and, after twenty-four hours, more than half of the glucose remained in the medium.

Cells were also harvested from the chemostat effluent for studies under growth conditions. The results are shown in Figures 148 and 149. The linear removal of glycerol in the control system is difficult to explain; however, it is seen by comparison of both control systems that

Figure 146 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 1-14-68).

Figure 147 - Metabolic Response under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 1-14-68).

Figure 148 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 1-15-68).

Figure 149 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 1-15-68).

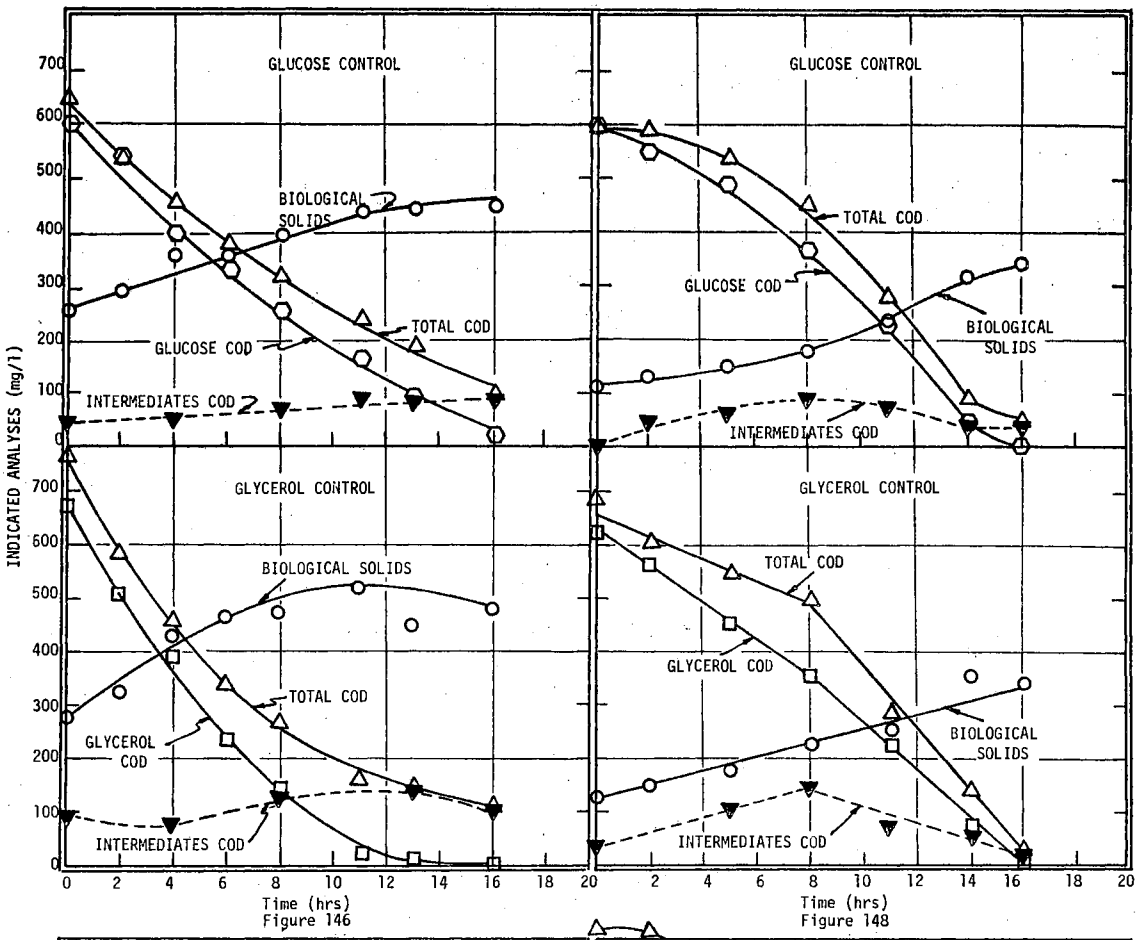



Figure 146




Figure 148

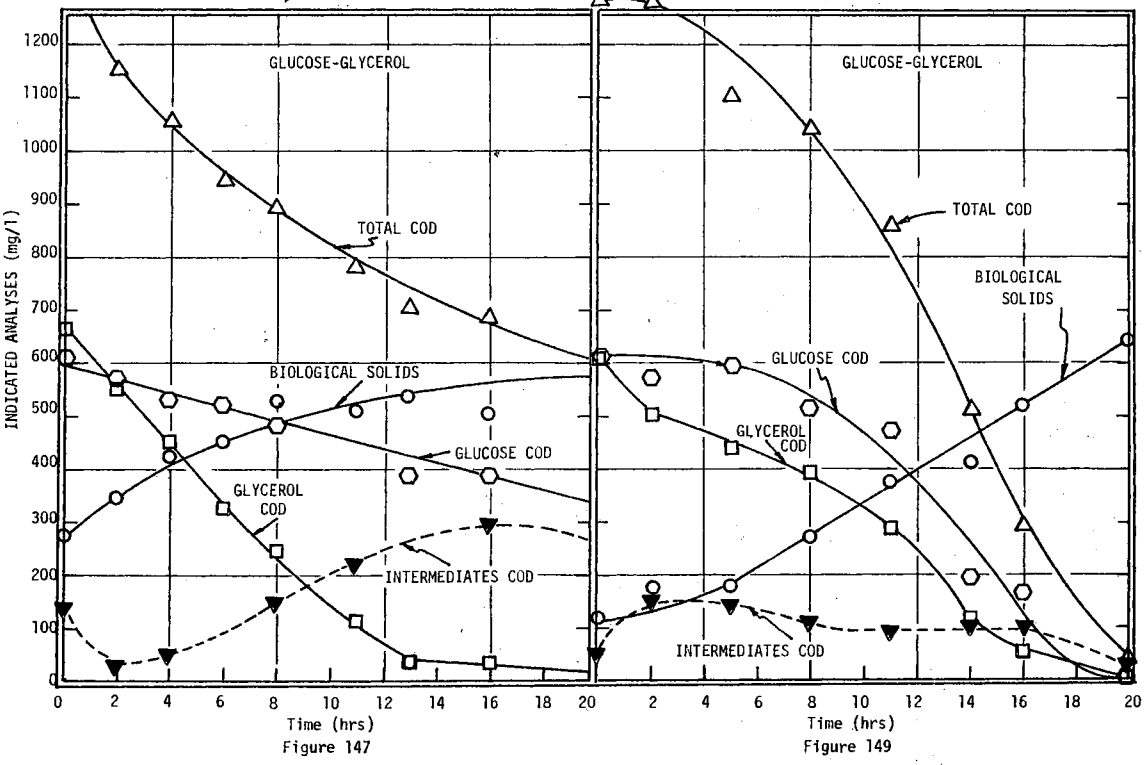



Figure 147




Figure 149

glucose and glycerol were removed in approximately the same time. It is also seen in the combined system that glucose removal was retarded for five hours, but thereafter glucose and glycerol were removed concurrently.

14. Effect of Changing Dilution Rate from 1/24 to 1/12 $hr^{-1}$

As can be seen in Figure 150, doubling the hydraulic loading to a dilution rate of 1/12 $hr^{-1}$ did not cause severe disruption of system efficiency. Indeed, if a transient can be said to exist, it was one in which the cell concentration rose slightly, and substrate removal efficiency improved. However, a significant change in microbial predominance did occur. On the third day after changing the dilution rate, the filamentous organisms which previously predominated began to disappear from the unit, and their disappearance coincided with a decrease in the biological solids concentration. At this time the COD in the effluent rose slightly. During the succeeding days of operation the mixed liquor developed a light blue color, biological solids returned to higher levels, and COD removal was extremely high. At this time, cells were harvested from the reactor effluent for batch experiments under nonproliferating conditions.

It is seen in Figures 151 and 152 that under nonproliferating conditions the cells prevailing in the continuous flow unit could assimilate glucose and glycerol with approximately the same proficiency. Glycerol removal proceeded in accordance with zero order kinetics, but substrate removal in the glucose control followed an "S" shaped pattern. However, when the substrates were used as a combined carbon source, the reverse pattern of removal developed and it seems clear that the presence of glycerol retarded the removal of glucose.

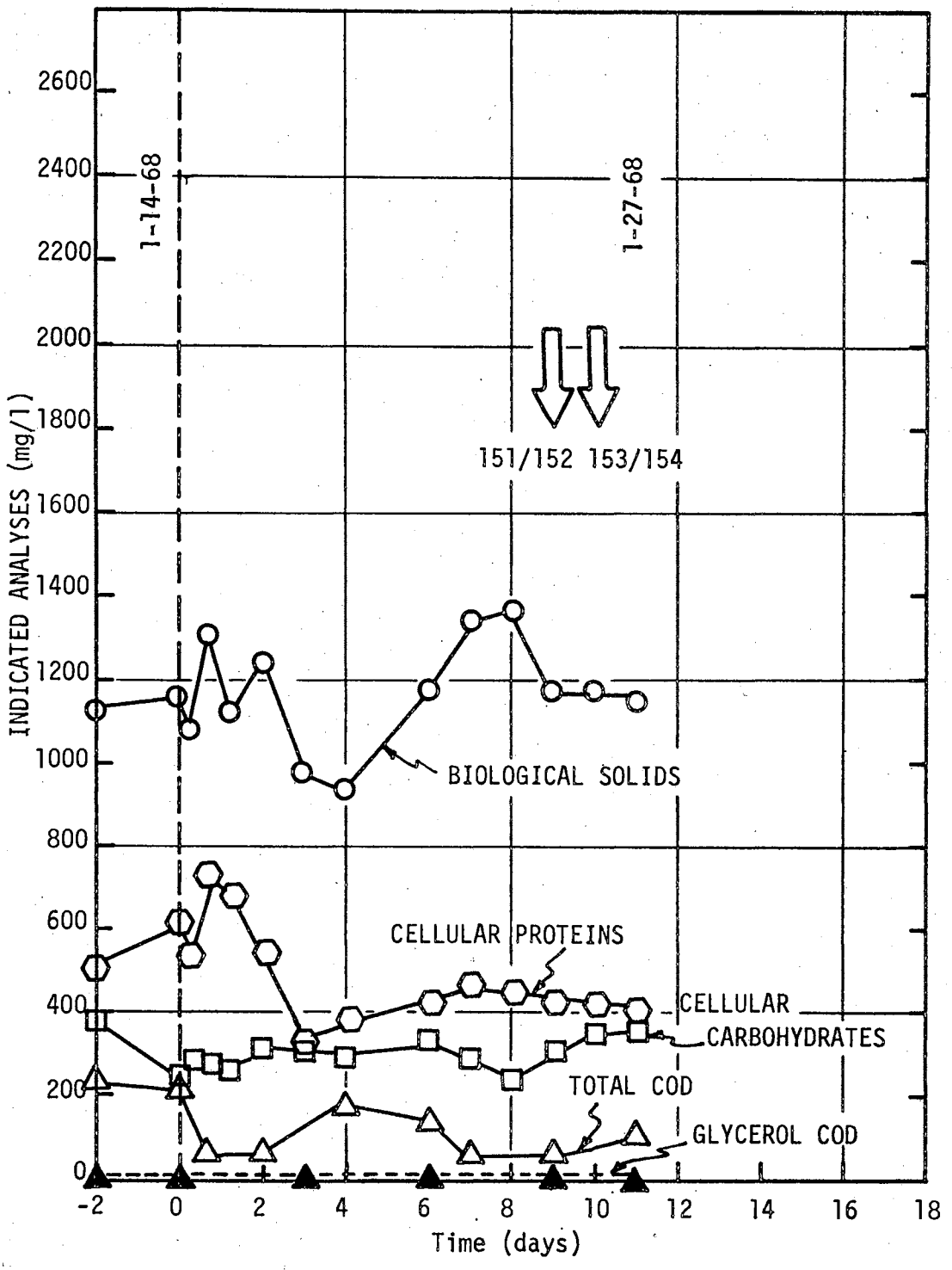



Figure 150 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/24 to 1/12 $hr^{-1}$ During Period of Operation From 1-14-68 to 1-27-68.

Figure 151 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 1-25-68).

Figure 152 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 1-25-68).

Figure 153 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 1-26-68).

Figure 154 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/12 $hr^{-1}$ on 1-26-68).

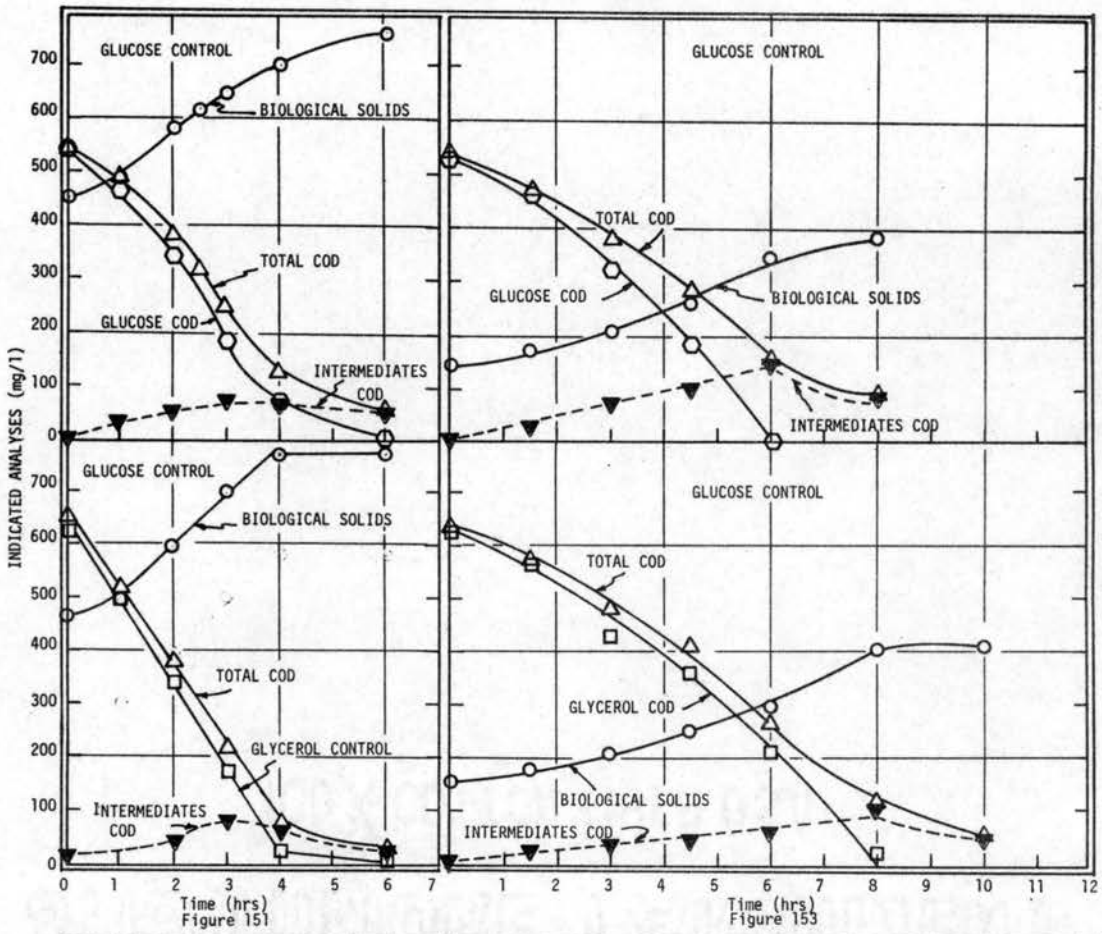



Figure 151




Figure 153

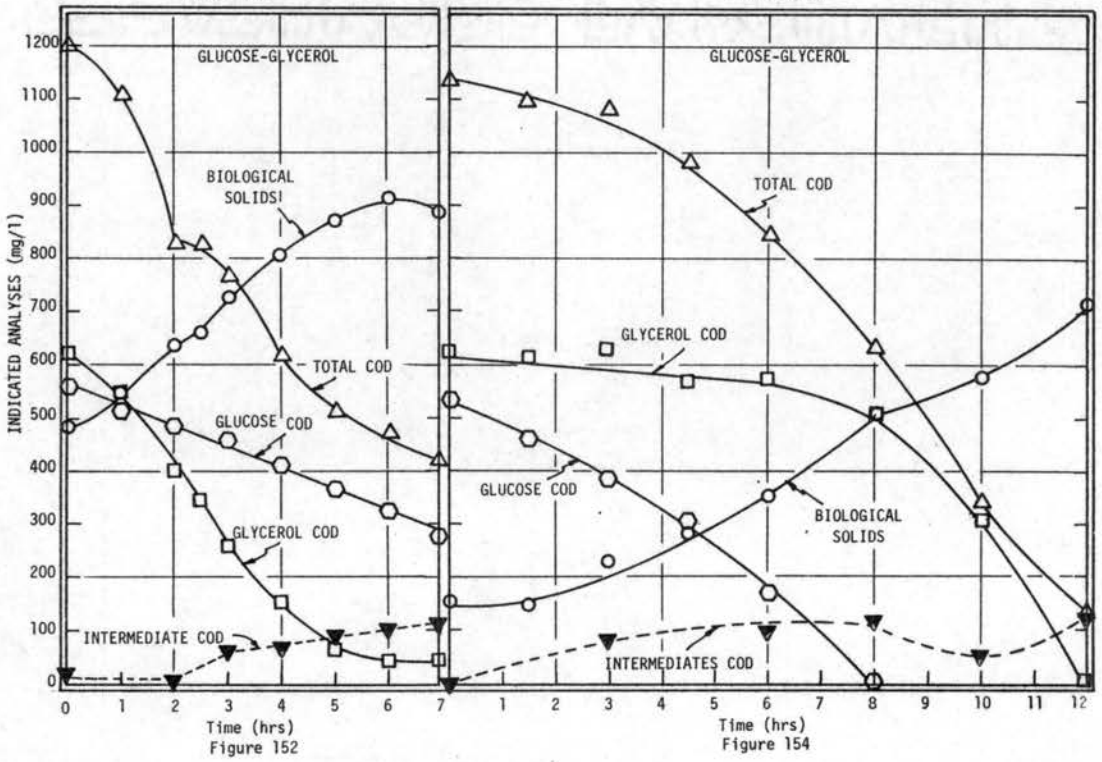



Figure 152




Figure 154

Cells harvested for experimentation under growth conditions exhibited the responses shown in Figures 153 and 154. The growth and substrate removal patterns shown for the control systems indicate that these glycerol-acclimated cells could readily metabolize glucose; indeed, glucose was metabolized at a slightly faster rate than was glycerol. When the compounds were used as a joint carbon source, glucose retarded glycerol removal. Glycerol was not rapidly removed until all of the glucose had been exhausted from the system.

15. Effect of Changing Dilution Rate from 1/12 to 1/4 $hr^{-1}$

Tripling the flow rate from a dilution rate of 1/12 $hr^{-1}$ to 1/4 $hr^{-1}$ caused a severe disruption of the steady state, as can be seen in Figure 155. Effluent COD rose to 1400 mg/l, 1100 mg/l of which was registered as glycerol. Biological solids concentration dropped from nearly 1200 mg/l almost to 400 mg/l. The maximum disruption of the system took place in approximately four hours; thereafter the system began to recover, and by the end of thirty hours had regained a steady state condition. After allowing a period of operation at the new steady state, cells were harvested from the reactor effluent and used to make batch experiments under nonproliferating conditions.

The results of the experimentation in batch systems under nonproliferating conditions are shown in Figures 156 and 157. The results for the control systems indicate that the cells exhibited approximately the same facility for oxidative assimilation of glucose and glycerol. As a combined carbon source, glucose and glycerol were removed concurrently.

On the following day, cells were harvested from the reactor for batch experimentation under growing conditions. The results are shown in Figures 158 and 159. In addition to the usual substrate and the

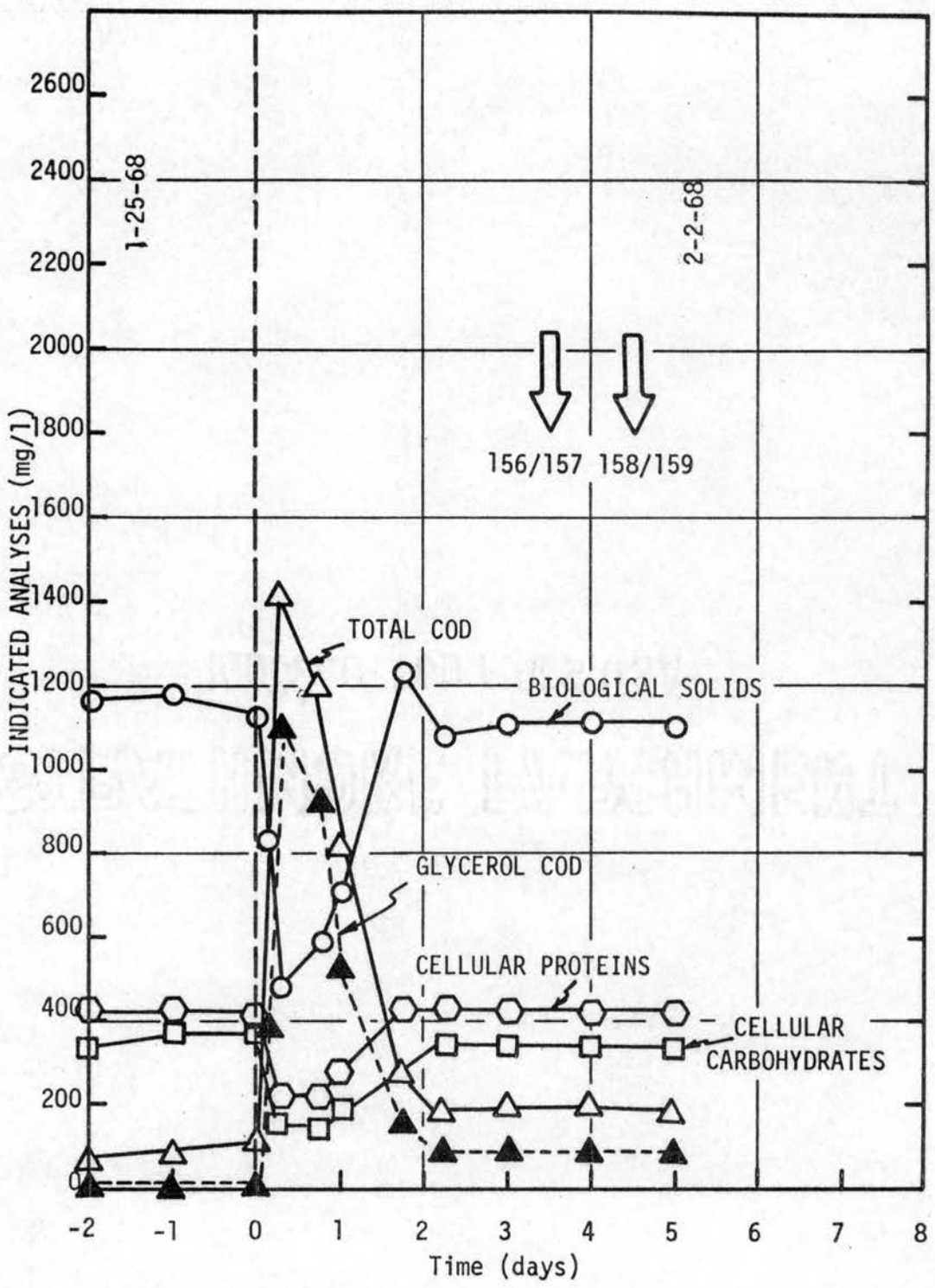



Figure 155 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/12 to 1/4 $hr^{-1}$ During Period of Operation From 1-25-68 to 2-2-68.

Figure 156 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 1-31-68).

Figure 157 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 1-31-68).

Figure 158 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-1-68).

Figure 159 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-1-68).

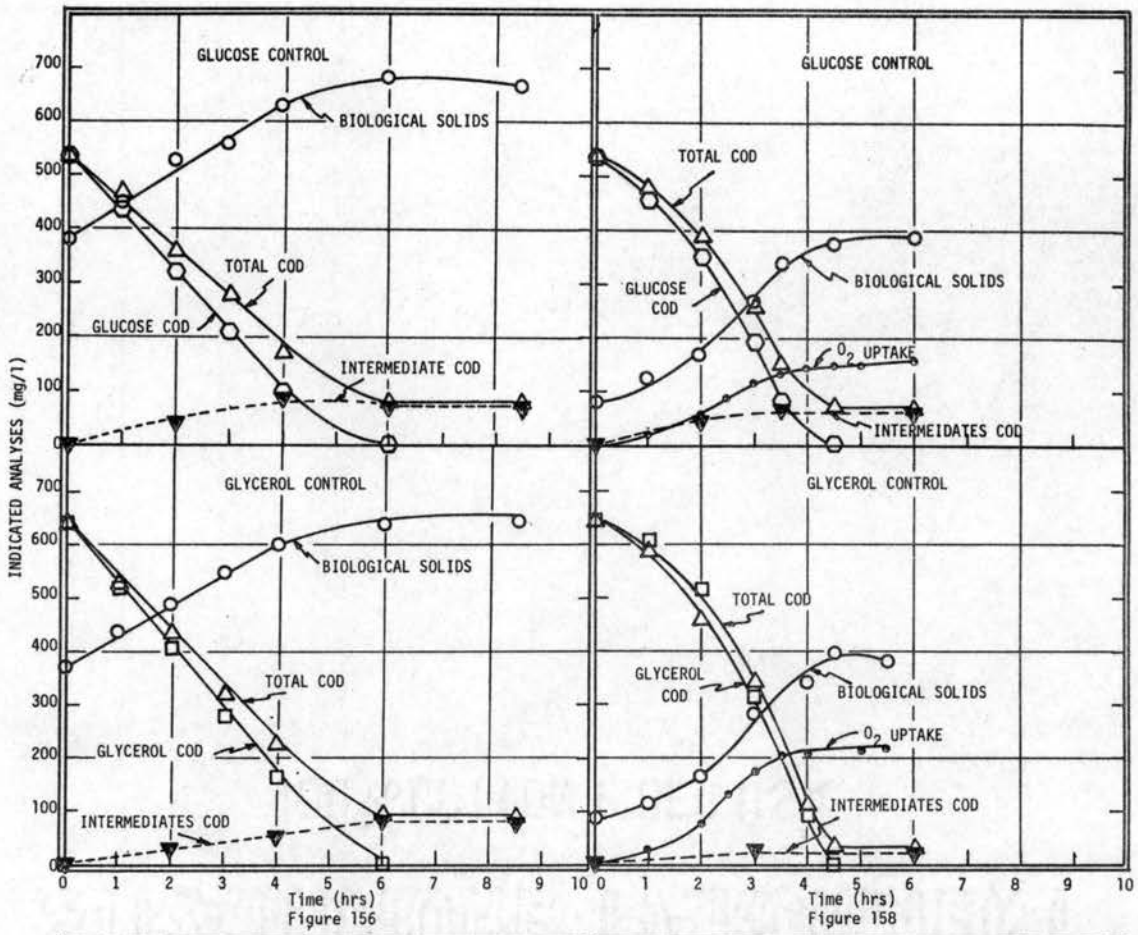



Figure 156




Figure 158

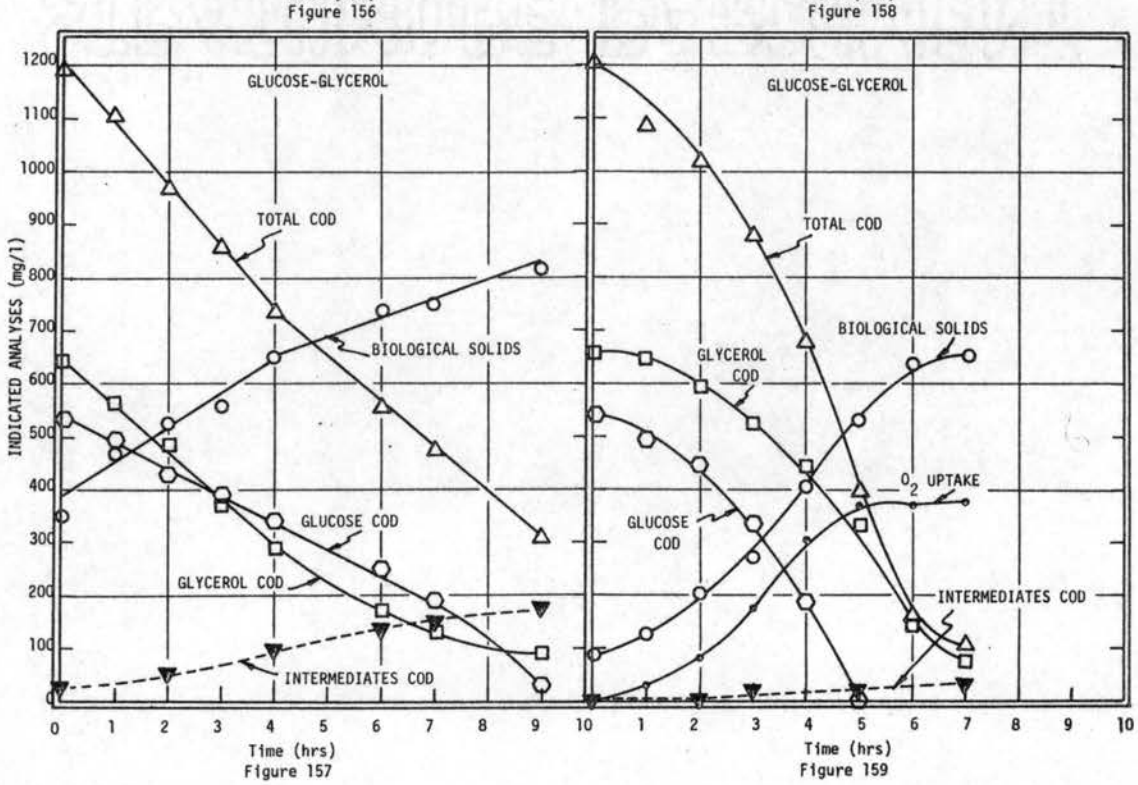



Figure 157




Figure 159

biological solids determinations, oxygen uptake in the Warburg respirometer was measured for the controls and combined substrate systems. The control systems indicate that both compounds could be readily used for growth. When the compounds were used as a combined carbon source they were removed concurrently.

