UTILIZATION OF MIXTURES OF CARBOHYDRATES BY HETEROGENEOUS POPULATIONS

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CHAPTER I

INTRODUCTION

A. Nature and Importance of the Study

In recent years the activated sludge process has become the most popular biological wastewater treatment process and has been extensively used by both municipalities and industries. There is every reason to believe that it will create more interest and find even greater use in the near future. Why does the activated sludge process pose such a definite challenge and great potential? This may be due to the fact that it has relatively high efficiency (rapid removal of the associated organic compounds, and short aeration and detention periods), requires smaller space for treatment than other biological processes, and provides a high degree of operational flexibility.

Activated sludge consists of macroscopic flocs produced by the growth of bacteria and other microorganisms in the presence of dissolved oxygen. The living masses of microorganisms in the activated sludge are essentially responsible for the purification of the waste water. They remove the soluble and suspended organic matter under aerobic conditions. After consumption of the associated

organic matter, the sludge flocs are allowed to settle and are separated from the carriage water which, now freed from 90-95 per cent of its organic contamination, leaves the settling tank as a relatively clear liquid.

In order to gain a better insight into operational criteria and various basic concepts concerning the kinetics and mechanisms of the activated sludge process, considerable research has been carried out toward understanding and establishing the basic mechanisms governing the metabolism of specific organic compounds by activated sludge. Mostly, this metabolic research has been related to compounds appearing in domestic sewage, significant industrial wastes, or potentially significant industrial wastes.

Recently, many attempts have been made to demonstrate the general occurrence of sequential substrate removal in a two-component growth medium. Of greater significance to the pollution control field is the finding that both enzyme inhibition and repression exist in heterogeneous populations during catabolism. Since the function of metabolic control mechanisms is a significant phenomenon for the activated sludge process, it is appropriate and necessary to extend the investigation using various combinations of carbon source. Carbohydrates were chosen as the class of compounds to be investigated in this study, because:

a. they are major components of domestic sewage and some types of industrial wastes;

b. a great deal of biochemical information on carbo-

hydrate metabolism is available for use in analyzing the results of the present study;

c. a wide variety of structurally different carbohydrates can be chosen as experimental substrates.

B. Purpose and Scope of the Present Investigation

This study was undertaken for the following purposes:

1) to gain a better understanding of metabolic control mechanisms;

2) to investigate the extent of interference or blockage of utilization between various carbohydrate substrates and to determine the generality of sequential or concurrent removal for various carbohydrate combinations;

3) to examine the pattern and rate of carbohydrate utilization by different activated sludge systems;

4) To examine the possibility of release of metabolic intermediates and/or end products during carbohydrate utilization by different activated sludge systems.

It is hoped that the results of this study will contribute significantly toward the understanding of the response of activated sludge to changes in carbohydrate components in a waste water and that it will provide additional bases for predicting the course of waste purification.

CHAPTER II

LITERATURE REVIEW

A. Carbohydrates and the Activated Sludge Process

1. Types of Wastes Which Contain Carbohydrates

Although considerable research has been conducted in the study of the characteristics of industrial wastes, not much information is available regarding either the amount or the types of carbohydrates contained in various wastes. Almost all of the investigations have been concerned primarily with the total amounts of organic matter, COD, or BOD contained in the major wastes. Table I shows some of the types of wastes containing carbohydrates which have been reported in the literature.

2. Effects of the Presence of Carbohydrates on the Operation of the Activated Sludge Processes

The presence of large amounts of carbohydrates has been cited to be the cause for various operational difficulties at treatment plants. Smith (9, 10) has indicated that overloading with carbohydrate wastes causes bulking. He pointed out that the possible reason for the bulking of sludge is that overloading could reduce the dissolved oxygen concen-

TABLE I

AMOUNT OF CARBOHYDRATES CONTAINED IN VARIOUS WASTES

Waste	Carbohydrate	Amount or Value	Reference
Domestic Sewage	Carbohydrates	240 mg/l	(1)
Beet Sugar Wastes			
Flume Water	Sucrose	100 mg/l	(2)
Process Waste Water	Sucrose	1500 mg/l	(2)
Dairy Wastes			
Whole Milk	Lactose	4.8 %	(3)
Pineapple Processing			
Composite Samples	Sugar	2000-5000 mg/l	(4)
Rice Wastes	Starch	1200 ppm	(5)
Pulp and Paper Mill Wastes			
Sulfite Waste Liquor	Sugar	16.9 %*	(6)
Semichemical Soda-pulping Wastes	Pentosans	18.9 % of BOD	(7)
Jute Cooking Liquor	Complex Polysaccharides	10.9 %**	(8)
Rope Cooking Liquor	"	10.6 %**	(8)
Rag Cooking Liquor	"	3.6 %**	(8)

*of total solids, bone-dry basis
**of volatile matter

tration in the aerator or could cause a pH drop creating conditions favorable for the predominance of filamentous organisms. However, Ingols and Heukelekian (11) have shown that at high glucose concentration, when excessive amounts of suspended solids concentrations were present in relation to feed concentration, and with an abundant supply of nitrogen source, sludge bulking may not occur for a relatively long period. When the bulking sludge exists, usually the

sludge volume index will increase enormously and the sludge will not settle in the final clarifiers. The above citation emphasizes the fact that the control of sludge bulking is one of the most important problems concerning the successful operation of the activated sludge process.

3. Sludge Production with Carbohydrate Substrates

As early as 1949, Monod (12) showed that the relationship between substrate consumption and protoplasmic accumulation for a given organism is a constant for a given substrate under similar conditions. This relationship is known as the "yield coefficient" (Y). Considerable work has been conducted in regard to the sludge production for carbohydrate substrates. Sawyer (13) has reported that 44 to 64 per cent of carbohydrate substrates would be expected to be channelled into cell synthesis. Placak and Ruchhoft (14) have shown a sludge yield of 65 to 85 per cent. Rao and Gaudy (15) reported 48 to 82 per cent, and concluded that the variation in yield might be due to predominance or selection of species. Rao (16) also emphasized that sludge production depends mainly upon the biochemical activities of the sludge. Therefore, when dealing with predominantly heterogeneous populations, the biochemical activities of the predominating species should be considered in making any comparison of sludge production for any particular waste.

B. Metabolic Control Mechanisms

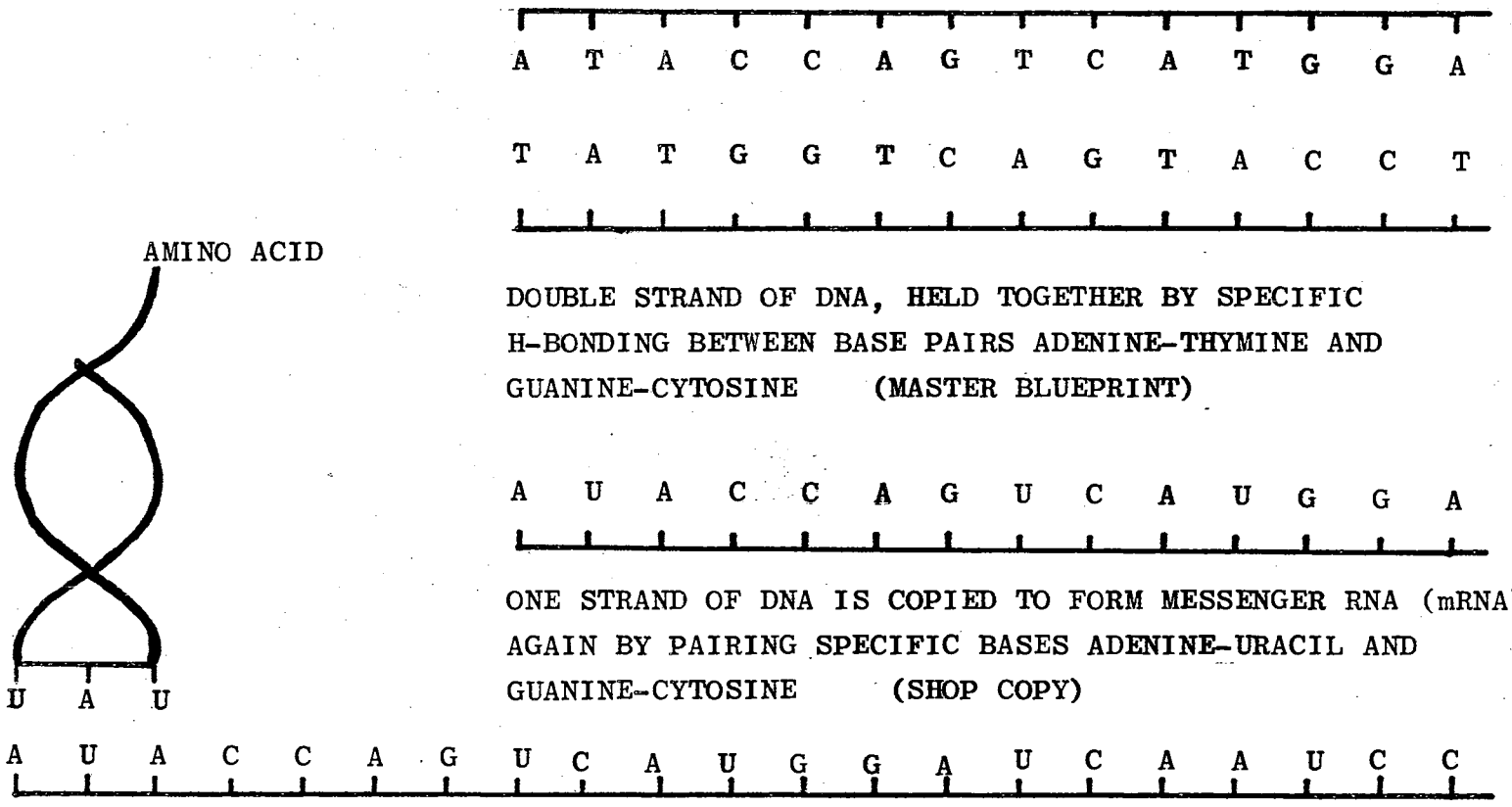
Since the activated sludge process is essentially a

biological process, it is appropriate and necessary to review the current knowledge of basic metabolic control mechanisms which are operative in bacteria and thus operate in activated sludge systems. A bacterium has the genetic capability to produce a specific spectrum of enzymes. This basic capability for enzyme synthesis can be induced or repressed by a change in the external environment. The two phenomena are known as enzyme induction and enzyme repression. The essential similarity of these two processes has been explicitly investigated by Jacob and Monod (17), who have presented the repressor-operator model for the regulation of enzyme synthesis. Enzyme induction is defined as the increase in the specific rate of enzyme synthesis upon addition of some nutrients, usually, but not necessarily, the substrate of the enzyme (18). While enzyme repression is defined as the decrease in the rate of synthesis of a particular enzyme or group of metabolically related enzymes (19), usually enzyme repression results from the presence of certain compounds in the cells. These substrates are called "repressors."

If repression and induction functioned efficiently, one would anticipate that the activities of the enzyme(s) in the living organisms would be nearly as fast as their maximum possible rates of action. From the applied standpoint such processes could play an important role in controlling the acclimation of activated sludge to various waste components.

Recently, Gaudy (20) has discussed the mechanisms of enzyme synthesis, induction and repression in both synthetic and catabolic pathways, feedback inhibition in biosynthetic pathways and a new mechanism, proposed by Gaudy and his co-workers, for feedback inhibition of enzyme activity in degradative pathways. The diagrams and the manuscript which are given below are taken from Gaudy's research seminar presentations at Notre Dame University, and the University of North Carolina (20).

> "Figure 1 is a diagrammatic representation of the mechanism for the synthesis of proteins of specific amino acid sequence (enzymes). The double-stranded DNA molecule is held together by specific H-bonding between the base pairs adenine-thymine and guanine-cytosine. If the living cell is considered analogous to a factory or manufacturing plant, the DNA may be considered as the master blueprint for all products produced in the factory. Indeed, it is the master blueprint for the production of an entire new factory. In the synthesis of new protein, one strand of DNA is copied to form a molecule of messenger RNA (mRNA). RNA is formed through the pairing of specific bases adenine-uracil and guanine-cytosine. Again, using the factory analogy, this single-stranded messenger RNA may be considered as a disposable shop copy taken from the master blueprint. Each triplet, i.e., three bases in series, provides the code for a specific amino acid in each protein to be manufactured by the cell. This code, which is now embodied in, or carried by, the messenger RNA, is translated to form protein with specific amino acid sequence by pairing specific bases on the messenger RNA with complementary bases on amino-acyl RNA (amino acid transfer RNA). In Figure 1, a molecule of amino acid transfer RNA is shown "docked" in proper position, at a molecule of messenger RNA. The specific amino acid which is to be joined in peptide linkage to another molecule of amino acid is shown at the other extremity of the amino acid transfer RNA. To make the sequence of amino acids in protein, another molecule of amino acid transfer RNA carrying the triplet GGU will pair with the triplet CCA on the mRNA, placing the two amino acids in the proper position to form a peptide bond. The formation of the peptide bond between the



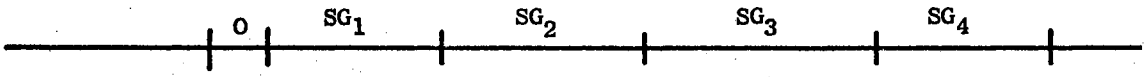
CODE IS TRANSLATED TO FORM PROTEIN WITH SPECIFIC AMINO ACID SEQUENCE BY PAIRING SPECIFIC BASES ON mRNA WITH COMPLEMENTARY BASES ON AMINOACYL-RNA (AMINO ACID TRANSFER RNA). EACH TRIPLET (THREE BASES) CODES FOR A SPECIFIC AMINO ACID.

Fig. 1 - MECHANISM FOR SYNTHESIS OF PROTEINS OF SPECIFIC AMINO ACID SEQUENCE

two amino acids will be catalyzed by a specific enzyme. The entire process will be repeated until the protein molecule is formed.

According to the "operon" model of protein synthesis, the information, or code, for the manufacture of specific enzymes for a particular metabolic pathway is sequentially strung along the DNA molecule. The DNA segments which code the amino acid sequence for each protein are called "structural genes." Figure 2 shows a diagram of the operon model for synthesis of enzymes required to catalyze the hypothetical pathway shown in the figure. SG_1, SG_2, SG_3, and SG_4 are structural genes, that is, segments of DNA carrying information specifying structure (amino acid sequence) for enzymes 1, 2, 3, and 4 of the biochemical pathway. E is the final product of the pathway, and each reaction is catalyzed by a specific enzyme. The segment of DNA is copied, as was shown in Figure 1, to form one molecule of messenger RNA. Under the operon model, copying must begin at O, the operator, shown at the left of the sequence of structural genes along the DNA molecule. This segment along the DNA molecule including the operator and the structural genes is called the "operon" for the pathway producing the product E. If the operator, O, is blocked, messenger RNA cannot be made and enzymes 1, 2, 3, and 4 cannot be produced. Let us assume that the particular cell under question has the genetic capability to use substrate A in the pathway and produce product E. Let us further assume that substrate A is not present in the medium; therefore, the factory would be uneconomically run if the enzymes for this metabolic pathway were manufactured, and there must be some metabolic control mechanism whereby the cell can prevent their manufacture. Such a mechanism for genetic repression of an inducible degradative pathway is shown in Figure 3. In this model, the operator is controlled by the "regulator gene," RG, which produces a molecule of active repressor, R, possessing the capability of blocking O, and thus the synthesis of messenger RNA. If inducer I (substrate or a compound of similar structure) is added, the repressor, R, is inactivated by combination with the inducer, producing inactive repressor, R', which cannot block the operator, and the required enzymes for the pathway are synthesized.

Now, let us assume that the hypothetical pathway with which we are dealing is a biosynthetic one, i.e., one which requires an input of energy and leads to the production of, for example, a structural component of the cell. To make the factory efficient, there must be some way to shut off the production of this syn-



SG_1, SG_2, SG_3 AND SG_4 ARE STRUCTURAL GENES, THAT IS, SEGMENTS OF DNA CARRYING INFORMATION SPECIFYING STRUCTURE (AMINO ACID SEQUENCE) FOR ENZYMES 1, 2, 3 AND 4 OF A BIOCHEMICAL PATHWAY.

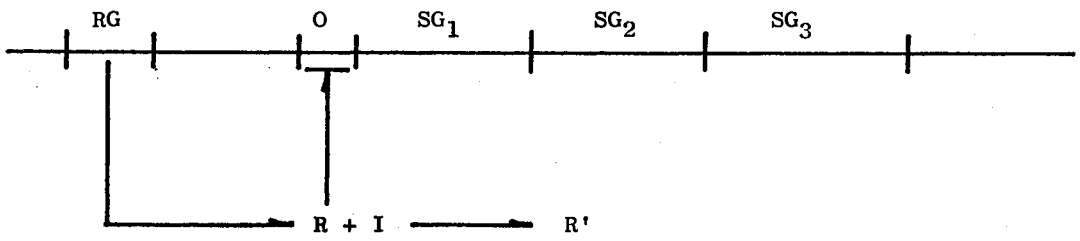
E IS THE FINAL PRODUCT OF THE PATHWAY AND EACH REACTION IS CATALYZED BY A SPECIFIC ENZYME.

$$A \xrightarrow{ENZ_1} B \xrightarrow{ENZ_2} C \xrightarrow{ENZ_3} D \xrightarrow{ENZ_4} E$$

THE SEGMENT OF DNA IS COPIED TO FORM ONE MOLECULE OF MESSENGER RNA. COPYING MUST BEGIN AT O (OPERATOR). THE SEGMENT INCLUDING THE OPERATOR AND THE STRUCTURAL GENES IS THE OPERON FOR THE PATHWAY PRODUCING E.

IF O IS BLOCKED, MESSENGER RNA CANNOT BE MADE AND ENZYMES 1, 2, 3 AND 4 CANNOT BE PRODUCED.

Fig. 2 - OPERON MODEL OF PROTEIN SYNTHESIS.



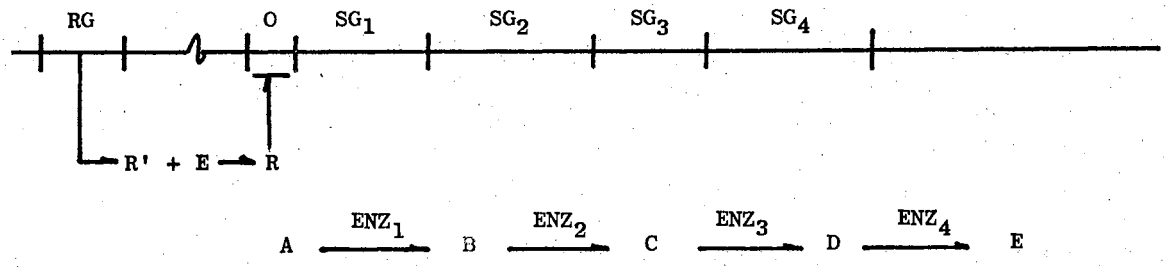
IN THE ABSENCE OF A SUBSTRATE, ENZYMES SPECIFIC FOR ITS DEGRADATION ARE NORMALLY REPRESSED. ACTIVE REPRESSOR, R, PRODUCED BY THE REGULATOR GENE, RG, BLOCKS SYNTHESIS OF MESSENGER RNA. IF INDUCER, I (SUBSTRATE OR A COMPOUND OF SIMILAR STRUCTURE) IS ADDED, THE REPRESSOR, R, IS INACTIVATED BY COMBINATION WITH THE INDUCER. THE INACTIVE REPRESSOR, R', CANNOT BLOCK THE OPERATOR AND THE ENZYMES ARE SYNTHESIZED.

Fig. 3 - GENETIC REPRESSION OF INDUCIBLE DEGRADATIVE PATHWAY.

thetic product when the proper amount has been made. A diagrammatic representation of the repression of this biosynthetic pathway is given in Figure 4. Accumulation of the final product, E, in the cell through overproduction (or from addition of compound E to the medium) represses synthesis of the enzymes specifically involved in the production of E. In this case, a regulator gene (RG) produces, normally, an inactive repressor, R', which is activated by reaction with E. This reaction produces active repressor, R, which attaches at the operator, O, blocking formation of the required molecule of messenger RNA.

Thus far we have seen (Figure 3) how an organism can shut off or turn on the synthesis of enzymes required to obtain energy from a particular starting material (substrate) and (Figure 4) how an organism can "shut down" the production line when it has produced enough of a particular product which it requires. However, one might ask, how does an organism when presented with two (or more) carbon sources, possess the ability to grow first on one then on another, thus exhibiting the pehnomenon of sequential substrate removal? Very often in studies using multicomponent media, one of the substrates used is glucose. The fact that in nearly all cases glucose blocks growth on other compounds has led to the use of the term "glucose effect" in describing the sequential removal phenomenon. However, compounds other than glucose have been observed to cause similar effects, and the more general term "metabolite repression" has been suggested by McFall and Mandelstam (21). This type of control of enzyme synthesis is shown diagrammatically in Figure 5. Here we see the operator controlled by the mechanism which was shown in Figure 3, and in addition, another overriding control emanating from the action of another regulator gene, RG_A. RG_A is the metabolic regulator gene which produces an inactive repressor, R'. RG_B is the genetic regulator gene (shown in Figure 3), which produces an inactive repressor subject to inactivation by an inducer. As we have seen before, the genetic repressor is inactivated in the presence of the substrate of this particular enzyme sequence. Now, let us assume that a second, more rapidly metabolized substrate is also present, and that P is a common product formed from both substrates. In this case, the inactive repressor, R', produced by the metabolic regulator gene RG_A, combines with P to produce an active repressor, R, preventing production of enzymes for degradation of the more slowly metabolized substrate.

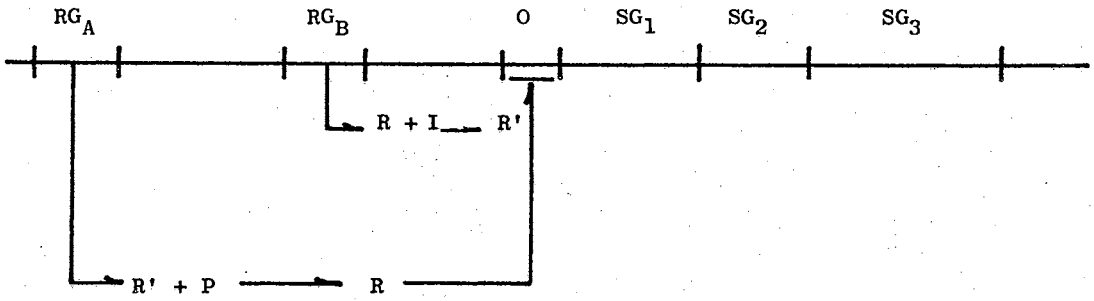
Thus far we have seen how the cell can control the synthesis of enzymes required for a particular meta-



ACCUMULATION OF THE FINAL PRODUCT, E, IN THE CELL, EITHER FROM THE MEDIUM OR THROUGH OVERPRODUCTION WITHIN THE CELL REPRESSES SYNTHESIS OF ENZYMES SPECIFICALLY INVOLVED IN THE PRODUCTION OF E.

THE REGULATOR GENE (RG) PRODUCES AN INACTIVE REPRESSOR, R', WHICH IS ACTIVATED BY REACTION WITH E. ACTIVE REPRESSOR, R, ATTACHES AT OPERATOR, O, BLOCKING FORMATION OF MESSENGER RNA FOR THE OPERON.

Fig. 4 - REPRESSION OF A BIOSYNTHETIC PATHWAY.



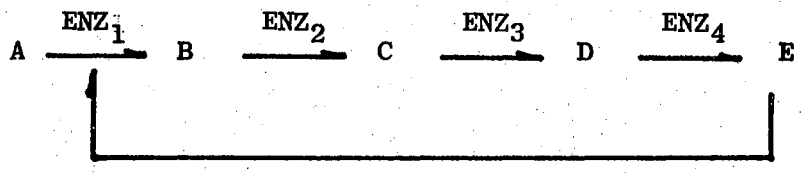
RG_A IS THE METABOLIC REGULATOR GENE WHICH PRODUCES AN INACTIVE REPRESSOR, R'. RG_B IS THE GENETIC REGULATOR GENE WHICH PRODUCES AN ACTIVE REPRESSOR, SUBJECT TO INACTIVATION BY AN INDUCER.

IN THE PRESENCE OF THE SUBSTRATE OF THIS ENZYME SEQUENCE, THE GENETIC REPRESSOR IS INACTIVATED. IF A SECOND, MORE RAPIDLY METABOLIZED SUBSTRATE IS ALSO PRESENT, AND IF P IS A COMMON PRODUCT FORMED FROM BOTH SUBSTRATES, THE INACTIVE REPRESSOR, R', IS COMBINED WITH P TO PRODUCE AN ACTIVE REPRESSOR, R, PREVENTING PRODUCTION OF ENZYMES FOR DEGRADATION OF THE MORE SLOWLY METABOLIZED SUBSTRATE.

Fig. 5 - METABOLITE REPRESSION OF "GLUCOSE EFFECT."

bolic pathway in the presence of sole or multicomponent carbon sources. We have also seen how the cell can stop the production of the tools (enzymes) it needs to make a particular synthetic product when it has produced enough of this product. Let us suppose that enough of this biosynthetic product has been made, and that the manufacture of enzymes needed to produce this synthetic product has been stopped. We have yet to see how the cell stops using the tools (enzymes) which it has already manufactured to make the particular product which it no longer needs to make. For biosynthetic pathways there is a rapid control mechanism which accomplishes this feat. This type of control has been termed "feedback inhibition" and is diagrammatically shown in Figure 6. Here we see again the hypothetical pathway starting with substrate A leading to product E. Accumulatibn of E in the cells prevents further synthesis of the enzyme specific for its production by the repression mechanisms previously discussed, and also immediately inhibits further formation of E by inhibiting the activity or the function of the first enzyme in the pathway. The product E combines with the enzyme at a site near to, and probably overlapping, the site at which substrate A must be bound (allosteric inhibition), preventing the binding of A, i.e., preventing the formation of the enzyme substrate complex. This mechanism provides for a very rapid cessation of the production of a particular product.

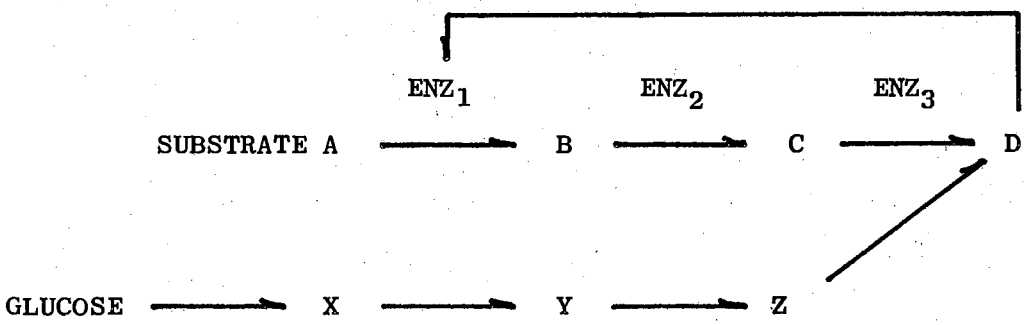
We may ask if such a rapid mechanism could also exist for the cessation of the use of a particular substrate, i.e., does a rapid mechanism exist for degradative pathways? Based upon work in our own laboratories, using both heterogeneous and pure cultures of microorganisms, we have concluded that such a mechanism does indeed exist. Our proposed mechanism is shown diagrammatically in Figure 7. Let us assume that D is a metabolic intermediate or degradative product made during degradation of either substrate A or, for example, glucose. Both substrates are metabolized via different pathways, but D is an intermediate common to both. Let us assume that D is produced more rapidly from glucose, and that as it accumulates as a result of this rapid breakdown of glucose, it inhibits the activity (via allosteric inhibition) of the first enzyme responsible for breakdown of substrate A, preventing any further overaccumulation of D. The existence of such a mechanism explains the experimental observation of sequential substrate removal in systems containing high concentrations of cells previously acclimated to substrate A, and observations of sequential substrate removal in nonproliferating systems."



ACCUMULATION OF E IN THE CELL PREVENTS FURTHER SYNTHESIS OF THE ENZYMES SPECIFIC FOR ITS PRODUCTION (REPRESSION) AND ALSO IMMEDIATELY INHIBITS FURTHER FORMATION OF E BY INHIBITING THE ACTIVITY OF THE FIRST ENZYME OF THE PATHWAY.

E COMBINES WITH THE ENZYME AT A SITE NEAR TO, AND PROBABLY OVER-LAPPING, THE SITE AT WHICH SUBSTRATE A MUST BE BOUND (ALLOSTERIC INHIBITION), PREVENTING THE BINDING OF A.

Fig. 6 - FEEDBACK INHIBITION IN BIOSYNTHESIS.



PRODUCT D IS FORMED DURING DEGRADATION OF EITHER SUBSTRATE, BUT IS PRODUCED MORE RAPIDLY FROM GLUCOSE. D ACCUMULATES AS A RESULT OF THE RAPID BREAKDOWN OF GLUCOSE AND INHIBITS ACTIVITY OF THE FIRST ENZYME RESPONSIBLE FOR BREAKDOWN OF SUBSTRATE A, PREVENTING FURTHER OVER-ACCUMULATION OF D.