16. Effect of Changing Dilution Rate from 1/4 to 1/24 $hr^{-1}$

Initially, it was planned that the experimentation would be terminated after the dilution rate had been returned by increments to the initial 1/4 $hr^{-1}$ rate. However, since it had been seen that at times in response to some of the changes in dilution rate there were severe changes in microbial predominance, it was decided to subject the continuous flow reactor to a series of rather severe hydraulic shock loads. The previous change represented one in which the cells were required to respond to a three-fold increase in growth rate, and it was next decided to make the cells in the unit respond to a six-fold decrease in growth rate. Accordingly, the dilution rate was changed from 1/4 to 1/24 $hr^{-1}$.

The system response is shown in Figure 160, wherein it is seen that the severe retardation of growth rate caused the cells to dilute out of the system and the effluent COD to rise rather sharply to approximatly 1000 mg/l. However, only 150 mg/l glycerol appeared in the medium at this time. Continued operation at this dilution rate caused severe fluctuations in both biological solids and effluent COD concentration. After eleven days of operation at the new dilution rate, it appeared that the system had regained an equilibrium with respect to biological solids concentration and effluent COD, and cells were harvested for batch experimentation.

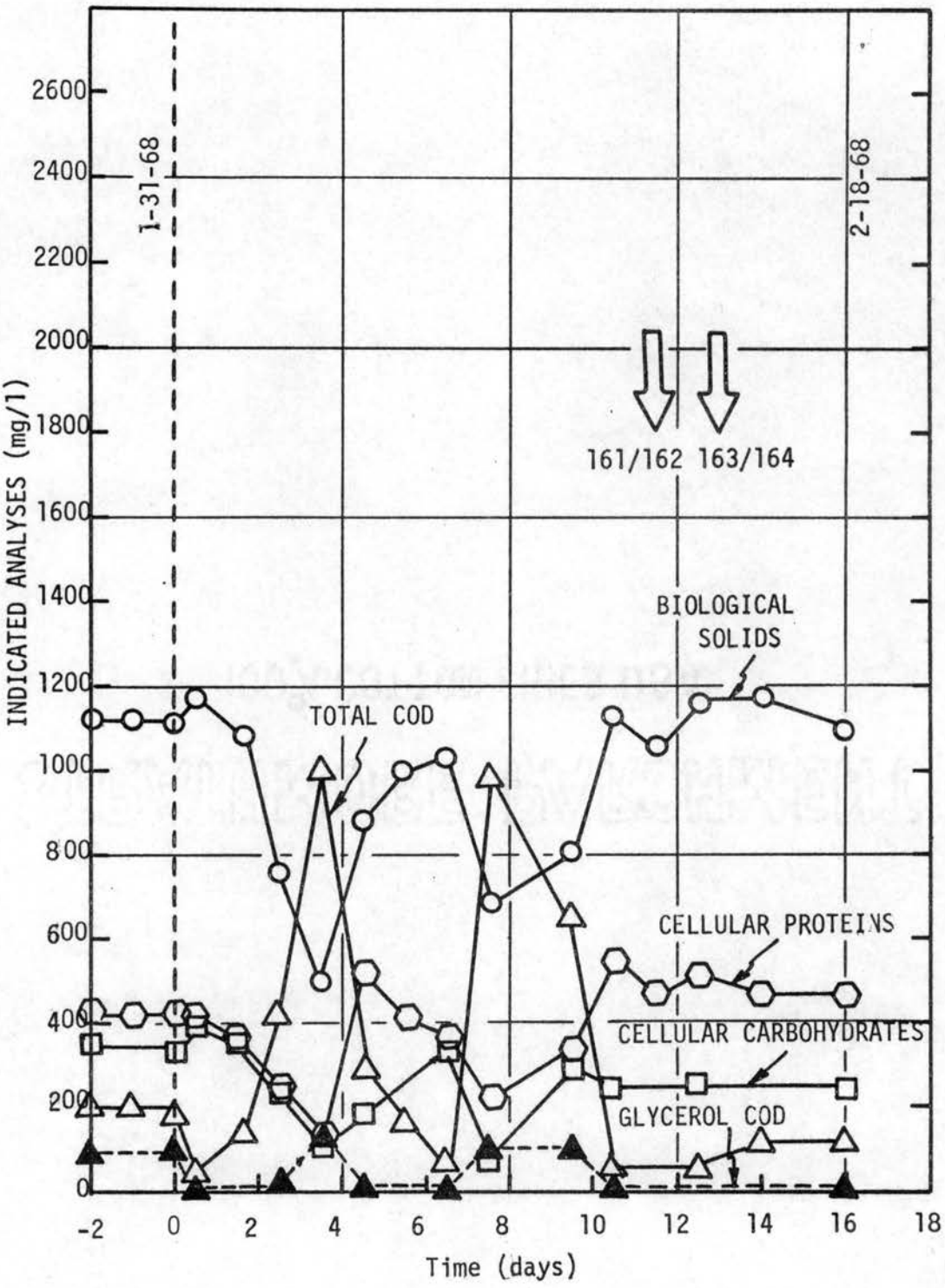



Figure 160 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/4 to 1/24 $hr^{-1}$ During Period of Operation From 1-31-68 to 2-18-68.

Under nonproliferating conditions it may be seen (Figures 161 and 162) that both substrates could be oxidatively assimilated by the glycerol-acclimated sludge with approximately equal facility. When used as combined carbon sources under nonproliferating conditions, the substrates were removed concurrently; however, glycerol was initially removed at a much faster rate than was glucose, and the total COD curve reflects primarily the effect of glycerol removal up to the fourth hour of the experiment. Thereafter, glycerol removal was sharply retarded while glucose continued to be removed at essentially the same rate as it had been removed during the first four hours of the experiment.

Both substrates were readily used by the glycerol-acclimated cells under growth conditions (see Figures 163 and 164); however, glucose was removed more rapidly. The compounds were removed concurrently when employed as a joint carbon source; however, glycerol removal was considerably retarded in the presence of glucose.

On the following day, 20 ml of effluent from the chemostat were used to seed a batch unit containing 2000 mg/l glycerol growth medium, and growth was followed by optical density measurements of the suspension. As the optical density curve approached the end of the log growth phase, cells were harvested from the batch unit, washed in buffer solution, and used for batch experiments to test for the mode of substrate removal. Results of the experiment are shown in Figures 165 and 166. The control systems for this experiment are similar in response to those of the previous batch experiment which was run one day earlier on cells harvested directly from the chemostat effluent (see Figures 163 and 164). The combined system, as in the previous experiment, exhibited concurrent removal of glucose and glycerol. Again, glucose was removed somewhat

Figure 161 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 2-13-68).

Figure 162 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 2-13-68).

Figure 163 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 2-14-68).

Figure 164 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/24 $hr^{-1}$ on 2-14-68).

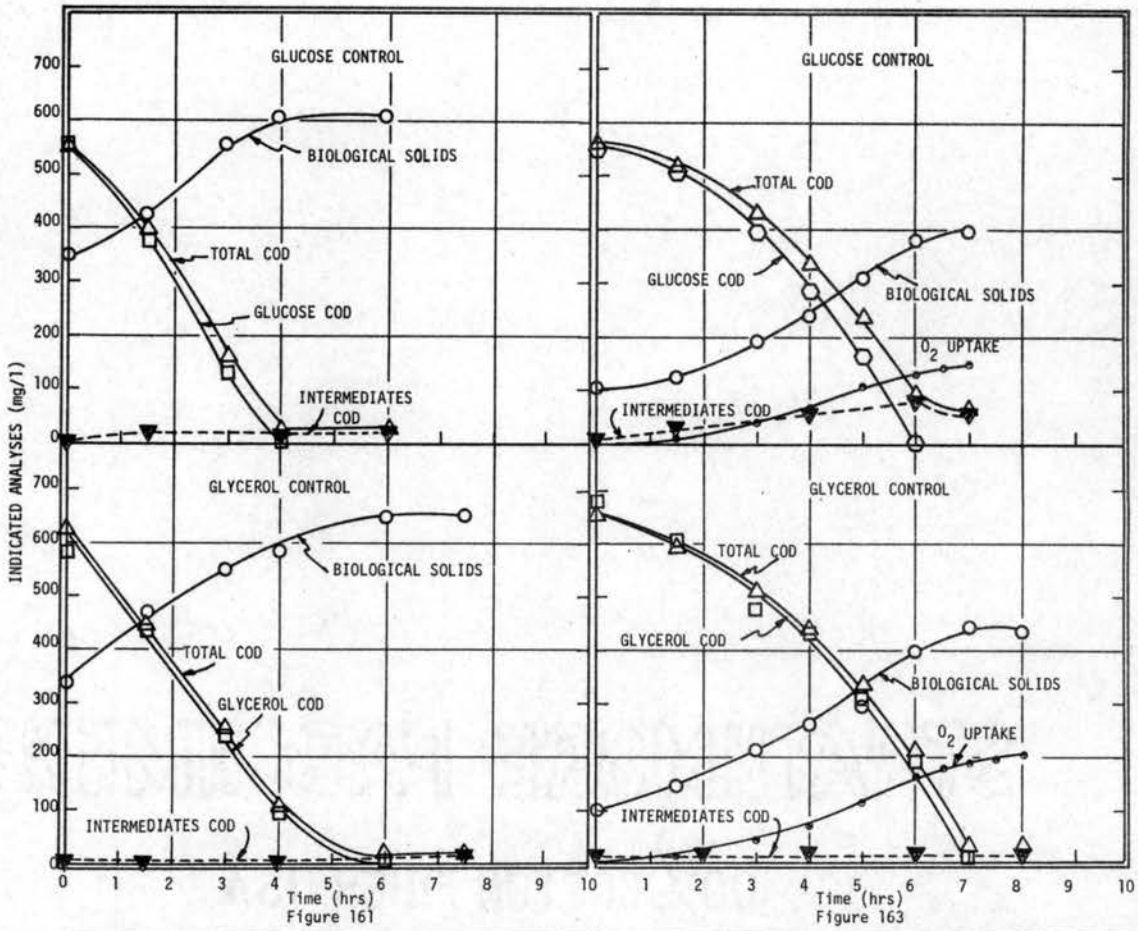



Figure 161




Figure 163

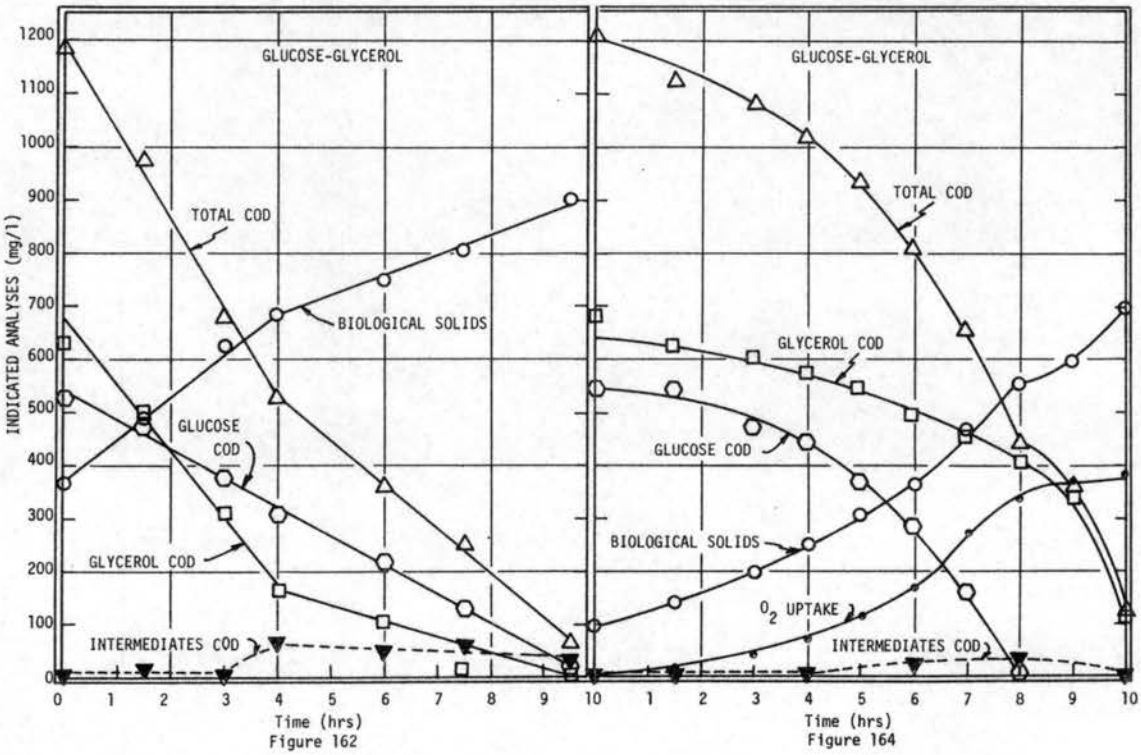



Figure 162




Figure 164

more rapidly than glycerol, but it is clear that glycerol metabolism proceeded while some glucose remained in the system. During the one-day interval between the experiments shown in Figures 163-164 and Figures 165-166, there were no discernible changes in species predominance. The cells taken from the continuous flow unit and used immediately for batch studies had been previously growing in a log growth phase at a rate of 1/24 $hr^{-1}$ ($\mu$ = 0.042 $hr^{-1}$), while the cells which were used for experimentation for the latter experiment were harvested at or near the maximum logarithmic growth rate on glycerol ($\mu$ = 0.143 $hr^{-1}$). Since the observed responses were similar in both cases, it would appear that previous growth rate, of itself, at least for the populations existing in the system at the time these experiments were run, did not affect the mode of substrate removal of glucose and glycerol.

17. Effect of Changing Dilution Rate from 1/24 to 1/4 $hr^{-1}$

After a period of steady state operation at a dilution rate of 1/24 $hr^{-1}$, the reverse shock loading was applied and the cells were forced to respond to a six-fold increase in growth rate. Also, at the time of changing the dilution rate, the airflow rate was returned to a rate of 4000 ml/min. It will be recalled that since the time of experiencing solids buildup in the unit, the airflow rate had been increased to 8000 ml/min. It was known that at either flowrate dissolved oxygen concentration in the reactor was sufficient to ensure aerobic conditions at all times. A return to the original airflow rate while the unit was operating at a relatively high dilution rate would provide a severe condition under which the dissolved oxygen could be continually monitored, thus allowing presentation of dissolved oxygen data. If the unit operated with a high dissolved oxygen content at the fastest growth rate

Figure 165 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (20 ml of Mixed Liquor Taken From Chemostat Effluent at D = 1/24 $hr^{-1}$ on 2-14-68 and Grown in One Liter of 2000 mg/l Glycerol Medium Until the End of Log Growth Phase, Then Washed and Resuspended for Batch Study).

Figure 166 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (20 ml of Cells Taken from Chemostat Effluent at D = 1/24 $hr^{-1}$ on 2-14-68 and Grown in One Liter of 2000 mg/l Glycerol Medium Until the End of Log Growth Phase, Then Washed and Resuspended for Batch Study).

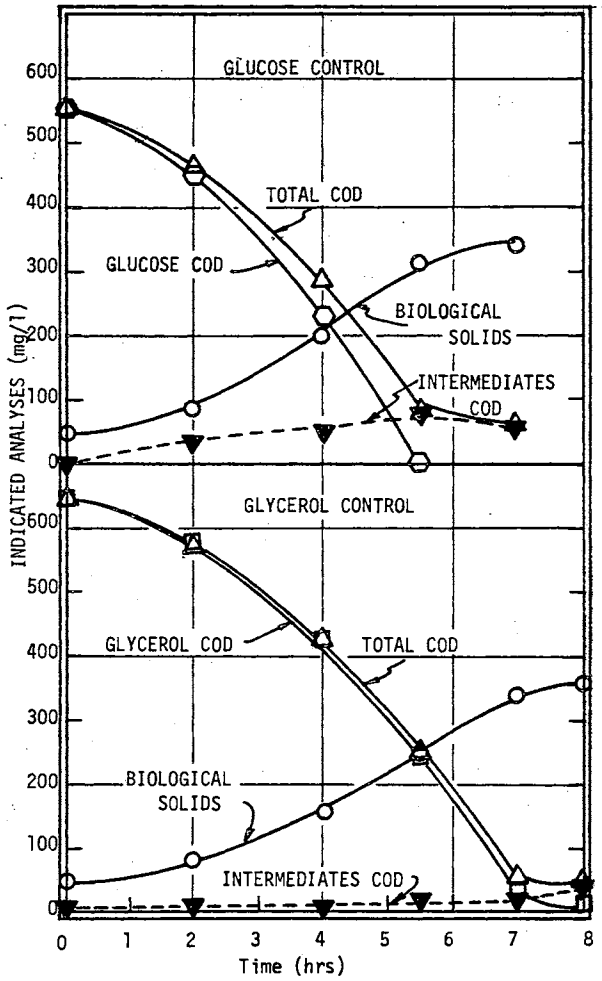



Figure 165

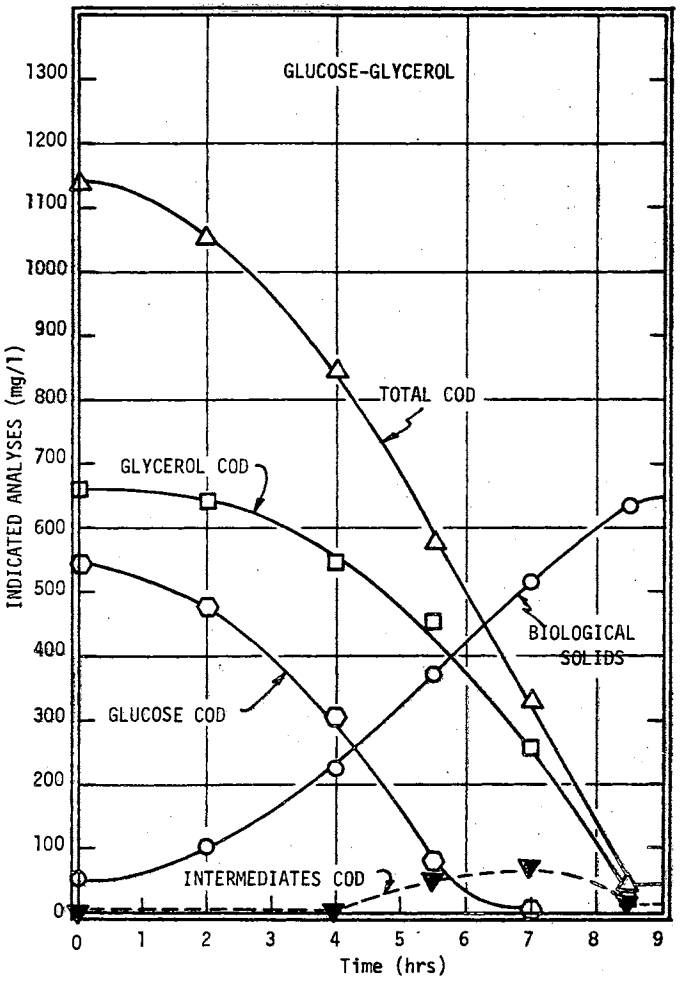



Figure 166

employed, assurance would be provided that it was aerobic at all times during the study.

The response to the increase in growth rate in the continuous flow reactor is shown in Figure 167. It is seen that this change in growth rate caused the greatest disruption of system efficiency yet experienced in the reactor. The biological solids concentration dropped very sharply from 1100 mg/l to 200 mg/l, and all of the incoming substrate passed through the system. However, by the end of the third day of operation the system had regained its initial efficiency. Dissolved oxygen concentration was never below 7 mg/l. During the period of severe disruption (extremely low solids concentration in the reactor), conditions of supersaturation of dissolved oxygen existed. The hydraulic shock loading caused a change in predominance. During the period of solids decrease, the organisms existing before the shock were diluted out of the system, and during the solids recovery a new species predominance was established, as adjudged by the appearance of the mixed liquor in the unit and the appearance of the centrifuged cell pellet. The carbohydrate content of the new population was somewhat higher than that for the population which existed before the change in dilution rate.

Cells were harvested and used for studies under nonproliferating conditions to test for the mode of substrate removal. As may be seen from the behavior of the control systems (Figure 168), the new population could remove both substrates under conditions of oxidative assimilation and on glucose a considerable amount of metabolic intermediates accumulated in the medium as glucose was removed. These intermediates were not metabolized within the 10-hour experimental period. When used as combined carbon sources, both glycerol and glucose were removed more

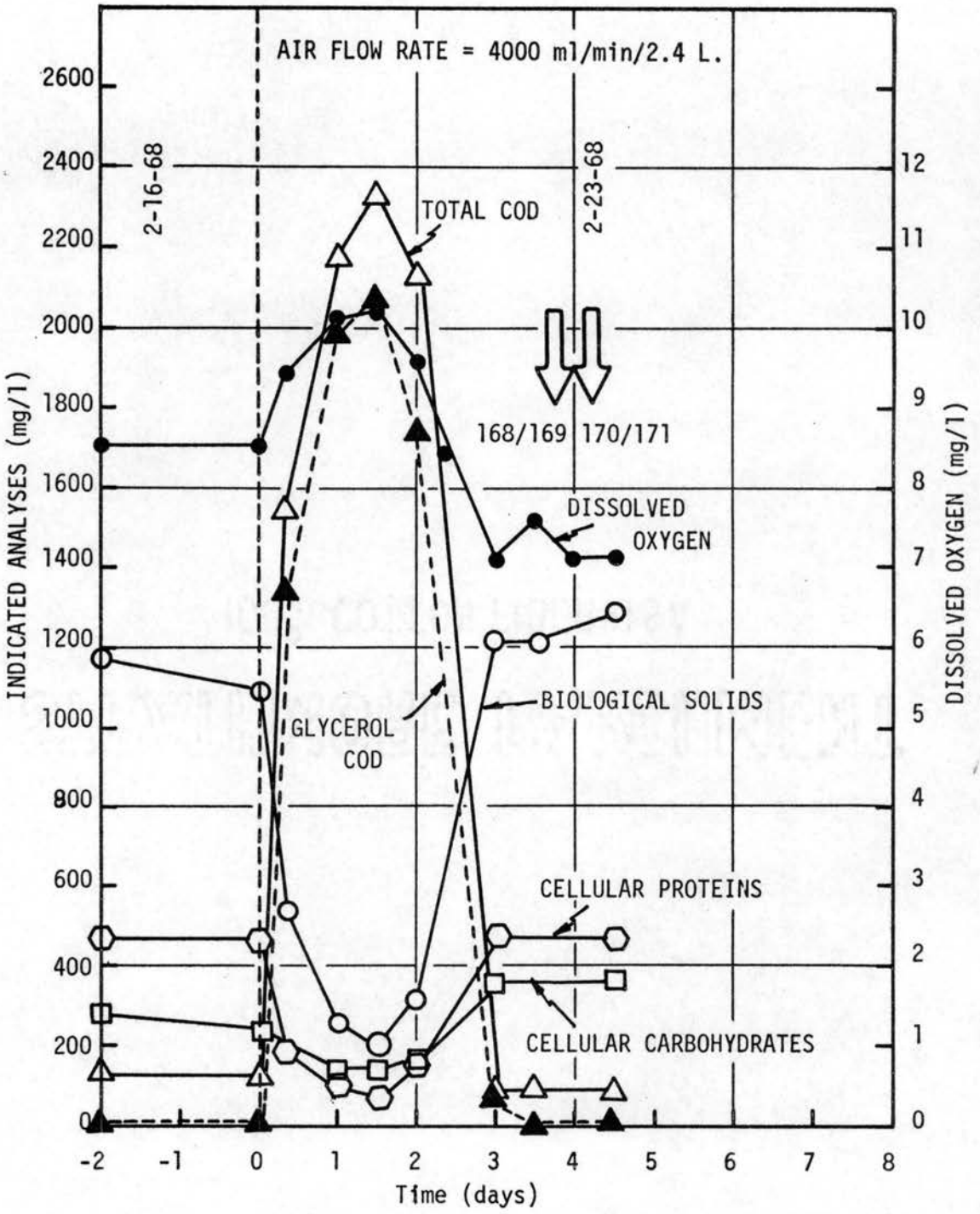



Figure 167 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/24 to 1/4 $hr^{-1}$ During Operation From 2-16-68 to 2-23-68.