Fig. 7 - FEEDBACK INHIBITION IN DEGRADATIVE PATHWAY (PROPOSED MECHANISM).

Gaudy and his co-workers (22, 23, 24, 25) have shown conclusively that sequential substrate removal can occur in heterogeneous populations, and this finding has been confirmed by Prakasam and Dondero (26), and by Stumm-Zollinger (27). The fact that substrates in a wastewater or growth medium can incite fairly rapid response and cause sequential substrate removal for entire heterogeneous populations has significant ramifications to the water pollution control field, since sequential substrate removal can cause somewhat severe kinetic discontinuities in the rate of wastewater purification. Changes in the types of compounds contained in incoming wastewaters to biological treatment processes have been termed "qualitative" shock loads.

C. Responses to Qualitative Shock Loads

A qualitative shock load may be defined as a change in chemical structure of the incoming substrate (28). According to Natarajan (29), the following are possible responses when carbon source changes from A, which is being actively degraded, to B:

1) increase in the rate of degradation of A and degradation of B;

2) no change in the rate of degradation of A and degradation of B;

3) increase in the rate of degradation of A and no degradation of B;

4) decrease in the rate of degradation of A and degradation of B;

5) no change in the rate of degradation of A and no degradation of B;

6) decrease in the rate of degradation of A and no degradation of B;

7) stoppage of degradation of A and degradation of B;

8) stoppage of degradation of A and no degradation of B.

In order to eliminate undesired responses and to attain the desired responses in activated sludge processes, it is necessary to understand the effects of control mechanisms on the substrate utilization.

Gaudy (3) has indicated that there are three possible mechanisms by which a heterogeneous population which has not been previously acclimated may successfully respond to a qualitative shock loading. These are selection of species, shift in metabolic pathway, and induction of required enzymes. He has also reasoned that the total response of a heterogeneous population to the qualitative shock load could be a combination of the response of the three types, and they could occur simultaneously but interdependently.

D. Kinetics of Microbiological Growth and Substrate Utilization

1. Exponential Growth and Exponential Substrate Utilization

The increase of microorganisms in a system is generally exponential, when the amount of substrate is very large com-

pared to the amount of microorganisms present, such as in the early stages of cultivation starting with a small inoculum. In this case, the kinetics of microbiological growth can be expressed as follows:

$$\frac{dx}{dt} = \mu \cdot x \tag{1}$$

$$\mu t = \ln (x/x_o) \tag{2}$$

where $\frac{dx}{dt}$ is the rate of change of cell concentration, μ is the specific growth rate ($time^{-1}$), x_o and x are the concentrations of cells initially and at time t.

The decrease of substrate is exponential, when the amount of microorganisms is very large compared to the amount of substrate such as in later stages of cultivation or in the case where a large inoculum is used. During this phase, the reaction is first order in the case of s; it is expressed as

$$-\frac{ds}{dt} = k_1 \cdot s \tag{3}$$

$$k_1 t = \ln (s_o/s) \tag{4}$$

where $-\frac{ds}{dt}$ is the rate of substrate utilization, k_1 is the specific substrate removal rate, s_o and s are the concentrations of substrate initially and at time t.

As the available food supply is exhausted, a negative acceleration phase exists. Usually this phase is called the "declining" growth phase, in which the microbiological growth rate and the substrate removal rate are still expressed by a first order reaction. But Equations (1) and (2) become as follows:

$$\frac{dx}{dt} = C \cdot x \tag{5}$$

$$C \cdot t = \ln (x/x_o) \tag{6}$$

where C is a variable function of remaining food. While substrate removal is expressed by the following relationship

$$-\frac{ds}{dt} = K_2 \cdot x \cdot s \tag{7}$$

$$K_2 \cdot x \cdot t = \ln (s_o/s) \tag{8}$$

where K_2 is logarithmic substrate removal rate when the growth rate becomes substrate concentration dependent.

2. Linear Growth and Linear Substrate Utilization

Fujimoto (33) has proposed that when a substrate of low solubility is supplied, the growth rate is determined by diffusion of substrate in the medium. If the supply of such a substrate is sufficient, the amount of the actual useful substrate in the medium is kept constant throughout the cultivation without any relation to the microorganisms. For this case he derived the following equation:

$$\frac{dx}{dt} = \mu \frac{x}{1 + kx} \tag{5}$$

where k is constant. If the amount of microorganisms is very large, then $\frac{x}{1 + kx}$ is held constant and therefore the cell growth and also the substrate consumption is linear and the rate is zero order.

According to Krishnan and Gaudy (34), the substrate consumption in batch systems follows zero order kinetics under three sets of conditions; in systems with high solids

and an adequate nitrogen supply; in systems with no external nitrogen supply; and in systems in which protein synthesis is inhibited. Zero order consumption of substrate may be expressed as:

$$-\frac{ds}{dt} = k \tag{6}$$

This equation states that substrate consumption rate is independent of the substrate concentration.

3. Kinetics of Substrate Consumption as Related to Growth

The relationship between bacterial growth and substrate consumption can be expressed by the equation

$$y = -\frac{dx}{ds} \tag{7}$$

where y is known as the yield coefficient.

Combinations of Equations (1) and (7) gives an expression that describes the rate of substrate consumption as related to growth

$$-\frac{ds}{dt} = \left(\frac{\mu}{y}\right) x \tag{8}$$

If the substrate is maintained at a concentration higher than the growth rate limiting concentration, the specific growth constant, μ, can be kept constant. If y is also assumed to be constant (e.g., $y \sim 0.5$), Equation (8) reveals that the rate of substrate consumption is proportional to x.

From Equation (2) we get

$$x = x_o e^{\mu t} \tag{9}$$

The combination of Equations (8) and (9) gives

$$-\frac{ds}{dt} = \left(\frac{\mu}{y}\right) \cdot x_o e^{\mu t} \tag{10}$$

This equation shows that if the initial cell concentration is small in comparison with the substrate concentration, the rate of substrate consumption increases logarithmically with time in accordance with the logarithmic increase of cell concentration. Upon integration of Equation (10), we get

$$-\Delta s = \left(\frac{x_o}{y}\right) \left(1 + e^{\mu t}\right) \tag{11}$$

where $-\Delta s$ is the substrate consumed at any time. Therefore, a plot of log $(-\Delta s)$ versus time gives a linear relationship for $t \gg 0$. Stumm-Zollinger (27, 35) has shown that the slope of the curve is related to the specific growth rate constant and corresponds to the slope of the semilogarithmic biological growth curve.

CHAPTER III

MATERIALS AND METHODS

A. Substrate Selection and Combination

Monosaccharides and disaccharides are two major subdivisions of carbohydrates. This investigation involved seven monosaccharides and four disaccharides. The monosaccharides used were two aldopentoses (xylose and lyxose), two ketohexoses (fructose and sorbose), and three aldohexoses (glucose, galactose, and mannose). The disaccharides used were

sucrose	α-glucosido-1,2-β-fructoside
maltose	α-glucosido-1,4-glucose
cellobiose	β-glucosido-1,4-glucose
β-lactose	β-galactosido-1,4-glucose

The hydrolysis of these disaccharides results in the cleavage of the component hexoses. Some basic data for these carbohydrates are listed in Table II.

For the first series of experiments, xylose and lyxose were selected for combined study with nine other carbohydrates (i.e., combined singly to make nine different two-component systems). However, the results of the lyxose-glucose system showed that no glucose-acclimated sludges

TABLE II

BASIC DATA ON CARBOHYDRATES STUDIED

Carbohydrate	Class	Formula	Hexoses yielded on Hydrolysis	Natural Source	Method of Determination
xylose	aldopentose	$C_5H_{10}O_5$	---	wood gums	orcinol test
lyxose	aldopentose	$C_5H_{10}O_5$	---	heart muscle	orcinol test
fructose	ketohexose	$C_6H_{12}O_6$	---	fruit juices, honey, hydrolysis of cane sugar	resorcinol test
sorbose	ketohexose	$C_6H_{12}O_6$	---	juice of mountain-ash fruits	resorcinol test
glucose	aldohexose	$C_6H_{12}O_6$	---	fruit juices, hydrolysis of starch and cane sugar	glucostat test
galactose	aldohexose	$C_6H_{12}O_6$	---	hydrolysis of lactose	galactostat test
mannose	aldohexose	$C_6H_{12}O_6$	---	hydrolysis of plant mannosans and gums	anthrone test
maltose	disaccharide (reducing)	$C_{12}H_{22}O_{11}$	glucose + glucose	diastatic digestion or hydrolysis of starch	anthrone test
lactose	disaccharide (reducing)	$C_{12}H_{22}O_{11}$	glucose + galactose	milk	anthrone test
sucrose	disaccharide (nonreducing)	$C_{12}H_{22}O_{11}$	glucose + fructose	cane and beet sugar, sorghum, pineapple, carrot root	anthrone or resorcinol test
cellobiose	disaccharide (reducing)	$C_{12}H_{22}O_{11}$	glucose + glucose	hydrolysis of starch	anthrone test

could employ lyxose as the sole carbon source. Therefore, sorbose was used instead of lyxose in this investigation.

B. Cell Acclimation

Bacterial populations were acclimated for the following purposes:

1) production of the necessary enzymes either through activation of latent characteristics (enzyme induction), or through genetic change (mutation);

2) selection of the species which can best utilize the substrate under the given environmental conditions (36).

1. Development of Young Cells Population for Low Initial Inoculum Studies

A sewage seed was obtained from the effluent of the primary clarifier of the sewage treatment plant in Stillwater, Oklahoma. The sewage was used to seed a 250 ml Erlenmeyer flask which contained 5 ml of sewage seed, 25 ml of standard synthetic medium, about 50 mg of substrate (the carbohydrate to which the sludge would be acclimated), and sufficient distilled water to yield a final volume of 50 ml of reaction fluid. A standard synthetic medium was prepared to provide the culture with the concentration of the compounds shown in Table III. The flask was aerated at room temperature (~22^{o}C) on a reciprocal shaker at 100 strokes/min for 36 hours. After 36 hours, 10 ml of the mixed liquor were transferred to 500 ml of freshly prepared synthetic growth medium of the same composition as that

TABLE III

STANDARD SYNTHETIC MEDIUM

Constituent	Concentration
$(NH_4)_2SO_4$	500 mg/l
$MgSO_4.7H_2O$	100 mg/l
$FeCl_3.6H_2O$	0.5 mg/l
$MnSO_4.H_2O$	10.0 mg/l
$CaCl_2$	7.5 mg/l
1.0 M Potassium Phosphate Buffer, pH 7.0 (KH_2PO_4-52.7 g/l) (K_2HPO_4-107 g/l)	10.0 mg/l
Tap Water (Trace Elements)	100 ml/l
Distilled Water	to volume

described above. Then this mixed liquor was distributed into seven or eight 250 ml Ehrlenmeyer flasks, and again aerated. After approximately 18 hours, the heterogeneous populations were used to seed the experimental units.

2. Development of Young Cell Populations for High Initial Inoculum Study

Galactose-acclimated cells were used in this study. In order to develop a galactose-acclimated sludge with a large population and to prolong the log growth phase, a special batch culture unit was operated. The cells obtained from the acclimation unit were inoculated into a fresh batch containing the same medium used for acclimation,

except that the concentration of galactose was increased five-fold, the buffer concentration was tripled, and the salts concentrations were doubled. After approximately 18 hours of incubation, the cells were harvested by centrifugation and washed once in 0.05 M phosphate buffer. Then the cells were used to seed the experimental batch units.

C. Methods of Analysis

Periodic sampling and subsequent analyses for specific carbohydrate content and total substrate removal were employed to examine the biological response of each system during the time course of the experiment.

1. Biological Solids Determination

a. Optical Density

To facilitate the estimation of biological growth, optical density measurement was employed to evaluate the growth pattern of each system. This method is well known, having been extensively used to evaluate the relative turbidity of colloidal suspensions. A correlation table of optical density (OD) and the percentage of light transmittance (%T) which had been prepared using the equation $D = -\log_{10} T$, was used to facilitate the measurement of optical density. All measurements were made at a wave length of 540 mμ, using 19 mm diameter tubes in a Bausch and Lomb spectrophotometer.

b. Membrane Filter Technique

In order to estimate the initial and peak biological

solids concentrations, a correlation curve of biological solids concentration and optical density was made for the heterogeneous populations. The curve is shown in Figure 8. As xylose and sorbose had been used in combination with nine other carbohydrates, xylose-acclimated, sorbose-acclimated, and glucose-acclimated cells were harvested from batch tubes and diluted to obtain various solid concentrations. The solid concentrations were determined by the membrane filter technique (39). Very light aluminum dishes (weighing approximately 0.2 g) were used to hold the Millipore filters. Filters were dried for two hours at 103°C, and equilibrated in a desiccator over night prior to obtaining the tare weight. Before final weighing, the same heating and desiccating procedures were followed. The optical densities were measured on the Bausch and Lomb Spectrophotometer.

2. Substrate Determination

a. Chemical Oxygen Demand (COD)

Total substrate removal was determined in accordance with the procedure given in Standard Methods (37). Potassium dichromate (0.25 N) and concentrated sulfuric acid were used. Silver sulfate was used at all times as catalyst.

Although the COD test does not differentiate between biologically oxidizable and biologically inert organic matter, it is widely used in the operation of treatment facilities because of the ease and speed in obtaining useful information on the behavior of the system, and its

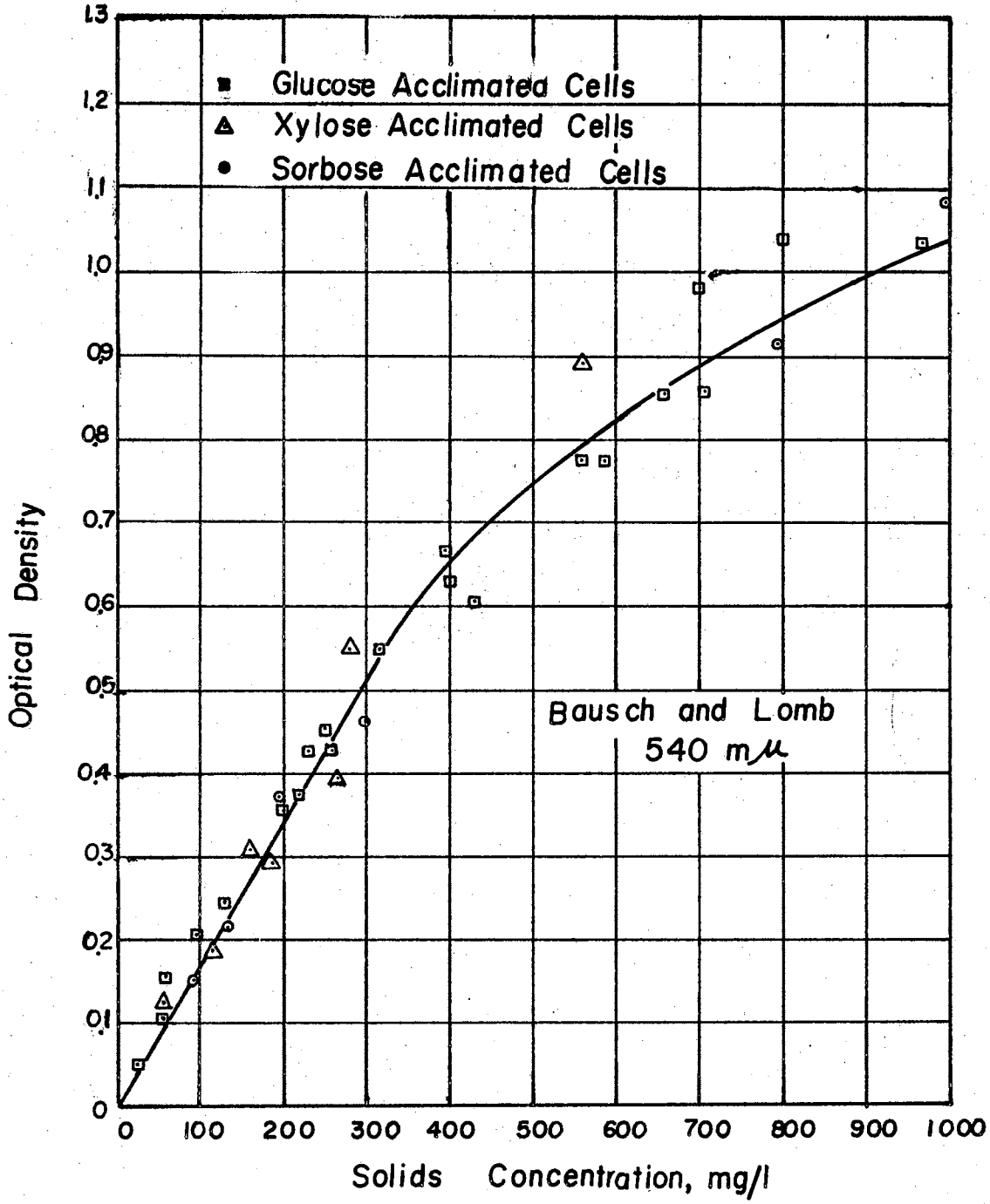


Fig. 8 - BIOLOGICAL SOLIDS CONCENTRATION VS. OPTICAL DENSITY FOR HETEROGENEOUS POPULATION.

helpfulness in indicating the presence of biologically resistant organic substance (38). However, in the studies of sequential substrate removal, the total COD could not be used as a measure of specific substrates. Hence, both nonspecific (COD) test and more specific analyses for carbohydrates were used in this study.

b. Anthrone Test (Total Carbohydrate)

The anthrone test was used to measure carbohydrates, such as mannose and lactose, which have no specific method for determination. The procedure employed was outlined and modified by Gaudy and his associates (39, 40). The amount of anthrone was increased to 9 ml to eliminate clouding of the sample, which sometimes occurs during or shortly after the boiling period. In the combined and shocked units, the concentration of carbohydrates for which there were no specific chemical methods of analysis, were determined approximately by subtracting the concentration of the other compound (determined specifically and converted to a COD value) from the total carbohydrate COD. The difference between total carbohydrate COD as measured by the anthrone test, and the COD due to the component as measured by a specific analytical method can only be taken as the COD due to the other specific component plus carbohydrate intermediates. Specific intracellular products of intermediary metabolism are sometimes excreted into the extracellular environment (41). This aspect will be discussed more fully in the results chapter.

c. Glucostat Method (Glucose Determination)

Glucose was determined by the glucostat test, which is quite specific for glucose. This enzymatic determination was run in accordance with the manufacturer's specifications, and standard method 1-A was employed (42, 43). The calculation of glucose concentration in the samples was based on glucose standards covering the range from 0.05 mg to 0.3 mg glucose.

d. Galactostat Test (Galactose Determination)

Since the galactostat test is very specific for galactose, determination of galactose concentration by this enzymatic technique was more suited for the present study than the conventional anthrone test. The test was performed in accordance with the procedure outlined by the Worthington Biochemical Corporation (44). The procedure is as follows:

1) dissolve contents of chromogen vial in 0.5 ml methanol and add distilled water to 30 ml;

2) dissolve contents of galactostat vial in distilled water and add to the chromogen solution, and adjust the final volume to 50 ml with distilled water;

3) store in a dark bottle and keep in the dark;

4) dilute sample solutions and standard galactose so that they contain 25-100 μg in 2.0 ml;

5) add 2.0 ml galactose reagent to each standard and sample tube;

6) incubate in a 37^{o}C water bath for one hour;

7) add 6.0 ml of 0.25M glycine buffer, pH 9.7, to stop the reaction;

8) read optical density at 425 mμ with the reagent blank set at 100 per cent transmittance.

The calculation of galactose concentration is similar to the calculation of glucose concentration. The standard curve for galactose solutions should cover the range from 0.02 to 0.12 mg galactose.

e. Orcinol Test (for Xylose and Lyxose)

This method, first proposed by Bial, has been extensively studied in a number of laboratories. The most widely used modification for pentose determination is that of Mejbaum (45), and it is the basis for the procedure used in the Bioengineering Laboratories of Oklahoma State University. The procedure is outlined as follows:

1) add 2 ml of 10% $FeCl_3.6H_2O$ to 400 ml of concentrated HCl to compose the HCl-$FeCl_3$ reagent;

2) dissolve 0.4734 gm of orcinol in 10 ml of 95% alcohol to make up the alcoholic-orcinol solution;

3) dilute pentose stock solution and samples with distilled water so that 3.0 ml contain 10-30 μg pentose;

4) add 6.0 ml of HCl-$FeCl_3$ reagent to each tube including a reagent blank, at least three standards and samples;

5) add 0.4 ml of alcoholic-orcinol solution;

6) heat the tubes for 20 minutes in a boiling water bath;

7) cool and read optical density at a wave length of 660 mμ with the reagent blank set at 100% transmittance.

The calculation of pentose concentration is similar to glucose except the standard curve covers the range from 0.01 to 0.025 mg of pentose.

f. Resorcinol Test (Fructose, Sorbose, and Sucrose)

This method, described by Roe in 1934, was employed to determine the quantity of ketohexoses such as fructose and sorbose. A great many improvements and modifications have been reported in adapting the test for ketohexose determination. The later modification performed in this study is given here. The procedure is as follows:

1) to prepare resorcinol reagent, dissolve 0.1 g of resorcinol and 0.25 g of thiourea into 100 ml of glacial acetic acid;

2) prepare HCl solution by diluting five parts of concentrated HCl into one part of distilled water;

3) dilute ketohexose stock solutions and samples so that 2.0 ml contains 20-80 μg ketohexose;

4) add 1 ml of resorcinol reagent into each tube;

5) add 7 ml of HCl solution and mix well by shaking;

6) place in a water bath at 80°C for exactly ten minutes;

7) remove and immerse the tubes in cold water for five minutes;

8) read optical density at 520 mμ within thirty minutes.

g. Nelson's Test (46) (Reducing Sugar Determination)

Determining the capacity of the carbohydrate containing sample to reduce Cu^{++} in an alkaline solution is the most common method for detecting the presence of free reducing groups in a carbohydrate. It is found that when the conditions are controlled, the amount of Cu^{++} reduced to Cu^{+} is directly proportional to the amount of reducing sugar in the sample analyzed. The Cu^{+} formed in the reaction precipitates as the rust-colored Cu_2O. In the Nelson test, the amount of Cu_2O formed is determined by addition of arsenomolybdic acid, which is qualitatively reduced to arsenomolybdous acid by the Cu^{+}. The rust-blue color of the arsenomolybdous acid is then measured colorimetrically. The following procedure is employed in the Bioenvironmental Laboratory of Oklahoma State University:

1) prepare Nelson's alkaline copper reagent and arsenomolybdate reagent; the detailed procedure for preparing these reagents is given in "Experimental Biochemistry" (46);

2) dilute standard and samples so that 1.0 ml contains 100 μg sugar;

3) add 1.0 ml of Nelson's alkaline copper reagent to each tube and shake well;

4) place the tubes simultaneously in a boiling water bath and heat for exactly twenty minutes;

5) remove the tubes simultaneously and immerse in cold water;

6) add 1.0 ml of arsenomolybdate reagent to each tube and shake well;

7) after five minutes, add 7.0 ml of distilled water to each tube and mix thoroughly;

8) read the optical density in the colorimeter at 540 mμ.

The standard curve for the calculation of reducing sugars covers the range from 0.01 to 0.06 mg.

h. Conversion of Carbohydrate Concentration to COD Values

For convenience in comparing the removal rates of different carbohydrates with the total COD removal rate, the concentration of each carbohydrate as obtained by its specific test was converted to equivalent COD by multiplying by the factor shown in Table IV.

TABLE IV

RELATIONSHIP BETWEEN CONCENTRATIONS AND COD VALUES FOR VARIOUS CARBOHYDRATES

mg/l xylose x 160/150	=	mg/l xylose COD
mg/l galactose x 192/180	=	mg/l glucose COD
mg/l galactose x 192/180	=	mg/l galactose COD
mg/l fructose x 192/180	=	mg/l fructose COD
mg/l sorbose x 192/180	=	mg/l sorbose COD
mg/l mannose x 192/180	=	mg/l mannose COD
mg/l sucrose x 384/342	=	mg/l sucrose COD
mg/l maltose x 384/342	=	mg/l maltose COD
mg/l lactose x 384/342	=	mg/l lactose COD
mg/l cellobiose x 384/342	=	mg/l cellobiose COD

The theoretical COD values of the above carbohydrates can be obtained from the following equations:

For pentose: $C_5H_{10}O_5 + 5\ O_2 \longrightarrow 5CO_2 + 5\ H_2O$

150 160

For hexose: $C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6CO_2 + 6\ H_2O$

180 192

For disaccharide: $C_{12}H_{22}O_{11} + 12\ O_2 \longrightarrow 12\ CO_2 + 11\ H_2O$

342 384

Excepting lyxose, sorbose, and cellobiose, the COD values of other carbohydrates have also been reported previously by Gaudy and Engelbrecht (28). Komolrit (47) has indicated that the experimental values for all of the carbohydrates he determined (glucose, galactose, lactose, and ribose) agreed quite well with the calculated theoretical values; and the differences were well within five per cent of the theoretical values. In accordance with previous studies, it would appear that Table IV is quite valid for conversion of the carbohydrate concentration values to corresponding COD values.

D. Experimental Protocol

1. Operation of Batch Units

The experiments in this study consisted first of inoculating three or four batch aeration tubes. Tube #1 was used as the control unit, and contained the carbohydrate to which the sludge was acclimated, at a substrate concentration of 500 mg/l. It also contained 100 mg/l of acclimated seed, and required standard synthetic wastes to yield a total volume of 1500 ml. Tube #2 was the control unit for the other carbohydrate used in the two-component system. The tube contents were the same as for tube #1, except that it contained a different substrate. Tube #3 contained equal concentrations of both carbohydrates used in tubes #1

and #2; thus it contained a total substrate concentration of 1000 mg/l. The seeds and inorganic components of the synthetic waste in the combined unit were the same as the controls. Tube #4, when employed, was used as the "shocked unit." The details of this system are described below under the subheading "Qualitative Shock Load."

At the beginning of the operation, zero hour, the substrates were fed to each unit, and 50 ml samples were removed from each unit and centrifuged for twenty minutes in a Servall centrifuge, Type SS-1. At the same time, samples were removed for optical density determination at 540 $m\mu$ on the Bausch and Lomb Spectrophotometer. After centrifugation the supernatant was carefully removed and put through a Millipore filter, 20 ml being used for COD analysis and 10 ml were frozen for the analysis of specific substrate. This procedure was repeated for the measurement of substrate removal and biological growth at various time intervals. The methods of analysis employed were described in detail in the section of "Methods of Analysis."

2. Qualitative Shock Load

In order to investigate the response of activated sludge to the introduction of structurally different carbon source than that to which it was acclimated, and to study the interference between the two components more thoroughly, a shock load unit (in addition to the two controls and one combined system) was operated in some experiments, e.g.,

sorbose-glucose systems. For a young cell system acclimated to sorbose, glucose was tipped in the sorbose unit when the sorbose-acclimated sludges were actively metabolizing sorbose. The concentration of the shock compound was prepared so that the unit would contain 500 mg/l of shock compound after the compound was tipped in. All other constituents of this unit were the same as for tube #1.

CHAPTER IV

RESULTS

A. Multicomponent Carbohydrate Studies

In order to determine the generality of sequential or concurrent substrate removal, eighteen combinations of carbohydrates were investigated in two-component carbon source media. A summary of the results for the various multicomponent substrate systems investigated is given in Table V.

Since a great deal of biochemical information is available on the metabolism of glucose, fructose, sucrose, and lactose, the results for experiments involving these substrates will be described and presented with accompanying figures in this chapter. In addition, the results for the sorbose-xylose system will also be shown graphically, because these two compounds were chosen as basic components to combine with other carbohydrates. The results for other systems will be presented briefly without showing figures; however, important experimental parameters are given for all experiments in Table VI at the end of this chapter.