Figure 168 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-22-68).

Figure 169 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-22-68).

Figure 170 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-23-68).

Figure 171 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/4 $hr^{-1}$ on 2-23-68).

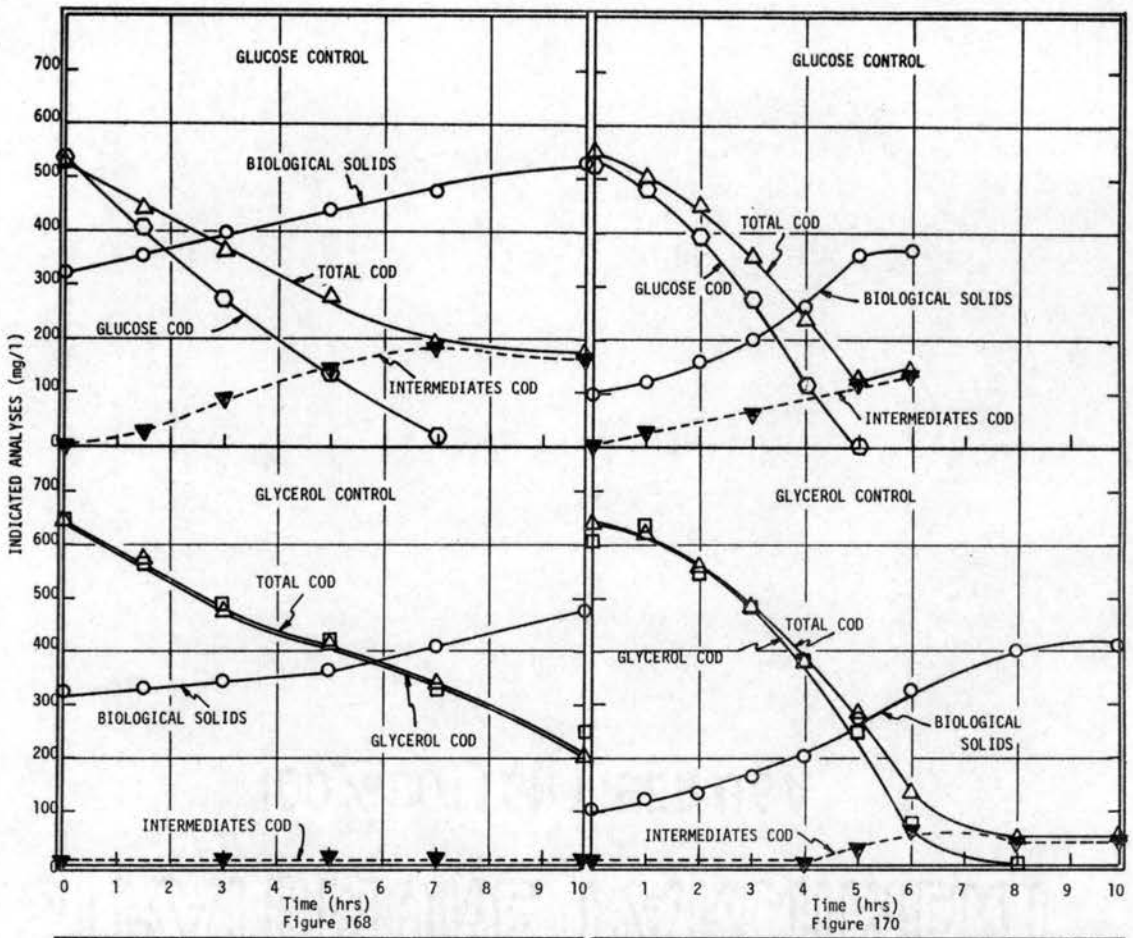



Figure 168




Figure 170

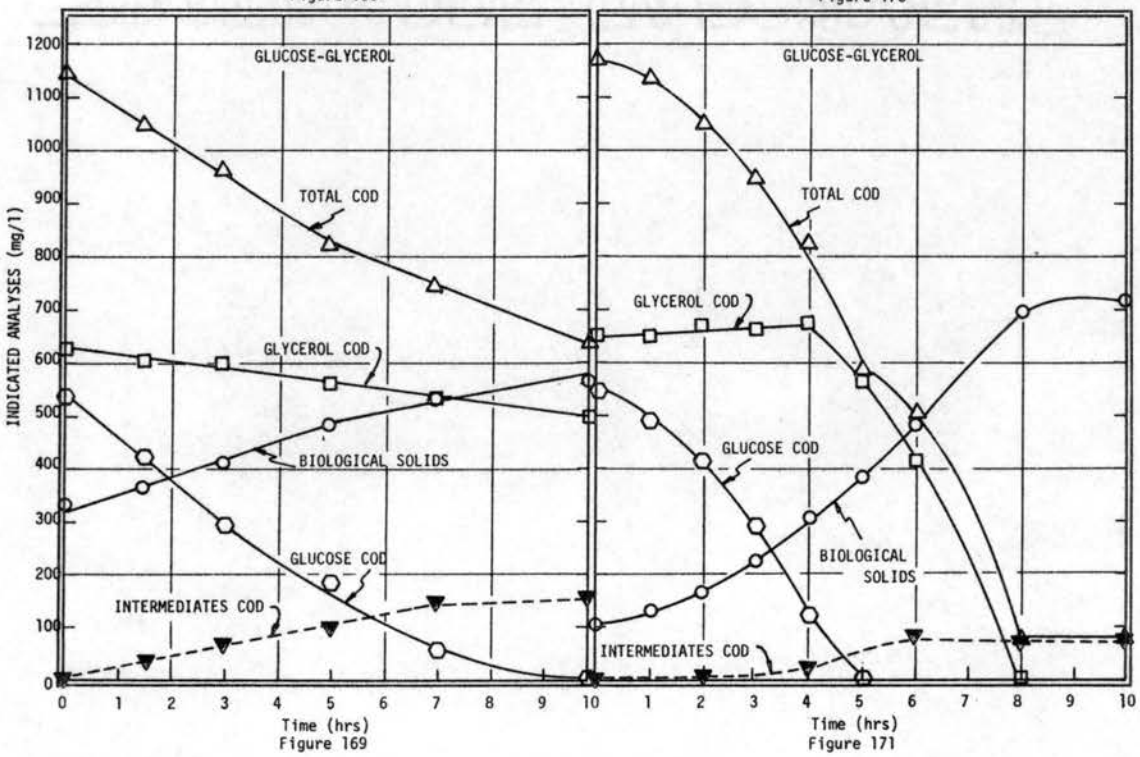



Figure 169




Figure 171

slowly than they were in the control systems, and it appears from the results shown in Figure 169 that glucose retarded glycerol removal to some extent.

Cells were then harvested from the reactor for use in batch studies under growth conditions to assess the mode of substrate removal. The results for the control systems (Figure 170) indicate that under growth conditions glucose was removed more rapidly than was glycerol, and when the two were used in combination (Figure 171), the presence of glucose prevented glycerol removal until all, or nearly all, glucose had been eliminated from the system. The sequential removal of these substrates was also reflected to a slight extent in the total COD curve.

18. Effect of Changing Dilution Rate from 1/4 to 1/96 $hr^{-1}$

The final dilution rate examined in the continuous flow unit was 1/96 $hr^{-1}$. This change in dilution rate required the organisms which had been growing at a logarithmic growth rate of 1/4 $hr^{-1}$ to respond to an imposed growth rate twenty-four times slower than the one to which they had been accustomed. Figure 172 shows this response. The first noticeable change was in the color of the reaction mixed liquor. It assumed a bluish hue six hours after changing dilution rates. After one day, the bluish hue disappeared. During the transient response the biological solids decreased from 1300 mg/l to approximately 620 mg/l. The total COD in the effluent rose from approximately 100 mg/l to approximately 400 mg/l. The system then recovered, and by the seventh day after applying the hydraulic shock loading it appeared that a new steady state condition was attained. The mixed liquor had a milky appearance, and filamentous forms predominated. It appeared that there was a marked change in species predominance compared to the population which existed

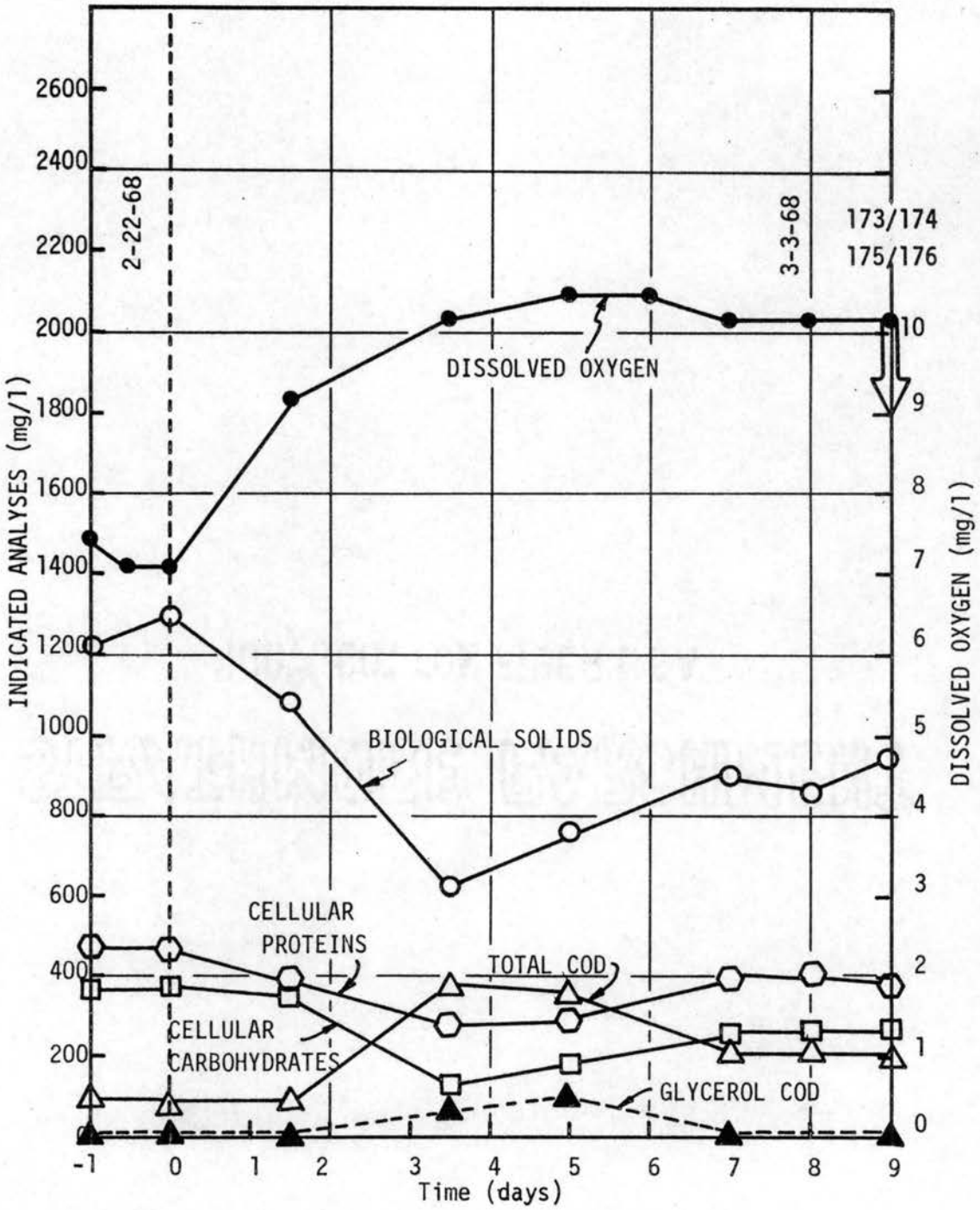



Figure 172 - Response of Continuous Flow Reactor to a Change in Dilution Rate From 1/4 to 1/96 $hr^{-1}$ During Operation From 2-22-68 to 3-3-68.

at the previous dilution rate.

On March 3, 1968, operation of the continuous flow reactor was terminated, and the cells were used for batch experimentation. Figures 173 and 174 show the results of batch experiments to determine the mode of substrate removal under nonproliferating conditions. Control systems indicate that both glucose and glycerol could be oxidatively assimilated with approximately the same facility; however, when the substrates were used as combined carbon sources, the presence of glycerol severely retarded the metabolism of glucose. The compounds were removed essentially in a sequential manner, and the sequential nature of carbon source removal was reflected in both the biological solids and total COD curves.

Cells were also used for studies under growth conditions, and the results are shown in Figures 175 and 176. As in the case of nonproliferating conditions, the results for the control systems indicate that both glycerol and glucose were used with approximately equal facility under growth conditions. When the compounds were used as combined carbon sources, glycerol retained the ability to block glucose removal which it had manifested under the nonproliferating conditions.

Prior to shutting down operation of the continuous flow unit, on March 3, 1968, 20 ml of suspension taken from the effluent of the chemostat were inoculated into one liter of growth medium containing 2000 mg/l glycerol, and aerated. At various times during the subsequent aeration period, optical density measurements were made, and after twenty-five hours of aeration the population had attained the end of the exponential growth phase ($\mu$ = 0.071 $hr^{-1}$). At this time the cells were harvested and washed, and resuspended in various growth media for the

Figure 173 - Metabolic Response Under Nonproliferating Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 3-3-68).

Figure 174 - Metabolic Response Under Nonproliferating Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 3-3-68).

Figure 175 - Metabolic Response Under Growing Conditions in Glucose and Glycerol Controls (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 3-3-68).

Figure 176 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (Cells Harvested at Dilution Rate 1/96 $hr^{-1}$ on 3-3-68).

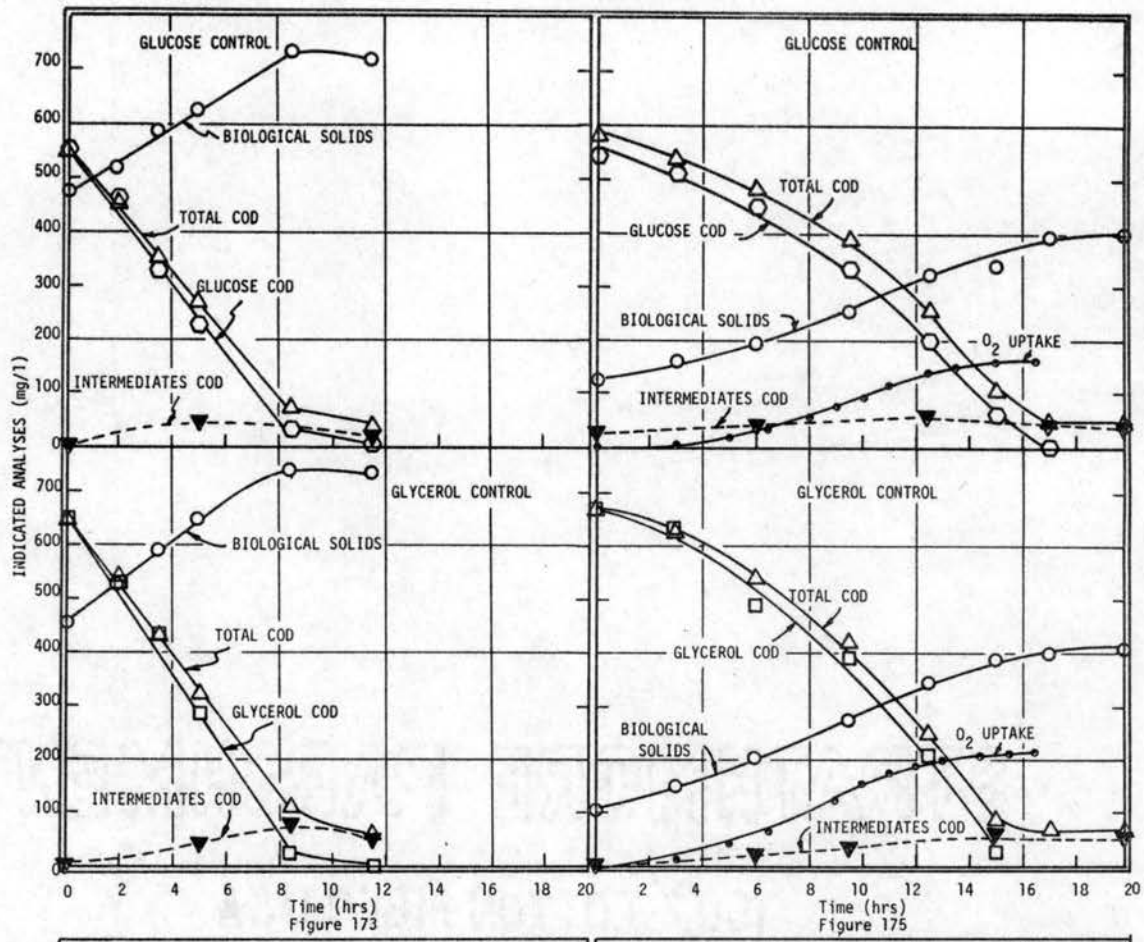



Figure 173




Figure 175

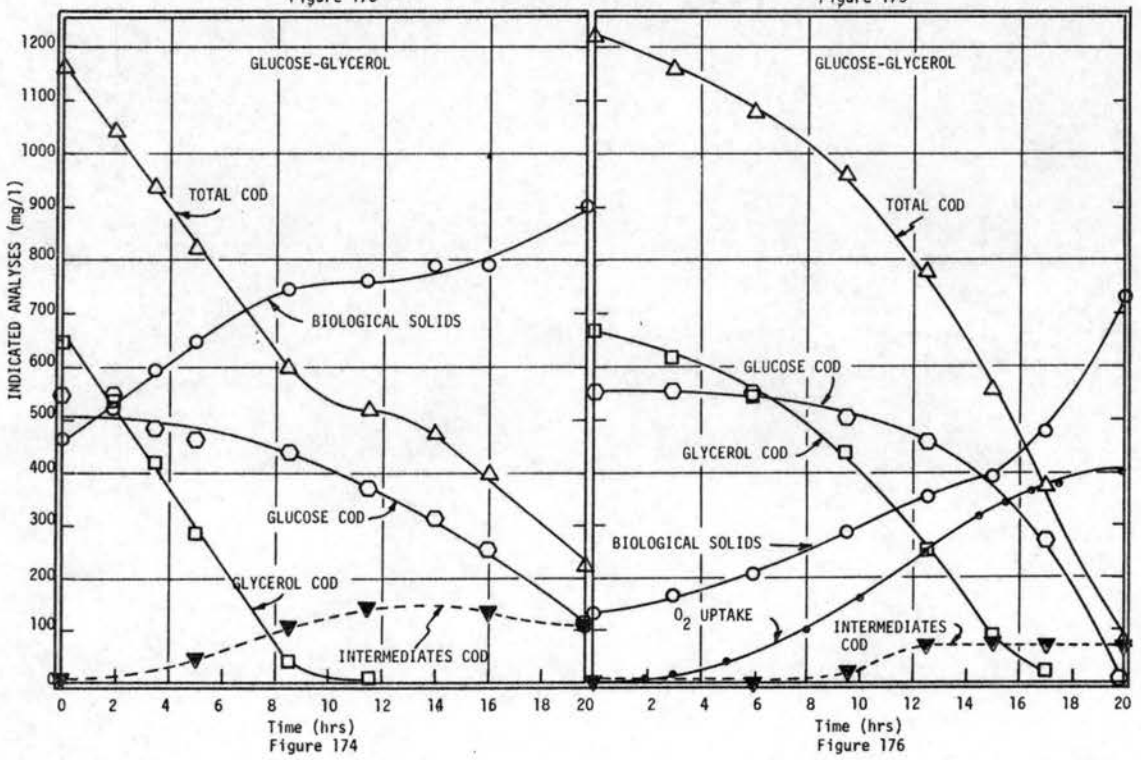



Figure 174




Figure 176

typical batch experiments to determine the mode of substrate removal. The results are shown in Figures 177 and 178. The behavior of the control systems was very much the same as that observed in the previous figure, i.e., both glucose and glycerol were used as sole carbon sources with approximately equal facility. However, when used as carbon sources, it was seen that glucose and glycerol were removed concurrently. There was no readily discernible difference in species predominance between the population used for the experimentation shown in Figures 175 and 176, and that used for the experimentation shown in Figures 177 and 178, but growth through one logarithmic growth cycle did affect the ability of glycerol to interfere with removal of glucose.

19. Summary of Data For Phase B

The biological responses of heterogeneous populations harvested from the chemostat at various dilution rates are summarized in Table VIII. Explanations of column headings, symbols, and methods of calculation are given following the table.

Figure 177 - Metabolic Response under Growing Conditions in Glucose and Glycerol Controls (20 ml of Mixed Liquor Taken From Chemostat at D = 1/96 $hr^{-1}$ on 3-3-68 and Grown in One Liter of 2000 mg/l Glycerol Medium Until the End of Log Growth Phase, Then Washed and Resuspended for Batch Study.

Figure 178 - Metabolic Response Under Growing Conditions in Glucose-Glycerol Combined Unit (20 ml of Mixed Liquor Taken from Chemostat at D = 1/96 $hr^{-1}$ on 3-3-68 and Grown in One Liter of 2000 mg/l Glycerol Medium Until the End of Log Growth Phase, Then Washed and Resuspended for Batcj Study.