1. Galactose and Glucose Systems

The results of three galactose-glucose experiments are presented as follows:

TABLE V

SUMMARY OF EXPERIMENTAL RESULTS

System	Methods of Analysis	Acclimated Young Cells	Pattern of Growth and Substrate Removal	Remarks:
Galactose and Glucose	COD Glucostat Galactostat	Galactose-acclimated Cells Glucose-acclimated Cells High Galactose-acclimated Cells	Monophasic Growth Sequential Removal Diphasic Growth Sequential Removal Monophasic Growth Concurrent Removal	1. Glucose represses the formation of galactose enzymes
Galactose and Maltose	COD Anthrone Glucostat Galactostat	Galactose-acclimated Cells Maltose-acclimated Cells	Monophasic Growth Concurrent Removal Diphasic Growth Sequential Removal	1. Maltose represses galactose enzymes formation
Xylose and Glucose	COD Glucostat Galactostat	Xylose-acclimated Cells Glucose-acclimated Cells	Monophasic Growth Concurrent Removal Diphasic Growth Sequential Removal	1. Glucose represses xylose enzymes formation
Xylose and Galactose	COD Galactostat Orcinol	Xylose-acclimated cells Galactose-acclimated Cells	Monophasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. No interaction
Xylose and Mannose	COD Orcinol Anthrone	Xylose-acclimated Cells Mannose-acclimated Cells	Monophasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. Mannose slightly repressed xylose enzymes formation
Xylose and Fructose	COD Orcinol Resorcinol	Xylose-acclimated Cells Fructose-acclimated Cells	Monophasic Growth Concurrent Removal Diphasic Growth Sequential Removal	1. Fructose represses the formation of xylose enzymes
Xylose and Sorbose	COD Orcinol Resorcinol	Xylose-acclimated Cells Sorbose-acclimated Cells	Diphasic Growth Concurrent Removal Diphasic Growth Sequential Removal	1. Metabolic intermediates inhibit the function of xylose enzymes 2. Sorbose may repress xylose enzymes formation, but data not conclusive
Xylose and Maltose	COD Orcinol Anthrone	Xylose-acclimated Cells Maltose-acclimated Cells	Diphasic Growth Sequential Removal Monophasic Growth Concurrent Removal	1. Xylose represses maltose enzymes formation
Xylose and Lactose	COD Orcinol Glucostat Galactostat Anthrone	Xylose-acclimated Cells Lactose-acclimated Cells	Monophasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. No interaction

TABLE V (Continued)

System	Methods of Analysis	Acclimated Young Cells	Pattern of Growth and Substrate Removal	Remarks:
Xylose and Sucrose	COD Orcinol Glucostat Nelson's Test Resorcinol	Xylose-acclimated Cells Sucrose-acclimated Cells	Monophasic Growth Concurrent Removal Diphasic Growth Sequential Removal	1. Sucrose may repress formation of xylose enzymes, but data not conclusive
Xylose and Cellobiose	COD Orcinol Glucostat Anthrone	Xylose-acclimated Cells Cellobiose-acclimated Cells	Monophasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. No interaction
Lyxose and Glucose	COD Glucostat Orcinol	Fresh Seeds Glucose-acclimated Cells	No Reganisms Growth and no Substrate Removal	1. A long time is required to induce lyxose enzymes
Sorbose and Glucose	COD Resorcinol Glucostat	Sorbose-acclimated Cells ---	Diphasic Growth Sequential Removal ---	1. Glucose represses sorbose enzymes formation 2. Glucose inhibits sorbose only when glucose concentration is higher than sorbose
Sorbose and Galactose	COD Resorcinol Galactostat	Sorbose-acclimated Cells Galactose-acclimated Cells	Monophasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. No interaction
Sorbose and Mannose	COD Resorcinol Anthrone	Sorbose-acclimated Cells Mannose-acclimated Cells	Monophasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. Sorbose represses the formation of mannose enzymes, but not completely
Sorbose and Maltose	COD Resorcinol Anthrone	Sorbose-acclimated Cells Maltose-acclimated Cells	Diphasic Growth Sequential Removal Monophasic Growth Concurrent Removal	1. Sorbose represses the formation of maltose enzymes
Sorbose and Lactose	COD Resorcinol Anthrone	Sorbose-acclimated Cells Lactose-acclimated Cells	Diphasic Growth Sequential Removal	1. Sorbose represses lactose enzymes formation 2. Lactose was exhausted before the cells were capable of using sorbose
Sorbose and Cellobiose	COD Resorcinol Anthrone	Sorbose-acclimated Cells Cellobiose-acclimated Cells	Diphasic Growth Concurrent Removal Monophasic Growth Concurrent Removal	1. Sorbose required long acclimation 2. Cellobiose was retarded by the presence of sorbose

a. Galactose-acclimated Cells

The curves for the control units of galactose and glucose, respectively, are presented in Figure 9. It was found that even though the initial cells used in this experiment had been acclimated to galactose, they still required acclimation to galactose and glucose. The possible explanation is that the prolonged acclimation of the sludge caused the loss of inducible enzymes in the organisms and impaired the ability of the organisms to remove substrates. The fact that the specific rate of glucose removal, Kg, in the glucose control was faster than the specific rate of total COD removal, Kt, showed that an amount of metabolic intermediates were excreted into the glucose medium during growth. The intermediate production is also shown by a comparison of the total COD curve and the glucose COD curve. It was found that when glucose was the sole source of carbon, it was metabolized faster than galactose, as seen by comparison of the growth rates and substrate removal rates for the two control units.

In Figure 10, the growth curve and COD removal curves for the combined unit of galactose and glucose are shown. These curves indicate that there was a lag in substrate utilization. The activation by glucose of the genes governing the synthesis of glucose-enzymes (induction) took place within the first four hours (the same as the glucose control unit). The galactose COD curve shows that the induction of galactose enzymes took place within ten hours,

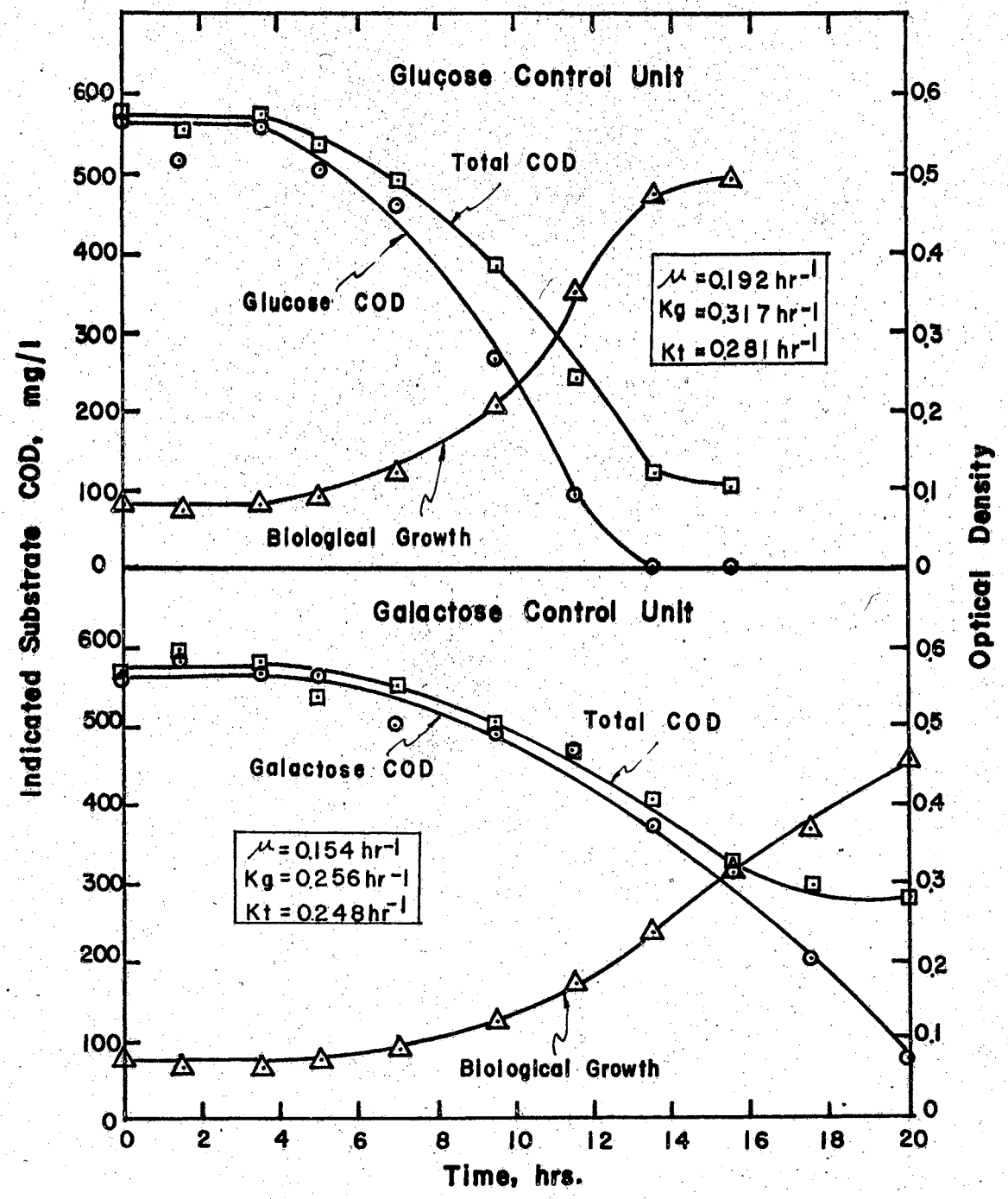


Fig. 9 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO GALACTOSE.

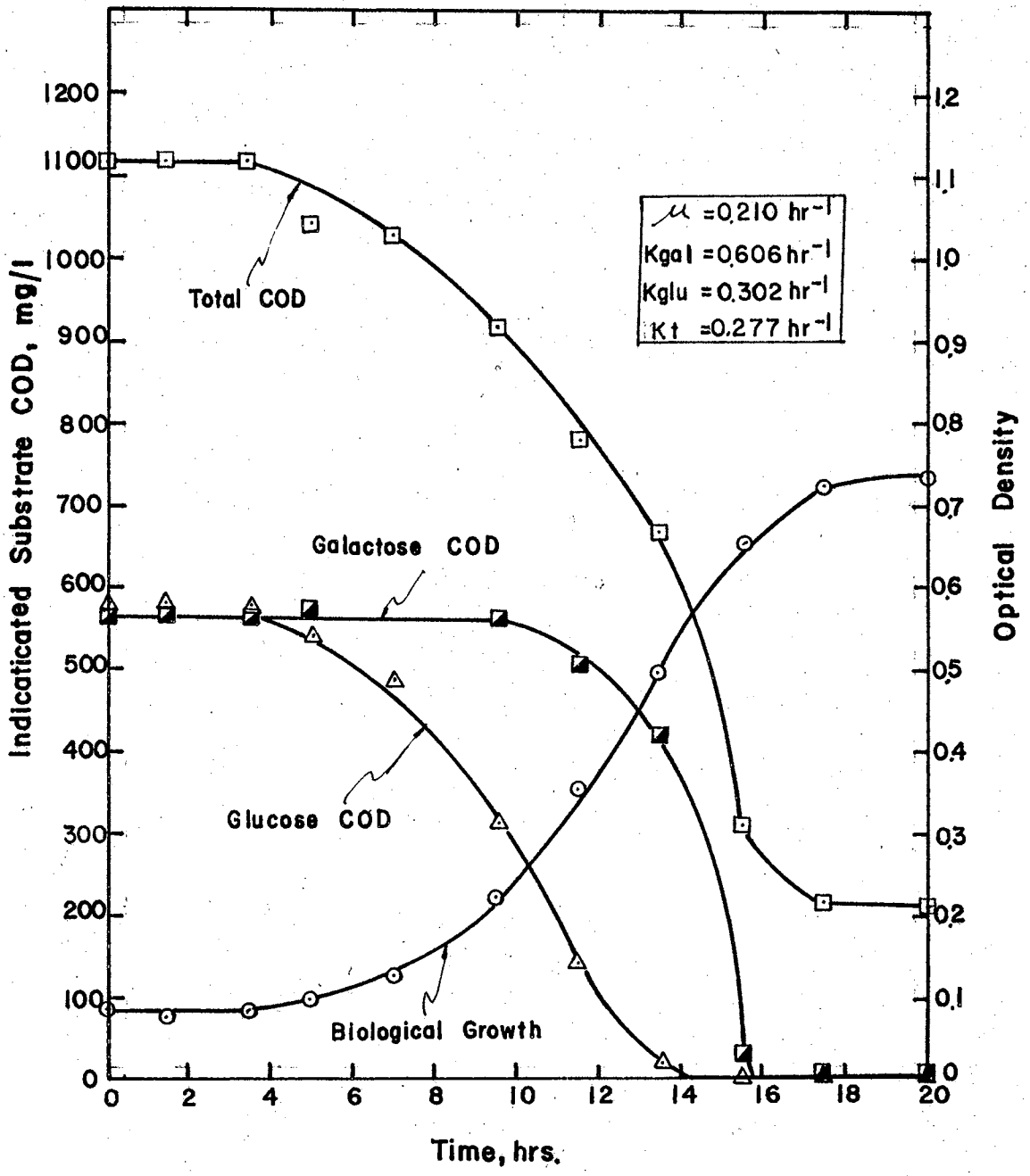


Fig. 10 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF GALACTOSE AND GLUCOSE. YOUNG CELLS ACCLIMATED TO GALACTOSE.

i.e., a 5-hour delay as compared to the galactose control. This would indicate that glucose repressed the formation of galactose-catabolizing enzymes. The formation and activities of galactose enzymes occurred only after almost half of the glucose had been eliminated from the solution. Glucose was eliminated at essentially the same rate in the control and in the combined unit; whereas galactose removal was enhanced greatly after release from the repression by glucose. This is probably due to the fact that an increased number of organisms, pre-grown on glucose, took part in the further growth on galactose. The essential similarity between the growth rate, μ, observed in the combined unit before galactose catabolism started and that observed in the glucose control again shows the shift from growth on glucose to growth on galactose.

The effect of the shock loading of glucose on the removal of galactose is shown in Figure 11. At 9.5 hours the galactose-catabolizing sludge was shock loaded with glucose. The introduction of glucose caused an immediate disruption of galactose metabolism. The continued rapid removal of total COD in the culture after the shock was due largely to glucose metabolism. Although some growth on galactose took place after the shock, it was not proportional to the increasing bacterial concentration. This would indicate that glucose repressed the formation of new galactose-catabolizing enzymes, and that the enzymes formed before the addition of glucose were responsible for elim-

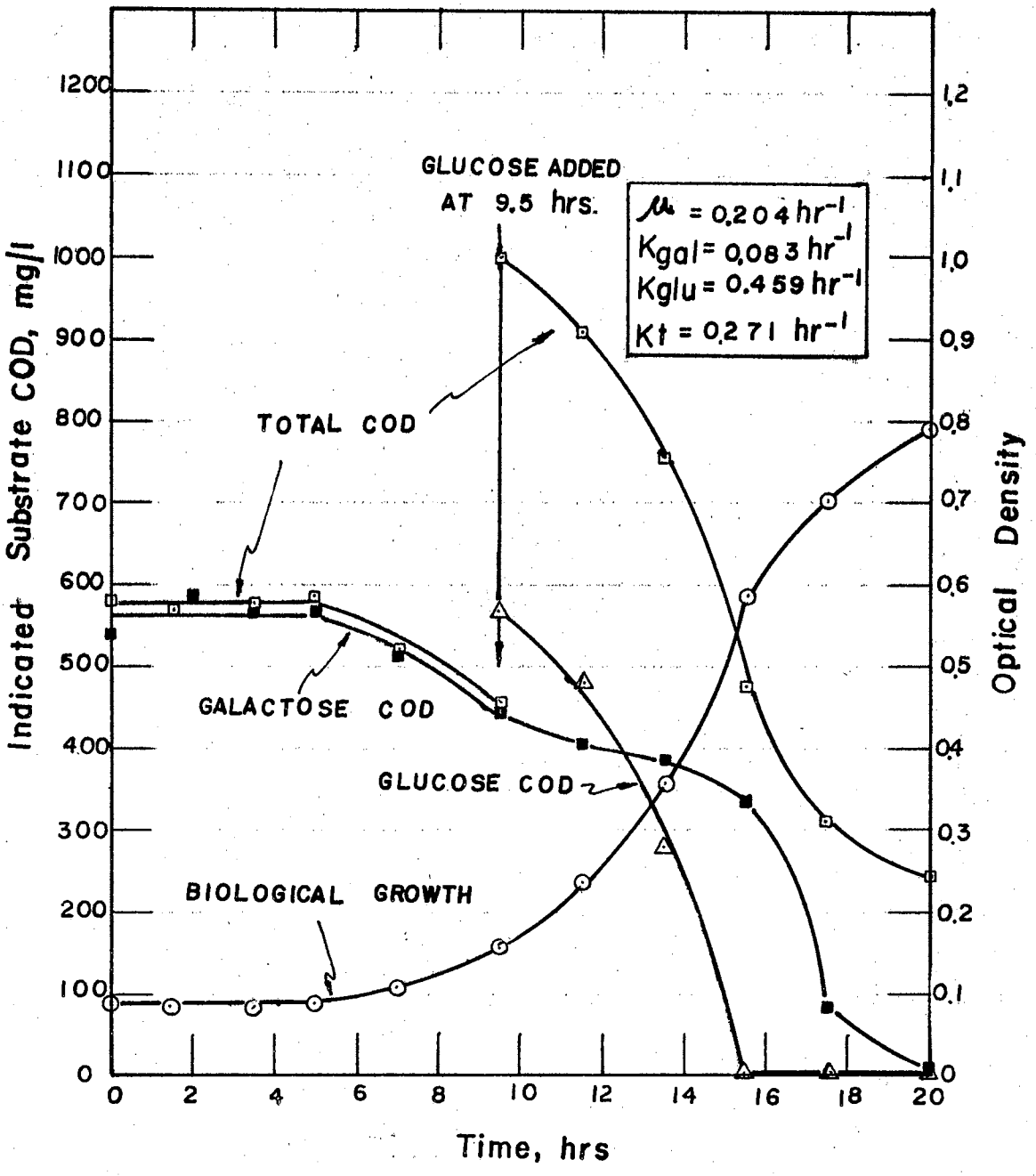


Fig. 11 - RESPONSE OF GALACTOSE-ACCLIMATED YOUNG CELLS TO SHOCK LOADING WITH GLUCOSE.

inating galactose from the medium. Since glucose was degraded immediately by the organisms already growing on galactose, the glucose-catabolizing enzymes were in all probability constitutive in these galactose-acclimated cells. Galactose was not again rapidly metabolized until half of the glucose had been removed. About 240 mg/l of residual COD was present in the medium at the cessation of this experiment.

b. Glucose-acclimated Cells

Figure 12 shows the substrate removal characteristics for galactose and glucose by glucose-acclimated sludge. Some metabolic intermediates were excreted into the medium when glucose was catabolized by glucose-acclimated sludges. About 120 mg/l of residual COD was present in the glucose medium at the end of the experiment. The results for the galactose control unit indicated that glucose-acclimated organisms required no long acclimation period before metabolizing galactose; i.e., the enzymes responsible for galactose catabolism were apparently constitutive in the glucose-acclimated sludge, or in any event, were induced rapidly. This suggests that glucose most probably inhibited the activity rather than the synthesis of galactose enzymes in Figure 10, because galactose enzymes already existed in the sludge after glucose metabolism started. A comparison of total COD and galactose COD curves shows that very few metabolic intermediates were excreted into the galactose medium.

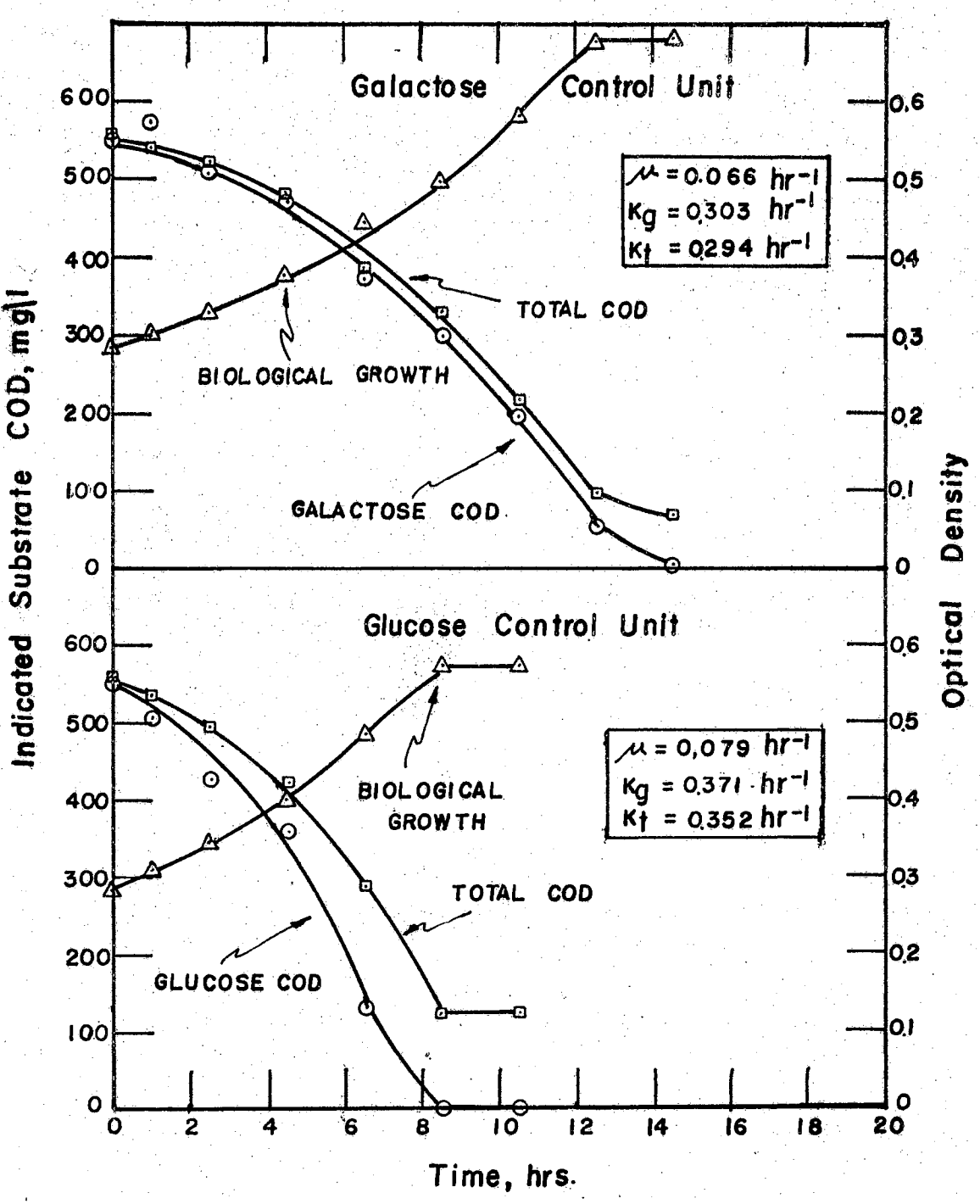


Fig. 12 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO GLUCOSE.

The effect of galactose on glucose metabolism by glucose-acclimated cells when both compounds are present at the start of the experimental period is shown in Figure 13. Glucose was eliminated essentially at the same rate in the control and in the combined units, while galactose removal was suppressed almost completely in the combined unit. Galactose metabolism started rapidly after about half of the glucose had been exhausted. The shift from growth on glucose to growth on galactose caused a change of the growth rate. Although diphasic growth on glucose and galactose was not apparent on the growth curve, two linear portions of different slope (μ_1, μ_2) did appear on a semilogarithmic plot used to determine growth rate. Also, two linear portions on a semilogarithmic plot of total COD removal showed characteristics of sequential substrate removal. A comparison of the total COD curve and the sum of two component substrate COD's showed that only a small release of intermediates during metabolism of glucose and galactose occurred. The residual COD in the combined unit was much less than the sum of the residual COD in the two control units.

The effect of a shock loading of galactose on the removal of glucose is shown in Figure 14. A comparison of the growth curve and glucose removal curve in the glucose control and galactose shocked unit indicates that both growth and glucose removal were retarded somewhat by the introduction of galactose. The activity of galactose

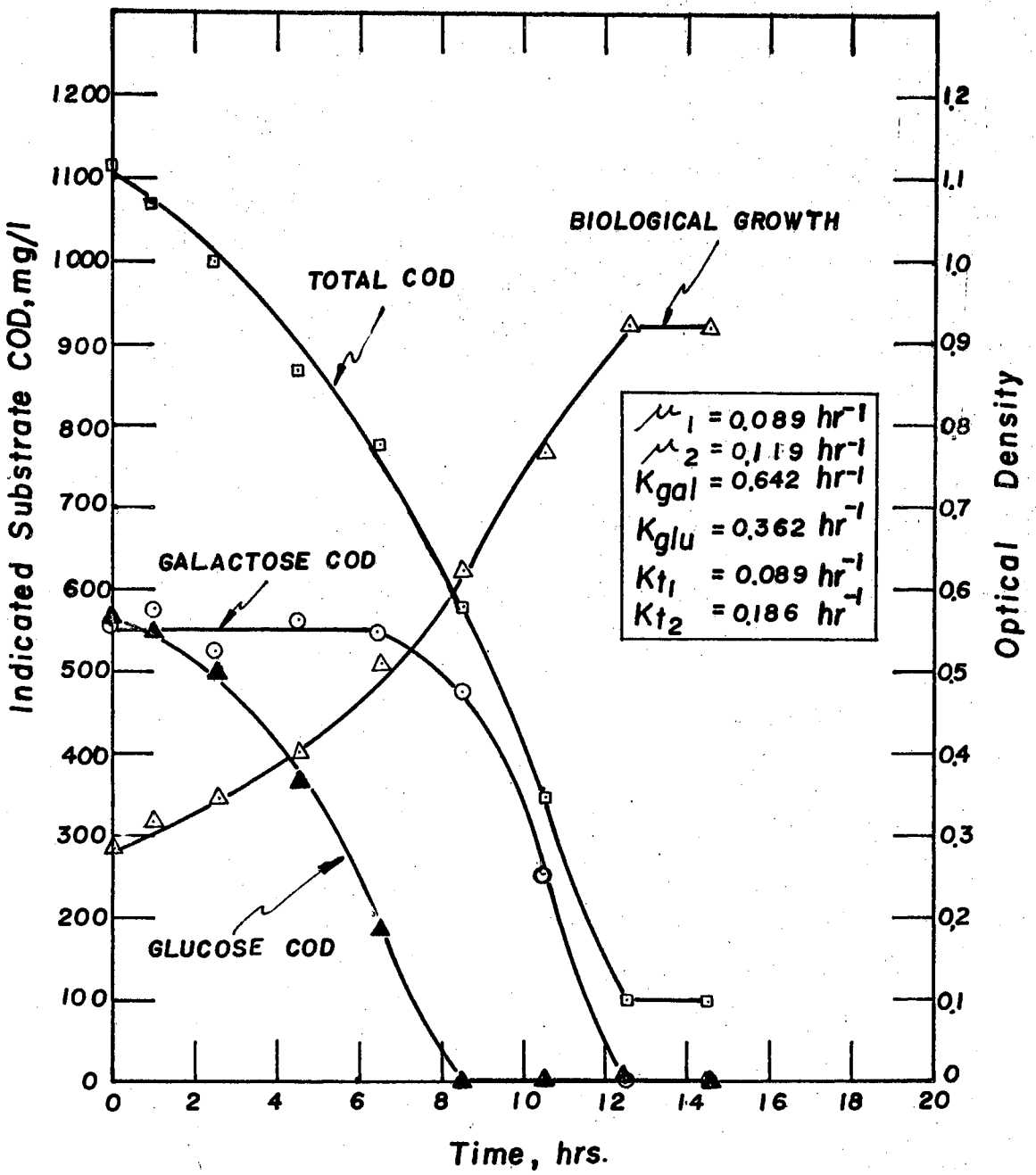


Fig. 13 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF GALACTOSE AND GLUCOSE. YOUNG CELLS ACCLIMATED TO GLUCOSE.

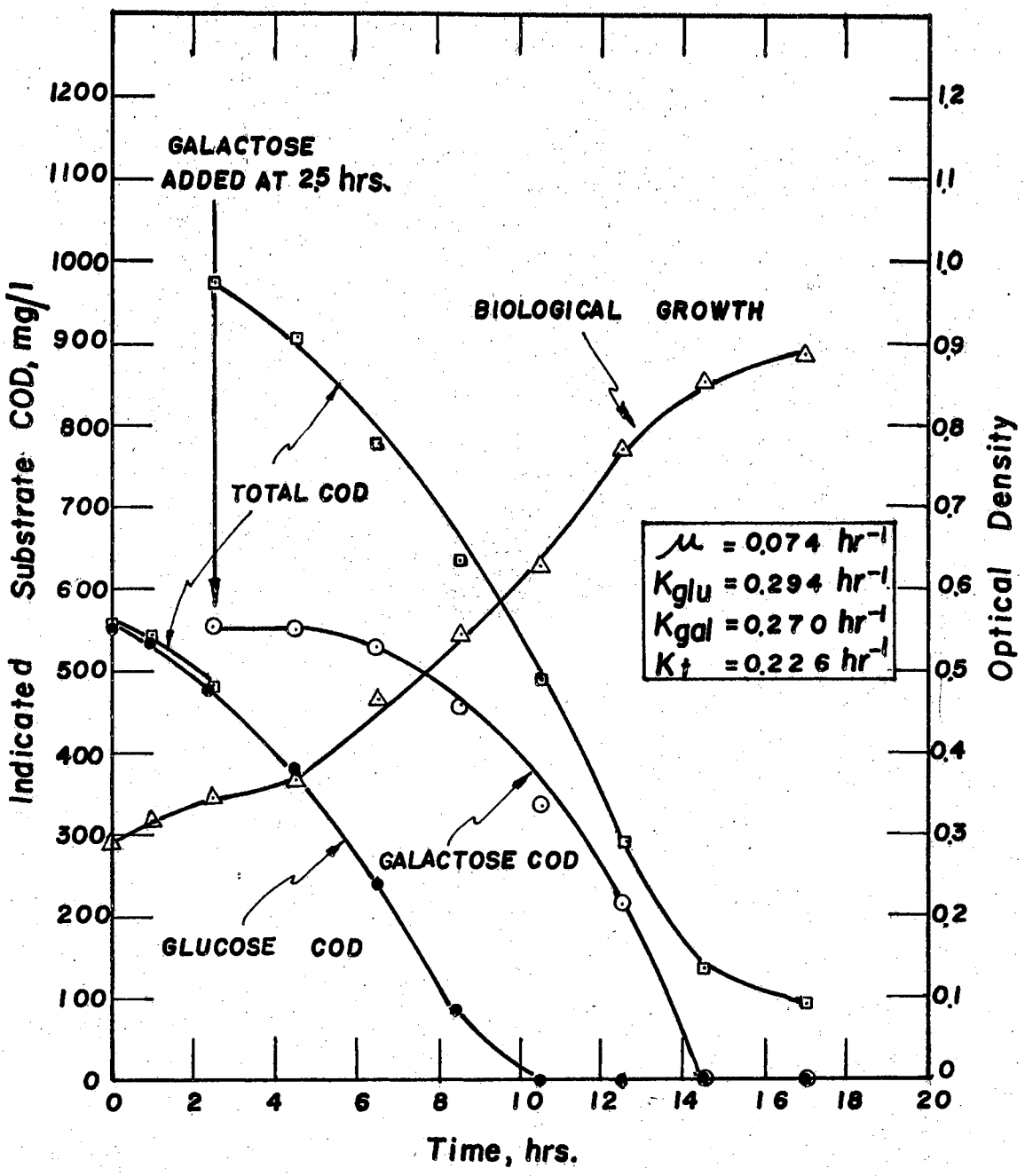


Fig. 14 - RESPONSE OF GLUCOSE-ACCLIMATED YOUNG CELLS TO SHOCK LOADING WITH GALACTOSE.

enzymes was suppressed as shown by the lag period which occurred in galactose metabolism. The approximate equality of the residual COD, shown in the combined and the shocked units, indicates that the production of intermediates was not changed appreciably when equal amounts of galactose and glucose were used in the growth medium.

c. Young Cells Acclimated to Galactose (Higher Initial Cell Concentration)

A further attempt was undertaken to investigate the effect of glucose on the utilization of galactose. The initial concentration of young cells used in this experiment was higher than those used in the other experiments; the initial concentration of biological solids was approximately 350 mg/l.