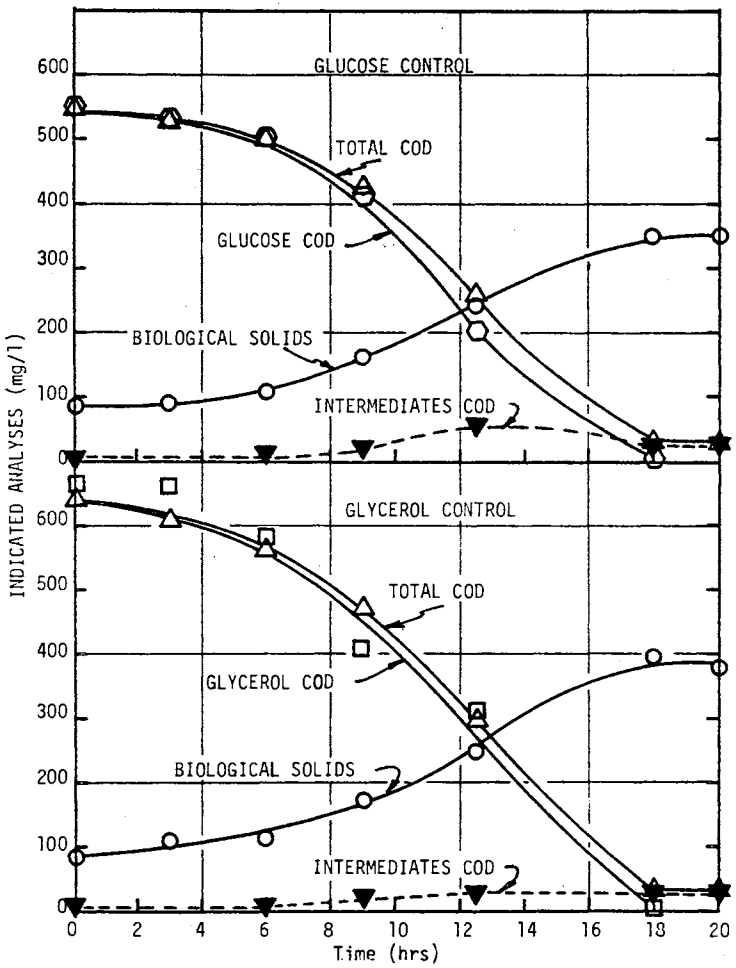



Figure 177

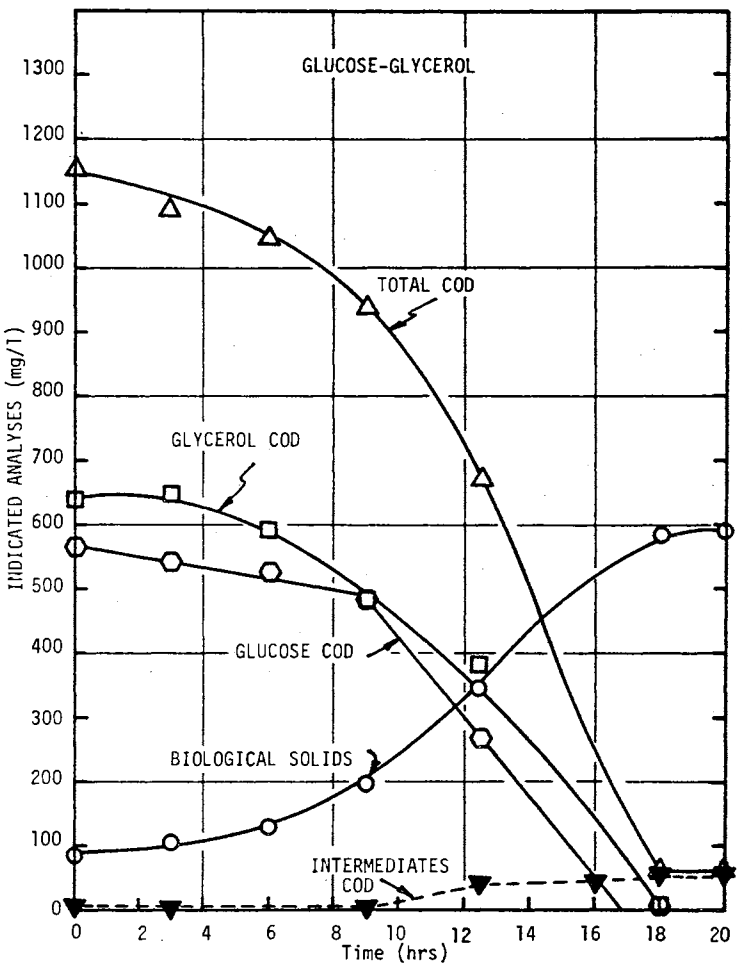



Figure 178

TABLE VIII

BIOLOGICAL RESPONSES OF HETEROGENEOUS POPULATIONS HARVESTED FROM THE CHEMOSTAT AT VARIOUS DILUTION RATES
(Batch Experiments: Glucose Control, Glycerol Control, Combined System)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Substrate COD Removal Rates | | | |
| Fig. No. | Dilution Rate $hr^{-1}$ | Biological Solids High or Low | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Growth Rate, $\mu$ $hr^{-1}$ or mg/l/hr | Total COD $hr^{-1}$ or mg/l/hr | Glucose $hr^{-1}$ or mg/l/hr | Glycerol $hr^{-1}$ or mg/l/hr | Mode of Removal |
| 78 | | | glucose | 430 | 640 | 210 | 460 | 45.7 | $\mu_{01}$=60 | $K_{01}$=230 | $K_{01}$=235 | | |
| 78 | | H | glycerol | 430 | 580 | 150 | 480 | 31.3 | $\mu_{01}$=75 | $K_{01}$=170 | | $K_{01}$=250 | Concurrent |
| 79 | | * | combined | 430 | 810 | 380 | 960 | 39.5 | $\mu_{01}$=70 | $K_{01}$=188 | $K_{01}$=235 | $K_{11}$=0.388 | |
| | 1/4 | | | | | | | | | | | | |
| 80 | | | glucose | 60 | 300 | 240 | 500 | 48 | $\mu_{11}$= 0.242 | $K_{11}$=0.438 | $K_{01}$=194 | | Partially |
| 80 | | L | glycerol | 60 | 240 | 180 | 530 | 34 | $\mu_{11}$=0.242 | $K_{11}$=0.510 | | $K_{11}$=0.595 | Sequential |
| 81 | | | combined | 60 | 500 | 440 | 1040 | 42.2 | $\mu_{11}$=0.414 | $K_{11}$=0.670 | $K_{01}$=104 | $K_{12}$=1.075 | |
| 82 | | | glucose | 340 | 510 | 170 | 545 | 31.1 | $\mu_{01}$=45 | $K_{01}$=110 | $K_{01}$=250 | | |
| 82 | | H | glycerol | 340 | 510 | 170 | 550 | 31.0 | $\mu_{11}$=0.074 | $K_{11}$=0.610 | | $K_{11}$=0.788 | Concurrent |
| 83 | | * | combined | 340 | 670 | 330 | 1030 | 32.0 | $\mu_{11}$=0.129; $\mu_{02}$=24 | $K_{11}$=0.548; $K_{02}$=94 | $K_{11}$=0.462 | $K_{11}$=0.600 | |
| | 1/12 | | | | | | | | | | | | |
| 84 | | | glucose | 120 | 390 | 280 | 510 | 55 | $\mu_{11}$=0.267 | $K_{11}$=0.625 | $K_{11}$=0.74 | | Concurrent |
| 84 | | L | glycerol | 120 | 350 | 230 | 520 | 44.3 | $\mu_{11}$=0.233 | $K_{11}$=0.73 | | $K_{11}$=0.73 | but |
| 85 | | | combined | 120 | 610 | 490 | 1060 | 46.2 | $\mu_{11}$=0.345 | $K_{11}$=0.73 | $K_{11}$=0.73 | $K_{11}$=0.457 | Inhibitory |
| 87 | | | glucose | 260 | 460 | 200 | 550 | 36.4 | $\mu_{11}$=0.069 | $K_{11}$=0.584 | $K_{11}$=0.815 | | |
| 87 | | H | glycerol | 260 | 540 | 280 | 570 | 49.2 | $\mu_{11}$=0.07 | $K_{11}$=0.42 | | $K_{11}$=0.477 | Sequential |
| 88 | | * | combined | 260 | 690 | 430 | 1110 | 38.8 | $\mu_{11}$=0.180 | $K_{11}$=0.598 | $K_{11}$=0.7 | $K_{12}$=0.7 | |
| | 1/24 | | | | | | | | | | | | |
| 89 | | | glucose | 65 | 265 | 200 | 500 | 40 | $\mu_{11}$=0.138 | $K_{11}$=0.343 | $K_{11}$=0.381 | | |
| 89 | | L | glycerol | 65 | 305 | 240 | 600 | 40 | $\mu_{11}$=0.138 | $K_{11}$=0.343 | | $K_{11}$=0.343 | Sequential |
| 90 | | | combined | 55 | 605 | 540 | 1130 | 47.8 | $\mu_{11}$=0.153; $\mu_{02}$= 90 | $K_{11}$=0.343; $K_{02}$=176 | $K_{11}$=0.153 | $K_{02}$=115 | |
| 93 | | | glucse | 780 | 940 | 160 | 360 | 44.5 | $\mu_{11}$=0.925 | $K_{11}$=0.264 | $K_{01}$=520 | | |
| 93 | | H | glycerol | 750 | 950 | 200 | 460 | 43.5 | $\mu_{01}$=75 | $K_{01}$=220 | | $K_{01}$=300 | Concurrent |
| 94 | | | combined | 780 | 1170 | 390 | 880 | 44.3 | $\mu_{01}$=78 | $K_{11}$=0.152; $K_{12}$=0.517 | $K_{01}$=255 | $K_{11}$=1.16 | |
| | 1/4 | | | | | | | | | | | | |
| 95 | | | glucose | 120 | 320 | 200 | 470 | 42.5 | $\mu_{01}$=25; $\mu_{02}$=21 | $K_{01}$=80; $K_{02}$=34 | $K_{11}$=0.885 | | |
| 95 | | L | glycerol | 120 | 320 | 200 | 520 | 38.5 | $\mu_{11}$=0.091 | $K_{11}$=0.765 | | $K_{11}$=0.775 | Concurrent |
| 96 | | | combined | 120 | 450 | 330 | 890 | 37.0 | $\mu_{11}$=0.203 | $K_{11}$=1.10 | $K_{11}$=0.707 | $K_{11}$=0.563 | |

Table VIII (continued)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Substrate COD Removal Rates | | | |
| Fig. No. | Dilution Rate $hr^{-1}$ | Biological Solids High or Low | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Growth Rate, $hr^{-1}$ or mg/l/hr | Total COD $hr^{-1}$ or mg/l/hr | Glucose $hr^{-1}$ or mg/l/hr | Glycerol $hr^{-1}$ or mg/l/hr | Mode of Removal |
| 97 | | | glucose | 590 | 790 | 200 | 460 | 43.5 | $\mu_{11}$=0.61 | $K_{11}$=0.472 | $K_{11}$=2.04 | | |
| 97 | | H | glycerol | 590 | 780 | 190 | 600 | 31.7 | $\mu_{11}$=0.131 | $K_{01}$=300 | | $K_{01}$=320 | Concurrent |
| 98 | | | combined | 590 | 980 | 390 | 1060 | 36.8 | $\mu_{01}$=80 | $K_{01}$=260<br>$K_{12}$=0.4 | $K_{11}$=0.745 | $K_{11}$=0.45 | |
| | 1/4 | | | | | | | | | | | | |
| 99 | | | glucose | 100 | 280 | 180 | 450 | 40 | $\mu_{11}$=0.214 | $K_{11}$=0.646 | $K_{11}$=0.674 | | |
| 99 | | L | glycerol | 100 | 330 | 230 | 580 | 39.7 | $\mu_{11}$=0.21 | $K_{11}$=0.75 | | $K_{11}$=0.75 | Concurrent |
| 100 | | | combined | 100 | 550 | 450 | 1050 | 42.8 | $\mu_{11}$=0.325 | $K_{11}$=0.98 | $K_{11}$=1.035 | $K_{11}$=0.585 | |
| 103 | | | glucose | 420 | 660 | 240 | 485 | 49.5 | $\mu_{01}$=48 | $K_{11}$=0.293 | $K_{11}$=1.15 | | |
| 103 | | H | glycerol | 420 | 575 | 155 | 500 | 31 | $\mu_{01}$=16<br>$\mu_{02}$=48 | $K_{01}$=125<br>$K_{02}$=65 | | $K_{11}$=0.77 | Concurrent |
| 104 | | | combined | 420 | 770 | 350 | 1090 | 32 | $\mu_{01}$=50<br>$\mu_{02}$=15 | $K_{01}$=150<br>$K_{02}$=90 | $K_{01}$=160 | $K_{01}$=175<br>$K_{02}$=33 | |
| | 1/12 | | | | | | | | | | | | |
| 105 | | | glucose | 135 | 335 | 200 | 420 | 47.6 | $\mu_{11}$=0.165 | $K_{11}$=0.564 | $K_{01}$=153 | | |
| 105 | | L | glycerol | 135 | 285 | 150 | 485 | 31 | $\mu_{11}$=0.224 | $K_{11}$=0.72 | | $K_{11}$=0.81 | Concurrent |
| 106 | | | combined | 135 | 495 | 360 | 900 | 40 | $\mu_{11}$=0.172<br>$\mu_{12}$=0.0875 | $K_{11}$=0.94<br>$K_{12}$=0.81 | $K_{11}$=0.8 | $K_{11}$=0.785 | |
| 107 | | | glucose | 20 | 150 | 130 | 380 | 34.2 | $\mu_{11}$=0.131 | $K_{11}$=0.216 | $K_{11}$=0.332 | | Partially |
| 107 | | L | glycerol | 20 | 210 | 190 | 575 | 33.0 | $\mu_{11}$=0.305 | $K_{11}$=0.417 | | $K_{11}$=0.417 | Sequential |
| 108 | | | combined | 20 | 420 | 400 | 1065 | 38.5 | $\mu_{11}$=0.255 | $K_{11}$=0.325 | $K_{11}$=0.407 | $K_{11}$=0.410 | |
| 110 | | | glucose | 150 | 360 | 210 | 510 | 41.2 | $\mu_{01}$=21 | $K_{01}$=55 | $K_{11}$=1.23 | | |
| 110 | | H | glycerol | 150 | 390 | 240 | 630 | 38.1 | $\mu_{11}$=0.202 | $K_{11}$=0.627 | | $K_{11}$=0.627 | Sequential |
| 111 | | * | combined | 150 | 600 | 450 | 960 | 46.8 | $\mu_{11}$=0.14 | $K_{11}$=0.68 | $K_{11}$=1.19 | $K_{12}$=0.693 | |
| | 1/24 | | | | | | | | | | | | |
| 112 | | | glucose | 60 | 275 | 215 | 510 | 42.2 | $\mu_{01}$=21.5 | $K_{01}$=58.5 | $K_{11}$=1.27 | | |
| 112 | | L | glycerol | 60 | 400 | 340 | 600 | 56.5 | $\mu_{11}$=0.234 | $K_{11}$=0.3 | | $K_{11}$=0.627 | Sequential |
| 113 | | | combined | 60 | 500 | 440 | 1060 | 41.5 | $\mu_{11}$=0.27 | $K_{11}$=0.38 | $K_{11}$=0.675 | $K_{12}$=0.693 | |
| 114 | | | glucose | 510 | 835 | 325 | 500 | 65 | $\mu_{01}$=103 | $K_{01}$=166 | $K_{01}$=190 | | |
| 114 | | H | glycerol | 510 | 810 | 300 | 580 | 51.7 | $\mu_{11}$=0.122 | $K_{11}$=0.242 | | $K_{11}$=0.242 | Concurrent |
| 115 | | | combined | 510 | 1110 | 600 | 1040 | 57.6 | $\mu_{01}$=115<br>$\mu_{02}$=60 | $K_{01}$=200<br>$K_{12}$=0.866 | $K_{11}$=0.445 | $K_{11}$=1.02<br>$K_{12}$=0.866 | |
| | #1 | | | | | | | | | | | | |
| 116 | | | glucose | 220 | 540 | 320 | 500 | 64 | $\mu_{01}$=52.5 | $K_{01}$=83.5 | $K_{01}$=87.5 | | |
| 116 | | L | glycerol | 220 | 560 | 340 | 600 | 56.6 | $\mu_{01}$=45 | $K_{01}$=67 | | $K_{01}$=68 | Concurrent |
| 117 | | | combined | 220 | 730 | 510 | 1050 | 48.5 | $\mu_{01}$=51 | $K_{01}$=93.3<br>$K_{02}$=125 | $K_{01}$=67.5<br>$K_{02}$=41 | $K_{01}$=35<br>$K_{02}$=58.4 | |

Table VIII (continued)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Substrate COD Removal Rates | | |
| Fig. No. | Dilution Rate $hr^{-1}$ | Biological Solids High or Low | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Growth Rate, $hr^{-1}$ or mg/l/hr | Total COD $hr^{-1}$ or mg/l/hr | Glucose $hr^{-1}$ or mg/l/hr | Glycerol $hr^{-1}$ or mg/l/hr | Mode of Removal |
| 119 | | | glucose | 110 | 370 | 260 | 500 | 52 | $\mu_{11}=0.162$ | $K_{11}=0.465$ | $K_{11}=0734$ | | |
| 119 | 1/36 | L | glycerol | 110 | 290 | 180 | 520 | 34.6 | $\mu_{11}=0.162$ | $K_{11}=0.422$ | | $K_{11}=0.525$ | Partially Sequential |
| 120 | | | combined | 110 | 430 | 320 | 900 | 35.6 | $\mu_{11}=0.14$ | $K_{11}=0.465$ | $K_{11}=0.64$ | $K_{01}=26.7$, $K_{12}=0.78$, $K_{03}=26.5$ | |
| 122 | | | glucose | 150 | 400 | 250 | 500 | 50 | $\mu_{11}=0.018$ | $K_{11}=0.334$ | $K_{11}=0.368$ | | |
| 122 | 1/48 | L | glycerol | 150 | 400 | 250 | 580 | 43.2 | $\mu_{11}=0.018$ | $K_{11}=0.334$ | | $K_{11}=0.334$ | Concurrent |
| 123 | | | combined | 150 | 640 | 490 | 1080 | 45.3 | $\mu_{11}=0.083$, $\mu_{12}=0.098$ | $K_{11}=0.334$ | $K_{11}=0.16$ | $K_{11}=0.177$ | |
| 125 | | | glucose | 100 | 310 | 210 | 500 | 50 | $\mu_{11}=0.0765$ | $K_{11}=0.278$ | $K_{11}=0.278$ | | Partially Sequential but Glucose is Inhibited |
| 125 | 1/72 | L | glycerol | 100 | 375 | 275 | 580 | 47.5 | $\mu_{11}=0.0788$ | $K_{11}=0.257$ | | $K_{11}=0.257$ | |
| 126 | | | combined | 100 | 740 | 640 | 1100 | 57 | $\mu_{11}=0.0841$ | $K_{11}=0.22$ | $K_{01}=3.9$, $K_{12}=0.214$ | $K_{11}=0.152$ | |
| 127 | | | glucose | 110 | 355 | 245 | 500 | 49 | $\mu_{11}=0.392$ | $K_{11}=0.457$ | $K_{11}=0.78$ | | |
| 127 | #2 | L | glycerol | 110 | 375 | 265 | 560 | 47.3 | $\mu_{11}=0.405$ | $K_{11}=0.655$ | | $K_{11}=0.655$ | Sequential |
| 128 | | | combined | 110 | 575 | 465 | 1010 | 46 | $\mu_{11}=0.381$ | $K_{11}=0.655$ | $K_{01}=175$ | $K_{01}=30$, $K_{12}=1.82$ | |
| 130 | | | glucose | 60 | 230 | 170 | 450 | 37.8 | $\mu_{11}=0.118$ | $K_{11}=0.155$ | $K_{11}=0.275$ | | |
| 130 | 1/96 | L | glycerol | 60 | 290 | 230 | 600 | 38.4 | $\mu_{11}=0.118$ | $K_{11}=0.155$ | | $K_{11}=0.155$ | Partially Sequential |
| 131 | | | combined | 60 | 480 | 420 | 1100 | 38.2 | $\mu_{11}=0.1$ | $K_{11}=0.181$ | $K_{01}=23.7$, $K_{12}=0.35$ | $K_{12}=0.231$ | |
| 132 | | | glucose | 35 | 285 | 250 | 560 | 44.6 | $\mu_{11}=0.333$ | $K_{11}=0.2$ | $K_{11}=0.2$ | | |
| 132 | #3 | L | glycerol | 35 | 350 | 315 | 530 | 59.5 | $\mu_{11}=0.247$ | $K_{11}=0.22$ | | $K_{11}=0.22$ | Concurrent |
| 133 | | | combined | 35 | 600 | 565 | 1160 | 48.7 | $\mu_{11}=0.277$ | $K_{11}=0.231$ | $K_{11}=0.204$ | $K_{11}=0.227$ | |
| 135 | | | glucose | 60 | 170 | 110 | 530 | 20.8 | $\mu_{11}=0.075$ | $K_{11}=0.110$ | $K_{11}=0.110$ | | Concurrent |
| 135 | 1/192 | L | glycerol | 60 | 150 | 90 | 520 | 17.3 | $\mu_{11}=0.036$ | $K_{11}=0.091$ | | $K_{11}=0.091$ | but |
| 136 | | | combined | 60 | 330 | 270 | 1080 | 25 | $\mu_{11}=0.046$ | $K_{11}=0.13$ | $K_{11}=0.0975$ | $K_{11}=0.14$ | Inhibitory |
| 138 | | | glucose | 20 | 220 | 200 | 510 | 39.2 | $\mu_{11}=0.011$ | $K_{11}=0.187$ | $K_{11}=0.180$ | | Concurrent |
| 138 | 1/192 | L | glycerol | 20 | 155 | 135 | 535 | 25.3 | $\mu_{11}=0.0102$ | $K_{11}=0.11$ | | $K_{11}=0.115$ | but |
| 139 | | | combined | 20 | 520 | 500 | 1090 | 45.8 | $\mu_{11}=0.157$ | $K_{11}=0.16$ | $K_{11}=0.180$ | $K_{11}=0.175$ | Inhibitory |

Table VIII (continued)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Substrate COD Removal Rates | | | |
| Fig. No. | Dilution Rate $hr^{-1}$ | Biological Solids High or Low | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Growth Rate, $hr^{-1}$ or mg/l/hr | Total COD $hr^{-1}$ or mg/l/hr | Glucose $hr^{-1}$ or mg/l/hr | Glycerol $hr^{-1}$ or mg/l/hr | Mode of Removal |
| 140 | | | glucose | 200 | 270 | 70 | 330 | 21.2 | $\mu_{01}$=2.4 | $K_{01}$=40 | $K_{01}$=65 | | |
| 140 | 1/96 | L | glycerol | 200 | 330 | 130 | 590 | 22 | $\mu_{11}$=0.0365 | $K_{11}$=0.0985 | | $K_{11}$=0.105 | Sequential |
| 141 | | | combined | 200 | 640 | 440 | 1100 | 40 | $\mu_{01}$=5<br>$\mu_{12}$=0.166 | $K_{01}$=27.5<br>$K_{12}$=0.144 | $K_{11}$=0.194 | $K_{12}$=0.214 | |
| 143 | | | glucose | 130 | 360 | 230 | 540 | 42.5 | $\mu_{11}$=0.0845 | $K_{11}$=0.193 | $K_{11}$=0.245 | | |
| 143 | 1/48 | L | glycerol | 130 | 400 | 270 | 620 | 43.5 | $\mu_{11}$=0.0455 | $K_{11}$=0.163 | | $K_{11}$=0.163 | Concurrent |
| 144 | | | combined | 130 | 670 | 540 | 1090 | 49.5 | $\mu_{11}$=0.0825 | $K_{11}$=0.201 | $K_{11}$=0.139 | $K_{11}$=0.210 | |
| 146 | | | glucose | 260 | 450 | 190 | 580 | 32.8 | $\mu_{11}$=0.173 | $K_{11}$=0.0905 | $K_{11}$=0.1 | | |
| 146 | | H | glycerol | 260 | 520 | 260 | 665 | 39.2 | $\mu_{11}$=0.22 | $K_{11}$=0.134 | | $K_{11}$=0.173 | Concurrent but Glucose is Inhibited |
| 147 | 1/24 | | combined | 260 | 570 | 310 | 860 | 36.2 | $\mu_{11}$=0.17 | $K_{11}$=0.0375 | $K_{01}$=13.8 | $K_{11}$=0.132 | |
| 148 | | | glucose | 120 | 340 | 220 | 540 | 40.8 | $\mu_{11}$=0.0593 | $K_{11}$=0.268 | $K_{11}$=0.268 | | |
| 148 | | L | glycerol | 120 | 360 | 240 | 660 | 36.4 | $\mu_{01}$=14.5 | $K_{01}$=17.5<br>$K_{02}$=60 | | $K_{01}$=33.7<br>$K_{02}$=43.7 | Concurrent but Glucose is |
| 149 | | | combined | 120 | 650 | 530 | 1240 | 42.8 | $\mu_{11}$=0.103 | $K_{11}$=0.26 | $K_{11}$=0.232 | $K_{11}$=0.119 | Inhibited |
| 151 | | | glucose | 470 | 770 | 300 | 490 | 61 | $\mu_{11}$=0.16 | $K_{11}$=0.788 | $K_{11}$=0.8 | | |
| 151 | | H | glycerol | 470 | 785 | 315 | 630 | 50 | $\mu_{01}$=95 | $K_{01}$=145 | | $K_{01}$=150 | Concurrent but Glucose is Inhibited |
| 152 | 1/12 | | combined | 470 | 915 | 445 | 785 | 57 | $\mu_{11}$=0.116<br>$\mu_{12}$=0.55 | $K_{11}$=1.41<br>$K_{12}$=0.19 | $K_{01}$=39.2 | $K_{11}$=0.642 | |
| 153 | | | glucose | 150 | 380 | 230 | 450 | 51.2 | $\mu_{11}$=0.142 | $K_{11}$=0.435 | $K_{11}$=0.517 | | |
| 153 | | L | glycerol | 150 | 400 | 250 | 580 | 43.2 | $\mu_{11}$=0.133 | $K_{11}$=0.49 | | $K_{11}$=0.49 | Sequential |
| 154 | | | combined | 150 | 730 | 580 | 1000 | 58 | $\mu_{11}$=0.137<br>$\mu_{11}$=0.084 | $K_{11}$=0.505 | $K_{11}$=0.33 | $K_{01}$=10<br>$K_{12}$=0.522 | |
| 156 | | | glucose | 380 | 680 | 300 | 455 | 66 | $\mu_{01}$=62.5 | $K_{01}$=86 | $K_{01}$=111 | | |
| 156 | | H | glycerol | 380 | 650 | 270 | 540 | 50 | $\mu_{01}$=60 | $K_{01}$=102 | | $K_{01}$=118 | Concurrent |
| 157 | 1/4 | | combined | 380 | 820 | 440 | 870 | 50.5 | $\mu_{01}$=68.6<br>$\mu_{02}$=36 | $K_{01}$=115<br>$K_{02}$=84 | $K_{01}$=48.6 | $K_{01}$=87.5<br>$K_{12}$=0.25 | |
| 158 | | | glucose | 80 | 390 | 310 | 470 | 65.8 | $\mu_{11}$=0.423 | $K_{11}$=0.85 | $K_{11}$=0.855 | | |
| 158 | | L | glycerol | 80 | 400 | 320 | 600 | 53.5 | $\mu_{11}$=0.33 | $K_{11}$=0.75 | | $K_{11}$=0.75 | Concurrent |
| 159 | | | combined | 80 | 650 | 570 | 1100 | 51.7 | $\mu_{11}$=0.423 | $K_{11}$=0.775 | $K_{11}$=0.458 | $K_{11}$=0.440 | |

Table VIII (concluded)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Substrate COD Removal Rates | | | |
| Fig. No. | Dilution Rate $hr^{-1}$ | Biological Solids High or Low | Carbon Source | Initial Solids mg/l | Peak Solids mg/l | Δ Solids mg/l | Total COD Removed mg/l | Cell Yield % | Growth Rate, $hr^{-1}$ or mg/l/hr | Total COD $hr^{-1}$ or mg/l/hr | Glucose $hr^{-1}$ or mg/l/hr | Glycerol $hr^{-1}$ or mg/l/hr | Mode of Removal |
| 161 | | | glucose | 350 | 620 | 270 | 530 | 51 | $\mu_{01}=65$ | $K_{01}=132$ | $K_{01}=138$ | | |
| 161 | | H | glycerol | 350 | 650 | 300 | 600 | 50 | $\mu_{01}=66.6$ | $K_{01}=130$ | | $K_{01}=130$ | Concurrent |
| 162 | | | combined | 350 | 900 | 550 | 1120 | 49 | $\mu_{01}=78.8$<br>$\mu_{02}=36.5$ | $K_{01}=164$<br>$K_{02}=121$ | $K_{01}=54$ | $K_{01}=122$<br>$K_{02}=30$ | |
| | 1/24 | | | | | | | | | | | | |
| 163 | | | glucose | 100 | 400 | 300 | 500 | 60 | $\mu_{11}=0.24$ | $K_{11}=0.584$ | $K_{11}=0.6$ | | Concurrent |
| 163 | | L | glycerol | 100 | 450 | 350 | 620 | 56.5 | $\mu_{11}=0.24$ | $K_{11}=0.584$ | | $K_{11}=0.584$ | but |
| 164 | | | combined | 100 | 700 | 600 | 1090 | 55 | $\mu_{11}=0.25$<br>$\mu_{12}=0.12$ | $K_{11}=0.375$<br>$K_{12}=1.375$ | $K_{11}=0.414$ | $K_{11}=0.433$ | Inhibitory |
| 165 | | | glucose | 50 | 340 | 290 | 480 | 60.5 | $\mu_{11}=0.2$ | $K_{11}=0.5$ | $K_{11}=0.58$ | | Concurrent |
| 165 | #4 | L | glycerol | 50 | 350 | 300 | 600 | 50 | $\mu_{11}=0.166$ | $K_{11}=0.55$ | | $K_{11}=0.55$ | but |
| 166 | | | combined | 50 | 630 | 580 | 1100 | 52.7 | $\mu_{11}=0.376$ | $K_{11}=0.6$ | $K_{11}=0.58$ | $K_{11}=0.55$ | Inhibitory |
| 168 | | | glucose | 320 | 525 | 205 | 355 | 57.7 | $\mu_{01}=24$ | $K_{01}=51$ | $K_{01}=83.4$ | | |
| 168 | | H | glycerol | 320 | 480 | 160 | 450 | 35.6 | $\mu_{01}=9$<br>$\mu_{02}=23$ | $K_{01}=55$<br>$K_{02}=44$ | | $K_{01}=55$<br>$K_{02}=44$ | Sequential |
| 169 | | | combined | 320 | 565 | 245 | 510 | 48 | $\mu_{01}=32$<br>$\mu_{02}=20$ | $K_{01}=64$<br>$K_{02}=38$ | $K_{01}=70$ | $K_{01}=13$ | |
| | 1/4 | | | | | | | | | | | | |
| 170 | | | glucose | 100 | 370 | 270 | 430 | 62.8 | $\mu_{11}=0.248$ | $K_{11}=0.626$ | $K_{11}=0.80$ | | |
| 170 | | L | glycerol | 100 | 400 | 300 | 590 | 50.7 | $\mu_{11}=0.215$ | $K_{11}=0.59$ | | $K_{11}=0.59$ | Sequential |
| 171 | | | combined | 100 | 720 | 620 | 1090 | 57 | $\mu_{11}=0.27$ | $K_{11}=0.692$<br>$K_{12}=0.935$ | $K_{11}=0.805$ | $K_{12}=0.995$ | |
| 173 | | | glucose | 475 | 735 | 360 | 510 | 70.5 | $\mu_{01}=42.4$ | $K_{01}=60$ | $K_{01}=59$ | | Partially Sequential but Glucose is Inhibited |
| 173 | | H | glycerol | 455 | 740 | 385 | 580 | 66.4 | $\mu_{01}=45.3$ | $K_{01}=68.3$ | | $K_{01}=73$ | |
| 174 | | | combined | 465 | 915 | 450 | 940 | 48 | $\mu_{01}=38$<br>$\mu_{02}=20$ | $K_{01}=66$<br>$K_{02}=43.4$ | $K_{11}=0.33$ | $K_{01}=70.5$ | |
| | 1/96 | | | | | | | | | | | | |
| 175 | | | glucose | 120 | 405 | 275 | 530 | 52 | $\mu_{11}=0.075$ | $K_{11}=0.275$ | $K_{11}=0.275$ | | Partially Sequential but Glucose is Inhibited |
| 175 | | L | glycerol | 120 | 420 | 300 | 600 | 50 | $\mu_{11}=0.103$ | $K_{11}=0.268$ | | $K_{11}=0.268$ | |
| 176 | | | combined | 120 | 720 | 600 | 1140 | 57.5 | $\mu_{11}=0.075$<br>$\mu_{11}=0.122$ | $K_{11}=0.268$ | $K_{12}=0.283$ | $K_{11}=0.268$ | |
| 177 | | | glucose | 80 | 350 | 270 | 510 | 53 | $\mu_{11}=0.142$ | $K_{11}=0.289$ | $K_{11}=0.289$ | | Partially Sequential but Glucose is Inhibited |
| 177 | #5 | L | glycerol | 80 | 400 | 320 | 610 | 52.5 | $\mu_{11}=0.142$ | $K_{11}=0.289$ | | $K_{11}=0.289$ | |
| 178 | | | combined | 80 | 590 | 510 | 1090 | 46.8 | $\mu_{11}=0.146$ | $K_{11}=0.247$ | $K_{01}=9$<br>$K_{02}=65$ | $K_{11}=0.316$ | |

## EXPLANATION OF COLUMNS IN TABLE VIII

1. Column 1 shows figure number in thesis.
2. Column 2 shows the chemostat dilution rate at which the cells were harvested for the batch experiment.
3. Column 3 shows the initial conditions with respect to biological solids concentration. "H" represents high initial solids inoculum with experiment run under nonproliferating conditions. "H*" represents experiments run under nitrogen-deficient conditions, i.e., no nitrogen added, but cells not washed. "L" represents low initial solids inoculum under proliferating conditions.
4. Column 4 shows the carbon sources in the batch units, either glucose, glycerol, or glucose and glycerol.
5. Column 5 shows initial solids concentration.
6. Column 6 shows peak solids concentration.
7. Column 7 shows the increase in biological solids concentration calculated by subtraction of column 5 from column 6.
8. Column 8 shows total COD removed at the time of peak solids concentration.
9. Column 9 shows the cell yield in percent calculated as column 7 divided by column 8.
10. Column 10 shows the rate of growth. When the biological growth followed first order increasing or decreasing kinetics, the unit of expression is $hour^{-1}$. When the biological growth followed zero order kinetics, the unit of expression is mg/l/hr.
11. Column 11 shows the rate of total COD removal. When the rate of total COD removal followed first order increasing or decreasing kinetics, the unit of expression is mg/l/hr.
12. Column 12 shows the rate of glucose COD removal.
13. Column 13 shows the rate of glycerol COD removal.
14. Column 14 shows the mode of substrate removal, i.e., either concurrent removal or sequential removal.