The growth and substrate utilization responses of a galactose-acclimated sludge to the presence of glucose or galactose are given in Figure 15. Figure 15a shows that glucose consumption was essentially linear, i.e., the rate was zero order. This was due to the high biological solids used at the start of the experiment. Also, some intermediates and/or end products were excreted into the glucose medium, and these intermediates were subsequently used by the cells (except for 50 mg/l of residual COD). Figure 15b indicates that very few intermediates were produced during galactose metabolism, and about 60 mg/l of residual COD was present in the medium at the end of the experiment.

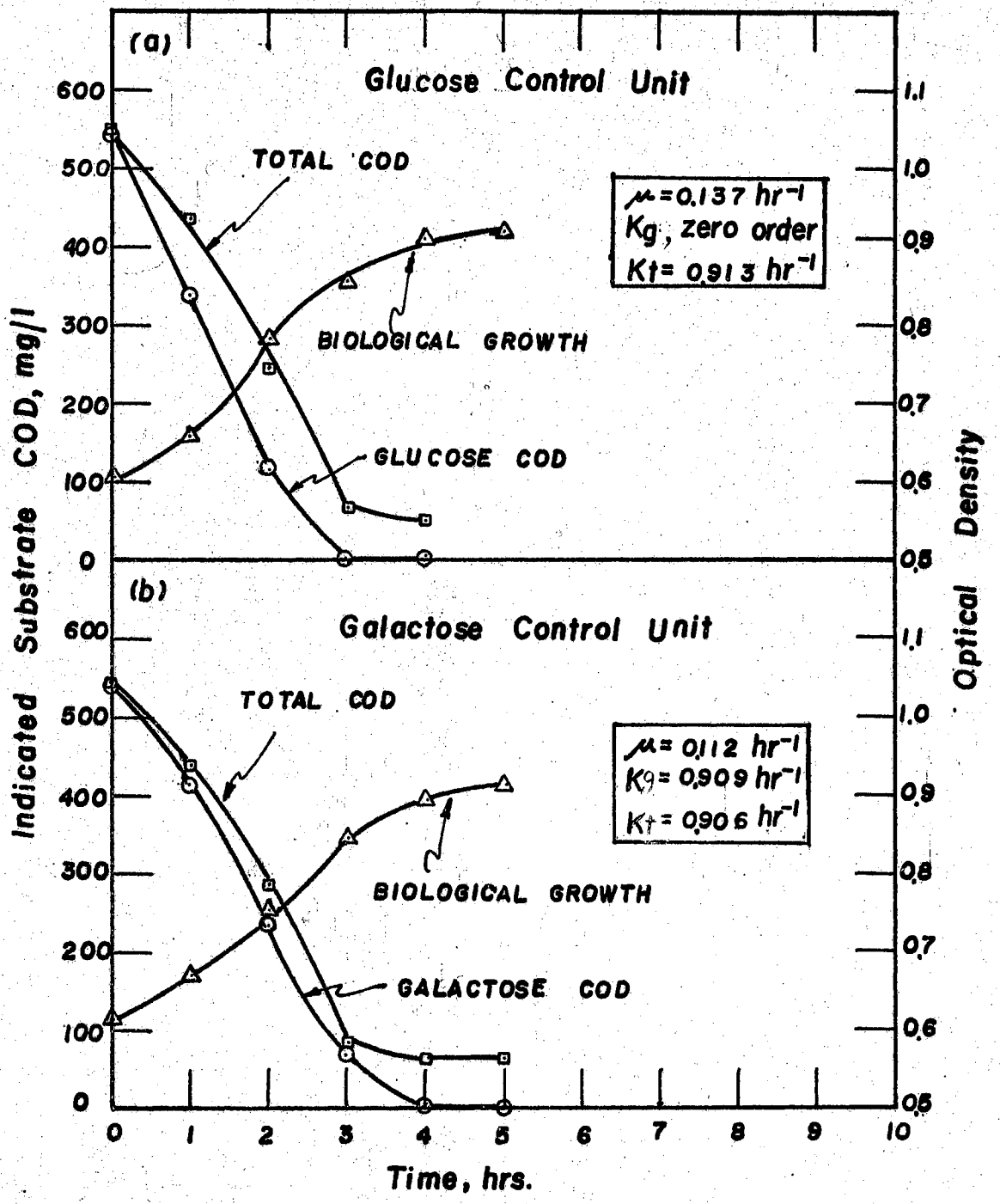


Fig. 15 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO GALACTOSE (HIGH INITIAL CELLS CONCENTRATION).

Concurrent substrate utilization of glucose and galactose is shown in Figure 16. In this case the presence of glucose decreased the galactose removal rate from 0.909 to 0.578 hr^{-1}, but did not fully inhibit the activity of galactose-catabolizing enzymes. Both glucose and galactose were eliminated at an appreciably slower rate in the combined unit as compared with the control units. An appreciable amount of intermediates was produced during metabolism of the substrates, as seen by a comparison of total COD and the sum of two specific substrate COD's. These intermediates were subsequently used by cells except about 30 mg/l of residual COD left in the medium.

According to the results of the galactose-glucose system described above, the following conclusions can be drawn:

1) Galactose-catabolizing enzymes were constitutive in glucose-acclimated sludges; similarly, glucose enzymes were contained in galactose-acclimated sludges.

2) Glucose repressed the formation of galactose-catabolizing enzymes, but did not inhibit galactose metabolism.

3) Sequential removal of glucose and galactose accompanied by diphasic growth occurred in the low initial concentration system, whereas concurrent substrate utilization accompanied by simultaneous growth on both substrates resulted when high concentrations of galactose-acclimated sludges were employed.

4) The accumulation of intermediates observed in the

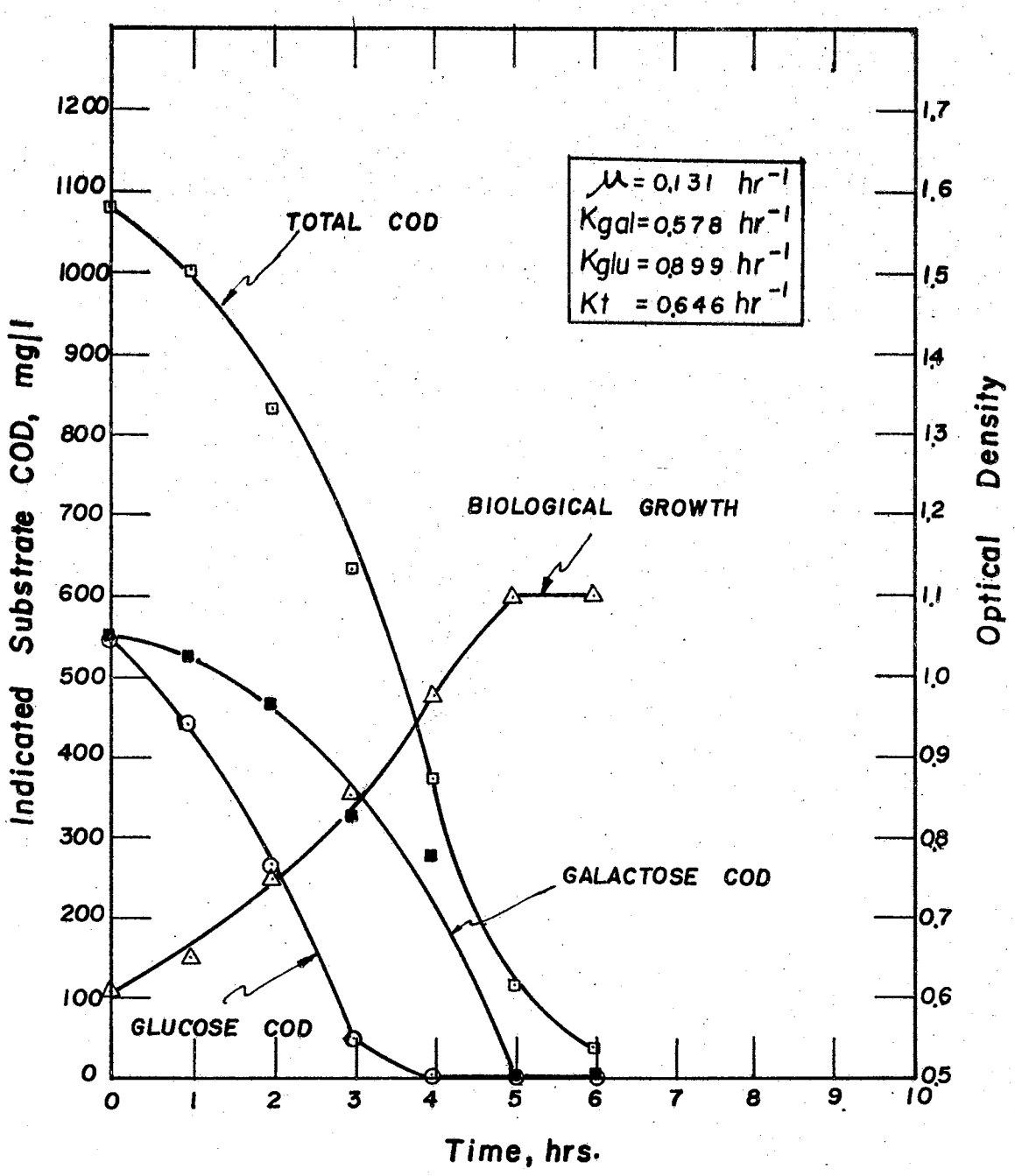


Fig. 16 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF GLUCOSE AND GALACTOSE. YOUNG CELLS ACCLIMATED TO GALACTOSE (HIGH INITIAL CELL CONCENTRATION).

substrate mixture was changed as compared to the sum of intermediate production observed in the medium containing one substrate component only; the amount of residual COD produced from the substrate mixture was lower when higher concentrations of initial biological solids were used.

5) The introduction of galactose into a system actively metabolizing glucose caused very small retardation of growth and glucose removal.

6) Glucose removal rate was linear when the high concentration of galactose-acclimated cells was used.

2. Galactose and Maltose System

Two experiments were undertaken to investigate the responses of either galactose-acclimated sludges or maltose-acclimated sludges to the presence of galactose and maltose in the culture medium. The following observations could be made from the data of these experiments:

1) Organisms pre-grown on maltose required no acclimation to galactose; cells grown on galactose required no acclimation to maltose.

2) Only a small amount of intermediates was produced in either of the controls or the combined units by the two different acclimated sludges. The residual COD in the mixture was approximately equal to the sum of the residual COD's in the controls.

Since little or no glucose was found in the medium, glucose was eliminated immediately, as maltose was cleavaged into glucose by the organisms, or maltose was taken directly into the cells.

4) The results of experiments using maltose-acclimated cells indicated that sequential substrate elimination accompanied by diphasic growth was caused by maltose repression of galactose enzymes, while concurrent removal and monophasic growth occurred in the experiment using galactose-acclimated cells.

5) In the experiment of galactose-acclimated sludge, the specific growth rate μ and the specific total COD removal rate Kt observed in the substrate mixture were smaller than those observed in the media containing one substrate component only.

The experimental parameters for this system are shown in Experiments 4 and 5 of Table VI.

3. Xylose and Glucose System

a. Xylose-acclimated Young Cells

The curves for the control units are presented in Figure 17. No lag was observed in glucose metabolism (Figure 17a) indicating that enzymes responsible for the metabolism of glucose were constitutive in xylose-acclimated cells. A great amount of intermediates was excreted into both xylose medium and glucose medium, as shown by a comparison of the COD removal curves and substrate removal curves. These intermediates were subsequently used by the organisms; only about 40 mg/l of residual COD was observed in both media at the cessation of this experiment. The specific rates of xylose and glucose removal in the control units were much faster than the total COD removal rates of

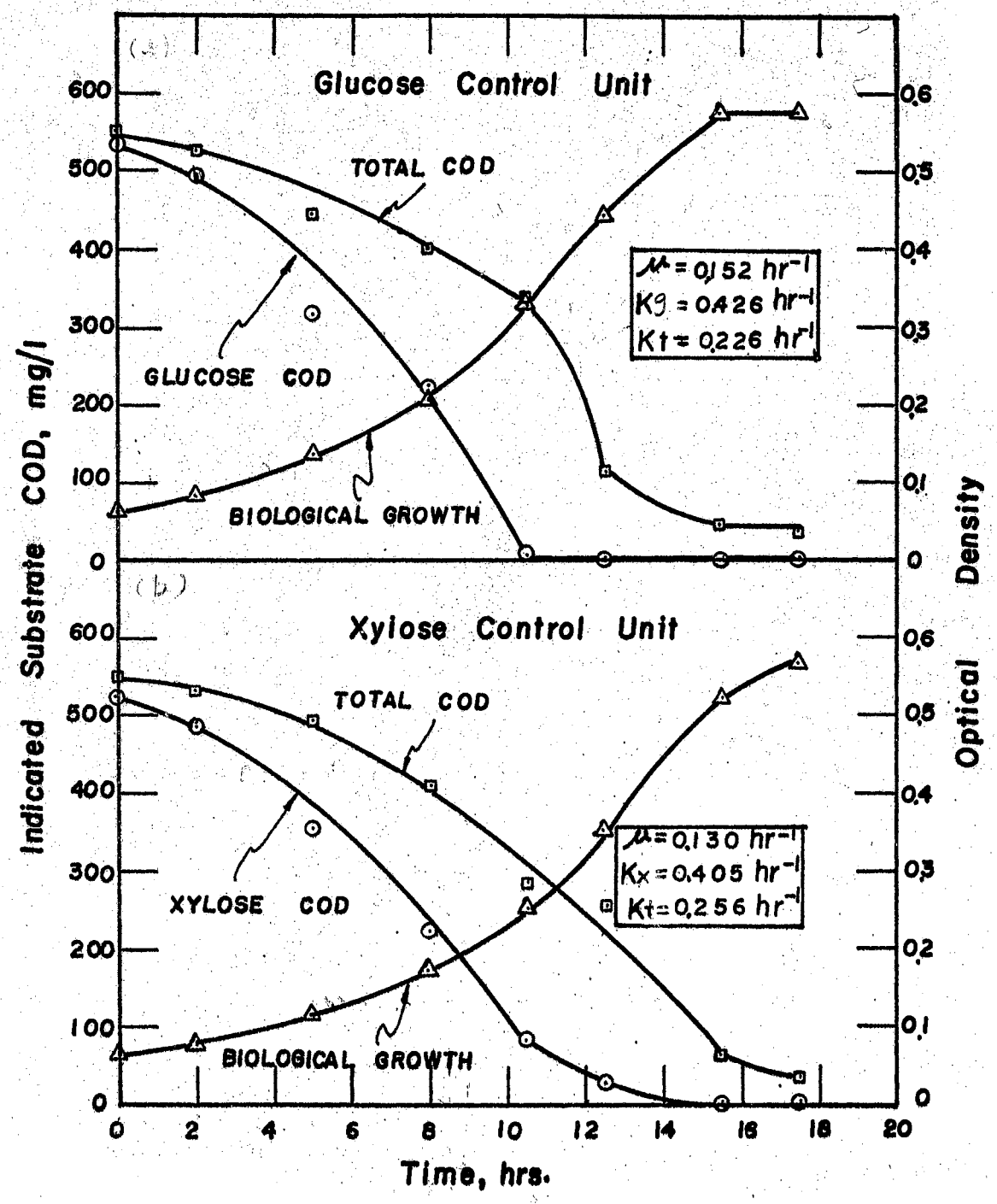


Fig. 17 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO XYLOSE.

respective units, again showing the large amounts of intermediates produced during growth.

Figure 18 presents the growth curve and COD removal curves in the combined unit of xylose and glucose. The fact that xylose utilization continued in the presence of glucose indicated that the enzymes necessary for xylose utilization were still being synthesized and still active; i.e., glucose did not affect the formation of enzmyes nor their activities. The specific growth rate observed in the combined unit was larger than those observed in the control units. This would indicate that xylose and glucose were removed concurrently and that the organisms grew on both substrates simultaneously. Nevertheless, both xylose and glucose removal were retarded by the presence of other components, as shown by a comparison of the rates of xylose and glucose removal in the combined and control units.

b. Glucose-acclimated Young Cells

Figure 19b for the glucose control unit shows that glucose removal and cell growth were essentially linear, thus the growth rate and glucose removal rates followed zero order kinetics. Approximately 60 mg/l of residual COD was observed in the glucose medium. Although the organisms had been pre-grown on glucose, it would appear from the results shown in Figure 19a that they contained xylose enzymes at the start of the experiment. A considerable amount of metabolic intermediates was released into the medium during xylose metabolism by glucose-acclimated cells.

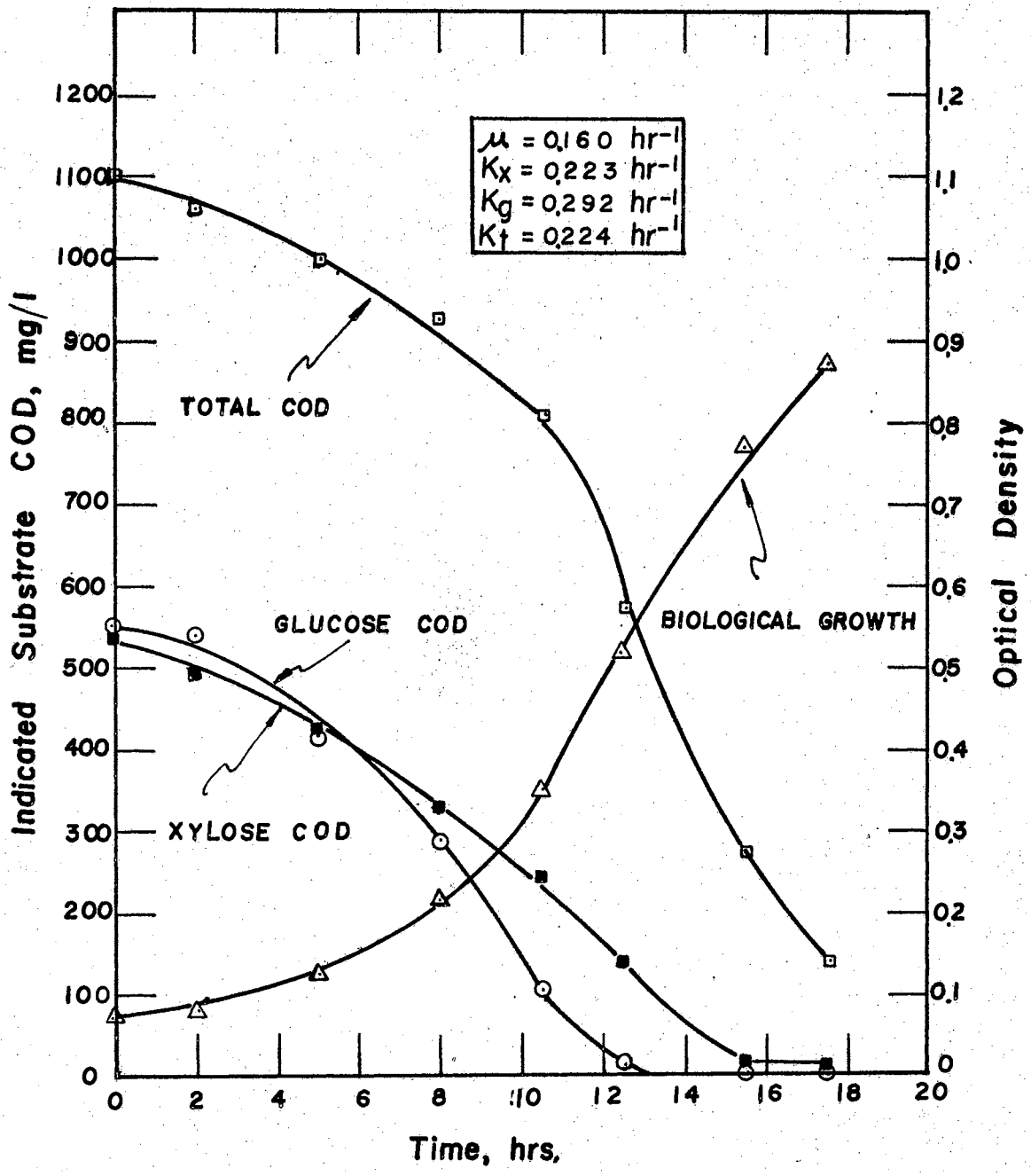


Fig. 18 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF XYLOSE AND GLUCOSE. YOUNG CELLS ACCLIMATED TO XYLOSE.

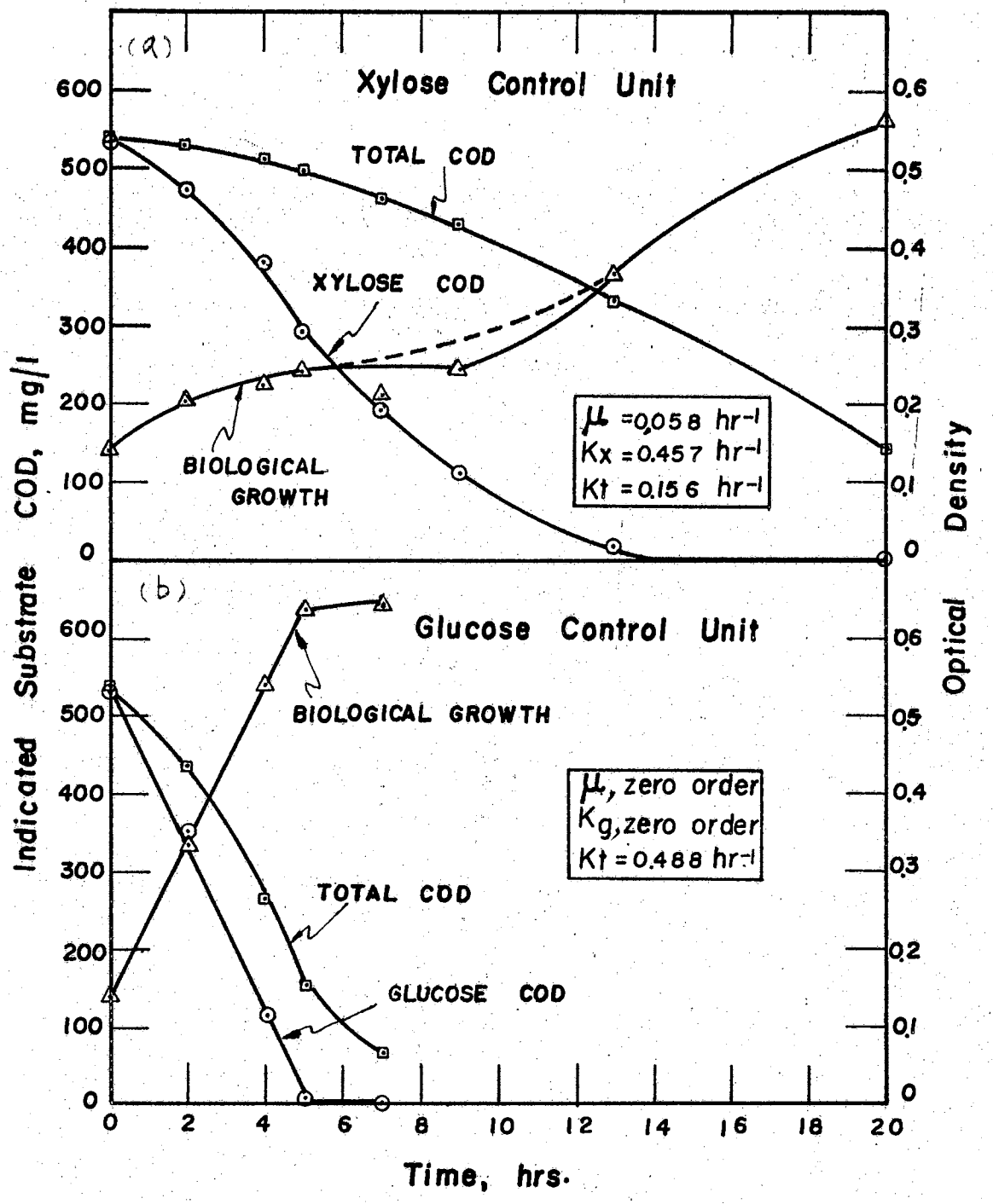


Fig. 19 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO GLUCOSE.

The residual COD (150 mg/l) observed in this experiment was extremely high. Since some carbon source (xylose) was consumed between five and nine hours, the stoppage of growth during this period could not be interpreted as a result of the induction of xylose-catabolizing enzymes. A semilogarithmic plot of total COD removal curve showed that total COD was removed exponentially between seven and thirteen hours. Thus, it seems reasonable to propose that the organisms also grew exponentially during this period. The dashed curve is the proposed growth curve of this period. Another possible explanation for the apparent growth plateau was that the COD consumed between five and nine hours was used for energy but not for growth. It is interesting to note that at the end of xylose removal about 60 per cent of the initial available xylose carbon was present in the medium as non-xylose organic material. A portion of this was apparently taken up by the cells during the declining growth phase after xylose metabolism had effectively ceased.

Diphasic growth was also observed in the medium containing both xylose and glucose (Figure 20). In this case, however, glucose prevented removal of xylose. Xylose removal did not begin until about half of the glucose had been eliminated. After the exhaustion of glucose, the patterns of growth and total COD removal in the combined system were essentially similar to those observed in the xylose control. Apparently these results indicated that the shift from growth on glucose to growth on xylose caused the

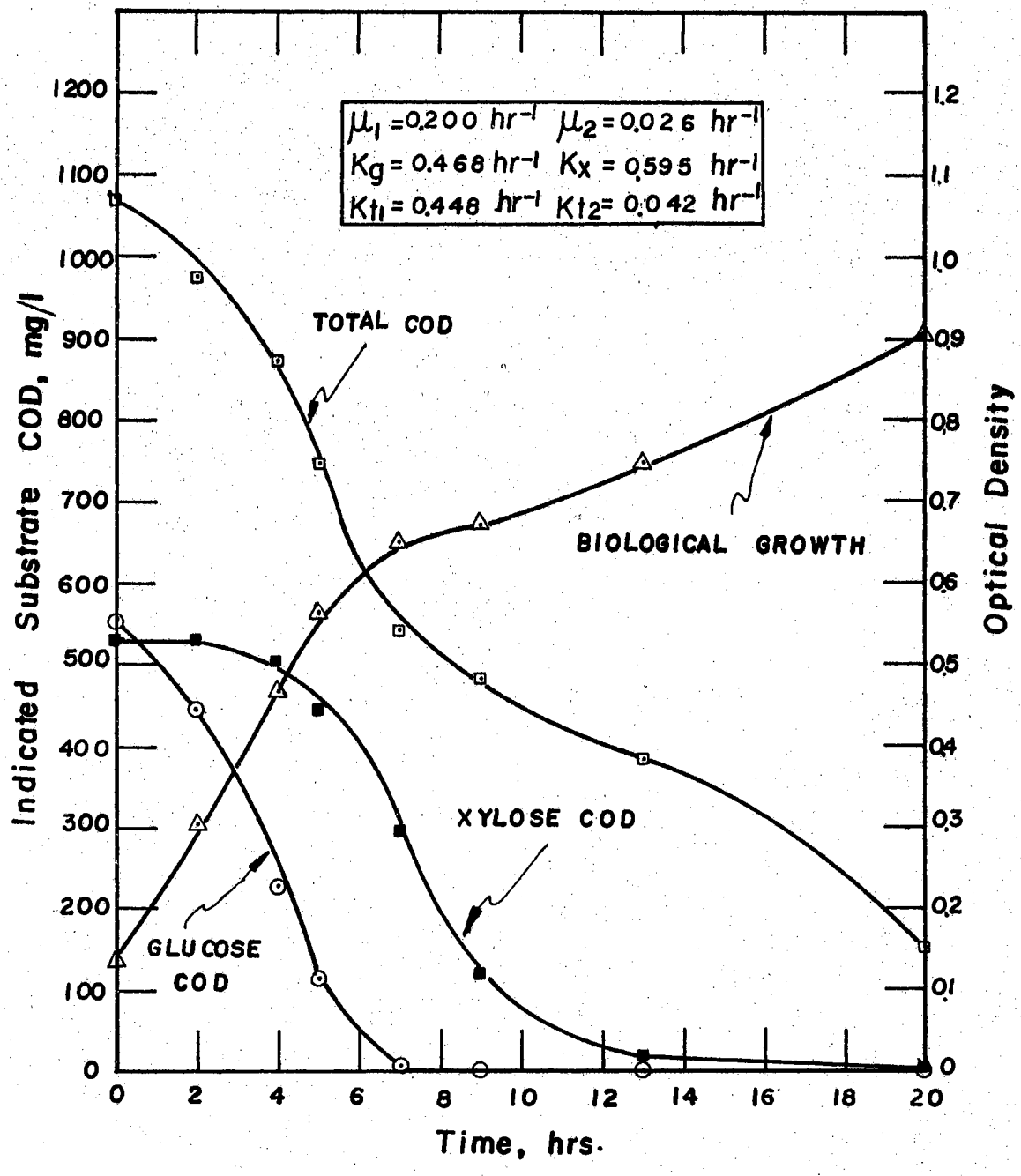


Fig. 20 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF XYLOSE AND GLUCOSE. YOUNG CELLS ACCLIMATED TO GLUCOSE.

diphasic growth. The sharp changes in the growth rates (μ_1, μ_2) and the total COD removal rates (Kt_1, Kt_2) emphasize the existence of diphasic growth and sequential substrate removal in this system. A great amount of metabolic intermediates accumulated in the xylose-glucose medium, and was subsequently used by the organisms except for 150 mg/l of residual COD. A comparison of the combined and the control units indicates that most intermediates produced in the mixed substrate system were due to the metabolism of xylose. Although glucose repressed the synthesis of xylose enzymes, glucose metabolism was also affected by the presence of xylose. Both rate of growth on glucose and rate of glucose removal in the combined unit were slightly retarded as compared to those observed in the glucose control unit.

The results for this system indicate that the following conclusions can be drawn:

1) The shape of the growth and COD curves for the xylose control suggests that some xylose-metabolizing enzymes were present in glucose-acclimated sludges.

2) Glucose repressed the formation of xylose enzymes, and did not block the activity of these enzymes.

3) Xylose-acclimated cells grew on both substrates simultaneously and removed substrates concurrently; whereas glucose-acclimated organisms grew on glucose and xylose diphasically and removed the substrates sequentially.

4) Glucose metabolism was slightly retarded by the presence of xylose.

5) A considerable amount of metabolic intermediates was produced from xylose metabolism by sludge acclimated to xylose or glucose.

4. Xylose and Galactose System

The following observations are made from the results of two experiments which were run for this system (see Experiments 8 and 9, Table VI):

1) The organisms pre-grown on either component contained enzymes necessary for the metabolism of the other component, because no acclimation period was required in the controls.

2) Neither substrate affected the formation or the activity of the enzymes responsible for the utilization of the other substrate.

3) The patterns of growth and substrate removal in the xylose-galactose medium were: monophasic growth and concurrent substrate removal.

4) The growth rate μ observed in the combined unit was larger than those observed in the controls, when xylose-acclimated cells were used. On the contrary, μ in the combined unit was smaller than those in the controls, when galactose-acclimated cells were employed. This finding is taken as an indication that the introduction of xylose into the galactose medium retarded the utilization of galactose.

5. Xylose and Mannose System

The following observations were made for the inter-

actions of xylose and mannose (see Experiments 10 and 11, Table VI):

1) Mannose-catabolizing enzymes were present in the xylose-acclimated sludge; however, it appeared that mannose-acclimated sludge required a short acclimation period to xylose.

2) Comparison of the xylose curves for the xylose control and the two-component system indicates that mannose repressed slightly the synthesis of xylose enzymes.

3) Xylose and mannose were removed concurrently by a heterogeneous population. The growth pattern was monophasic.

4) Comparing xylose and mannose removal in the combined medium with the removal of each in the control systems demonstrates that the presence of xylose enhanced mannose removal, while xylose removal was relatively retarded in the combined system.

5) The residual COD in all systems was approximately the same.

6. Xylose and Fructose System

a. Xylose-acclimated Young Cells

Figure 21 shows the microbial growth and substrate utilization patterns for the control units. It is seen that no acclimation was required for fructose utilization; i.e., the xylose-acclimated cells apparently contained fructose enzymes at the start of the experiment as evidenced by the absence of an appreciable lag period. Since the

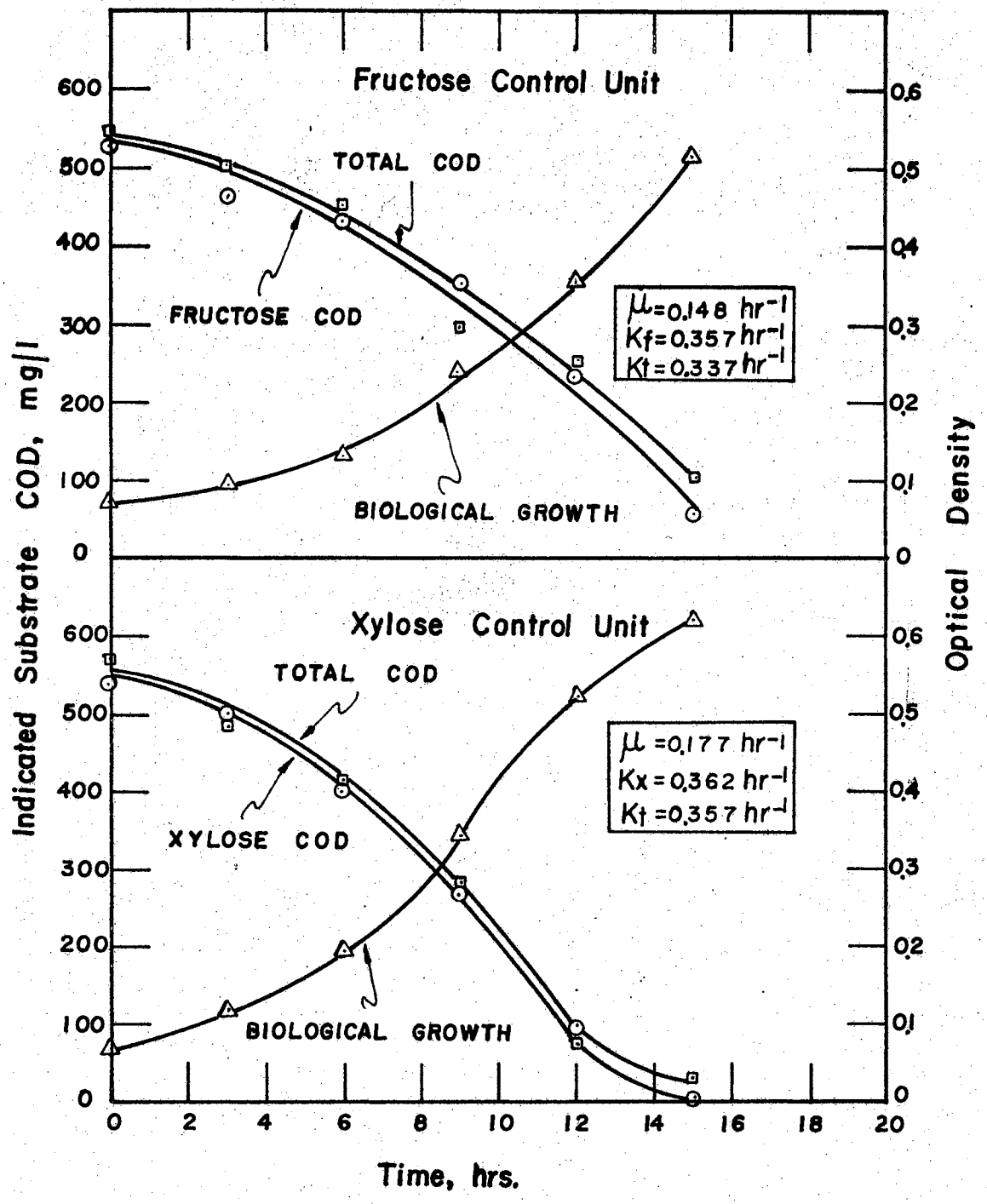


Fig. 21 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO XYLOSE.

organisms had been pre-grown on xylose, they were capable of growing rather rapidly on xylose without acclimation.

The growth response of a xylose-acclimated microbial population to the presence of xylose and fructose in the culture medium is given in Figure 22. It is noted that the organisms grew on fructose and xylose simultaneously; i.e., they utilized the component substrates concurrently. The facts that growth rate μ observed in the combined unit was larger than observed in the controls, and that the substrate removal rates Kx and Kf were smaller in the combined than those in the individual controls also indicate the pattern of simultaneous growth and concurrent substrate removal. It is evident that the intermediates produced in the medium were negligible.

b. Fructose-acclimated Young Cells

The curves of the control units are given in Figure 23. It is seen that about eight hours of acclimation was required for the fructose-acclimated organisms to metabolize xylose. This indicated that the bulk of the organisms in the system contained no xylose-catabolizing enzymes at the start of the experiment.

The result for the combined unit (Figure 24) shows that fructose completely repressed the synthesis of enzymes required for xylose metabolism. The xylose COD curve indicates that the activation by xylose of the genes governing the synthesis of xylose enzymes did not take place until 14-16 hours after initiation of the experiment (the time

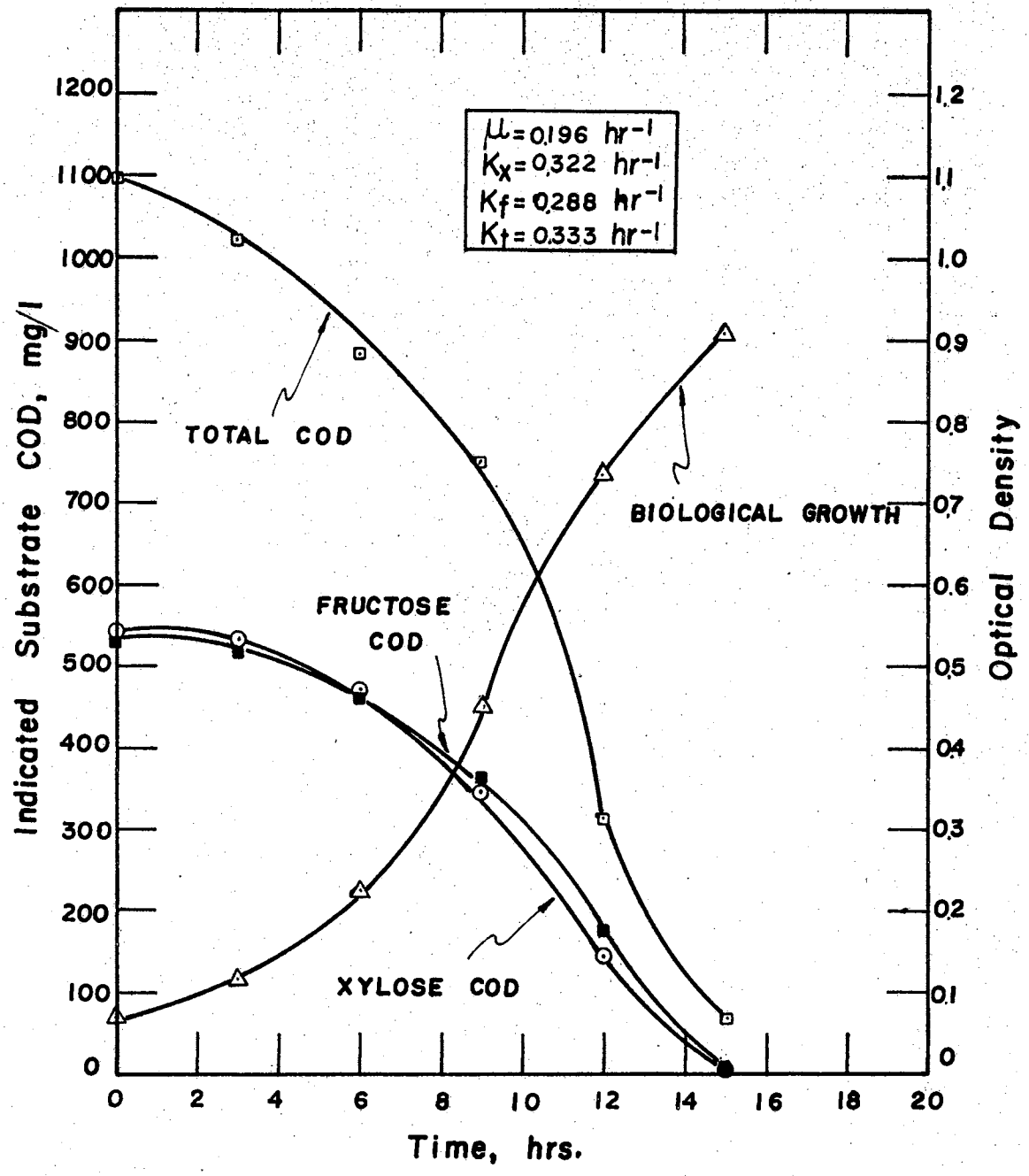


Fig. 22 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF XYLOSE AND FRUCTOSE. YOUNG CELLS ACCLIMATED TO XYLOSE.

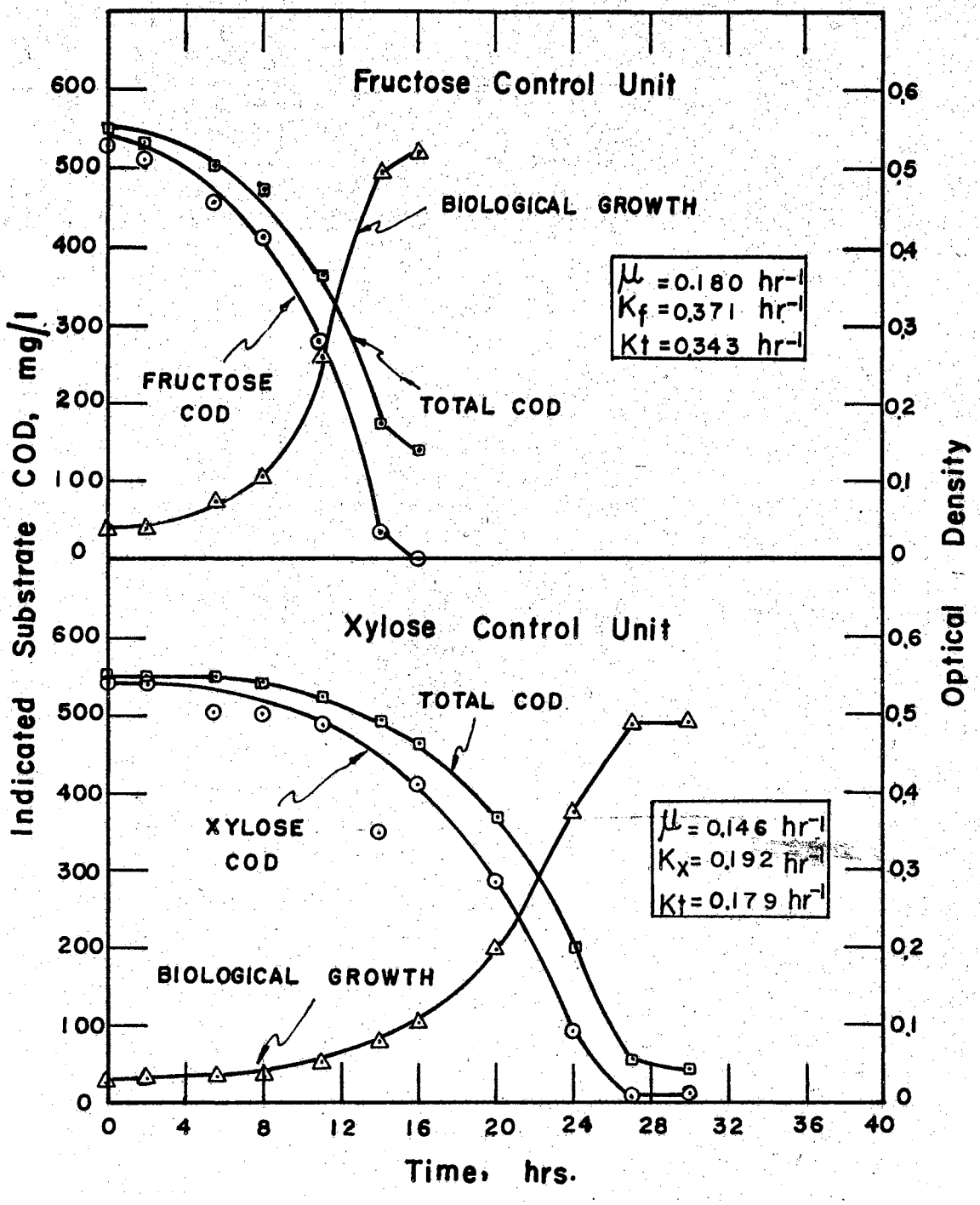


Fig. 23 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO FRUCTOSE.

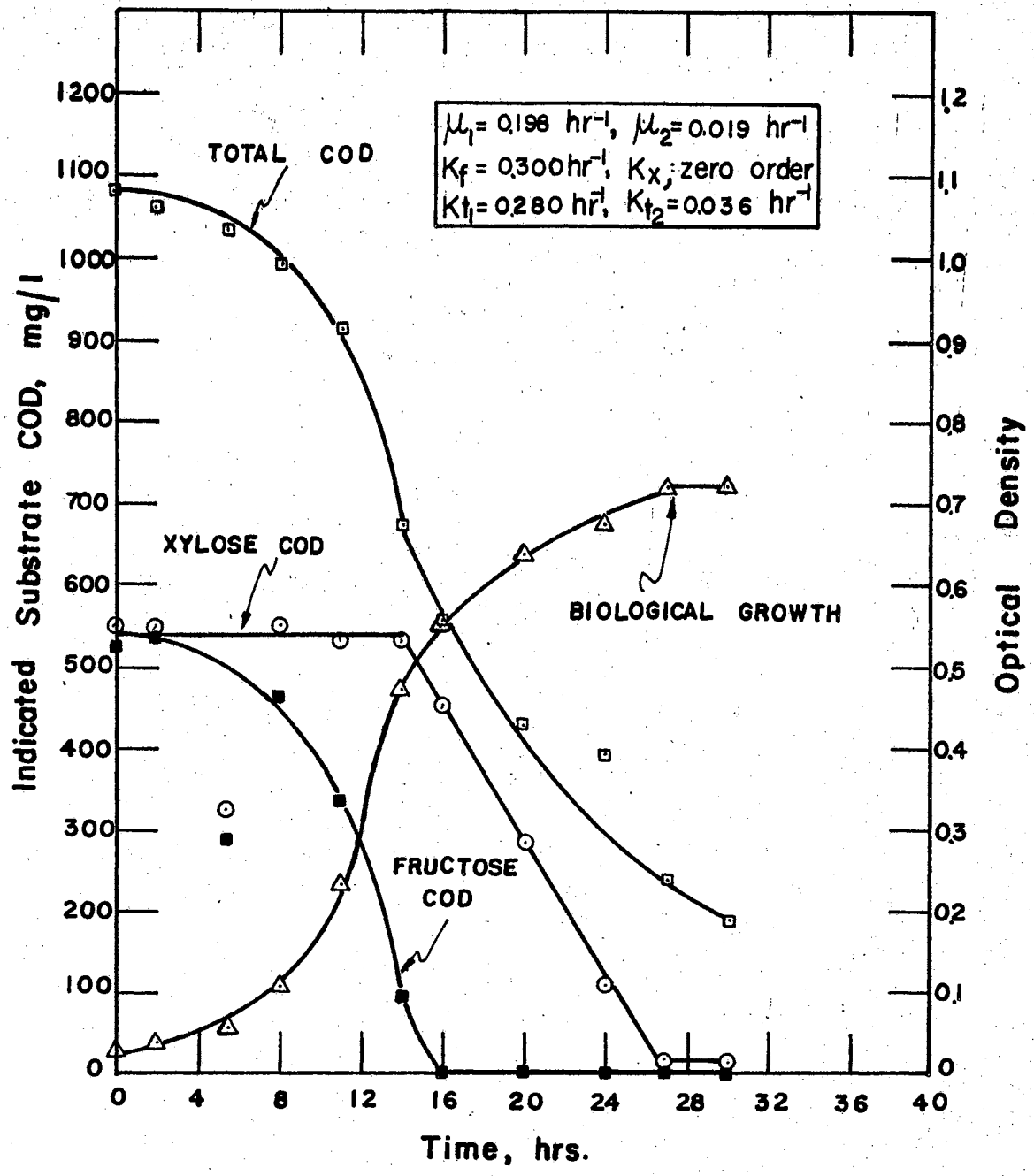


Fig. 24 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF XYLOSE AND FRUCTOSE. YOUNG CELLS ACCLIMATED TO FRUCTOSE.

required to eliminate fructose). It is seen that xylose utilization was initiated rapidly and was removed linearly as soon as fructose was exhausted. The results also show that two growth rates (μ_1 and μ_2) and two total COD removal rates (Kt_1 and Kt_2) were caused by diphasic growth and sequential substrate removal. The residual COD in the mixture was approximately equal to the sum of the residual COD's in the controls, indicating that the production of intermediates was not appreciably affected. According to these investigations, the following conclusions can be drawn for the interactions and utilization of a xylose-fructose medium:

1) Xylose-acclimated populations contained fructose-catabolizing enzymes, while fructose-acclimated organisms required acclimation to xylose.

2) For xylose-acclimated cells, the presence of fructose in the culture medium did not affect the activity of the xylose enzymes, nor did xylose affect fructose metabolism. Thus, xylose-acclimated organisms grew on xylose and fructose simultaneously and removed the substrates concurrently.

3) Fructose repressed the formation of xylose-catabolizing enzymes. Therefore, the growth pattern of fructose-acclimated cells on a fructose-xylose medium was diphasic; the utilization of the substrate mixture was sequential.

4) The production of intermediates was not changed appreciably by combining the substrates.

7. Xylose and Sorbose System

a. Xylose-acclimated Young Cells

Xylose-acclimated organisms were used to seed four units to investigate the patterns of utilization of xylose and sorbose respectively, and of both substrates combined, and the effect of the shock loading of sorbose on the active metabolism of xylose.

Figure 25 shows the growth and COD removal curves for the control units. It is noted that the production of intermediates in the sorbose control was somewhat larger than for the xylose control. Although the xylose-acclimated organisms did not require acclimation to sorbose, they removed sorbose very slowly (small growth rate μ and long substrate removal period, 34 hours).

Substrate utilization and growth in a medium containing xylose and sorbose are shown in Figure 26. It seems that the presence of sorbose did not affect the activity of xylose enzymes, nor did the presence of xylose affect the formation of sorbose enzymes, since both substrates were removed concurrently in the early steps of the experiment. This is a particularly interesting experiment, since after an early period of concurrent removal, xylose began to block sorbose removal. However, at 16 hours this trend was reversed and xylose was blocked by sorbose. The results suggest that metabolic intermediates released by the cells may have been responsible for the atypical response observed. The growth pattern in this xylose and sorbose mixture

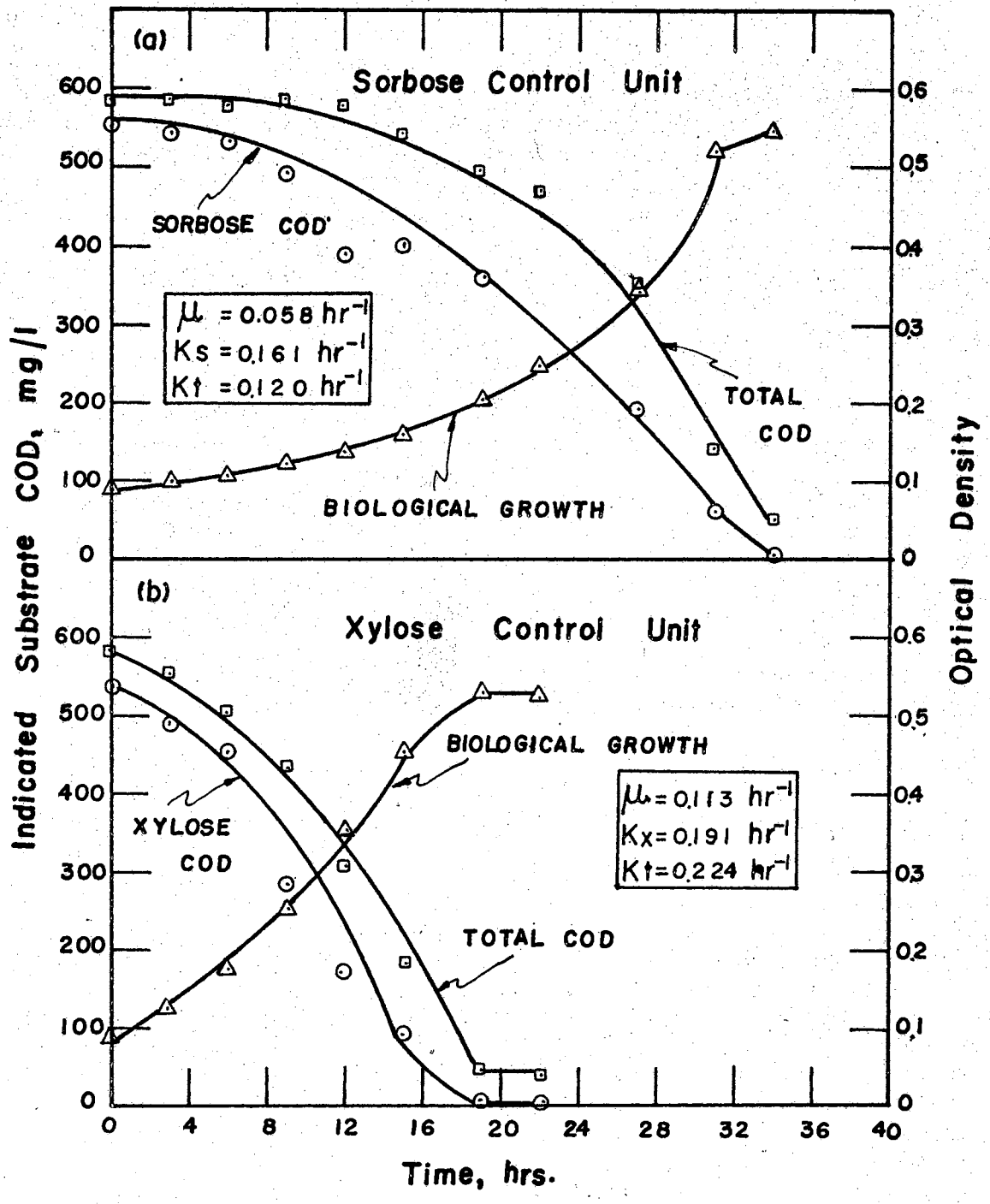


Fig. 25 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO XYLOSE.

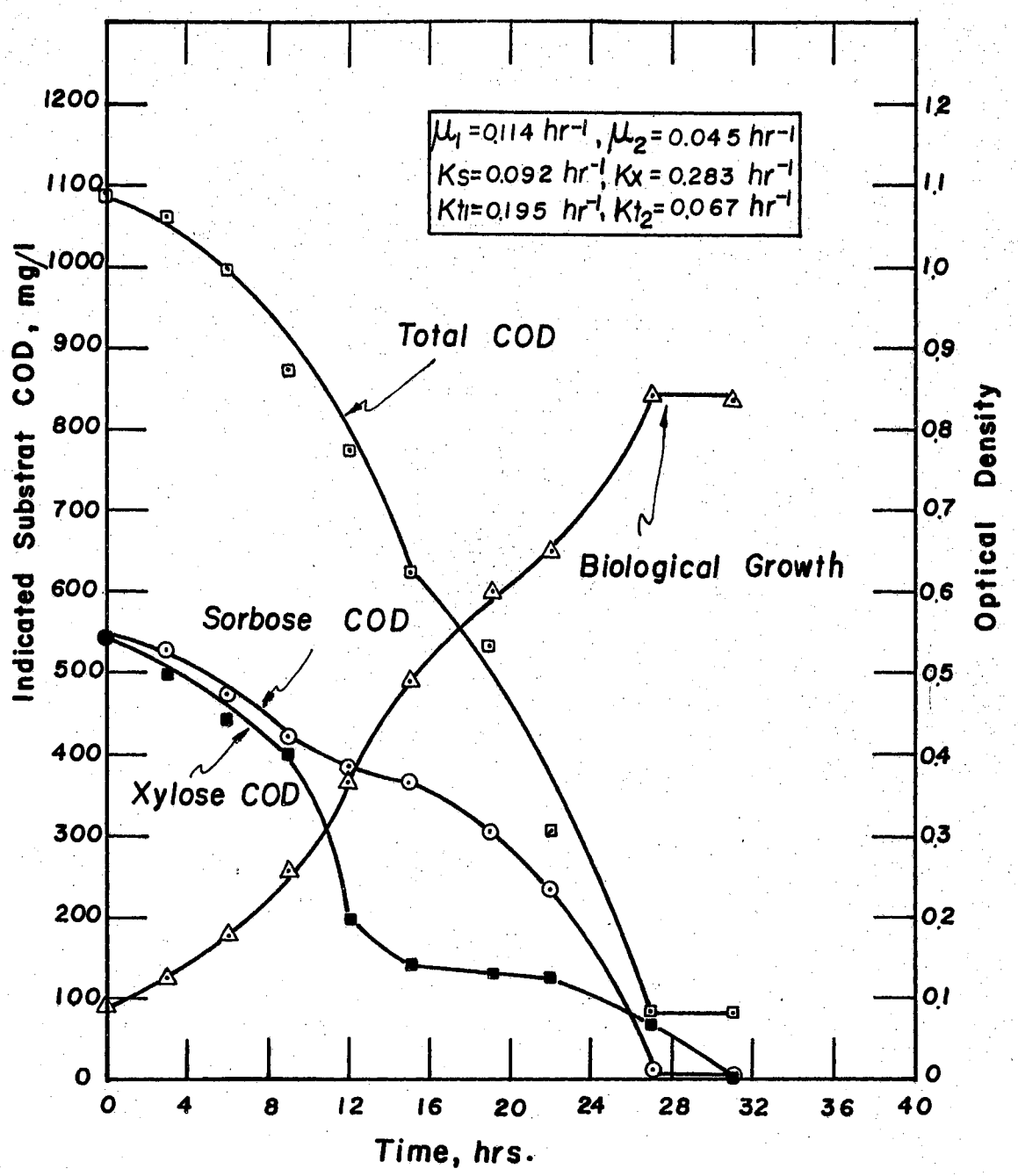


Fig. 26 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF SORBOSE AND XYLOSE. YOUNG CELLS ACCLIMATED TO XYLOSE.

was diphasic, even though the substrates were, in a gross sense, removed concurrently. The change of total COD removal rate (Kt_1, Kt_2) was caused by the phenomenon of diphasic growth.