Note: Explanation of subscripts for rate symbols, columns 10 through 13: the first number of the subscript indicates the type of kinetics: 1 and -1 represent first order increasing kinetics and first order decreasing kinetics, respectively. 0 represents zero order kinetics. The second number of the subscript indicates the sequence of kinetic phases: 1 denotes either the first phase or that only a single phase occurred, whereas 2 refers to the second phase.

In column 2 some experiments are labeled #1, etc., instead of dilution rate in the chemostat for which the cells were harvested. These were special experiments and the source of seed is described below:

#1 - Cells were taken from chemostat effluent at D = 1/24 $hr^{-1}$ and grown in a batch reactor with 2000 mg/l glycerol on a 24-hour feeding cycle for two weeks. Cells were then harvested and used in the batch study.

#2 - Cells were taken from chemostat effluent at D = 1/72 $hr^{-1}$. (At this time the mixed liquor exhibited an orange color.) The cells were grown in a batch reactor on a 24-hour feeding cycle for one week; the cells were then harvested and used in the batch study. (At this time the mixed liquor was light yellow.)

#3 - Cells were taken from chemostat effluent at D = 1/96 $hr^{-1}$ and grown in a batch reactor with 2000 mg/l of glycerol for one day. Cells were then harvested and used in the batch study. (No change of the color in the mixed liquor was observed.)

#4 - Cells were taken from chemostat effluent at D = 1/24 $hr^{-1}$. Twenty ml were placed in one liter of medium containing 2000 mg/l glycerol. Cell growth was followed by measuring the optical density at 540 mμ until the end of the log growth phase; then the cells were washed and used in the batch study. (There was no change in the color of the mixed liquor from the time of taking cells from the chemostat to the time of using the cells for batch study.)

#5 - Cells were taken from chemostat effluent at D = 1/96 $hr^{-1}$. Twenty ml were placed in one liter of medium containing 2000 mg/l glycerol. Cell growth was followed by measuring the optical density at 540 mμ until the end of the log growth phase, then the cells were washed and used in the batch study. (There was no change in the color of the mixed liquor from the time of taking cells from the chemostat to the time of using the cells for batch study.)

# CHAPTER V

# DISCUSSION

## A. Phase A: Studies on Substrate Removal in Systems Containing Two Carbon Sources

### 1. Combinations of Glucose with Various Carbohydrates (Source of Heterogeneous Population:Direct Inoculum of Municipal Sewage)

The microbial population employed in this portion of the research was presumably the most heterogeneous initial seeding population used throughout the entire investigation, because the organisms were not previously acclimated to one of the carbon sources; thus selection of species best suited for the carbon source was not a prerequisite. Any selection which did occur was accomplished during the substrate removal period. These experiments were run with the specific aim of determining the mode of substrate removal when the highly diversified population represented by a municipal sewage was subjected to a multicomponent carbon source.

It was seen in Figures 3 through 12 and in Table I that sequential removal occurred in all five dual carbon source systems, i.e., glucose in combination with galactose, mannose, ribose, lactose, and sucrose. In all cases it was found that the logarithmic growth rate on glucose was greater than on the compound with which it was combined (see control

systems, column 11, Table I). This occurrence is in accord with the theories of metabolite repression. In all cases except in the ribose experiment (Figures 7 and 8), there was essentially no lag in initiation of growth on glucose whereas there was a discernible lag for the other carbohydrates. However, the lags in initiation of metabolism of the other carbohydrates were significantly increased when they were used in combination with glucose (see column 9, Table I). Thus it may be discerned that enzymes needed for metabolism of these compounds were repressed by glucose or products of glucose metabolism. In the case of ribose, the natural lag was so long that the occurrence of repression could not be assessed for this experiment. In this case it is interesting to note that the occurrence of sequential substrate removal can not always be employed as a test for repression.

2. Mixed Substrate Systems Consisting of Glucose in Combination with Various Compounds (Heterogeneous Microbial Seed Acclimated to the Various Compounds)

This series of experiments included, among others, the same substrate combinations employed previously; however, the population was pre-acclimated to the substrate under investigation. In comparison with the previous results, it was found that glucose exhibited a repressive effect on galactose, mannose, lactose, and ribose, but not on sucrose. It is possible that the use of the rather high initial inoculum of pre-induced cells in the sucrose experiment (160 mg/l) prevented detection of repression. The presence of glucose did, however, slow the rate of sucrose removal compared with its removal rate in the control system (compare sucrose removal rates in columns 13 and 14, Table II).

The only other compound which was not removed sequentially in the presence of glucose was arabinose. Arabinose is usually broken down via a metabolic route not common to glucose and many other carbohydrates (34), and it seems possible that common metabolic intermediates essential for the operation of metabolite repression were not produced in this system.

In assessing these results it is important to comment on the effectiveness of the various analytical parameters as indicators of the occurrence of sequential substrate removal. In the glucose-ribose system, sequential substrate removal caused both diphasic total COD and biological growth curves to be generated (Figure 20), whereas in the glucose-fructose and glucose-galactose systems sequential substrate removal occurred but did not cause diphasic total COD and growth curves (Figures 14 and 16). It seems apparent that the only way to detect unequivocally the existence of sequential substrate removal is by actually analyzing for the substrate in question. It is also appropriate to point out that demonstration of sequential substrate removal is not necessarily proof that repression of enzyme synthesis has occurred, as was seen in the case of the glucose-ribose system using sewage seed. On the other hand, the demonstration of concurrent substrate removal is not proof that enzyme repression has not occurred. This aspect has recently been discussed by Grady (35) who observed concurrent removal of fructose and lysine, but was able to show that fructose repressed formation of lysine-degrading enzymes in lysine-acclimated cells.

3. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Galactose in Combination with Various Compounds

(Heterogeneous Populations Acclimated to Galactose)

Both glucose and sucrose blocked removal of galactose to the extent that sequential substrate removal was observed. In the sucrose-galactose system, removal of galactose began before all sucrose had been metabolized; however, the repressive effect of sucrose on galactose was clearly seen. From the results shown in Figure 36, it may be discerned that little or no glucose and fructose accumulated in the medium as hydrolysis products of sucrose.

Neither ribose nor fructose caused serious disruption of galactose removal. Growth on either of these compounds as sole carbon source was approximately as rapid as on galactose, and acclimation to the latter compound conferred acclimation to ribose and to fructose. The galactose-acclimated cells could not grow on arabinose as sole source of carbon (Figure 33), and it is apparent (Figure 34, combined system) that the failure to acclimate to arabinose was not due to lack of a source of energy for synthesis of arabinose-degrading enzymes. It would, therefore, appear that either there were no cells in the initial sewage seed with the genetic capability to metabolize arabinose, or that acclimation to galactose selected against such cells during the acclimation period prior to employing the cells in the experiment.

4. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Ribose in Combination with Various Compounds (Heterogeneous Populations Acclimated to Ribose)

Acclimation to ribose conferred acclimation to glucose (Figure 19), fructose (Figure 37), galactose (Figure 39), and sucrose (Figure 43), but not to lactose (Figure 41). However, the presence of ribose repressed acclimation to lactose (compare Figures 41 and 42). Both glucose

and sucrose caused severe blockage of ribose metabolism, whereas fructose retarded ribose removal to only a small extent and the presence of galactose enhanced the rate of ribose removal (compare columns 13 and 14 for the galactose-ribose system, Table IV).

5. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Arabinose in Combination with Various Compounds (Heterogeneous Populations Acclimated to Arabinose)

It was found that neither glucose, fructose, galactose, mannose, lactose, nor sucrose seriously affected utilization of arabinose by cells which were pre-induced by growth on arabinose. Acclimation to arabinose conferred acclimation to glucose, but the growth rate on glucose was not significantly greater than the growth rate on arabinose. For all other substrates examined (fructose, galactose, mannose, lactose, and sucrose), the growth rates observed were lower than those on arabinose in the comparable control systems. In such cases one might predict that sequential substrate removal would not be observed, since utilization of one compound would not be expected to accumulate metabolite repressor any faster than utilization of the other. The results using lactose and arabinose (Figures 51 and 52) indicate that arabinose repressed acclimation to lactose. Although for all other substrate combinations concurrent substrate removal was observed, it is interesting to note that the relative speed of removal can still cause discontinuity in the overall COD removal and growth curves. For example, there was some evidence for diphasic total COD removal in the arabinose-fructose system (Figure 46) because of the slowness of fructose removal. Also because of the slowness of galactose removal (Figure 48), there was kinetic discontinuity in the growth curve. The results for the

arabinose-mannose system (Figures 49 and 50) are particularly interesting because, although mannose was removed more rapidly in the combined system (Figure 50) than it was in the control, the relative slowness of mannose removal caused a pronounced discontinuity in the total COD and growth curves.

6. Studies on Substrate Removal in Two-component Carbon Source Systems Consisting of Glycerol in Combination with Various Compounds (Heterogeneous Populations Acclimated to Glycerol)

Glucose and galactose repressed glycerol utilization (Figures 28 and 58). Acclimation to glycerol conferred acclimation to both glucose and galactose. Both were removed as sole carbon sources somewhat faster than was glycerol, and significant amounts of metabolic intermediates accumulated in the medium (Figures 27 and 57). Glycerol in combination with sucrose (Figure 68), fructose (Figure 56), mannose (Figure 60), or ribose (Figure 62) yielded concurrent substrate removal. Fructose, ribose, and mannose when used as sole carbon sources were metabolized more slowly than was glycerol, and no accumulation of metabolic intermediates was noted in the medium. The growth rate on sucrose was approximately the same (see Table VI) as the growth rate on glycerol, but a considerable quantity of metabolic intermediates accumulated in the medium during growth on sucrose (Figure 67) and it was observed (Figure 68) that while removal of glycerol and sucrose was concurrent, a fairly high concentration of glycerol remained when most of the sucrose had been removed (see 8-hour sample, Figure 68). The data indicate that both accumulation of metabolic intermediates due to metabolism of one of the compounds in a multicomponent substrate system and the relative growth rates play a significant role in determining

whether sequential or concurrent removal will be observed. The former consideration is probably a more useful one in predicting the mode of removal. For example, in the glycerol-galactose system (Figures 57 and 58, and Table VI), growth on galactose was much slower than on glycerol. From this information it might be predicted that concurrent removal would ensue when the compounds were used in combination; however, the results clearly indicate that galactose repressed glycerol removal. If one examines the glycerol COD and galactose COD removal curves in Figure 57, it is seen that the curve for galactose removal is not too unlike the glycerol removal curve even though the total COD removal in the galactose system is significantly slower than that of the glycerol system. The difference between the galactose curve and the total COD curve provides an indication of the degree of accumulation of metabolic intermediate products produced during degradation of galactose. With this information one might expect the possibility that sequential removal would ensue when glycerol and galactose were used as a combined carbon source, since the concept of metabolite repression requires some accumulation of an intermediate product common to degradation of both compounds. However, it should be noted the production and accumulation of metabolic products does not provide an unequivocal basis for predicting the occurrence of sequential removal since there is no guarantee that the particular metabolite repressor will be included in the accumulated intermediates.

Arabinose could not be metabolized by the glycerol-acclimated population, a result similar to that observed for cells acclimated to galactose. Glycerol retarded acclimation to lactose. In this respect, the glycerol-acclimated population gave results similar to those

observed for ribose- and for arabinose-acclimated cells when grown in a combined substrate system consisting of lactose and either ribose or arabinose.

7. Studies on Substrate Removal in Two-component Carbon Source Systems (Heterogeneous Populations Acclimated to One of the Compounds)

From the results using either galactose-acclimated or fructose-acclimated cells in systems combining these two carbon sources (Figures 29-30 and 69-70, and Table VII), it appears that these compounds are compatible, i.e., they are removed concurrently and neither affects removal of the other. It is interesting to compare the response of microbial populations acclimated to lactose when placed in media containing lactose and the monosaccharides of which lactose is composed (lactose-glucose, Figures 23 and 24; lactose-galactose, Figures 71 and 72), as well as when placed in combination with another monosaccharide, ribose (Figures 75 and 76). Glucose caused blockage of lactose removal (sequential removal), whereas galactose did not (concurrent removal), and in the lactose-ribose system, lactose blocked ribose removal, i.e., sequential removal ensued but removal of the added compound (ribose) was repressed. The growth rates on lactose and on glucose were approximately the same (0.126 $hr^{-1}$ versus 0.122 $hr^{-1}$), but a considerable amount of intermediates was produced from glucose and sequential removal ensued. The growth rate on galactose was somewhat lower than on lactose (0.143 $hr^{-1}$ versus 0.179 $hr^{-1}$); also, less intermediates accumulated from galactose than from glucose utilization, and concurrent substrate removal ensued. The growth rate on ribose was less than half that on lactose, and the result was repression of ribose utilization in the combined system. These results augur well for the predictability of

sequential removal and of the order of removal based upon comparative growth rates on either compound as sole source of carbon and the accumulation of metabolic intermediates.

With sucrose-acclimated cells, glucose did not cause sequential removal of glucose and sucrose (Figures 25 and 26), and it can be seen from Table VII that the growth rate on glucose was approximately the same as on sucrose when each was employed as a sole carbon source. Also, in the glucose control very little metabolic intermediates accumulated in the medium; thus the resultant concurrent substrate removal was predictable from the behavior of the control systems. Using sucrose-acclimated cells in the sucrose-galactose system (Figures 73 and 74), it was found that galactose partially blocked sucrose removal even though the growth rate on galactose was slower than on sucrose in the controls. However, in the galactose control, metabolite repression was manifested.

In summary, it may be stated that in general, glucose exerts a repressive effect on the synthesis of enzymes required for the utilization of a variety of other compounds regardless of whether the initial population is highly heterogeneous (sewage seed) or has been subjected to species selection by growth on the specific compound used as a joint carbon source with glucose. In cases where glucose exerted this effect, either the growth rate or accumulation of intermediates (or both) using glucose as a sole carbon source was greater than for the other compound in the medium. Also for combinations of compounds not involving glucose, when sequential removal was observed, the growth rate on the compound which exhibited the repression was greater than the growth rate on the compound which was repressed, or growth on the repressor substrate

lead to accumulation of intermediates. Since compounds other than glucose have been shown to cause repression of the utilization of second substrates in heterogeneous microbial populations, it may be concluded that this is not an effect uniquely caused by glucose, and that the reason glucose represses utilization of such a wide variety of compounds is because most microorganisms grow faster on glucose than on other carbon sources. Also, because growth on glucose often leads to the accumulation of metabolic intermediates, the metabolite repressor can be expected to be present in sufficiently high concentration to stop or retard synthesis of enzymes needed to degrade other substrates. It would therefore appear that whenever one compound in a mixture of compounds is dissimilated faster than its breakdown products can be channelled into synthetic pathways, it may be suspected that its presence might cause blockage of metabolism of other carbon sources in the medium. If the carbon sources have at some point in the metabolic pathway of their dissimilation the same sequence of reactions, repression and possible sequential substrate removal can be expected. This reasoning is in accordance with the basic concepts of "metabolite repression." Most of the basic research on this control mechanism has been accomplished using single species of bacteria. However, the work presented in this report, as well as other work in our laboratories employing heterogeneous populations all obtained from municipal sewage and used directly or pre-acclimated to a specific carbon source, indicate that this metabolic control mechanism is present in many different species and that it tends to act <u>en masse</u>. It is therefore a phenomenon which has important ramifications to biological treatment of waste waters, particularly industrial wastes; it is a phenomenon which can help

explain some of the discontinuities in the kinetic course of purification when manifested as sequential substrate removal. Furthermore, the work presented here leads to the conclusion that such manifestations of metabolite repression may be predictable on the base of relative growth rates and production of metabolic intermediates.

## Phase B: Effect of Dilution Rate on Metabolism of Glycerol, and on the Sequential Removal of Glucose and Glycerol

### 1. Response to Changes in Dilution Rate

#### a. General Observations

Other research workers have observed changes in microbial predominance during operation of continuous flow reactors, and in general, it has been concluded by these workers that the detention time or dilution rate provides a strong selective pressure and exerts a considerable effect upon species predomination in a continuous flow reactor (36). Also, it has been concluded by other workers that regardless of substrate composition or degree of organic loading, the microbial population present in the sludge is one of the major factors influencing the performance of an activated sludge system, and that species predominance can change unexpectedly after a continuous flow unit receives a shock loading (37).

The present study on the behavior of continuous flow reactors is perhaps the most extensive one yet accomplished in this field, since the unit was operated and rather extensively observed for a one-year period rather than for the much shorter periods employed by other investigators. Throughout this time rather severe changes in bacterial predominance were noted, and the system certainly could not be said to be

in the "steady state" for extended periods of time. For example, Figure 160, which depicts the response of the unit after a change in dilution rate from 1/4 to 1/24 $hr^{-1}$, showed successive transitions due largely to changes in bacterial predominance which caused the unit to function in a decidedly unsteady condition for a period of over ten days following the change in dilution rate.

It was generally observed that the color of the mixed liquor changed as a consequence of a change in dilution rate, and concurrent observation under the microscope indicated morphological differences in the population. In general, at rather high dilution rates (1/4 and 1/12 $hr^{-1}$), either a milky or light bluish tint was observed in the mixed liquor, and under the microscope the population appeared to consist primarily of spheres and rods. At dilution rates from 1/24 to 1/48 $hr^{-1}$ filamentous organisms usually predominated, and the mixed liquor exhibited a yellowish color. At dilution rates above 1/48 $hr^{-1}$, filaments did not predominate. It should be emphasized that these are very gross indications of changes in predominance and that many changes could occur which would not be detected without extensive taxonomic studies. Thus, the changes observed represent the minimum measure of predominance variation.

b. Observations on the Patterns of Biochemical Response with Increasing Dilution Rate

From the results obtained by Mateles, et al. (38), it seemed apparent that the biological system could undergo drastic disturbance during the transition from one dilution rate to another. Also, the recent studies on hydraulic shock loading by George (39) indicated that severe hydraulic shock loading would create deleterious transient

responses resulting in decreased biological solids concentration and considerable increase in effluent COD during the transient state.

In the present study, the range of dilution rates employed was rather broad, and an attempt has been made to classify the resultant biochemical or biological responses into three patterns, i.e., drastic or severe responses, medium responses, and mild responses.

(1) Drastic Response

When the increase in dilution rate was of considerable magnitude (Figures 91, 142, 155, and 167), the immediate response was a rapid washout of biological solids, and severe leakage of effluent COD. The COD appearing in the effluent was accounted for almost totally as the primary exogenous substrate, i.e., glycerol, and not as metabolic intermediates.

(2) Medium Response

The most critical parameter from the viewpoint of assessing the ability of a system to function as a pollution control process is the effluent COD rather than the biological solids concentration. The response obtained when the dilution rate was changed from 1/48 to 1/24 $hr^{-1}$ was presented in Figure 145. Although this change in dilution rate halved the detention time, it might certainly be expected that the 24-hour detention time would be ample for the removal of 2000 mg/l glycerol and, in accordance with this expectation, effluent glycerol was not increased. It was further seen that the biological solids, although fluctuating somewhat, did remain relatively steady. However, as an after-effect of the change in dilution rate, the effluent COD fluctuated for more than a week, and at times the concentration of COD in the

effluent was in the range of 300-400 mg/l. Thus, the response did not cause severe washout of cells or primary carbon source, but did cause undesirable fluctuations in COD removal efficiency.

(3) Mild Response

The mild response is envisioned as one which does not lead to a severe or even a detectible transient when the dilution rate is increased. Instead, the shock seems to produce an after-effect which causes a period of unsteady operation for some time after the flowrate has been changed. The results shown in Figure 150 typify such a response. After changing the dilution rate from 1/24 to 1/12 $hr^{-1}$, there was no appreciable change in solids concentration and, indeed, the effluent COD concentration became lower than it had been in the previous steady state condition. However, on the third day after changing the flowrate, filamentous organisms disappeared from the unit, and the mixed liquor turned a bluish color. The biological solids began to dilute out somewhat, and the effluent COD rose to approximately 200 mg/l. Eight to nine days after changing the dilution rate, the unit again approached steady state conditions.

c. Observations on the Patterns of Biochemical Response with Decreasing Dilution Rate

(1) Drastic Response

Drastic reductions in the dilution rate, i.e., changes in dilution rate which require the organisms to adjust to a much slower growth rate can cause severe after-effects, as exemplified by the results shown in Figure 160 when the dilution rate was changed from 1/4 to 1/24 $hr^{-1}$. The results of the application of an even more severe "reverse hydraulic

shock load" were shown in Figure 172. In this experiment the dilution rate was changed from 1/4 to 1/96 $hr^{-1}$, and this change led to a severe decrease in biological solids concentration and a significantly high increase in COD in the effluent. While most of the effluent COD was not attributable to the primary substrate, glycerol, it is significant to note that there was an increase in the glycerol concentration in the effluent.

(2) Medium Response

The medium response characterized primarily by an increase in effluent COD but little or no leakage of the primary substrate is exemplified by the results shown in Figures 77 and 86 for changes in dilution rate from 1/4 to 1/12 $hr^{-1}$ and from 1/12 to 1/24 $hr^{-1}$, respectively. The difficulty of trying to categorize shock load response by the type of substrate leakage is apparent by the response shown in Figure 109. In this case the dilution rate was changed from 1/12 to 1/24 $hr^{-1}$, and no glycerol was detected in the effluent in response to the shock. However, the effluent COD concentration rose to 600 mg/l, which in any circumstance would appear to be a rather drastic deleterious response.

(3) Mild Response

In general, responses which might be termed "mild" are observed when two-fold (or less) changes in detention times were applied. In such cases, although there were usually discernible fluctuations in the biological solids concentration, the quality of the effluent remained fairly constant. Examples of such responses were shown in Figure 121 (dilution rate changed from 1/36 to 1/48 $hr^{-1}$), and Figure 124 (dilution rate changed from 1/48 to 1/72 $hr^{-1}$). For slower growing systems (1/72

to 1/96 $hr^{-1}$, Figure 129, and 1/96 to 1/192 $hr^{-1}$, Figure 134), there were no significant changes in the biological solids concentration or the effluent COD in response to the changes in dilution rate.

d. Comparison of Responses to Increasing and Decreasing Dilution Rate

It has been observed in these studies that under continuous flow conditions heterogeneous microbial populations can be expected to undergo changes in microbial predominance as well as metabolic disturbances under the influence of changing dilution rate. The extent of the disruption in substrate removal efficiency depends, to a large measure, on the severity of the hydraulic shock load applied and on the particular metabolic behavior of the organisms predominating in the unit at the time the shock is applied, and to a lesser extent upon the age of the cells, i.e., the dilution rate before the shock is applied. Whether the shock represents an increase or a decrease in the dilution rate, disturbances in the level of biological solids and the efflciency of COD removal efficiency can be expected.

Since the cells (apparently) cannot immediately respond to an increase in dilution rate by an equivalent immediate increase in the growth rate, such a shock has a tendency to wash out constituents of the mixed liquor and exaggerates or increases the concentration of the exogenous substrate in the reactor. What might be termed "medium response" is one in which the dissimilating capacity of the cells responds more rapidly than synthetic capacity of the cells, and this situation leads to rather high leakage of effluent COD but rather low leakage of primary carbon source. As the severity of the hydraulic shock is increased, even the dissimilating capacity of the cells cannot keep pace, and this situation results in greater leakage of the primary

carbon source.

It is somewhat more difficult to envision the biological occurrences taking place when disruption of system efficiency is observed due to decrease in dilution rate. In this case the demand placed upon the cells is simply that they grow slower than they were growing previous to applying the shock loading. However, under the shock loading conditions applied during these studies, the flux of the carbon source through the system is also decreased. It is noted that under the general carbon-limiting growth conditions imposed throughout these studies, cells may be said to exist under starvation conditions and, indeed, when they are made to exist under even greater carbon-limiation (as the dilution rate is further decreased), it seems possible that a significant portion of the population may be subject to die-off and, indeed, lysis. Under such shock loading conditions, when the effluent COD rises and there is no leakage of primary substrate, it seems reasonable to postulate that the effluent COD consists essentially of materials released from lysed cells. Also, under shock loading conditions it might be expected that a greater diversity of species can exist in the reactor due to the fact that there is less selective pressure against slow growers. Also, due to the likelihood of a greater degree of lysis of fast-growing organisms, vital cell constituents may be available for auxotrophic organisms which may exist as a satellite population.