The results for the unit shocked with sorbose (Figure 27) also suggest that the accumulation of metabolic intermediates blocked the utilization of xylose at 15-16 hour. It is seen that the production of intermediates from the metabolism of xylose and sorbose mixture was not changed considerably as compared with those of the control units.

b. Sorbose-acclimated Young Cells

The results for the control units are given in Figure 28. The 14-hour lag in the xylose control indicated that the population, pre-grown on sorbose, contained little or no xylose-catabolizing enzymes.

Figure 29 shows the growth responses of the sorbose pre-grown organisms to the presence of xylose and sorbose. It is noted that the organisms were not capable of removing xylose in the presence of sorbose. The formation of xylose enzymes apparently did not start until almost all of the sorbose had been exhausted. Since the sorbose-acclimated organisms took approximately 14 hours to use xylose in both the xylose control and combined units, the pronounced diphasic character in COD removal may be due to repression by sorbose of xylose enzymes, or simply to the time required for induction of xylose enzymes in the sorbose-acclimated sludge. Furthermore, it is noted that the presence of

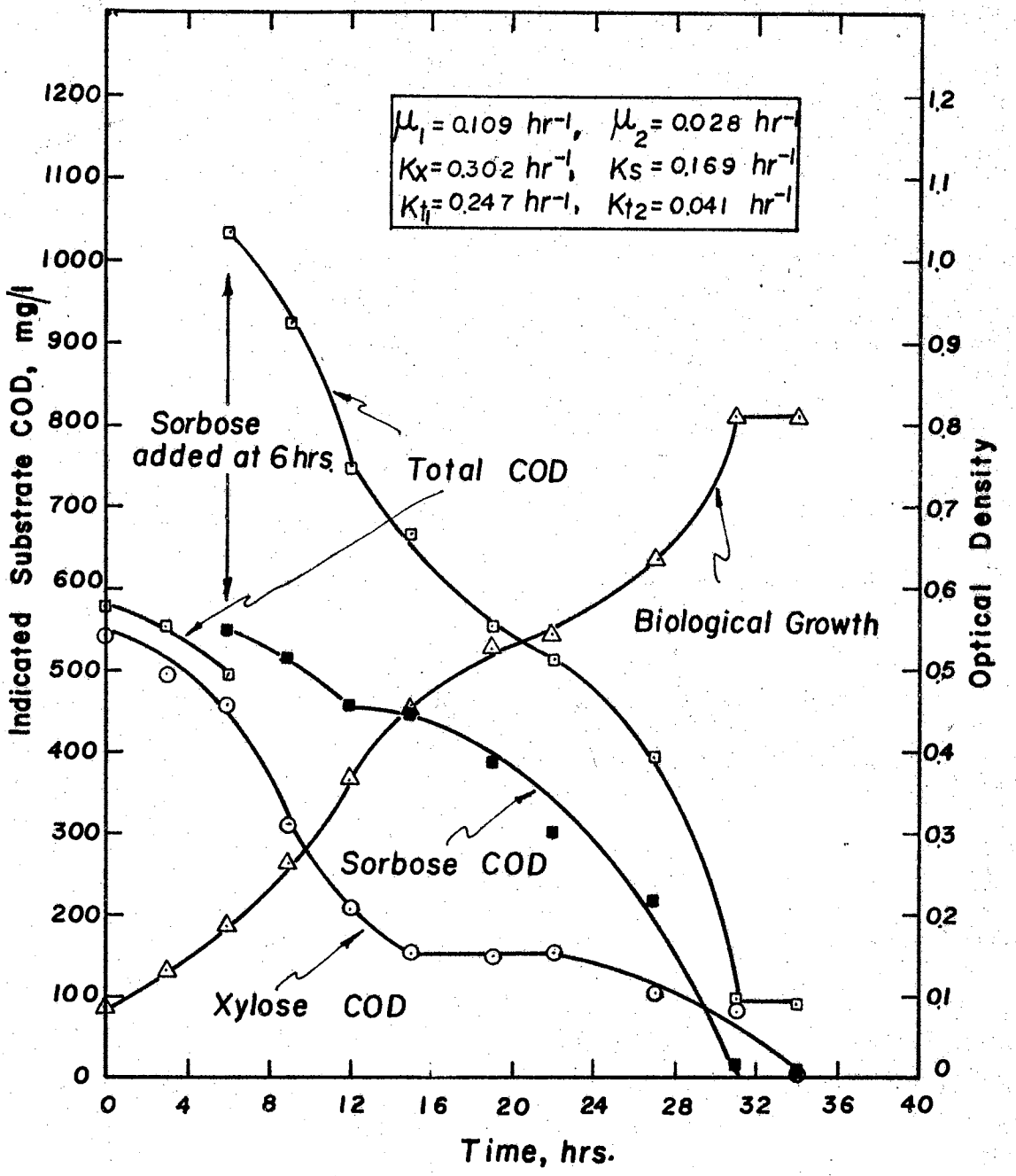


Fig. 27 - RESPONSE OF XYLOSE-ACCLIMATED YOUNG CELLS TO SHOCK LOADING WITH SORBOSE.

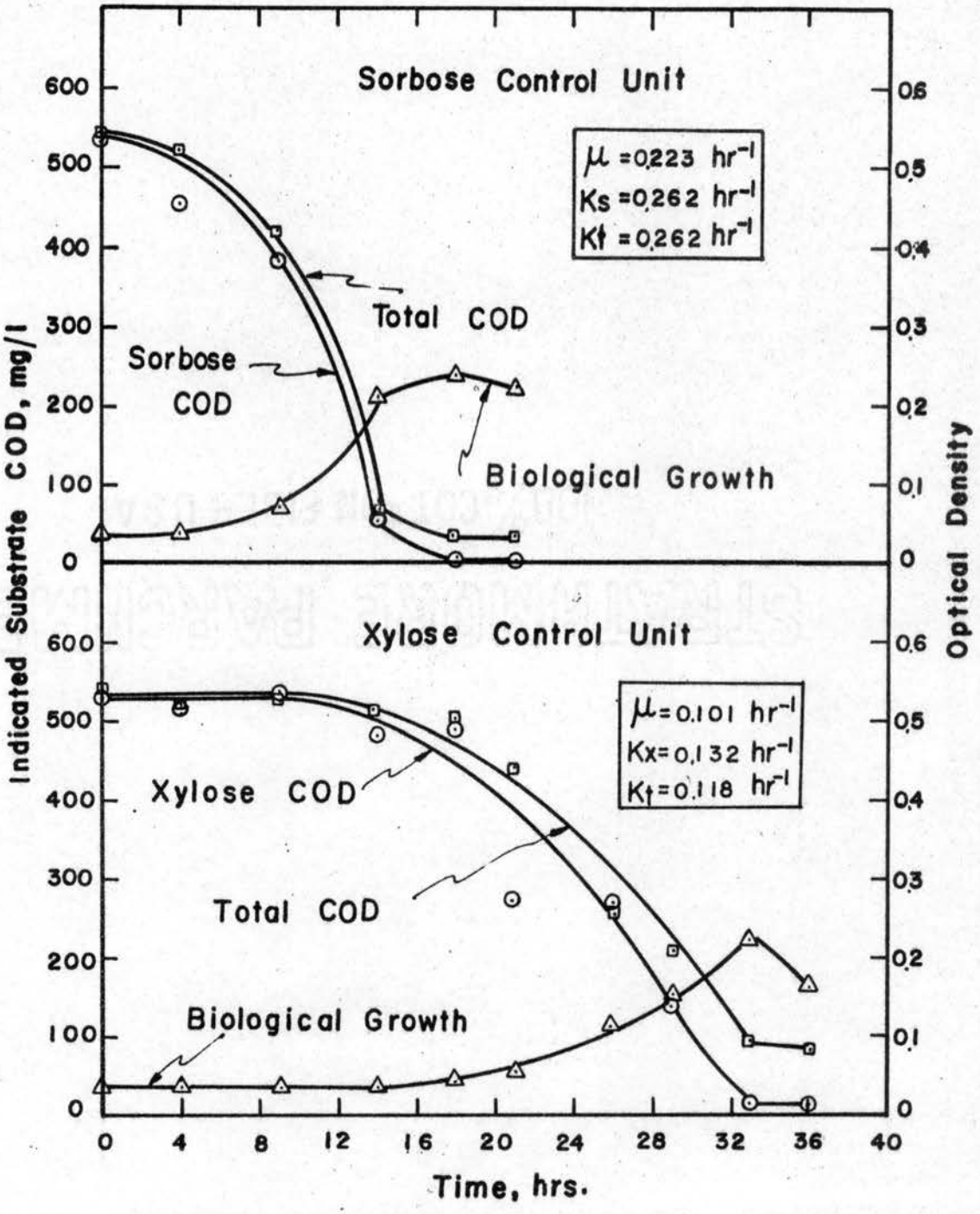


Fig. 28 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO SORBOSE.

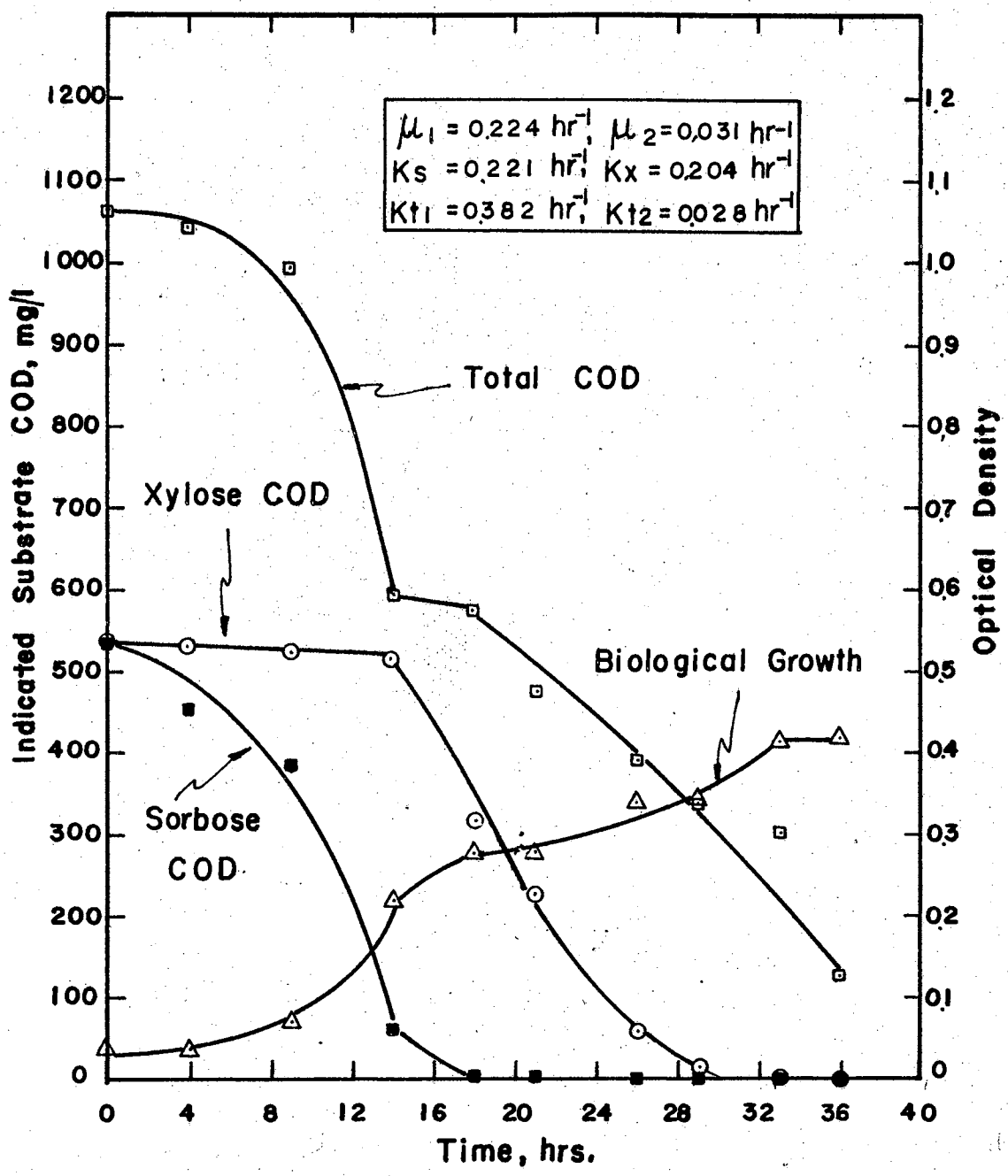


Fig. 29 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF XYLOSE AND SORBOSE. YOUNG CELLS ACCLIMATED TO SORBOSE.

xylose did not alter the sorbose removal rate. For the result shown in Figure 29, the lag between substrate removal cycles was more pronounced than those shown in figures of previous experiments (Figures 10, 13, 20, and 24). A comparison of intermediates production from the three units reveals that more intermediates were accumulated in the combined system than in the medium containing only the single substrates. These results suggest the following tentative conclusions:

1) Xylose-grown organisms contained sorbose-catabolizing enzymes, whereas sorbose-acclimated organisms did not contain xylose-utilizing enzymes.

2) For xylose-acclimated cells it would appear that metabolic intermediates inhibited the activity of xylose enzymes rather than sorbose itself.

3) Xylose-acclimated cells removed sorbose and xylose in atypical but grossly concurrent manner, and grew on the substrates simultaneously. However, the results suggest that accumulation of metabolic intermediates caused diphasic COD removal.

4) Sorbose-acclimated cells removed xylose and sorbose sequentially and grew on the substrates diphasically.

8. Xylose and Maltose System

The following observations are made from the results of two experiments using young cells acclimated to xylose and to maltose (see Experiments 16 and 17, Table VI):

1) The organisms pre-grown on either of the substrates

required acclimation to the other substrate, as evidenced by the presence of an appreciable lag period for the control.

2) Xylose was capable of repressing the formation of maltose enzymes, thus the pattern of substrate removal by xylose-acclimated cells was sequential with xylose preceding maltose.

3) For maltose-acclimated sludge, the similar maltose removal in the maltose control and combined system, and the similar acclimation period to xylose in the xylose control and combined system show that xylose did not affect the activity of xylose enzymes nor did maltose affect the formation of xylose enzymes. Therefore the maltose-acclimated cells utilized xylose and maltose concurrently, and grew on tne substrates simultaneously.

4) The growth rates observed in the combined units were greater than those observed in the media containing only one substrate component.

5) The production of intermediates was negligible.

9. Xylose and Lactose System

Both xylose and lactose-acclimated young cells were used to investigate the interaction of xylose and lactose. Tne following information was obtained from the results of these experiments (see Experiments 18 and 19, Table VI):

1) Since short lag periods were required by xylose-acclimated sludge before using lactose, and lactose-acclimated sludge before using xylose, lactose-catabolizing enzymes were not constitutive in the organisms pre-grown on

xylose, nor did lactose-acclimated cells contain a complement of xylose-catabolizing enzymes. However, the acclimation occurred rather rapidly.

2) By comparing xylose and lactose removal in the controls and in the combined units it can be seen that the presence of xylose slightly hastened the acclimation of xylose-acclimated sludge to lactose, and that lactose enhanced the acclimation of lactose-acclimated sludge to xylose.

3) The presence of lactose apparently decreased the rate of xylose removal for xylose-acclimated sludge, while the presence of xylose did not affect the rate of lactose metabolism to any significant degree as evidenced by the shape of the substrate removal curves for the control and the combined units.

4) The growth rate observed in the combined substrate medium was larger than those observed in the systems containing one substrate only.

5) Xylose and lactose were utilized concurrently by the activated sludges acclimated to both substrates.

6) A small amount of galactose and glucose were present in the synthetic medium during the metabolism of lactose.

10. Xylose and Sucrose System

a. Xylose-acclimated Young Cells

The growth and COD removal curves for the control units are given in Figure 30. It is seen that there was no lag in the sucrose utilization. This indicated that the

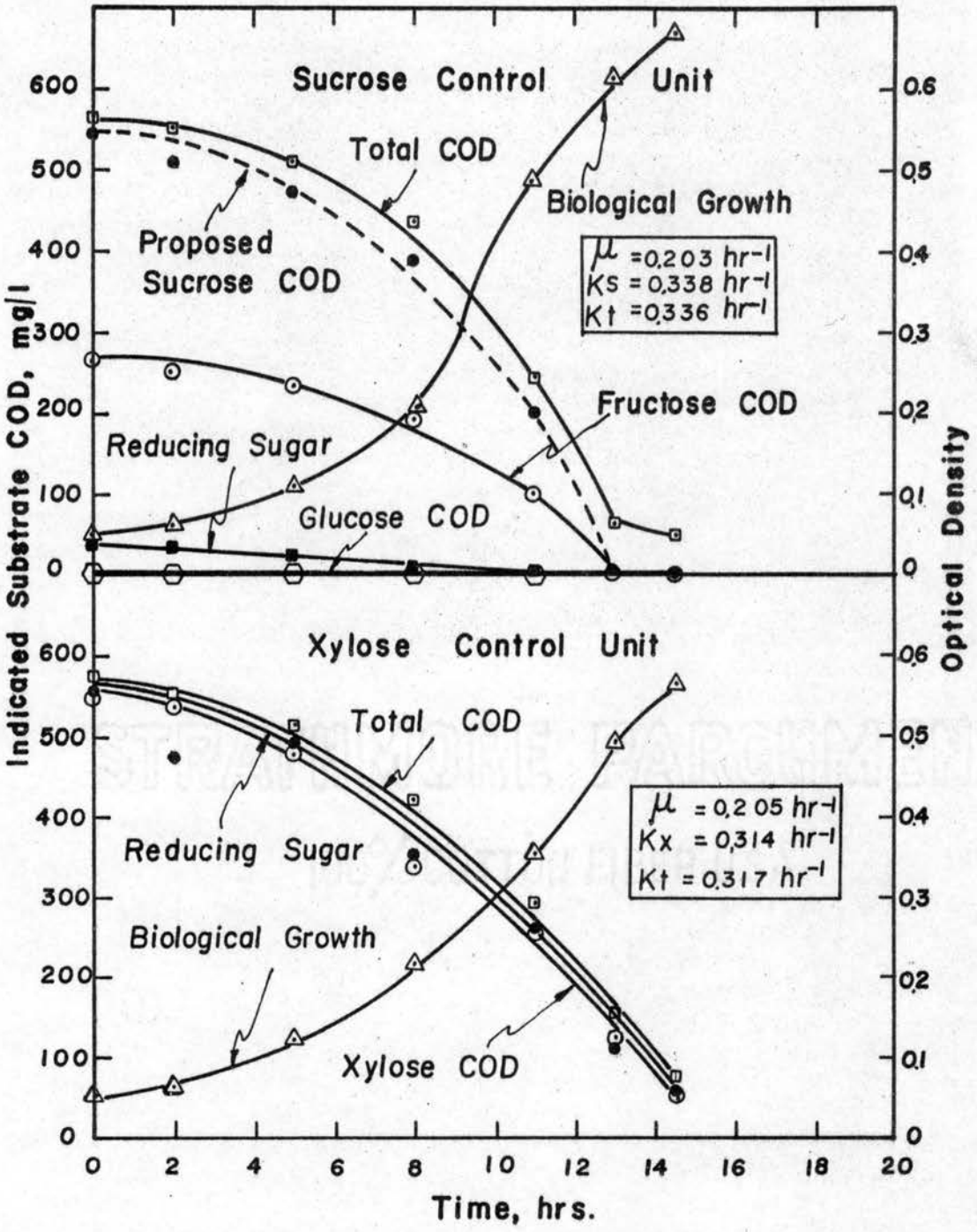


Fig. 30 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO XYLOSE.

sludge pre-grown on xylose contained or could rapidly synthesize the enzymes responsible for the removal of sucrose. The sucrose COD was estimated by doubling the fructose COD concentration at any given sampling time.

Figure 31 shows the course of xylose and sucrose removal in the xylose-sucrose synthetic waste. The sucrose curve and xylose curve were approximately the same as those shown in Figure 30; whereas the growth rate μ, observed in the combined unit, was larger than those observed in the controls. These results clearly indicated that sucrose and xylose were removed concurrently but the rate of sucrose removal was faster than the rate of xylose removal even though the sludge had been acclimated to xylose. The reducing sugar curve shown in Figure 31 was essentially equal to the xylose curve. These results indicate that little free fructose was present in the medium which contained sucrose as sole carbon source, and provide verification that sucrose concentration present in the culture medium is approximately equal to twice the concentration of fructose determined by the resorcinol test.

b. Sucrose-acclimated Young Cells

The results for the xylose control unit (Figure 32) show that xylose-catabolizing enzymes were not constitutive in the sludge acclimated to sucrose. Xylose was removed by the sucrose sludge at a very low rate; more than 48 hours were required to consume 500 mg/l of the xylose synthetic waste.

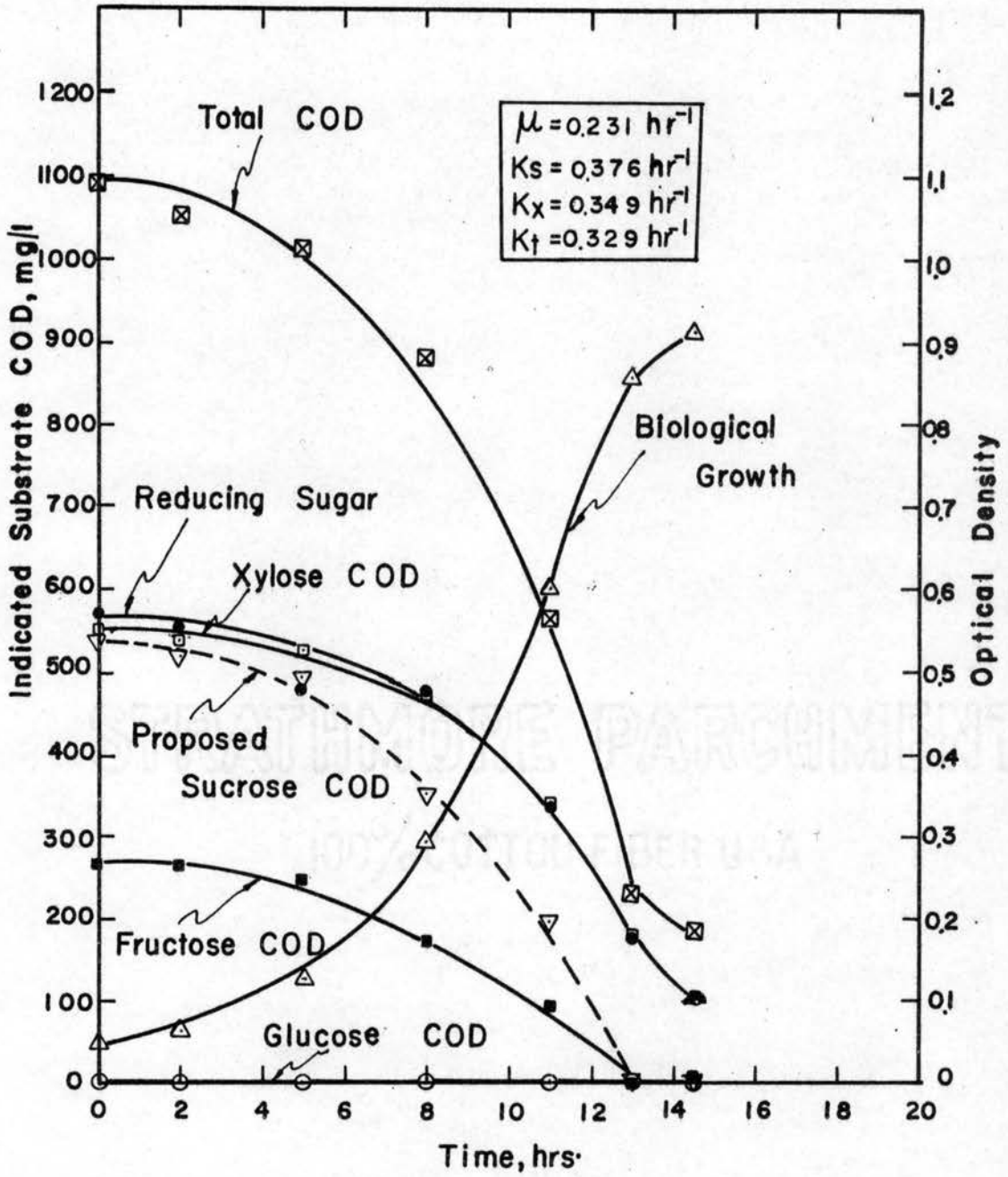


Fig. 31 - SYSTEM PERFORMANCE IN THE COMBINED UNITS OF XYLOSE AND SUCROSE. YOUNG CELLS ACCLIMATED TO XYLOSE.

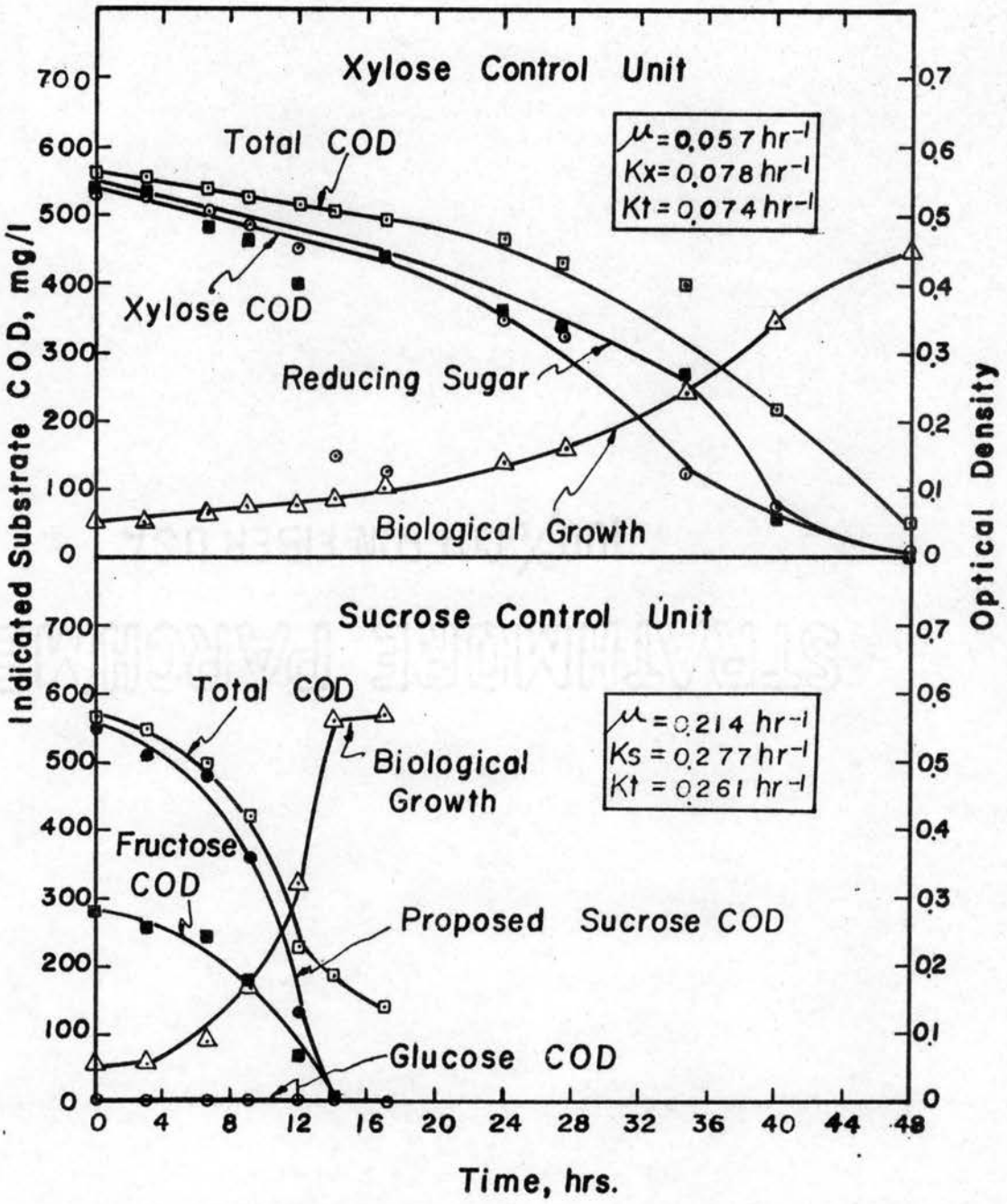


Fig. 32 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO SUCROSE.

The effect of xylose on sucrose metabolism by sucrose-acclimated sludge when both compounds are present at the start of the experimental period is shown in Figure 33. The superimposability of the growth curves in the sucrose control and in the combined system up to the time of sucrose exhaustion indicates that the presence of xylose did not affect the rates of growth and sucrose removal. The results also show that the substrate removal was sequential with sucrose preceding xylose and the growth pattern was diphasic with a pronounced lag between the two stages of growth. This pattern of sequential removal and diphasic growth might be attributable to repression by sucrose of xylose enzymes, but is more logically attributable to the time required to synthesize xylose enzymes by these sucrose-acclimated cells. The diphasic character was verified further by the occurrence of two separate growth rates (μ_1, μ_2) and two total COD removal rates (Kt_1, Kt_2) in Figure 33. A comparison of the controls and combined units indicates that the considerable amount of intermediates produced in the combined unit were due largely to the catabolism of xylose.

The following statements seem warranted from the experimental results for this system:

1) Xylose-catabolizing enzymes were inducible in the sludges pre-grown on sucrose; their production was dependent upon the presence of substrate xylose (inducer). Sucrose enzymes were constitutive in the xylose-acclimated

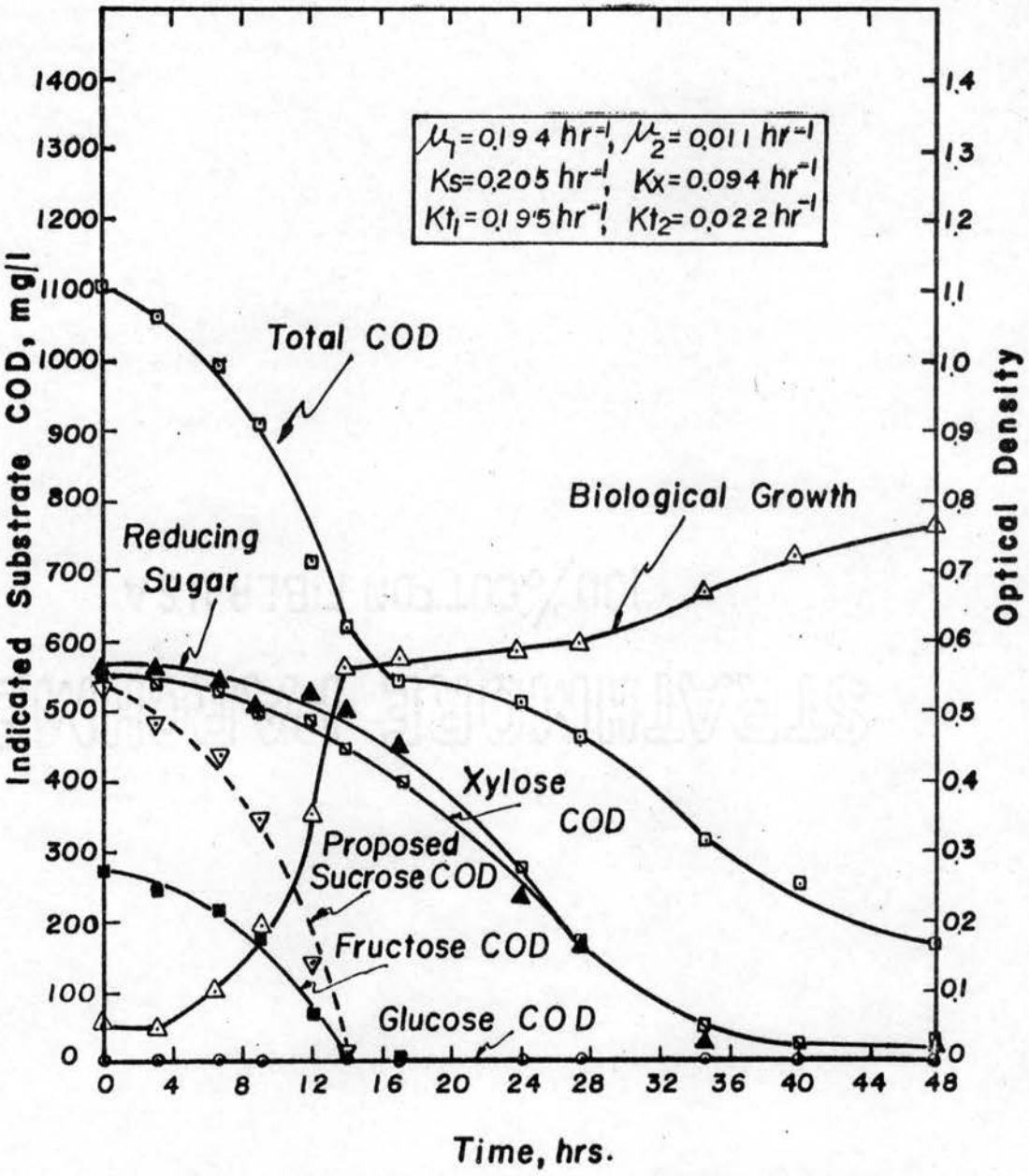


Fig. 33 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF XYLOSE AND SUCROSE. YOUNG CELLS ACCLIMATED TO SUCROSE.

sludges, as indicated by no lag period involved in sucrose metabolism.

2) Xylose had no effect on the formation and activity of sucrose-catabolizing enzymes, as indicated by the fact that the sucrose removal curves for the sucrose control and the combined units were approximately the same whether the sludges were acclimated to xylose or sucrose.

3) Since a long lag occurred in the xylose control for sucrose-acclimated sludge and no significant amount of xylose had been removed by the time sucrose was exhausted in the combined unit, sucrose might have repressed the formation of xylose-catabolizing enzymes.

4) The xylose-acclimated cells grew on xylose and sucrose concurrently. On the other hand, sucrose-acclimated sludges grew on the substrates sequentially with sucrose removal preceding xylose; there was a pronounced lag between the two stages of growth.

5) The considerable amount of intermediates produced in the substrate mixture was due largely to the catabolism of xylose.

11. Xylose and Cellobiose System

The following information was obtained from the results of two experiments using activated sludges acclimated to xylose and cellobiose (see Experiments 22 and 23, Table VI):

1) The xylose-acclimated sludges required acclimation to cellobiose, and cellobiose-acclimated sludges required acclimation to xylose.

2) Neither substrate effectively blocked the formation or activity of the enzymes responsible for the utilization of the second substrate. The basis of such a conclusion was that the metabolism of one substrate was not affected to any significant degree by the presence of the other substrate.

3) Xylose and cellobiose were removed concurrently by a heterogeneous population pre-grown on either xylose or cellobiose.

4) The growth rate μ observed in the substrate mixture was larger than those observed in either medium containing one substrate only. This result could be due to the increase of substrate (carbon) concentration in the combined unit.

12. Lyxose and Glucose System

Both fresh sewage seeds and glucose-acclimated cells were used to investigate the characteristic patterns of lyxose utilization (see Experiment 24, Table VI). It was found that there were no organisms capable of utilizing lyxose as substrate when lyxose alone was present, or when lyxose was present in combination with glucose. This would indicate that the enzymes responsible for the removal of lyxose will not be easily induced in the heterogeneous populations. Probably the lyxose-catabolizing enzymes can be induced only in a narrow range of microorganisms; in any event, not in those present in the sewage seed employed in this experiment.

13. Sorbose and Glucose System

Sorbose-acclimated young cells were employed to investigate possible substrate interactions in the sorbose-glucose synthetic waste (Figure 34). It is seen that the enzymes necessary for the metabolism of glucose were constitutive in the organisms pre-grown on sorbose. Although these cells were acclimated to sorbose, they were capable of growing faster on glucose than on sorbose. It is seen that a considerable amount of intermediates was produced in the glucose control. Evidently these intermediates were subsequently utilized by cells except for 100 mg/l of residual COD.

The data shown in Figure 35 give information on the activity of sorbose and glucose utilizing enzyme systems in the presence of both substrates. It is seen that a slight utilization of sorbose took place in the presence of glucose, but the rate of sorbose elimination from the medium was constant and in all probability the slight sorbose removal was due to the residual concentration of sorbose enzymes formed before the start of the experiment. This would indicate that glucose repressed the formation of new sorbose enzymes but did not inhibit the activity of the preformed enzymes. The growth and COD curves showed that the patterns of substrate removal and growth in sorbose-glucose medium were sequential with glucose preceding sorbose; Diphasic growth is not apparent in the graph; however, there was a small change in growth rate. The shift

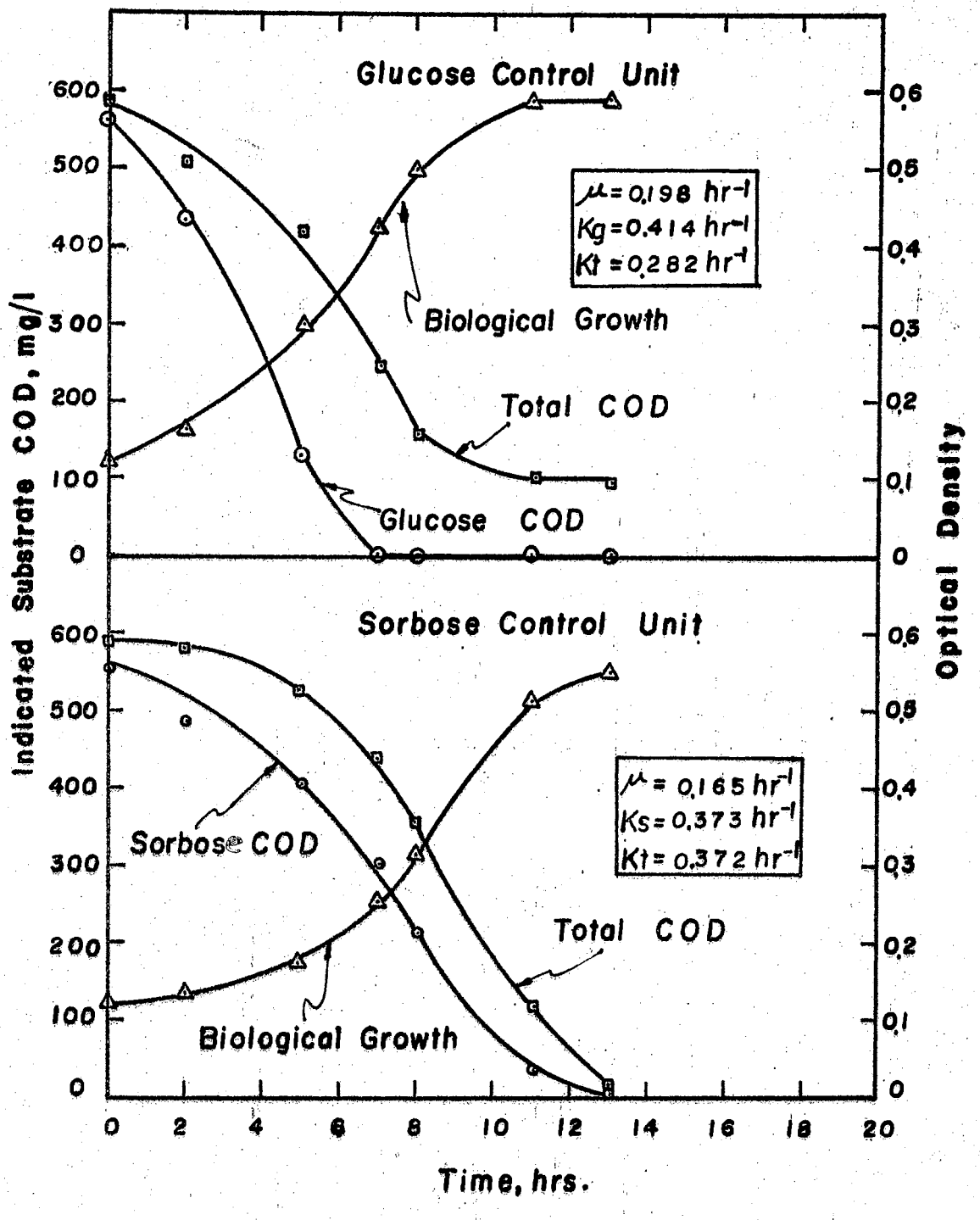


Fig. 34 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO SORBOSE.

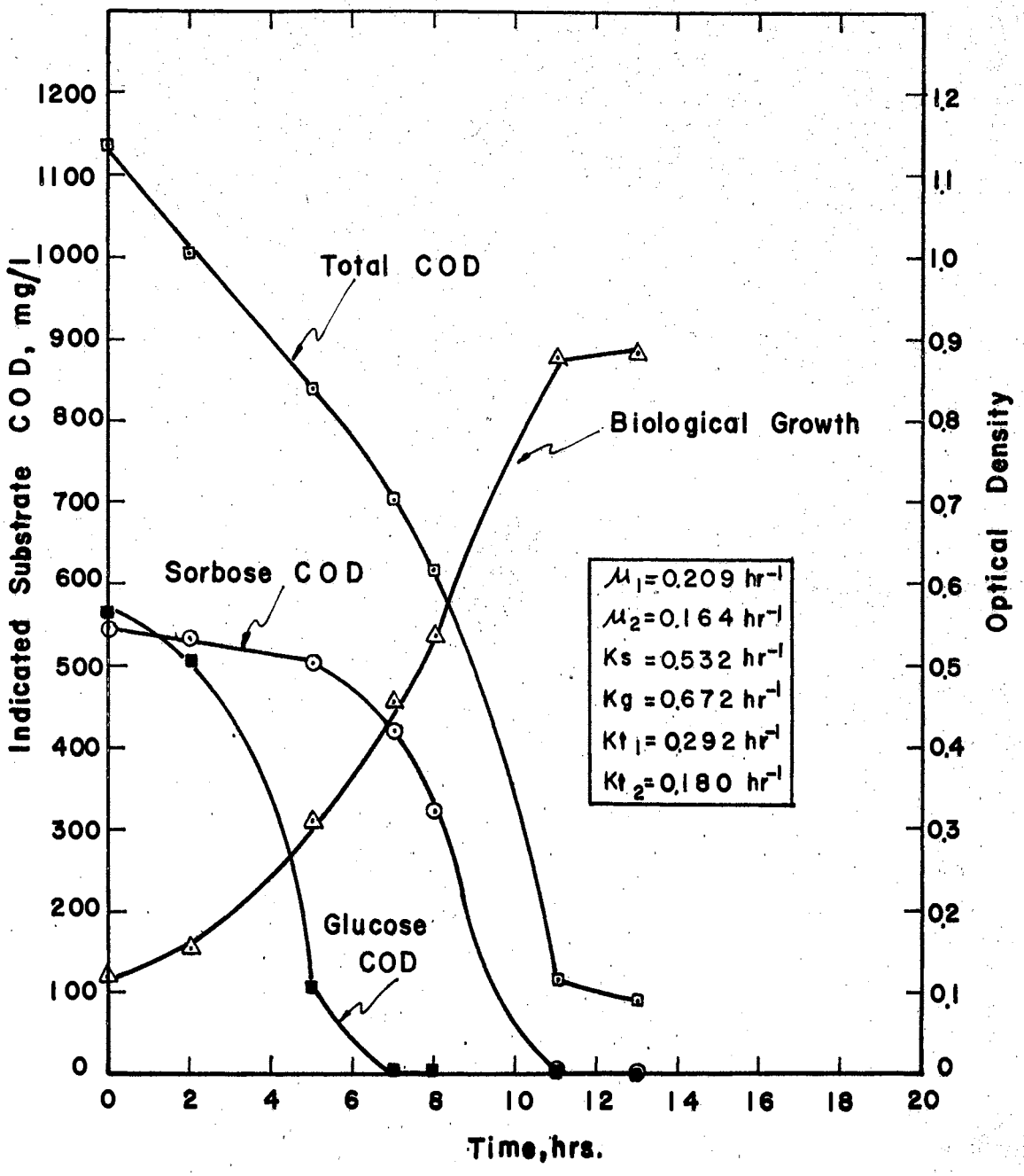


Fig. 35 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF SORBOSE AND GLUCOSE. YOUNG CELLS ACCLIMATED TO SORBOSE.

in the utilization of carbon sources in this case occurred very rapidly.

The results of an experiment in which young sorbose-acclimated cells were subjected to a severe glucose shock loading condition are given in Figure 36. It can be seen that immediate cessation of sorbose removal ensued from the glucose shock load; the removal of sorbose started again when the concentration of glucose was about the same as the concentration of the sorbose. This fact would indicate that glucose competed with sorbose and inhibited the activity of sorbose enzymes only when the glucose concentration was higher than sorbose concentration. This result seems coincidental with the result shown in the combined unit (Figure 35). Since glucose and sorbose were equally present in the combined medium at the start of the experiment, glucose repressed the formation of new sorbose enzymes but may not have inhibited the activity of preformed sorbose enzymes. From the comparison of residual COD produced in all four units, it would seem that the production of intermediates from sorbose and from glucose were not changed appreciably when used in combination or as sole carbon source, and that the intermediates produced in the combined and shock units were due primarily to the metabolism of glucose. The following conclusions can be made from the preceding results for this system:

1) The metabolism of glucose by sorbose-acclimated sludge proceeded without a lag period.

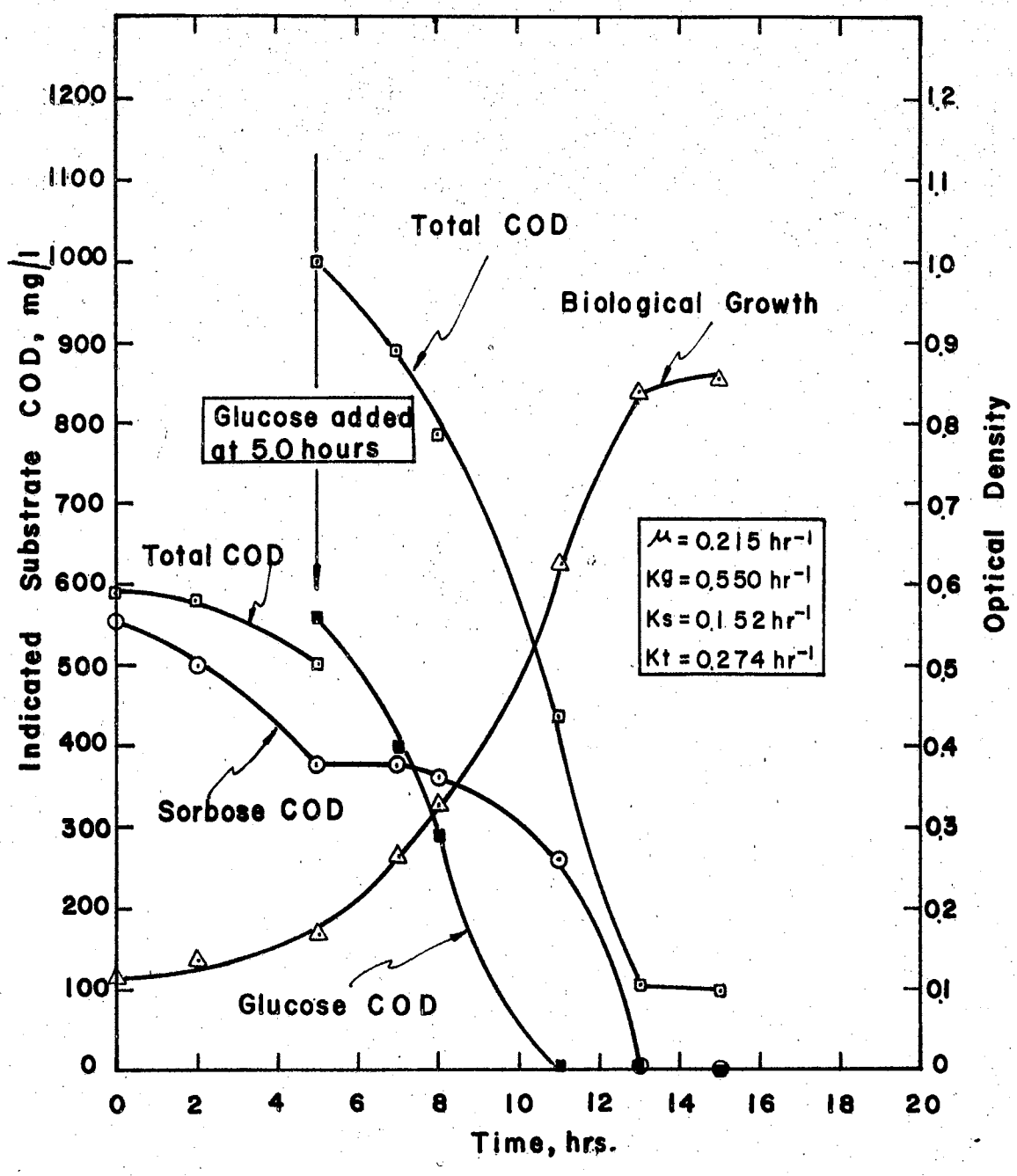


Fig. 36 - RESPONSE OF SORBOSE-ACCLIMATED YOUNG CELLS TO SHOCK LOADING WITH GLUCOSE.

2) Glucose repressed the formation of sorbose enzymes; it inhibited the activity of sorbose enzymes when glucose concentration was higher than sorbose.

3) The sequential removal of glucose and sorbose was accompanied by a diphasic growth with a small change of growth rate.

4) The production of intermediates was not changed appreciably when the substrates were used singly or in combination; the intermediates produced in the substrate mixture were essentially due to the metabolism of glucose.

14. Sorbose and Galactose System

The following information concerning the interactions of sorbose and galactose were obtained from the results of two experiments using sorbose and galactose-acclimated sludges (see Experiments 26 and 27, Table VI):

1) The enzymes responsible for the metabolism of either substrate were constitutive in the sludges acclimated to either substrate. This conclusion was based on the absence of an appreciable lag period for the metabolism of both substrates.

2) Sludges, acclimated to either sorbose or galactose, removed sorbose and galactose concurrently and grew on both substrates simultaneously. However, galactose was removed at a faster rate than sorbose.

3) Sorbose did not affect the activity or the formation of enzymes responsible for galactose metabolism, as shown by the fact that no significant interference or

retardation of galactose metabolism occurred due to the presence or introduction of sorbose.

4) Upon introduction of galactose, sorbose removal was not blocked, but was retarded. It seems that galactose competed with sorbose.

5) Since an appreciable lag period was required by galactose-acclimated sludge to metabolize sorbose in the combined and sorbose-shocked units, galactose might have repressed the formation of sorbose-catabolizing enzymes.

15. Sorbose and Mannose System (see Experiments 28 and 29, Table VI)

The results of this system indicated the following:

1) There was no lag in the metabolism of sorbose and mannose by the sludges acclimated to either substrate.

2) Sorbose repressed the formation of mannose-catabolizing enzymes; the synthesis of mannose enzymes did not start until sorbose had been removed to a certain concentration. Sorbose did not affect the activity of a sludge actively metabolizing mannose.

3) The presence of mannose (at the start of the experiment) and the introduction of a mannose shock loading did not affect the course of sorbose removal. That is to say, mannose did not affect the formation or activity of sorbose enzymes.

4) Sorbose and mannose were removed concurrently. However, sorbose was removed faster than mannose even though the cells had been acclimated to mannose.

16. Sorbose and Maltose System

Information concerning the responses of a heterogeneous population to the presence of sorbose and maltose and to the presence of both substrates combined is given below (see Experiments 30 and 31, Table VI):

1) Sorbose-acclimated sludges required no acclimation to maltose, whereas maltose-acclimated sludges required about eight hours of acclimation to sorbose.

2) A slight utilization of maltose by the sorbose-acclimated sludge took place in the presence of sorbose, but the rate of maltose elimination from the medium was constant. This would indicate that sorbose repressed the formation of new maltose enzymes, but did not inhibit the activity of the pre-formed enzymes.

3) Comparing sorbose removal by maltose-acclimated sludge in the control and in the combined and shocked units, it can be seen that the presence of maltose enhanced the induction of sorbose enzymes.

4) Sequential substrate removal and diphasic growth with sorbose preceding maltose occurred for sorbose-acclimated sludge; whereas maltose-acclimated cells removed maltose and sorbose concurrently.

5) Shock loading with maltose did not affect sorbose metabolism, nor did a sorbose shock loading affect maltose metabolism.

6) The production of intermediates was negligible in both one-component systems and in the combined systems.

17. Sorbose and Lactose System

a. Sorbose-acclimated Young Cells

The patterns of utilization of sorbose and lactose by sorbose-acclimated sludges are shown in Figure 37. It is seen that an acclimation period was required for lactose utilization. No accumulation of intermediates was detected in the sorbose control; growth on sorbose was much faster than on lactose.

Figure 38 shows the course of sorbose and lactose removal in the sorbose-lactose synthetic waste. Comparison of Figures 38 and 37 shows that sorbose was eliminated at essentially the same rate in the control and in the combined units, while lactose removal was initiated only after cessation of sorbose removal. The lactose and carbohydrate intermediates COD curve was obtained by subtracting the sorbose COD from the total carbohydrate COD (anthrone COD) remaining at any time. The pronounced lag between the two cycles of total COD removal and biological growth also provide evidence of diphasic growth and sequential substrate utilization.

Figure 39 shows the response to a shock loading of lactose of a sludge which was actively metabolizing sorbose. It is seen that lactose had no effect on sorbose removal, and lactose removal was initiated ten hours after the exhaustion of sorbose.

b. Lactose-acclimated Young Cells

The results for the control units are given in Figure

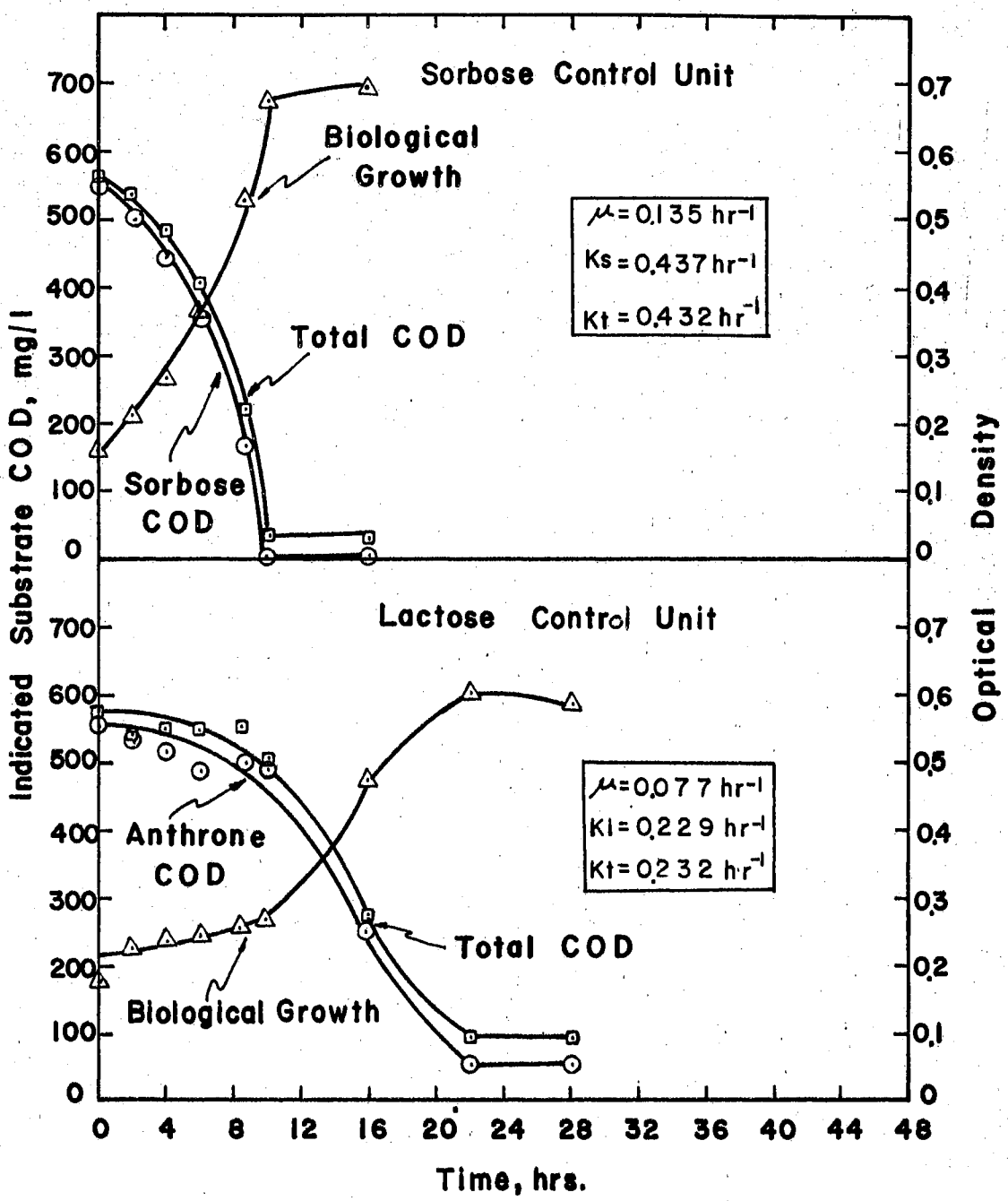


Fig. 37 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO SORBOSE.