Table IX was prepared with the aim of summarizing, in a quantitative manner, the biological responses obtained during steady and transient states for the various dilution rates throughout the entire year during which the continuous flow reactor was operated. The following generalizations seem valid. With respect to steady state operation

TABLE IX

BIOLOGICAL RESPONSES TO HYDRAULIC SHOCK LOADINGS FOR A CONTINUOUS FLOW COMPLETELY MIXED REACTOR FED 2000 mg/l GLYCEROL (2440 mg/l Total COD)

| Items | Sequences of Dilution Rates ($hr^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/4 | T.S. | 1/12 | T.S. | 1/24 (I) | 1/24 (II) | T.S. | 1/4 (I) | 1/4 (II) | T.S. | 1/12 (I) | 1/12 (II) | T.S. | 1/24 (I) | 1/24 (II) |
| Figure No. | 77 | 77 | 77 | 86 | 86 | 86 | 91 | 91 | 101 | 101 | 101 | 109 | 109 | 109 | 118 |
| Solids Conc. (mg/l) | 920 | 450 | 1400 | 700 | 1500 | 1850 | 550 | 1430 | 1180 | 1250 | 1300 | 980 | 650 | 900 | 2500 |
| Effluent Total COD (mg/l) | 100 | 250 | 150 | 450 | 200 | 200 | 1780 | 80 | 150 | 150 | 200 | 200 | 600 | 220 | 150 |
| Effluent Glycerol COD (mg/l) | 0 | 0 | 0 | 0 | 0 | 0 | 1500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Maximum Intermediates COD (mg/l) | 100 | 250 | 150 | 450 | 200 | 200 | 280 | 80 | 150 | 150 | 200 | 200 | 600 | 220 | 150 |
| Total COD Removed (mg/l) | 2340 | 2190 | 2290 | 1990 | 2240 | 2240 | 660 | 2360 | 2290 | 2290 | 2240 | 2240 | 1800 | 2220 | 2290 |
| Purification Efficiency, % | 95.8 | 89.8 | 94 | 81.7 | 92 | 92 | 27 | 96.6 | 94 | 94 | 92 | 92 | 73.2 | 91 | 94 |
| Yields, % | 38.5 | 20.5 | 61 | 35.2 | 67 | 82.5 | 84.2 | 60.6 | 51.5 | 54.5 | 58 | 43.7 | 36.3 | 40.5 | 109 |
| Cellular Proteins, % | 24 | 33.4 | 42.8 | 57 | 36.7 | 48.7 | 51 | 51.7 | 51.5 | 52 | 46.3 | 63.3 | 69.3 | 55.5 | 38 |
| Cellular Carbohydrates % | 13 | 15.6 | 17.8 | 28.5 | 18.7 | 19 | 32.8 | 23.5 | 21.2 | 24 | 23.2 | 18.4 | 23.1 | 22.2 | 16 |
| Time to Reach Critical Disruption, Days | | 1 | | 1 | | | 0.5 | | | 0.67 | | | 2 | | |
| Transient Period, Days | | 4 | | 4 | | | 1.67 | | | 1.33 | | | 3 | | |

TABLE IX (continued)

| Items | Sequences of Dilution Rates ($hr^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T.S. | 1/36 | T.S. | 1/48 | T.S. | 1/72 | T.S. | 1/96 | T.S. | 1/192 (I) | 1/192 (II) | T.S. | 1/96 (I) | 1/96 (II) | T.S. |
| Figure No. | 118 | 118 | 121 | 121 | 124 | 124 | 129 | 129 | 134 | 134 | 137 | 137 | 137 | 142 | 142 |
| Solids Conc. (mg/l) | 1400 | 1950 | 1370 | 1700 | 1030 | 1200 | 1050 | 1100 | 880 | 850 | 1280 | 1150 | 1050 | 950 | 550 |
| Effluent Total COD (mg/l) | 100 | 100 | 0 | 50 | 150 | 80 | 50 | 100 | 100 | 100 | 320 | 250 | 100 | 120 | 1270 |
| Effluent Glycerol COD (mg/l) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1040 |
| Maximum Intermediates COD (mg/l) | 100 | 100 | 0 | 50 | 150 | 80 | 50 | 100 | 100 | 100 | 320 | 250 | 100 | 120 | 230 |
| Total COD Removed (mg/l) | 2340 | 2340 | 2440 | 2390 | 2290 | 2360 | 2390 | 2340 | 2340 | 2340 | 2120 | 2190 | 2340 | 2320 | 1170 |
| Purification Efficiency, % | 95.8 | 95.8 | 100 | 97.5 | 94 | 96.6 | 97.5 | 95.8 | 95.8 | 95.8 | 86.5 | 89.8 | 95.8 | 95 | 48 |
| Yields, % | 60 | 82.3 | 56 | 71 | 45 | 50.8 | 44 | 47 | 37.5 | 36.4 | 60.3 | 52.5 | 46 | 41 | 47 |
| Cellular Proteins, % | 57 | 51 | 51 | 45.8 | 43.5 | 33.3 | 38 | 41 | 45.5 | 41.2 | 43 | 43.5 | 44.7 | 45.3 | 40 |
| Cellular Carbohydrates % | 21.4 | 15.6 | 18.2 | 17.6 | 24.2 | 16.7 | 19 | 18.2 | 17 | 17.6 | 15.6 | 19.1 | 28.5 | 25.2 | 27.3 |
| Time to Reach Critical Disruption, Days | 11 | | 5 | | 3 | | 2 | | 14 | | | 7 | | | 2 |
| Transient Period, Days | 18 | | 11 | | 8 | | 4 | | 24 | | | 15 | | | 4 |

TABLE IX (concluded)

| Items | Sequences of Dilution Rates ($hr^{-1}$) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/48 | T.S. | 1/24 | T.S. | 1/12 | T.S. | 1/4 | T.S. | 1/24 | T.S. | 1/4 | T.S. | 1/96 |
| Figure No. | 142 | 145 | 145 | 150 | 150 | 155 | 155 | 160 | 160 | 167 | 167 | 172 | 172 |
| Solids Conc. (mg/l) | 1100 | 1100 | 1200 | 950 | 1200 | 470 | 1100 | 500 | 1150 | 200 | 1220 | 630 | 900 |
| Effluent Total COD (mg/l) | 100 | 400 | 200 | 180 | 50 | 1400 | 200 | 1000 | 100 | 2340 | 80 | 380 | 200 |
| Effluent Glycerol COD (mg/l) | 0 | 0 | 0 | 0 | 0 | 1100 | 100 | 140 | 0 | 2050 | 0 | 80 | 0 |
| Maximum Intermediates COD (mg/l) | 100 | 400 | 200 | 180 | 50 | 300 | 100 | 860 | 100 | 290 | 80 | 300 | 200 |
| Total COD Removed (mg/l) | 2340 | 2040 | 2240 | 2260 | 2390 | 1040 | 2240 | 1440 | 2340 | 100 | 2360 | 2060 | 2240 |
| Purification Efficiency, % | 95.8 | 83.5 | 92 | 92.5 | 97.5 | 42.5 | 92 | 59 | 95.8 | 4.1 | 96.7 | 84.5 | 92 |
| Yields, % | 47 | 54 | 53.5 | 42 | 50.3 | 45 | 49.2 | 34.7 | 49.2 | 200 | 51 | 30.5 | 40.3 |
| Cellular Proteins, % | 40 | 31.2 | 41.7 | 40 | 35 | 46.8 | 38.2 | 30 | 43.5 | 35 | 38.3 | 44.5 | 44.5 |
| Cellular Carbohydrates % | 27.3 | 32 | 25 | 31.5 | 29.2 | 32 | 31.8 | 20 | 21.7 | 60 | 30.8 | 19 | 17.8 |
| Time to Reach Critical Disruption, Days | | 3 | | 4 | | 0.33 | | 3.5 | | 1.5 | | 3.5 | |
| Transient Period, Days | | 8 | | 9 | | 2 | | 10 | | 3 | | 7 | |

## EXPLANATION OF COLUMNS IN TABLE IX

Columns headed by numerical fractions, e.g., 1/4, indicate the average steady state values for the parameters shown on the left at the designated dilution rates. In cases when the steady state data appeared to produce two steady states, these are distinguished by use of Roman numerals in the column heading.

Columns labeled "T.S." designate the value in the transient state after each change in dilution rate for the parameters given on the left. The values given represent the maximum change from the previous steady state conditions.

with an influent COD (glycerol) of 2440 mg/l, the effluent COD varied from 100 to 200 mg/l, yielding a COD purification efficiency from 92 to 95.8 per cent. In general, glycerol was not detected in the medium during "steady state" operation. In general, the cell yields obtained during steady state operation were higher than those obtained during the transient state; however, it should be noted that the average yields computed for the steady state data were based upon many more samples than were available during the transient states. For shocks consisting of increases in dilution rate, the cellular carbohydrate content of the cells was subject to noticeable increase during the transient state, whereas the protein content remained fairly constant, or in some cases was slightly decreased. In general, it may be stated that the results of these studies are in accord with the findings of George (39).

e. Significance of Hydraulic Shock Loading in the Operation of Activated Sludge Processes

In the course of the average day, the strength and the quantity of domestic sewage varies to a considerable extent. The volume of flow generally reaches a maximum in the early forenoon, and the strength of the sewage is generally greatest at this time (40). In addition to changes in hydraulic rate of flow and total quantity of organic matter, there may be considerable variation in the components of the waste. Gaudy and Komolrit have reported studies pertinent to the effect of qualitative shock loadings on continuous flow biological systems (14).

Even though all of these types of environmental changes have been shown to cause fluctuations in system efficiency, the design engineer usually must select an average daily flow and an average total strength of the waste, and has not at least until recently, even given

consideration to changes in the composition of the waste. Thus, at present the procedures used by designers certainly do not provide treatment facilities which optimally provide for the needs of the treatment plant operator. However, the results of the present study and the past studies on shock loadings indicate to the author that many of the operational problems encountered due to various shock loadings (in particular, the hydraulic shock loadings) can be overcome by the simple expedient of providing an equalization basin between the primary clarifier and the aeration tank.

2. Effect of Dilution Rate on the Occurrence of Sequential Substrate Removal

a. Relationship Between Dilution Rate and Cell Age

It can be shown that in a steady state system the logarithmic growth rate of the population is equal to the dilution rate. Since the dilution rate is equal to the reciprocal of the detention time in the reactor, it may be stated that cells growing in the steady state in a completely mixed continuous flow reactor at the higher detention times will have, on the average, greater cell ages. Thus, in the present studies as a basis for comparison, the dilution rate or detention time was considered as a measure of the cell age. Cells growing at high dilution rates were considered to be young cells, whereas cells growing at low dilution rates were considered to be old cells.

b. Effect of Cell Age on the Occurrence of Sequential Substrate Removal

The effect of Cell age on the patterns of substrate removal is shown in Table X. In general, four substrate removal patterns were discerned; nearly complete sequential removal with glucose blocking

TABLE X

EFFECT OF CELL AGE ON THE PATTERNS OF SUBSTRATE REMOVAL

| Cell Age | No. Cases | 1 Complete Glucose Inhibition on Glycerol | | 2 Partial Glucose Inhibition on Glycerol | | 3 Concurrent Removal | | 4 Glycerol Inhibition on Glucose | |
|---|---|---|---|---|---|---|---|---|---|
| Hours | | Cases | % | Cases | % | Cases | % | Cases | % |
| 4 | 5 | 1 | 20 | 1 | 20 | 3 | 60 | 0 | 0 |
| 12 | 4 | 1 | 25 | 0 | 0 | 3 | 75 | 0 | 0 |
| 24 | 4 | 2 | 50 | 0 | 0 | 1 | 25 | 1 | 25 |
| 36-48 | 3 | 0 | 0 | 1 | 33.3 | 1 | 33.3 | 1 | 33.3 |
| 72-96 | 4 | 1 | 25 | 1 | 25 | 0 | 0 | 2 | 50 |
| 192 | 2 | 0 | 0 | 0 | 0 | 2 | 100 | 0 | 0 |

glycerol, partial blockage of glycerol removal by glucose, concurrent removal of glucose and glycerol, and blockage of glucose removal by glycerol. From the results of these studies it would appear that prediction of the mode of substrate removal on the basis of cell age or previous growth rate history is not attainable. In studies employing batch-grown cells, Gaudy, et al. (5) showed that "old" cells yielded concurrent substrate removal, whereas "young" cells yielded sequential removal with glucose blocking sorbitol removal. On the basis of these preliminary studies using batch-grown systems, it was suggested that control over the age of the population might enhance control over the occurrence of sequential substrate removal. The results of the present study indicate that there is some substantiation for this suggestion, since the tendency for the cells to exhibit sequential removal was decreased as the age of the culture increased. However, an increased cell age does not provide any guarantee that concurrent substrate

removal will be exhibited. On the contrary, the results of the present study indicate that concurrent removal can occur for very young cell populations. It would appear that the changes in predominance which occurred during this long-term study provided a complicating factor which does not allow ultimate conclusions to be drawn concerning the effect of cell age on the occurrence of sequential substrate removal. It does seem from these studies, however, that one must conclude that species predominance plays a role of equal significance with cell age, and that the cell age should not be used as a predictor of the mode of substrate removal.

In some respects it would seem that a researcher who uses heterogeneous populations takes the path of least resistance when he atttributes differences in experimental results to changes in microbial predominance in the experimental system. Such an approach was taken by Stumm-Zollinger (12), who observed concurrent removal in a glucose-galactose system run at a temperature of $7^{o}C$, and sequential removal of the same substrates when the system was run at $20^{o}C$. The difference in results was attributed to different predominating species at the two temperatures. It is unfortunate that her data cannot be analyzed with respect to the rate of glucose removal in the control systems. A similar approach was taken by Prakasam and Dondero (11), who observed sequential removal of glucose and sorbitol in a laboratory-grown population acclimated to sorbitol, but observed concurrent removal of the same substrates when the population consisted of a sample of activated sludge taken from a nearby treatment plant operating on domestic sewage. The differences in the results were again attributed to differences in the microbial species present and, indeed, to the restricted nature of the

sorbitol-acclimated population. However, it should be noted that in their studies using activated sludge, a considerably higher initial concentration of cells was employed than was used for the experiment with sorbitol-acclimated cells. Also, there is no way to compare the relative growth rates or substrate removal rates in control systems. It is interesting to note that their results with activated sludge seed could have been attributed to the effect of cell age, since it would certainly be expected that the activated sludge they employed consisted of a much older population than the laboratory-grown sorbitol cells. Stumm-Zollinger (13) has concluded that metabolic activity of a microbial community is dependent upon the species present in the community, shifts in the types of species present, the physiological response of individual organisms to multisubstrate environment, and the interaction of various microbial species. Such a general statement covers all possibilities without clearly pointing out any key influencing factor, and statements such as these can certainly be made even without the benefit of experimentation.

Perhaps the best indication that cell age should not be used as a predictor for the occurrence of sequential substrate removal has been obtained by Tsay (41). She found that growing cultures of Escherichia coli or Achromobacter sp. exhibited sequential removal of glucose and sorbitol for both young and old populations. Also, Heidman (42), using an unidentified pure culture, found sequential removal of glucose and sorbitol for young cells and for old cells. From these two studies it sould seem that for the species investigated, cell age had little effect upon the observation of sequential removal, and on the basis of these studies and the present results it is recommended that cell age

not be employed as a predictor of the mode of substrate removal. An indication that the species present in the population can exert an effect upon the occurrence of sequential substrate removal was obtained by Tsay (41), who found with a glucose-arabinose sybstrate system that Escherichia coli 45 exhibited concurrent substrate utilization although inhibition was evidenced, whereas Achromobacter exhibited sequential substrate removal.

3. Kinetics of Biological Growth and Substrate Removal

Any attempt to generalize kinetics for heterogeneous systems, particularly those prevalent in activated sludge systems, is extremely difficult since the observed kinetic mode is dependent upon the type of organisms present, the nature of the carbon source, the biological solids concentration in relation to the concentration of carbon source or growth-limiting nutrient, and physical factors such as mixing, etc. Also, various interactions between the different organisms which constitute the heterogeneous population do not enhance definitive mathematical formulation of kinetics of growth and substrate removal. However, from an engineering standpoint, it is essential that some attempt be made to categorize the type of kinetics which may exist in various systems under particular operational conditions, since such information is vitally needed if adequate design formulations and kinetic models are to be successfully employed.

During the course of the present investigation, a large amount of kinetic data was amassed, and it was possible to distinguish three distinct modes or types of kinetic behavior. These are discussed below.

a. Types of Kinetic Behavior Exhibited in Heterogeneous Systems

(1) First Order Increasing Kinetics

When growth and substrate removal proceed at a first order increasing rate, the rate of biological growth can be expressed as follows:

$$\mu_1 = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

and the rate of substrate removal can be expressed as follows:

$$K_1 = \frac{\ln \Delta S_2 - \ln \Delta S_1}{t_2 - t_1}$$

In the above formulas, $\mu_1$ is the logarithmic growth rate constant for exponential multiplication of the cells, and $K_1$ is the logarithmic substrate removal rate constant. The values of $X_2$ and $X_1$ are the concentrations of biological solids at times $t_2$ and $t_1$; the values $\Delta S_2$ and $\Delta S_1$ are the concentrations of substrate which have been removed at times $t_2$ and $t_1$. The values of the growth rate constant and the substrate removal rate constant are usually obtained from semilogarithmic plots of experimental data. The concentrations of substrate and of biological cells are usually expressed as mg/l, and time is usually expressed in hours. This type of kinetics might be expected in a system in which conditions were optimum for growth and the initial substrate concentration was high in relationship to the initial biological population.

(2) Zero Order Kinetics

In cases where biological growth (accumulation of biological solids) and removal of the carbon source can be plotted versus time yielding a straight line on arithmetic paper, zero order kinetics can

be said to exist. In such a case the rate of biological growth may be expressed as follows:

$$\mu_o = \frac{X_2 - X_1}{t_2 - t_1}$$

and the rate of substrate removal may be expressed as follows:

$$K_o = \frac{S_2 - S_1}{t_2 - t_1}$$

Such a kinetic mode might be expected when the concentration of the initial population is high in relation to the available substrate, or when the system is artificially limited (e.g., by nitrogen limitation or in the absence of an essential growth factor). Under such conditions, growth in the sense of replication of cells may not even occur; however, sludge accumulation is possible. If concentrations of cells and substrate are expressed in mg/l and time in hours, the units of $\mu_o$ and $K_o$ are mg/l/hr.

(3) First Order Decreasing Kinetics

This kinetic order is the one traditionally employed to depict the exertion of biochemical oxygen demand in the BOD test. Applying this equation to growth, the biological solids in the system at a given time can be expressed as follows:

$$X_t = X_o(1 - e^{-\mu t})$$

In accordance with such an expression, the biological solids concentration would become asymptotic to some upper limit at infinite time. The growth rate constant $\mu_{-1}$ may be expressed as follows:

$$\mu_{-1} = \frac{\ln \frac{X_o}{X_o - X_t}}{t}$$

In the above expression it should be emphasized that the value of $X_o$ does not represent the initial solids concentration, but the ultimate increase in solids concentration, i.e., the difference between solids concentration at time zero and infinite time. $X_t$ represents the increase in solids concentration at time t.

Under such a kinetic mode, the substrate concentration at a given time may be expressed as follows:

$$S_t = S_o \cdot e^{-Kt}$$

and the first order decreasing rate constant for substrate removal may be expressed as follows:

$$K_{-1} = \frac{\ln \frac{S_o}{S_t}}{t}$$

In the above equation $S_o$ represents the amount of substrate present in the system initially, and $S_t$ represents the substrate concentration remaining in the system at any time. This mode of kinetic expression might be expected to prevail in long-term experiments using nonproliferating systems, i.e., it would be expected to follow the period of zero order kinetics.

In general, most of the kinetic data obtained during the course of the present experimentation could be depicted, at least within the limits of engineering practicality, by the kinetic formulation for either first order increasing rate, zero order rate, or first order decreasing rate.

b. Kinetic Relationship Between Total Organic Carbon (Total COD) Removal and Biological Growth

In all of the above formulations for biological growth and

substrate removal, it is evident that the substrate removal curves which could be generated using these formulations would be mirror images of the sludge accumulation curves; that is, the mathematical formulations imply that there is a constant relationship between the rate of substrate removal and the rate of sludge accumulation during the period when these particular kinetic expressions are applicable. Thus it is tacitly assumed that during logarithmic growth of a biological population there is for each incremental utilization of the carbon source a specific (and a proportional) incremental addition to the biological solids concentration. Thus, the ratio of the weight of total carbon source (total COD) removed to the concentration of biological solids produced in the same time period is constant for all incremental time periods during which a particular kinetic order prevails. The reciprocal of this ratio is the sludge, or cell, yield.

In nearly all cases observed in the present experimentation, the substrate removal curve was indeed the complement of the biological solids accumulation curve. There were, however, some notable exceptions. For example, in Figure 94 it is felt that the best representation of solids accumulation is in accordance with linear kinetics, whereas the best representation of the total COD removal is somewhat diphasic and in an overall sense, represented as first order decreasing rate removal. However, it might be argued that the total COD removal could have been plotted as a linear function during the period of solids accumulation. Also, in Figure 149, the total COD removal curve is represented as first order increasing throughout most of the experiment, whereas biological solids accumulation is represented by linear kinetics. Also, in Figure 148, it is seen that for the glycerol control, removal of COD is best

represented as two sequential zero order modes of removal, whereas the biological solids accumulation curve would appear to be best fitted by a single straight line.

It is important to note that the general complementary nature for the kinetics of sludge accumulation and substrate removal applies only when the total COD (as the measure of the total available carbon source) is used as the guiding parameter for substrate. When a test specific for the carbon source was employed, it was often observed that the kinetics of carbon source utilization and of growth did not proceed in a complementary manner. In many experiments there was evidence that metabolic intermediates and/or endproducts accumulated in the medium. It would thus appear that various species of organisms can dissimilate a specific carbon source at a significantly faster rate than they can channel the dissimilation products into synthesis of cellular constituents.

c. Some Factors Affecting the Mode of Kinetic Expression in Heterogeneous Populations

(1) Structural Configuration of the Carbon Source

It would be expected that the type of carbon source available to the population would play a significant role in determining the type of overall growth and carbon source removal kinetics. In the present study the cells were acclimated to glycerol, and it is interesting to make comparisons between growth kinetics on glycerol and on glucose, a compound which is usually considered to be one to which no acclimation is needed. As an example of the gross differences in growth kinetics

which can exist, dependent upon the type of carbon source, attention is directed to Figure 95. The initial population was the same in both cases (with respect to type of organisms present and initial concentration of biological solids). However, the growth kinetics on glycerol were entirely different from the growth kinetics observed on glucose. On glucose, linear growth kinetics and linear removal of total COD were observed, whereas on glycerol, typical order first increasing rate kinetics were observed. For a similar experiment using a heterogeneous population grown on glycerol, the same general trend in kinetic expression was observed (Figure 112). Again, in Figure 140, the same general trend in the mode of kinetic expression was observed using cells harvested from the continuous flow unit. It is emphasized that a considerable period of time had elapsed between running the experiments shown in Figures 95, 112, and 140; there was ample time for changes in predominance to occur. However, the same general mode of kinetic expression was observed, and from such results it might be concluded that it is possible to generalize for heterogeneous populations that the type of substrate plays a major role in determining the type of kinetic expression which will be observed. However, the need for caution in making unequivocal conclusions in this regard is shown by the results presented in Figure 148. The experiments were run in precisely the same manner as those previously cited; however, in this case glucose gave a typical growth response whereas growth on glycerol (the substrate to which the cells were acclimated) as well as total COD removal could be best approximated as following linear kinetic order. Thus it would appear that while it is possible to accord a major role to the type of carbon source as a determinative factor for the mode of kinetic

expression, one cannot overlook the role which can be played by changes in predominating species in the population.

(2) The Role of Substrate Interactions

In multiple carbon source media it can be expected that substrate interactions leading to metabolite repression will often occur. Such occurrences can lead to severe discontinuities in the overall kinetics of growth and substrate removal and, at times, although the disruptions in overall kinetic mode may not be too severe, the presence of one compound can affect or shift the kinetics for the removal of another, even when the removal of the compounds proceeds concurrently. For an example of a change in the kinetic mode of removal of glycerol when used as a sole carbon source as compared to its removal when used in combination with glucose, attention is directed to Figures 89 and 90. In Figure 89 it is clearly seen that the removal of glycerol and growth on glycerol proceed in accordance with first order kinetics. In Figure 90 it can be seen that glycerol, which was removed after glucose was eliminated from the system, was eliminated in accordance with zero order kinetics. Again, in Figures 119 and 120, it is seen that in the multicomponent system (Figure 120) the presence of glucose caused three successive modes of glycerol removal, i.e., zero order, first order increasing, and zero order. Under nonproliferating conditions it is seen from the results shown in Figure 173 that the zero order kinetics expected under such conditions were observed in both control systems; however, when glucose and glycerol were used in combination, the kinetics of glucose removal and of solids accumulation, as well as of total COD removal, were considerably changed.

(3) The Effect of Intermediates on the Mode of Kinetic Expression

In these studies as well as in other studies in our laboratory, the effect of intermediate production, accumulation, and subsequent metabolism has been amply demonstrated. The accumulation of metabolic intermediates and/or endproducts in the medium quite naturally converts a single carbon source system into a multiple carbon source system, and it follows that substrate interactions leading to metabolite repression can exist in "single carbon source systems" as well as in initially multicomponent systems. A rather dramatic example of the effect that the accumulation of metabolic intermediates can have on the overall kinetics of growth and total COD removal is provided by the results shown in Figure 95 for the glucose control. Also in Figure 106 it is seen how, in a multicomponent carbon source medium, accumulation of metabolic intermediates from either glucose or glycerol (in the case shown, most probably from both) can lead to a gross discontinuity in total COD removal as well as in the kinetics of solids accumulation.

(4) The Effect of Exogenous Nitrogen Concentration and Initial Cell Concentration on the Kinetic Mode of Growth and Substrate Removal

The effects of nitrogen deficiency and of high initial biological solids concentration are considered together, since they both exhibit a general tendency to cause zero order kinetics with respect to growth and with respect to substrate removal. In some studies performed in the present investigation, cells were harvested from the chemostat, washed, and used under nonproliferating conditions. In other studies, especially when the dilution rate was low (high detention times), cells were taken from the continuous flow unit and used in nonproliferating experiments without the benefit of cell washing to remove traces of the

nitrogen source. This was necessary, since at the high detention times it was difficult to collect enough cells to run an experiment initiated with high biological solids concentration, and it was felt that the usual losses in solids concentration during the washing procedure would militate against the attainment of the desired initial biological solids concentration. The results shown in Figures 78 and 79 demonstrate the expected linear kinetics under nonproliferating conditions. In cases where the cells were not washed but the experiment employing the cells was run under nonproliferating conditions and the initial biological solids concentration was not particularly high, it would appear that the nitrogen carryover with the seed population provided enough nitrogen source to cause a divergence from the expected zero order kinetics. Thus in Figures 82 and 83, 87 and 88, and 110 and 111 the kinetic response was more typical of a growth system than a nonproliferating system.

(5) Effect of Cell Age on the Rate of Biological Solids Accumulation and the Rate of Total COD Removal

In the present study, dilution rates of 1/4 to 1/192 $hr^{-1}$ were employed, and it was of interest to determine whether the growth rate (or dilution rate) in the continuous flow unit exerted some effect on COD removal rate and growth rate when cells of different "cell age" were used in the batch experiments. In Figure 179 the rates of removal of total COD obtained using low biological solids concentrations under nonproliferating conditions, i.e., the batch control systems at different dilution rates, are plotted versus the cell age (assuming detention time in the continuous flow reactor as a measure of cell age). As the cell age increased from four to 48 hours, there was a marked decreasing

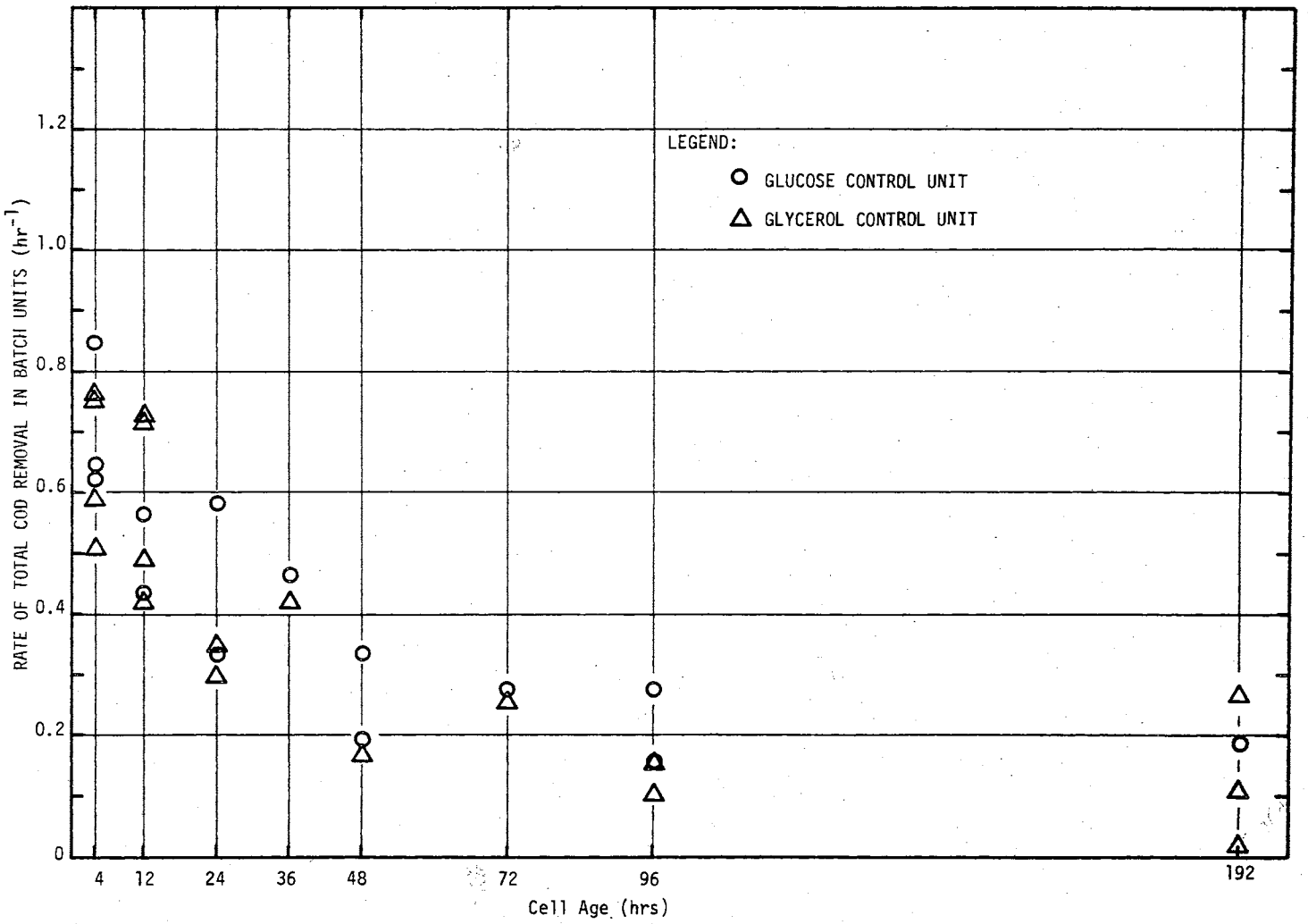



Figure 179 - Total COD Removal Rates Exhibited in 500 mg/l Glucose Batch Units and in 500 mg/l Glycerol Batch Units at Various Cell Ages.

trend in the observed rate of COD removal. At higher cell ages there appeared to be a tendency for the rate of substrate removal to become asymptotic to a lower limit.

Variation in log growth rate of the glycerol control units with cell age is shown in Figure 180. The data were quite scattered; however, there was a decreasing trend in growth rate as the cell age of the population increased.

(6) Factors Affecting Cell Yield

Cell yield may be defined in various ways; however, from the standpoint of the pollution control engineer, the cell yield is best described as the weight of sludge produced for a given amount of carbon source (COD) removed, expressed as a percentage. The sludge yield on a particular carbon source or waste water is an extremely important design factor for sizing the sludge handling facility at a treatment plant. Perhaps the most useful method for calculating the sludge yield is simply to determine the increase in biological solids which has taken place during the substrate removal period; the incremental increase in biological solids divided by the amount of COD removed (expressed as a percent) is the cell yield for the particular system involved. The cell yield value can also be based upon the increase in solids for a given amount of the original exogenous carbon source removed. However, because of the production of metabolic intermediates and their accumulation in the medium, this method is not of great utility in the pollution control field. It is also possible to calculate the cell yield as the ratio of the specific growth rate to the specific rate of total COD removal. This method would apply particularly during logarithmic growth. Another way in which the yield has been calculated is as the

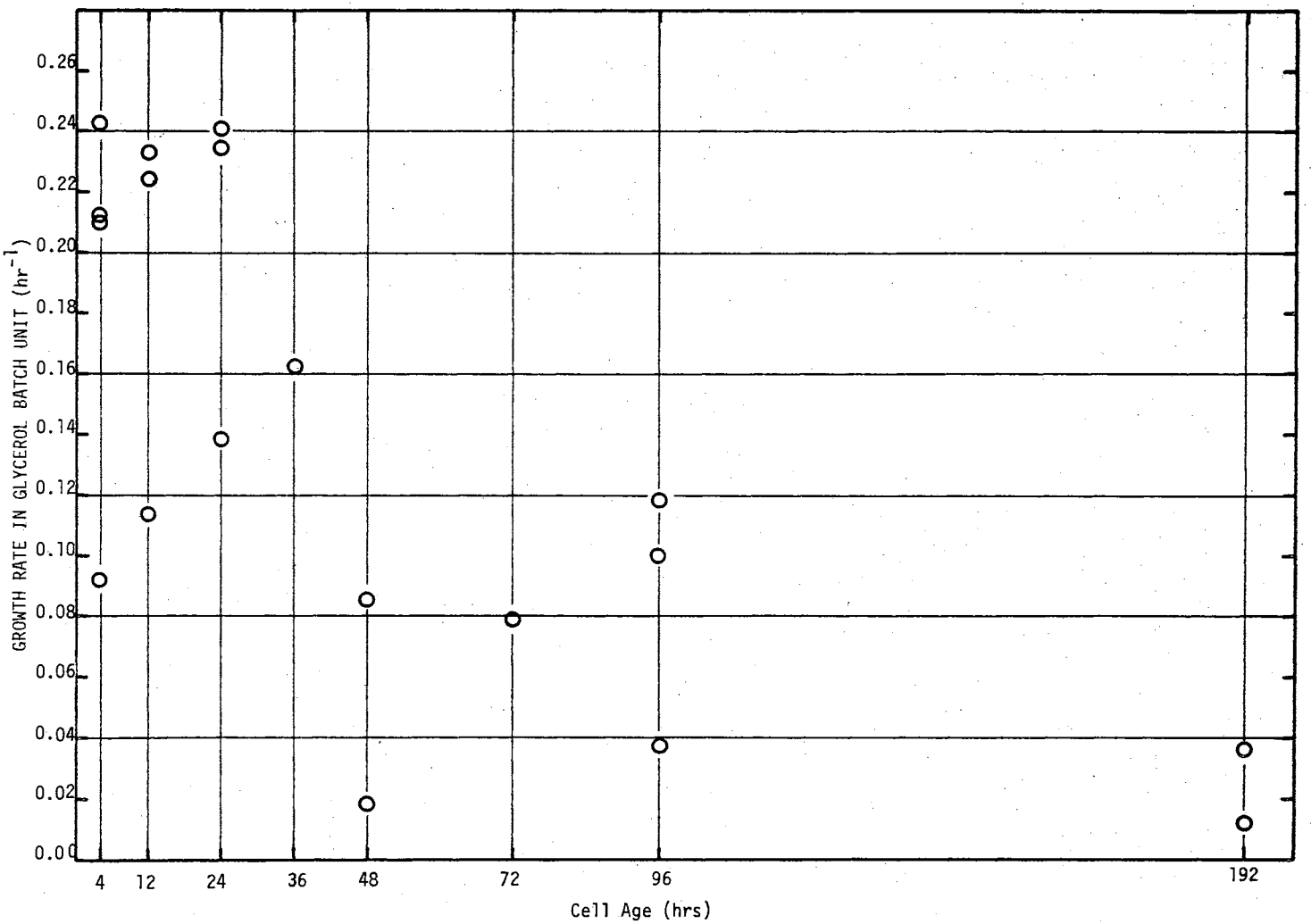



Figure 180 - Microbial Growth Rates Exhibited in 500 mg/l Glycerol Batch Units at Various Cell Ages.

average of the ratios of increases in biological solids concentration to corresponding amounts of COD removed at various times along the biological solids and COD removal curves. Since there is usually a constant relationship between biological solids produced and COD removed, the latter two methods are valid and also provide a check on the constancy of the cell yield throughout the substrate removal period (43). Rao and Gaudy have shown that all of the methods for calculating yield involving the ratio of the increase in solids to amount of COD removed give values which differ only slightly from each other. The method of choice in the present study was the first one cited, i.e., the increase in biological solids concentration divided by the amount of COD removed at the end of the COD removal period.

Recently there has been some controversy concerning the prediction of cell yield. Servizi and Bogan (44) maintained that compounds with higher molar free energy values gave greater cell yields than compounds with less molar-free energy. Gaudy and Gaudy (19) have debated this hypothesis, and have pointed out that in aerobic systems it is the amount of carbon available to the cells rather than the amount of energy from that carbon source which should tend to provide a limiting factor for the biological solids accumulation. Also, Rao and Gaudy (43) have shown that the cell yield can vary widely when heterogeneous populations are grown on a single carbon source. In the present work, yields were computed for the two compounds employed, i.e., glycerol and glucose. Since glycerol is a more reduced compound than is glucose (two extra hydrogen atoms), one might expect that the average yields on glycerol would be higher than on glucose. However, the average of forty-one experiments indicates that the average yield obtained in the

glucose controls was 47.9 per cent, and in the glycerol controls, 42.5 per cent. The average yield of the combined systems was 45.2 per cent. Thus it would appear that one could not conclude a priori that compounds which exhibited the greatest potential for oxidation would necessarily provide the highest cell yield.

It is interesting to examine the effect of cell age on cell yield. In the present study, two types of data lend themselves to such analysis. Computing the cell yield from the steady state data in the continuous flow (glycerol) unit, it was found that the yields varied from 40 to 70 per cent, and there was a slight tendency for the cell yield to decrease as the cell age increased, i.e., as detention time in the reactor increased. Figure 181 is a plot of cell yield in the chemostat versus detention time. There is considerable scatter, but the trend of the data indicates that there is a decreasing cell yield as detention time increases. The yields for the batch studies (glucose, glycerol, and the combined system) are shown in Figure 182. Again there is scatter in the data, but it may be discerned that there is a tendency for the cell yield to decrease as the cell age increased.

From the rather high degree of scatter in cell yield for each substrate at a given dilution rate, it can be seen that one cannot predict cell yield on the basis of substrate or substrate-type. In this regard the present data substantiate the conclusions of Rao and Gaudy, who found a wide scatter in cell yield in long-term studies using one substrate, glucose. In general, regardless of the experimental conditions, most of the yields obtained in the present study lie between 30 and 60 per cent, with the greatest concentration in the range of 40 to 50 per cent.

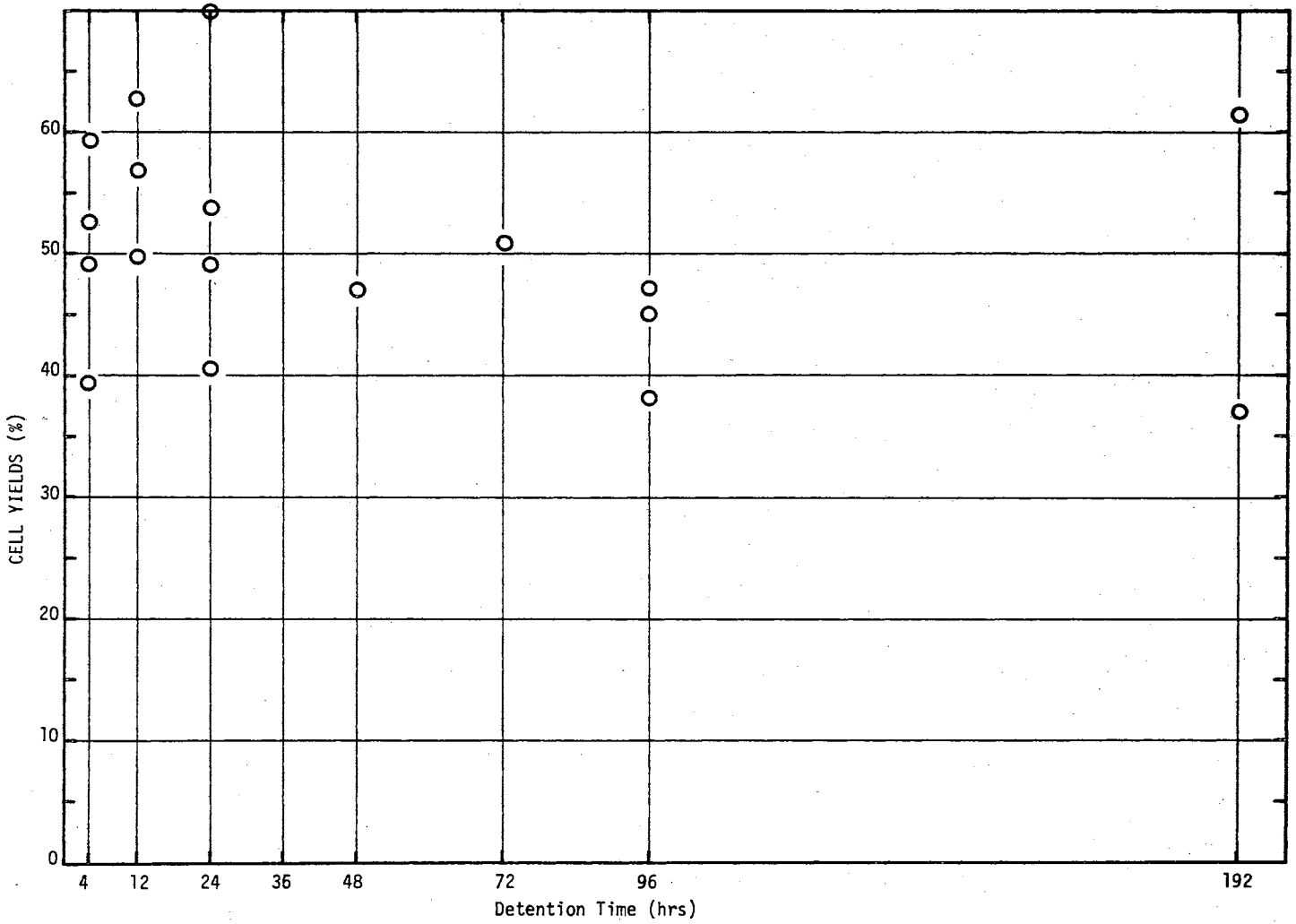



Figure 181 - Cell Yields Exhibited in 2000 mg/l Glycerol Continuous Flow Unit at Various Detention Times.

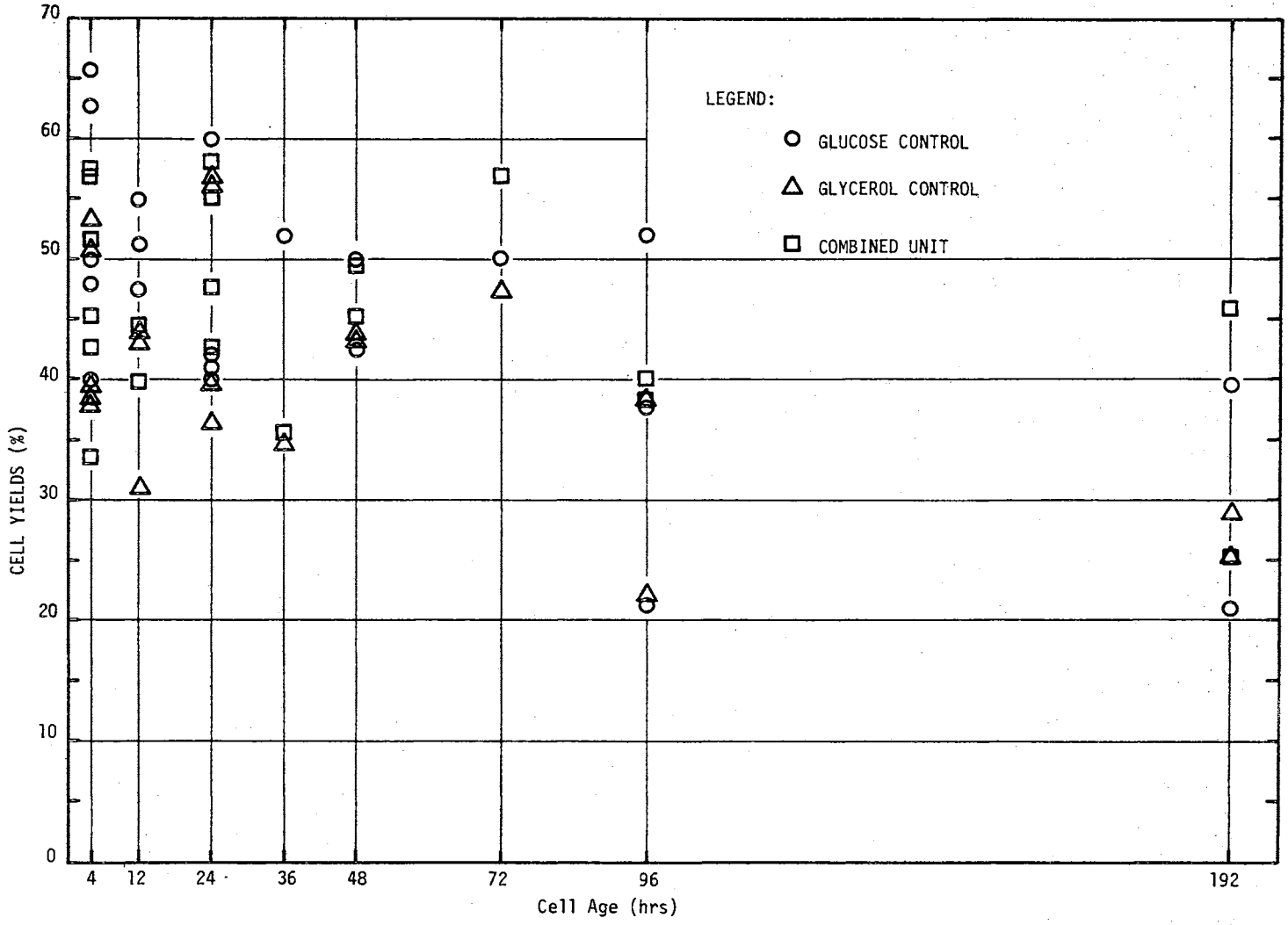



Figure 182 - Cell Yields at Various Cell Ages Exhibited in Batch Units.

(7) The Effect of Accumulation of Metabolic Intermediates and/or End-products on the Mode of Substrate Removal and the Effect of Cell Age on the Production of Metabolic Intermediates

In the discussion of the results presented in Phase A it was brought out that sequential removal or partial blockage of one compound by another occurred when the compound which exhibited the blocking effect was metabolized with considerable accumulation of metabolic intermediates in the medium. This finding is consistent with the hypothesis of metabolite repression, and it is interesting to analyze the studies on combined systems containing glucose and glycerol (combined units) which were performed in this latter phase of the research. In order to analyze the effect of the production of metabolic intermediates on the mode of substrate removal, the maximum intermediates in the glycerol control were subtracted from the maximum intermediates produced in the glucose control for experiments at the various dilution rates (detention times or cell ages). In Figure 183 the difference in intermediates produced is plotted versus cell age for the three major types of response which were exhibited, i.e., glucose blockage of glycerol, concurrent removal, and glycerol blockage of glucose. In the figure, points identified as glucose inhibition of glycerol include those in which the removal was nearly a totally sequential one, or a partially sequential removal. It is seen that glucose seriously interfered with glycerol removal when the production and accumulation of metabolic intermediates on glucose was considerably greater than that on glycerol. When the difference was somewhat lower, there was a tendency for concurrent removal of glucose and glycerol. When the controls yielded an insignificant amount of intermediates or when intermediates on glycerol were

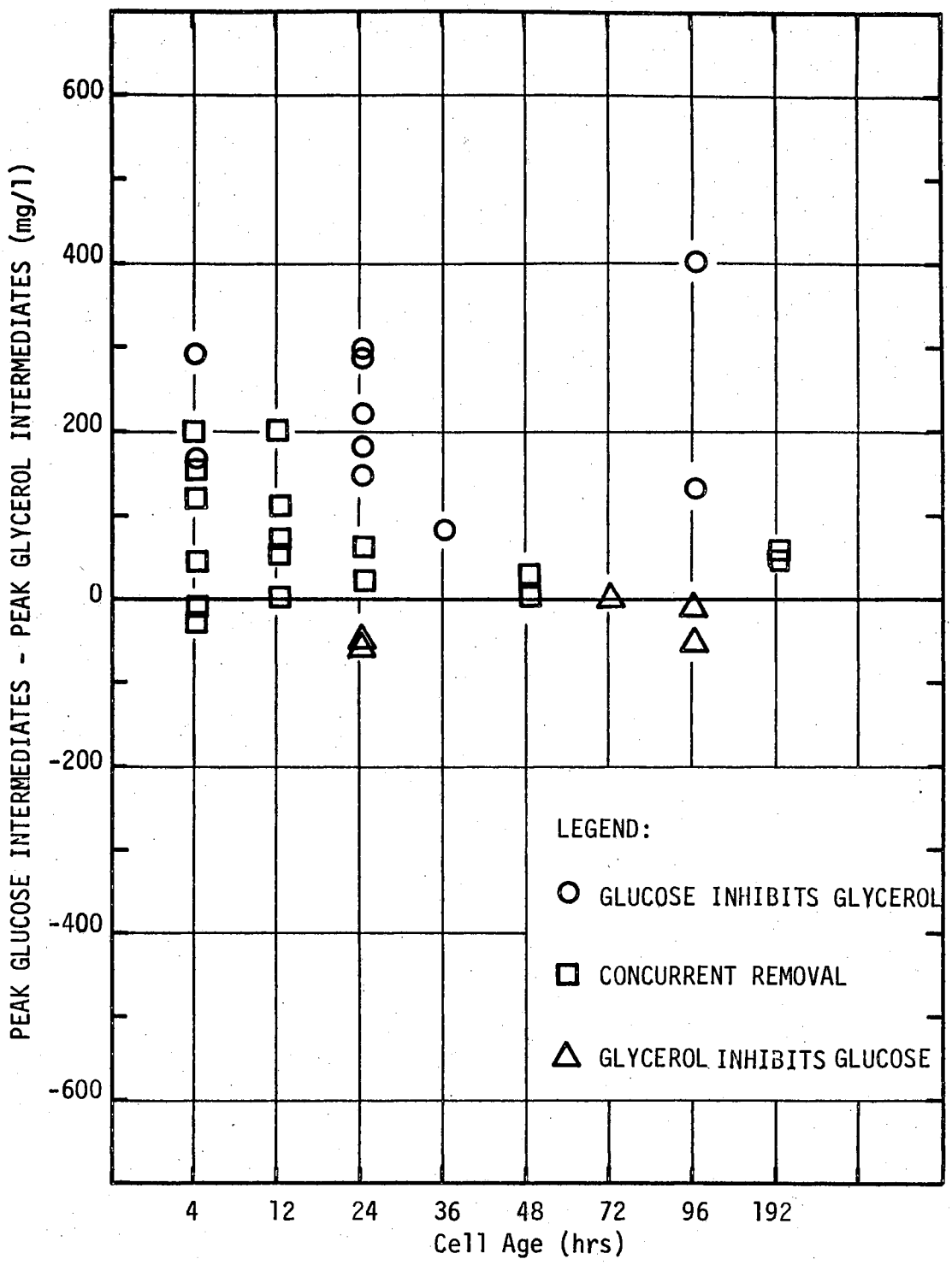



Figure 183 - Difference in Concentrations of Peak Metabolic Intermediates Produced in 500 mg/l Glucose Batch Unit and in 500 mg/l Glycerol Batch Unit at Various Cell Ages.

greater than intermediates produced on glucose, there was a tendency for glycerol to interfere with glucose removal.

It is interesting to examine the effect of cell age on the production of metabolic intermediates in all of the control systems under the various growth conditions employed (i.e., proliferating and nonproliferating conditions). The peak value for intermediate accumulation for each system is plotted versus cell age or detention time in Figure 184. These data illustrate quite strikingly that there was a tremendous variation in the amount of intermediates which were accumulated in the medium at any detention time (for example, see glucose control, proliferating conditions at the 4-hour detention time). It would thus appear that the previous growth history is not an unequivocal predictor of the amount of metabolic intermediates which can be expected to be produced or accumulated on a given substrate. It is also interesting to note that in some cases as much as 400 mg/l (as COD) of intermediates were produced on 500 mg/l of glucose. In general, a higher amount of metabolic intermediates was produced in the glucose system for younger cells than for older cells.

(8) Observations on the "Steady State" Parameters as Dilution Rate was Varied from 1/4 to 1/192 $hr^{-1}$

It is believed that the studies undertaken for this thesis research represent the compilation of continuous flow reactor data over the widest range of dilution rates ever examined. Also, steady state data at each dilution rate were obtained a number of times, i.e., the unit was often returned to operation at the same dilution rates used previously. It was therefore interesting to plot the average solids concentration, cell yield, and effluent COD values for each dilution rate

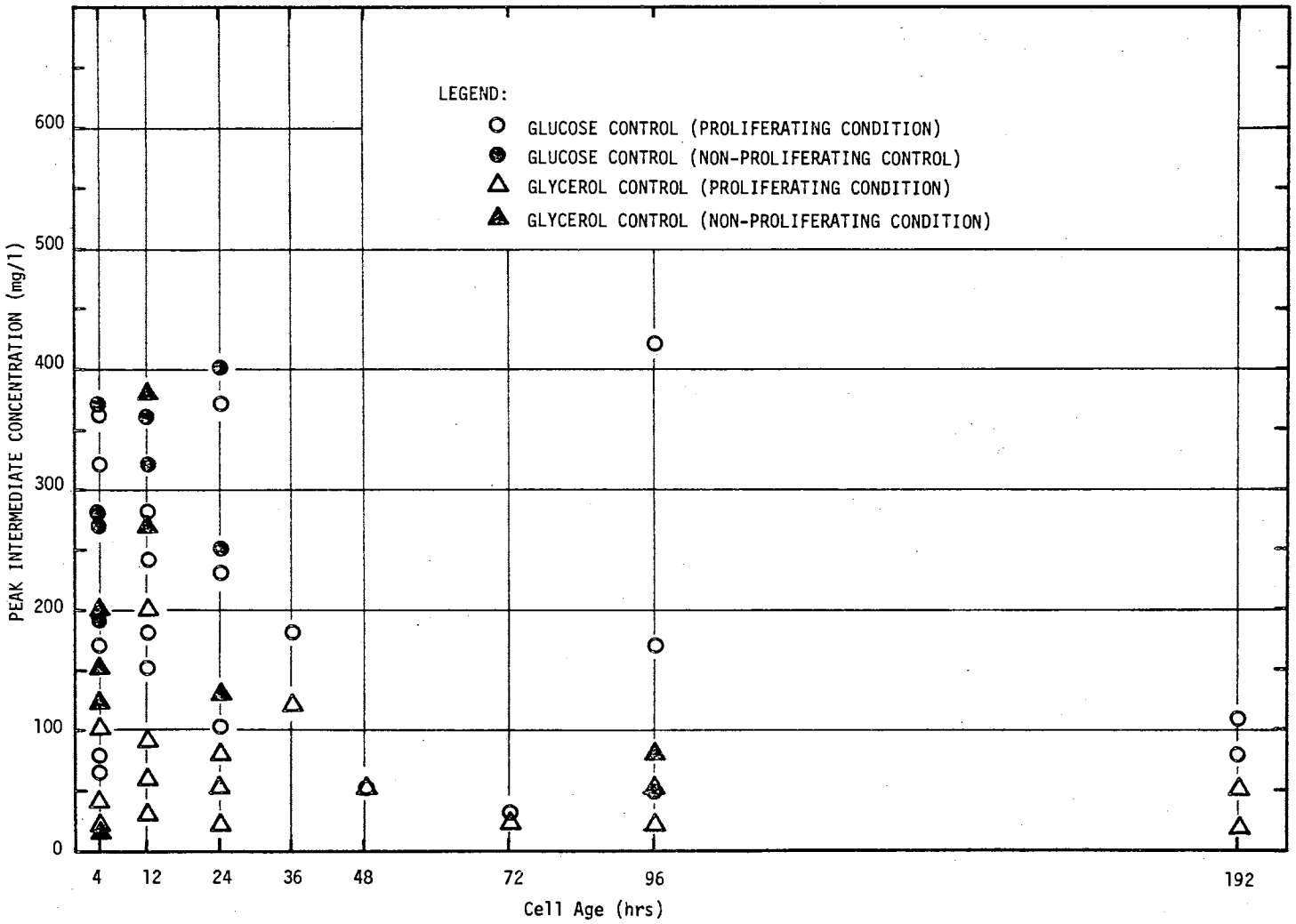



Figure 184 - Concentrations of Metabolic Intermediates Produced at Various Cell Ages.

to determine whether there were discernible trends in the average values of these parameters as dilution rate increased. Such a plot is shown in Figure 185. The solid symbols represent the averages of the various parameters plotted for each dilution rate; it should be remembered, however, that the figures used to obtain these average values are themselves averages of multiple samples taken in steady state operation at each dilution rate. It is seen that at the fastest dilution rate employed, 1/4 $hr^{-1}$, the unit had not yet begun to show evidence of approaching the region of cell dilute-out. The average conditions indicate that from dilution rates of 1/4 to 1/24 $hr^{-1}$, the system was relatively stable with respect to average biological solids concentration, cell yield, and effluent COD. At very low dilution rates there appeared to be a decrease in cell yield and in average biological solids concentration in the unit, and an increase in effluent COD. The decrease in biological solids at the very low dilution rates (or high detention times) would seem to be most probably due to autodigestion and cell lysis at these very slow growth rates (dilution rate of 1/192 $hr^{-1}$ = $\mu$ of 0.005 $hr^{-1}$). Thus the slight rise in COD would appear to be most likely due to nonbiodegradable lysis products. It is also important to note that the decrease in biological solids at the very high detention times was actually somewhat more severe than was registered by the biological solids concentration. Evaporation rate in the unit was measured and averaged approximately 80 ml/day. At a dilution rate of 1/192 $hr^{-1}$, the flowrate to the reactor was 250 ml/day. Thus, one-third of the water in the mixed liquor was evaporated. Since evaporation would tend to concentrate the cells, the decrease in cell concentration which was registered was attenuated by evaporation. It is surprising that the

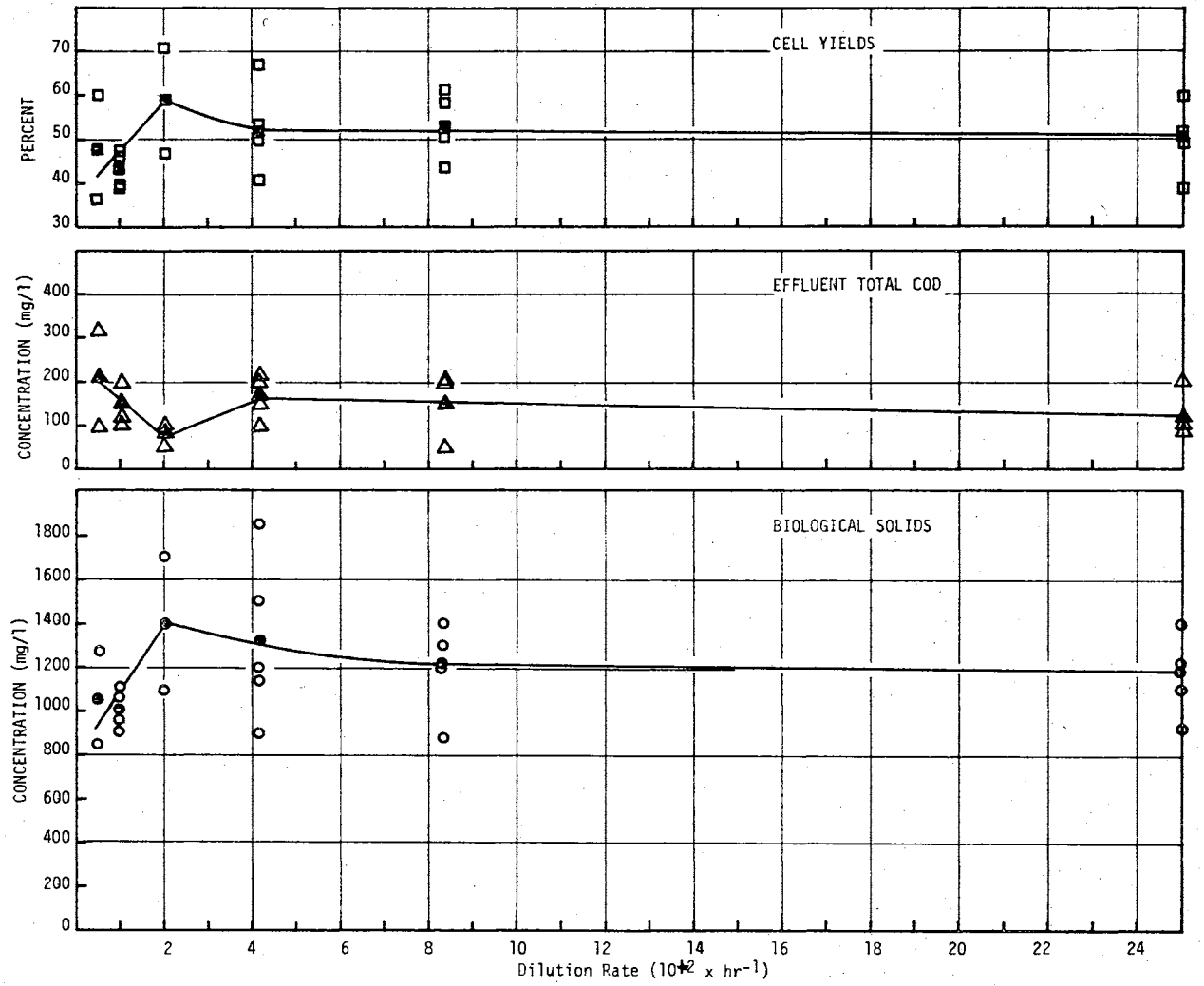



Figure 185 - Concentration of Biological Solids, Effluent COD, and Cell Yields in the 2000 mg/l Glycerol Continuous Flow Unit at Various Dilution Rates.

continuous flow unit could be operated at conditions approaching the steady state for a growth rate as slow as 0.005 $hr^{-1}$. However, from the results of the present study, it would be predicted that even slower growth rates would be possible and it seems reasonable to predict that steady state kinetic formulations might, therefore, be applied to the so-called aerated lagoons which employ somewhat higher detention times.

# CHAPTER VI

## CONCLUSIONS

1. Glucose exerts a repressive effect on the synthesis of enzymes required for the utilization of a variety of other compounds regardless of whether a direct sewage seed is employed or the microbial population is acclimated to a specific compound other than glucose.

2. The repressive effect of one compound upon another in a mixed substrate system is not a unique property of glucose. Sucrose, galactose, and other compounds which allow rapid growth or which cause a considerable concentration of metabolic intermediates and/or endproducts to accumulate in the medium also exhibit repressive properties.

3. Lactose, an important constituent of some dairy wastes, was particularly subject to blockage of removal by a variety of other compounds.

4. Using a sewage seed, a heterogeneous population which could metabolize arabinose was readily developed. However, heterogeneous populations developed from a sewage seed on other compounds could not readily metabolize arabinose.

5. Sequential substrate removal in a multicomponent carbon source system is not necessarily a manifestation of enzyme repression or suppression. In some cases the normal induction period required for the synthesis of enzymes for degradation of the substrate to which the cells

have not been previously acclimated may be so long that sequential removal is observed. In such cases it is essential to have available data on the control systems.

6. Neither a diauxic biological growth curve nor a diphasic total COD removal curve is an absolute parameter for evaluating the occurrence of sequential substrate removal, since substrate can be removed sequentially without the existence of diauxie. The only reliable indicator is analysis specific for the compounds under study.

7. The kinetics of biological growth is usually in accordance with the kinetics of total COD removal, but is not necessarily in accordance or agreement with the kinetics of removal of the specific substrate in the system.

8. Changes in microbial predominance in a continuously cultured system occur without the exertion of any known external pressure. Changes in the flowrate of the incoming carbon source also can bring about changes in microbial predominance.

9. The extent of disruption of system efficiency during the period of receiving a hydraulic shock load is dependent in large measure upon the severity of the hydraulic shock load which is applied, and in large measure upon the rapidity with which the particular predominating organisms in the unit at the time of applying the shock load can respond to the required change in growth rate. Of lesser importance in determining the severity of response is the age of the cells, i.e., the dilution rate at which the cells were growing before the shock was applied.

10. The typical response to a severe increase in dilution rate is a rapid washout of biological solids and a rise in substrate concentration in the effluent.

11. The response to a severe decrease in dilution rate is somewhat similar to the response to a severe increase in dilution rate. There is a decrease in the cell concentration and an increase in the COD concentration in the effluent; however, the increase in effluent COD concentration usually does not correspond to an increase in concentration of the original exogenous substrate. It appears that when the cells are forced to grow slower in response to the hydraulic shock load than they were growing previous to it, the starvation conditions imposed on the system cause some cell die-off and lysis. It seems reasonable to postulate that a significant portion of the COD of the effluent consists of lysis products.

12. In general, the cell yields obtained during steady state operation were higher than those obtained during transient states.

13. When an increase in dilution rate was applied to the continuous flow unit, the cellular carbohydrate content increased during the transient state, whereas the protein content remained fairly constant or, in some cases, was slightly decreased.

14. In the batch studies accomplished using glucose and glycerol, with cells harvested from the continuous flow unit at various dilution rates (cell ages), there was a tendency toward decreased occurrence of sequential substrate removal as the age of the culture increased. However, an increase in cell age did not provide any guarantee that concurrent substrate removal would be exhibited. Concurrent substrate removal was also observed for fairly young populations.

15. The kinetics of substrate removal and biological growth depend upon many factors, and from an engineering standpoint it would appear a fruitless exercise to attempt to devise sophisticated

mathematical formulations for all types of kinetics. However, a suitable fit to the growth and COD removal curves can be obtained with essentially three kinetic modes, i.e., first order increasing rate, first order decreasing rate, and zero order kinetics.

16. When cells of different ages were harvested from the continuous flow unit and used in batch studies it was noted that as the cell age increased, total COD removal rate tended to decrease.

17. In the continuous flow reactor, cell yields on glycerol varied from 40 to 70 per cent. Using cells harvested from the continuous flow reactor for batch studies, the cell yields calculated during the batch study generally varied between 30 and 60 per cent, and most of the cell yields were in the range of 40 to 50 per cent.

18. In forty-one experiments the cell yield for the glucose controls averaged 47.9 per cent, and for the glycerol control systems, 42.5 per cent. The average yields in the combined systems was 45.2 per cent. The higher yield on glucose, as compared to glycerol, would indicate that one cannot predict cell yield on the basis of energy content of the substrate. Glycerol contains more oxidizable hydrogen than does glucose and, if yield in aerobic systems could be adequately predicted on the basis of energy content of the substrate, one would have expected to observe greater yields on glycerol than on glucose.

19. The effect of metabolic intermediates on the occurrence of sequential substrate removal was ideally demonstrated in the glucose-glycerol systems. In cases where the accumulation of metabolic intermediates was considerably greater in the glucose control than in the glycerol control, glucose interfered with glycerol metabolism in the combined system. When the reverse situation occurred, there was a

tendency for glycerol to interfere with glucose removal.

20. In general, for the glucose controls, a greater amount of metabolic intermediates was produced when cells of younger age were used than when cells of older age were used. This finding tends to explain, in accordance with the principles of metabolite repression, why there is a tendency for the occurrence of sequential substrate removal to decrease as the cell age increases.

21. At dilution rates of 1/4 to 1/24 $hr^{-1}$ there were no appreciable differences in the steady state values of biological solids, effluent COD concentration and cell yields, whereas at very low dilution rates there appeared to be a decrease in cell yields and in the average solids in the unit, as well as a slight increase in effluent COD. The causes for this trend were not determined, but it seems reasonable to postulate that the decrease in biological solids concentration at very low dilution rates is most probably due to "aerobic digestion" and that the slight rise in effluent COD is due to non-biodegradable cell lysate products.

22. It was found in these studies that continuous culture of a microbial population could be successfully carried out at a growth rate as low as 1/192 $hr^{-1}$. Furthermore, it is believed that if evaporation could be prevented, it could be shown that even lower steady state growth rates could be attained.

# CHAPTER VII

## SUGGESTIONS FOR FUTURE WORK

The present study as well as much of the work which has preceded it and the work which is still in progress by other investigators in the bioengineering laboratories pertinent to substrate removal in multicomponent carbon source systems should be continued on a wide variety of combinations of substrates, since this work has formed the basis for possible future understanding of the complex and interrelated factors governing kinetics and mechanism of waste water purification. The following specific suggestions for future work would appear to provide fruitful avenues for further investigation:

1. The future studies, such as the type accomplished in Phase A of the present thesis work, should be continued and extended to a wider variety of compounds.

2. All available cell yield data collected by various investigators in the bioengineering laboratories should be summarized and subjected to statistical analysis. A very large amount of data of this type is available, and it should be possible to draw generalized conclusions since all of the yield data were collected under controlled conditions. It would also be desirable to conduct special studies on cell yield using heterogeneous populations for a variety of substrates with widely varying free energy content. It would also be advisable to

select a few substrates with widely varying free energy content for use in cell yield studies with selected pure culture to which these substrates were readily available as carbon and energy source.

3. Much has been learned in the bioengineering laboratories concerning the kinetics and mechanisms of substrate removal under a variety of operational conditions using heterogeneous populations. It would be ideal to select a well-studied pure culture such as Escherichia coli and to perform studies similar to those which have been accomplished using heterogeneous populations. While such a study would not add materially to the knowledge already gained concerning the kinetics and mechanism of waste water purification, such experimentation could make a contribution to the basic microbiological field, and would provide confirmation of conclusions drawn using heterogeneous populations.

4. Studies now under way by J. J. Su in which multicomponent substrate systems are being fed to continuous flow reactors form an essential extension of the present work on multicomponent substrate media.

5. Since in these studies and in others in our laboratories it has been found that heterogeneous populations are subject to rather wide fluctuations in predominating species, the studies now under way by D. E. Modesitt can provide extremely useful information. In particular, studies using known mixtures of species isolated from sewage in experiments wherein the feed consists of a multicomponent carbon source would provide an extremely useful extension of the present studies.

# BIBLIOGRAPHY


1. Monod, J., "The Phenomenon of Enzymatic Adaptation," Growth, 11, 223-289 (1947).

2. Su, J. J., "Utilization of Mixtures of Carbohydrates by Heterogeneous Populations," Masters Thesis, Oklahoma State University (May, 1968).

3. McFall, E., and Mandelstam, J., "Specific Metabolic Repression of Induced Enzymes in Escherichia coli," Nature, 197, 880-881 (1963).

4. Zwaig, N., and Lin, E. C. C., "Feedback Inhibition of Glycerol Kinase, a Catabolic Enzyme in Escherichia coli," Science, 153, 755-757 (1966).

5. Gaudy, A. F., Jr., Komolrit, K., and Bhatla, M. N., "Sequential Substrate Removal in Heterogeneous Populations," Journal Water Pollution Control Federation, 35, 903-922 (1963).

6. Gaudy, A. F., Jr., Gaudy, E. T., and Komolrit, K., "Multicomponent Substrate Utilization by Natural Populations and a Pure Culture of Escherichia coli," Applied Microbiology, 11, 157-162 (1963).

7. Gaudy, A. F., Jr., "Studies on Induction and Repression in Activated Sludge Systems," Applied Microbiology, 10, 264-271 (1962).

8. Gaudy, A. F., Jr., Komolrit, K., and Gaudy, E. T., "Sequential Substrate Removal in Response to Qualitative Shock Loading of Activated Sludge Systems," Applied Microbiology, 12, 280-286 (1964).

9. Prakasam, T. B. S., and Dondero, N. C., "Observations on the Behavior of a Microbial Population Adapted to a Synthetic Waste," Proceedings 19th Industrial Waste Conference, Purdue University, Extension Series, 835-845 (1964).

10. Komolrit, K., and Gaudy, A. F., Jr., "Substrate Interaction During Shock Loadings to Biological Treatment Processes," Proceedings 19th Industrial Waste Conference, Purdue University, Extension Series, 796-810 (1964).

11. Prakasam, T. B. S., and Dondero, N. C., "Aerobic Heterotrophic Bacterial Populations of Sewage and Activated Sludge. III. Adaptation in a Synthetic Waste," Applied Microbiology, 15, 1128-1137 (1967).

12. Stumm-Zollinger, E., "Effects of Inhibition and Repression on the Utilization of Substrates by Heterogeneous Bacterial Communities," Applied Microbiology, 14, 654-664 (1966).

13. Stumm-Zollinger, E., "Substrate Utilization in Heterogeneous Bacterial Communities," Journal Water Pollution Control Federation, 40, R213-R229 (1968).

14. Komolrit, K., and Gaudy, A. F., Jr., "Biochemical Response on Continuous Flow Activated Sludge Processes to Qualitative Shock Loadings," 37th Annual Conference Water Pollution Control Federation, Bal Harbour, Florida (September, 1964); also Journal Water Pollution Control Federation, 38, 85-101 (1966).

15. Gaudy, A. F., Jr., Komolrit, K., Gaudy, E. T., and Bhatla, M. N., "Multicomponent Substrate Removal by Activated Sludge and by Pure Culture Systems," Bacteriological Proceedings, xvii-xviii (1963).

16. Bhatla, M. N., and Gaudy, A. F. Jr., "Studies on the Causation of Phasic Oxygen Uptake in High Energy Systems," Proceedings 19th Industrial Waste Conference, Purdue University, Extension Series, 871-886 (1964); also Journal Water Pollution Control Federation, 38, 1441-1451 (1966).

17. Bhatla, M. N., and Gaudy, A. F. Jr., "Sequential Substrate Removal in a Dilute System by Heterogeneous Populations," Applied Microbiology, 13, 345-347 (1965).

18. Krishnan, P., and Gaudy, A. F. Jr., "Studies on the Response of Activated Sludge to Shock Loadings," Proceedings 12th Ontario Industrial Waste Conference (June, 1965); also Biotechnology and Bioengineering, VII, 455-470 (1965).

19. Gaudy, A. F. Jr., and Gaudy, E. T., "Microbiology of Waste Water Purification," Annual Review of Microbiology, 20, 319-336 (1966).

20. Grady, C. P. L., Jr., and Gaudy, A. F., Jr., "Substrate Interaction in Natural Populations," 55th Annual Meeting Oklahoma Academy of Science, Norman, Oklahoma (December, 1966).

21. Gaudy, A. F., Jr., "Metabolic Control Mechanisms in Heterogeneous Microbial Populations," Research Seminar, Notre Dame University, West Bend, Indiana (November 8, 1966); also University of North Carolina, Chapel Hill (March 4, 1967).

22. Kincannon, D. F., Gaudy, A. F., JR., and Gaudy, E. T., "Sequential Substrate Removal by Activated Sludge after a Change in Salt Concentration," Biotechnology and Bioengineering, VIII, 371-378 (1966).

23. Gaudy, A. F., Jr., Su, J. J., and Gaudy, E. T., "Studies on the Interaction of Organic Waste Components," VTEI - Vodohospodarake Technicko-Ekonomicke Informace (Czechoslovakia), 10, 32-38 (1968).

24. Gaudy, E. T., Tsay, S. S., and Gaudy, A. F., Jr., "Rapid Metabolic Control in Catabolic Pathways," Bacteriol. Proc., 1968:142 (1968).

25. Standard Methods for the Examination of Water and Waste Water, 12th Edition, American Public Health Association, New York (1965).

26. Experimental Biochemistry, Ed. by John M. Clark, Jr., W. H. Freeman and Company, San Francisco and London (1964).

27. Gaudy, A. F., Jr., "Colorimetric Determination of Protein and Carbohydrates," Industrial Water and Wastes, 7, 17-22 (1962).

28. "Glucostat for the Enzymatic Determination of Glucose," Worthington Biochemical Corporation, Freehold, New Jersey (1963).

29. "Galactostat - A Coupled Enzyme System for the Determination of Galactose," Worthington Biochemical Corporation, Freehold, New Jersey.

30. Ashwell, G., "Colorimetric Analysis of Sugars," Methods in Enzymology, Academic Press 3, 75-88, Ed. by S. P. Colowick and N. O. Kaplan (1957).

31. Jaeger, K., and Niemitz, W., "BOD Determination of Sewage by the Direct Method Using a Warburg Apparatus," Sewage and Industrial Wastes, 25, 631 (1953).

32. Umbreit, W. W., Burris, R. H., and Stauffer, J. I., Manometric Techniques, Burgess Publishing Company, Minneapolis, Minnesota (1957).

33. Komolrit, K., "Biochemical Response of Activated Sludge Processes to Organic Shock Loads," Doctoral Thesis, Oklahoma State University, Stillwater, Oklahoma (1965).

34. Thimann, K. V., The Life of Bacteria, 2nd Edition, The Macmillan Company, New York, and Collier-Macmillan, Ltd., London (1966).

35. Grady, C. P. L., Jr., "Metabolic Control Mechanisms Operative in Natural Microbial Populations Selected by Their Ability to Degrade Lysine," Doctoral Thesis, Oklahoma State University, Stillwater, Oklahoma (May, 1969).

36. Cassell, E. A., Sulzer, F. T., and Lamb, J. C., III, "Population Dynamics and Selection in Continuous Mixed Cultures," Journal Water Pollution Control Federation, 38, 1398-1409 (1966).

37. Genetelli, E., and Heukelekian, H., "The Influence of Loading and Chemical Composition of Substrate on the Performation of Activated Sludge," Proceedings 17th Industrial Waste Conference, Purdue University, Extension Series, 152-164 (1962).

38. Mateles, R. I., Ryu, D. Y., and Yasuda, T., "Measurement of Unsteady State Growth Rate of Microorganisms," Nature, 208, 263-265 (1965).

39. George, T. K., "Biochemical Response of Activated Sludge Processes to Hydraulic, pH, and Temperature Shock Loads," Doctoral Thesis, Oklahoma State University, Stillwater, Oklahoma (May, 1968).

40. Fair, G. M., Geyer, J. C., and Morris, J. C., Water Supply and Waste-Water Disposal, John Wiley and Sons, Inc., New York, London (1963).

41. Tsay, S. S., "Feedback Inhibition of Catabolic Pathways," Masters Thesis, Oklahoma State University, Stillwater, Oklahoma (May, 1968).

42. Heidman, J. A., Unpublished Data, Personal Communication.

43. Rao, B. S., and Gaudy, A. F., Jr., "Effect of Sludge Concentration on Various Aspects of Biological Activity in Activated Sludge," Proceedings, 5th Industrial Wastes Conference, Texas Pollution Control Association, 216 (1965); also Journal Water Pollution Control Federation, 38, 794-811 (1966).

44. Servizi, J. A., and Bogan, R. H., "Free Energy as Parameter in Biological Treatment," Journal Sanitary Engineering Division, ASCE, 89, SA3, 17-40 (1963).

# VITA

Ta-Shon Yu

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES ON THE EFFECT OF BACTERIAL GROWTH RATE ON SUBSTRATE REMOVAL IN MULTICOMPONENT SYNTHETIC WASTES

Major Field: Engineering

Biographical:

Personal Data: Born October 16, 1940, in Chang-Hwa, Taiwan, the Republic of China, the son of Hsi-tsung Yu and Shaing Lee.

Education: Attended Taiwan Provincial Cheng Kung Middle School in Taipei, Taiwan, the Republic of China; received the degree of Bachelor of Science from Taiwan Provincial Cheng Kung University, Tainan, Taiwan, the Republic of China, in June, 1963; received the degree of Master of Science in Sanitary and Public Health Engineering from the Oklahoma State University, Stillwater, Oklahoma, U. S. A., in May, 1966; completed requirements for the degree of Doctor of Philosophy in Engineering at the Oklahoma State University, in May, 1969, specializing in the bioengineering aspects of water pollution control.

Professional Experience: Served in the Chinese Navy as Civil Engineering Officer from July, 1963, to July, 1964; Bioengineering Graduate Research Assistant at the Oklahoma State University from September, 1965, to October, 1968; Staff Assistant at the Oklahoma State University from November, 1968, to March, 1969.

Membership in Professional Societies: American Water Works Association, Oklahoma Water and Pollution Control Association, Chinese Civil Engineering Association.

Publications:

Master Thesis: Effect of Polyelectrolyte Coagulant Aid on Bacterial Cells.