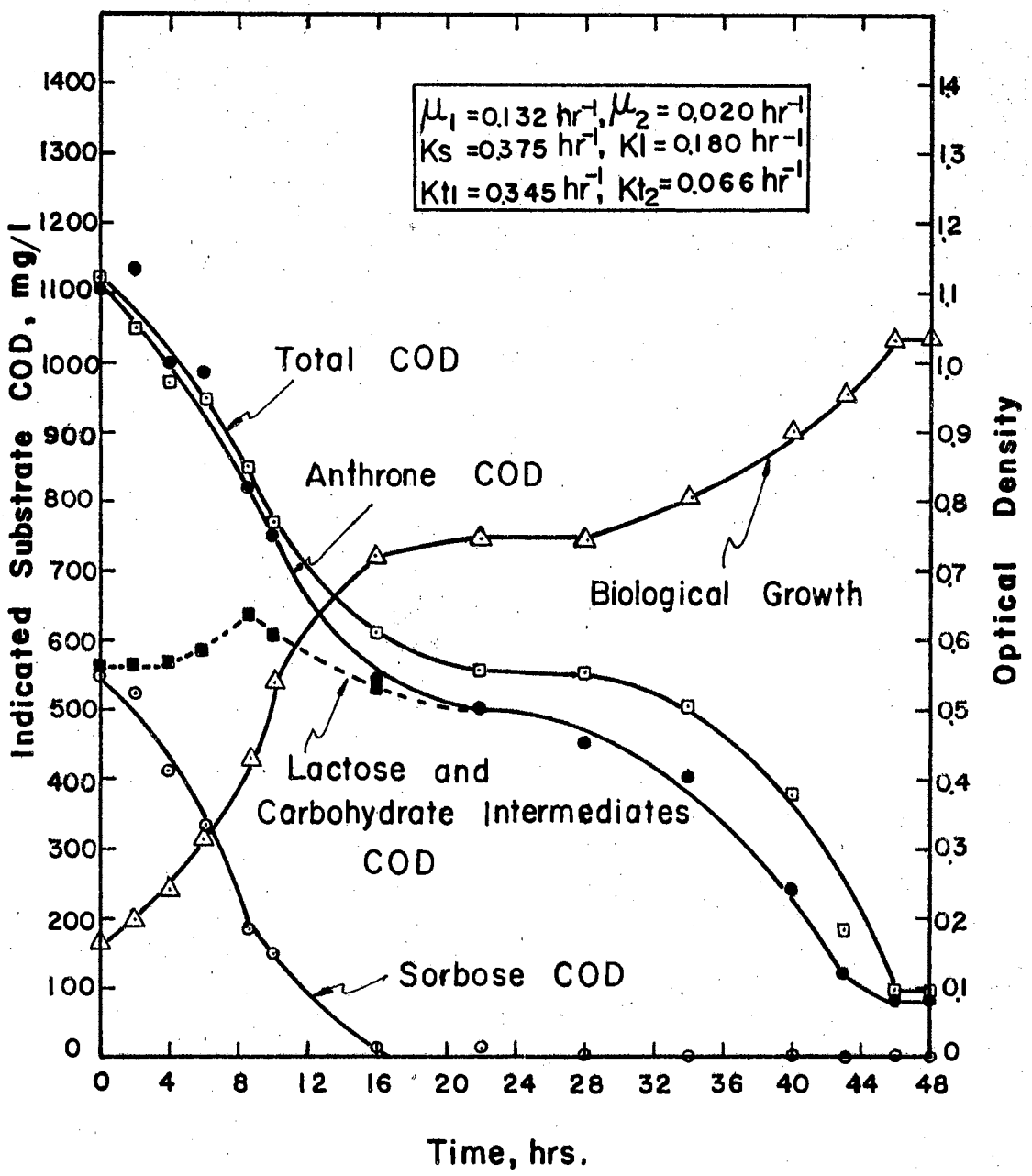


Fig. 38 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF SORBOSE AND LACTOSE. YOUNG CELLS ACCLIMATED TO SORBOSE.

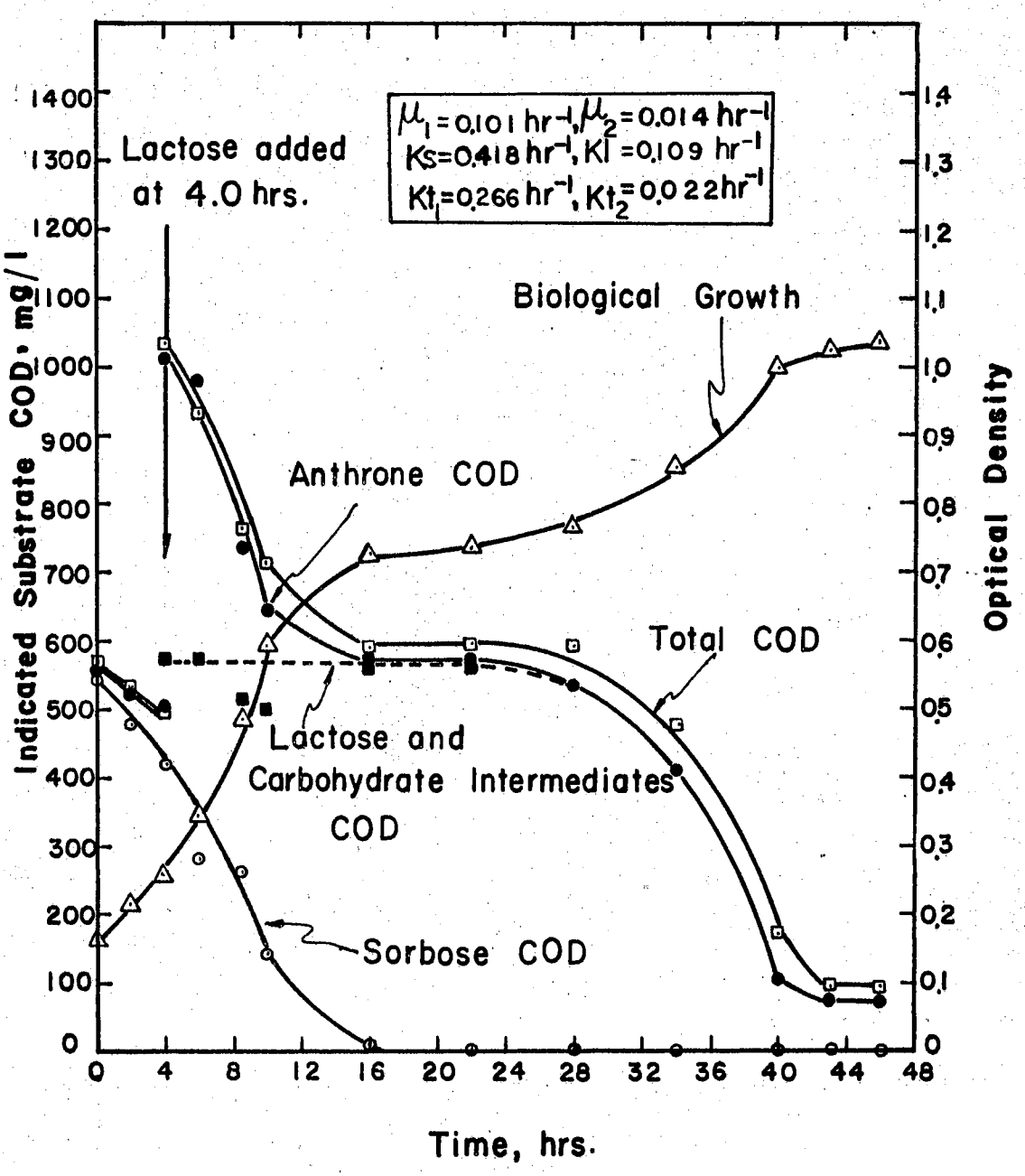


Fig. 39 - RESPONSE OF SORBOSE-ACCLIMATED YOUNG CELLS TO SHOCK LOADING WITH LACTOSE.

40. It is seen that an acclimation period of approximately eight hours was required for the lactose-acclimated sludge to initiate the utilization of sorbose. It should be noted that a rapid decrease in sludge mass and a rapid increase in COD occurred after the depletion of sorbose. This would indicate that lysis of sorbose microorganisms was accompanied by a release of compounds which could react with resorcinol and exhibit a COD.

Figure 41 shows the course of sorbose and lactose removal in the sorbose-lactose synthetic waste. The presence of sorbose caused a slight retardation of lactose removal and cell growth, as can be seen by a comparison of Figures 40 and 41. Sorbose metabolism was initiated at eight hours, i.e., the same time as in the sorbose control unit. It would appear that lactose did not interfere with the induction of enzyme(s) required for sorbose metabolism. The fact that sorbose removal, after the acclimation period, was much faster in the combined unit than in the sorbose control provides some indication that the organisms which had actively metabolized lactose took part in the further growth on sorbose. Since the shift from growth on lactose to growth on sorbose was so rapid, the COD and growth curves were not diphasic.

Figure 42 shows the results for a system which was shock loaded with sorbose. It is seen that the introduction of sorbose did not affect the course of lactose removal and that acclimation was required for sorbose metabolism.

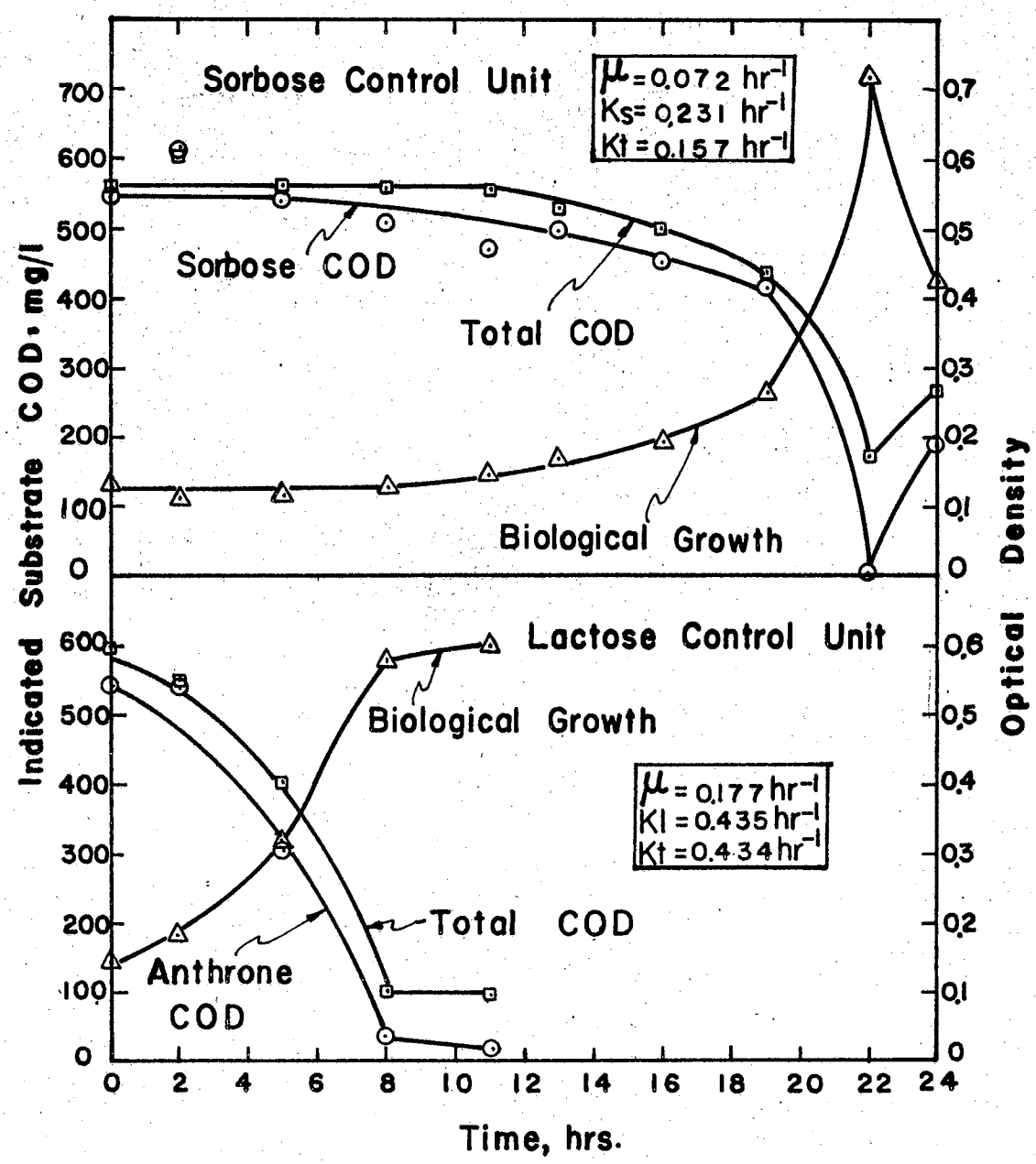


Fig. 40 - SYSTEM PERFORMANCE IN CONTROL UNITS. YOUNG CELLS ACCLIMATED TO LACTOSE.

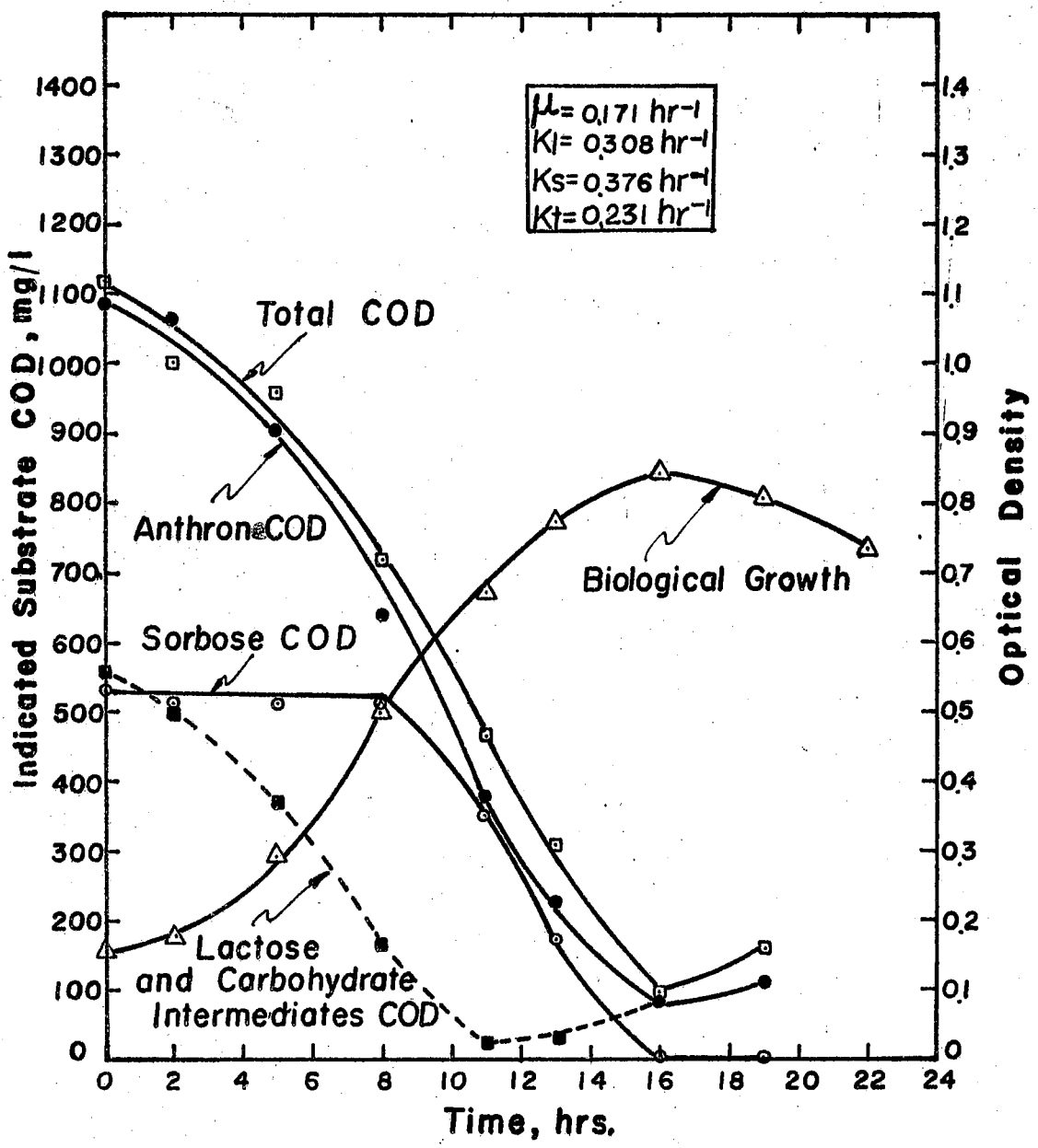


Fig. 41 - SYSTEM PERFORMANCE IN THE COMBINED UNIT OF LACTOSE AND SORBOSE. YOUNG CELLS ACCLIMATED TO LACTOSE.

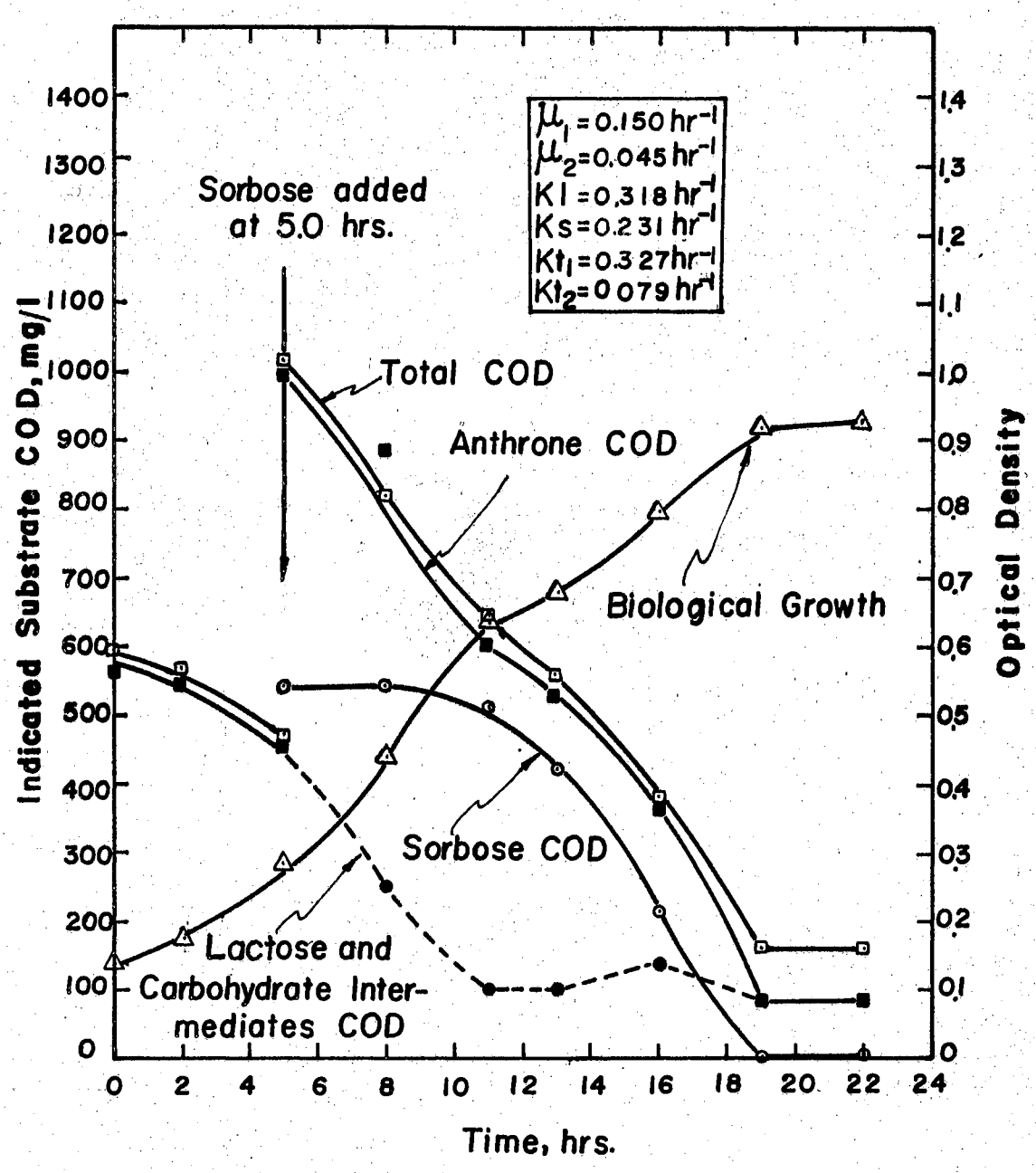


Fig. 42 - RESPONSE OF LACTOSE-ACCLIMATED YOUNG CELLS TO SHOCK LOADING WITH SORBOSE.

The diphasic growth and COD removal indicated that lactose was exhausted before the organisms were capable of metabolizing sorbose.

From data obtained on this substrate system, the following tentative conclusions have been drawn:

1) Sorbose-acclimated sludges did not require a long acclimation period to metabolize lactose, but lactose-acclimated sludges require a very long acclimation period before growth on sorbose.

2) Sorbose repressed the synthesis of lactose enzymes, thus sorbose and lactose were removed sequentially by sorbose-acclimated cells.

3) Lactose did not affect the formation of sorbose enzymes nor did it affect their activity for sludges acclimated to sorbose.

4) Lactose was exhausted before the organisms, which had been pre-grown on lactose, were capable of metabolizing sorbose. Hence, sorbose and lactose were removed sequentially also by lactose-acclimated cells.

18. Sorbose and Cellobiose System

The following results were obtained for two experiments using young cells acclimated to either sorbose or cellobiose (see Experiments 34 and 35, Table VI):

1) Sorbose-acclimated cells required acclimation to cellobiose. Cellobiose-acclimated cells required long acclimation (more than sixteen hours) to sorbose.

2) Sorbose and cellobiose were removed concurrently

by the cells acclimated to either substrates, but cellobiose removal was retarded in the combined and sorbose-shocked units compared to the rate of removal in the control.

3) The induction of sorbose enzymes by cellobiose-acclimated sludge would appear to be enhanced by the presence of cellobiose, as shown by comparison of sorbose metabolism in the control and in the combined and shocked units.

4) Using sorbose-acclimated cells, the growth rate in the substrate mixture was larger than growth rates for each individual substrate, while for the cellobiose-acclimated cells the growth rate in the substrate mixture was smaller than on cellobiose alone.

B. Experimental Parameters--Growth Rate, Substrate Removal Rate, and Sludge Yield

In order to compare utilization of carbohydrates in the various systems, the rates of growth and substrate removal, and sludge yield for all experiments are tabulated in Table VI.

Since in the log phase of bacterial growth the sludge mass increases exponentially, a plot of log of cell mass versus time will give a linear relationship. The specific growth rate μ is the slope of this linear portion. The specific total COD removal rate and specific substrate COD removal rate are the slopes of the linear portion of the total COD removal curve and substrate COD removal curve, respectively, on the semilog plot. As discussed in Chapter II, the period during which substrate removal increases at an exponential rate usually corresponds to the period dur-

TABLE VI

SYSTEM PARAMETERS FOR THE STUDY OF CARBOHYDRATES UTILIZATIONS BY DIFFERENT ACCLIMATED CELLS

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Expt. No.	System	Unit.	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
1	Galactose and Glucose (Galactose acclimated cells)	Galactose Control	0.0770	48	0.456	266	295	0.154	0.248	0.256	62.2	44.4	44.4	73.8
		Glucose Control	0.0875	52	0.498	290	470	0.192	0.281	0.317	68.3	40.2	42.6	50.6
		Combined	0.0875	52	0.733	482	905	0.210	0.277	kgal = 0.606 kglu = 0.302	75.8	30.0	35.8	47.6
		Glucose Shocked	0.0875	52	0.789	550	880	0.204	0.271	kgal = 0.083 kglu = 0.459	75.4	44.5	44.5	62.3
2	Galactose and Glucose (Glucose acclimated cells)	Glucose Control	0.2819	166	0.573	333	430	0.079	0.352	0.371	22.4	30.9	30.9	38.9
		Galactose Control	0.2840	168	0.683	428	480	0.066	0.294	0.303	22.5	47.3	47.3	55.2
		Combined	0.2840	168	0.921	755	1005	0.089 0.119	0.315 0.186	kgal = 0.642 kglu = 0.362	28.2 64.0	53.8	53.8	58.4
		Galactose Shocked	0.2882	170	0.886	695	960	0.074	0.226	kgal = 0.270 kglu = 0.294	32.8	42.8	47.3	54.7
3	Galactose and Glucose (High Galactose acclimated cells)	Galactose Control	0.615	362	0.912	740	495	0.112	0.906	0.909	12.4	64.4	70.0	76.3
		Glucose Control	0.602	351	0.921	755	480	0.137	0.913	*	15.0	54.5	74.8	84.1
		Combined	0.606	356	1.097	1160	1045	0.131	0.646	kgal = 0.578 kglu = 0.899	20.3	73.7	73.7	76.9
										*linear removal, rate is zero order				
4	Galactose and Maltose (Galactose acclimated cells)	Galactose Control	0.0386	24	0.465	272	530	0.158	0.346	0.348	45.7	43.8	44.3	46.8
		Maltose Control	0.0386	24	0.509	297	515	0.147	0.237	0.296	62.0	52.0	52.0	53.0
		Combined	0.0398	25	0.854	645	1015	0.139	0.168	kg = 0.238 km = 0.085	82.7	58.5	58.5	61.1

TABLE VI (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Exp. No.	System	Unit	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
5	Galactose and Maltose (Maltose acclimated cells)	Maltose Control	0.0942	56	0.538	313	485	0.207	0.438	0.473	47.2	48.4	50.4	53.0
		Galactose Control	0.0744	45	0.469	268	470	0.150	0.224		66.9	42.1	42.1	47.4
		Combined	0.0848	52	0.803	570	930	0.206 0.073	0.366 0.102	km = 0.388 kg = 0.678	56.2 71.6	51.1	51.1	55.7
6	Xylose and Glucose (Xylose acclimated cells)	Xylose Control	0.0680	41	0.569	331	515	0.130	0.256		50.8	50.3	55.2	56.3
		Glucose Control	0.0693	43	0.577	336	505	0.152	0.226		67.2	29.2	55.3	58.0
		Combined	0.0693	43	0.870	670	960	0.160	0.224	kx = 0.223 kg = 0.292	71.4	45.4	59.1	65.4
7	Xylose and Glucose (Glucose acclimated cells)	Glucose Control	0.1412	83	0.643	390	470	*	0.488	*	*	56.4	47.9	65.3
		Xylose Control	0.1397	82	0.569	331	350	0.058	0.156	0.457	39.4	25.6	47.9	71.2
		Combined	0.1382	81	0.903	723	815	0.200 0.026	0.488 0.042	kg = 0.468 kx = 0.595	44.7 61.8	39.1	60.6	78.8
								* linear removal or growth, rate is zero-order						
8	Xylose and Galactose (Xylose acclimated cells	Xylose Control	0.1024	61	0.542	316	500	0.102	0.305	0.317	34.6	47.2	74.2	51.0
		Galactose Control	0.1010	60	0.538	313	505	0.102	0.257	0.277	39.7	43.3	48.7	50.2
		Combined	0.0996	59	0.796	560	1015	0.119	0.208	kx = 0.234 kg = 0.218	52.2	57.8	47.8	49.4
9	Xylose and Galactose (Galactose acclimated cells)	Galactose Control	0.0942	67	0.495	288	465	0.200	0.363	0.394	55.1	40.9	40.9	47.6
		Xylose Control	0.0942	67	0.456	272	450	0.076	0.160	0.175	47.6	38.0	38.0	45.6
		Combined	0.0942	67	0.721	469	930	0.150	0.227	kg = 0.231 kx = 0.094	66.1	36.8	36.8	43.3
10	Xylose and Mannose (Xylose acclimated cells)	Xylose Control	0.0531	33	0.387	226	500	0.173	0.272	0.274	63.1	36.4	36.4	38.6
		Mannose Control	0.0531	33	0.441	257	495	0.156	0.267	0.299	58.4	41.8	41.8	45.2
		Combined	0.0531	33	0.648	393	985	0.231	0.320	kx = 0.326 km = 0.327	72.2	34.6	34.6	36.6

TABLE VI (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Exp. No.	System	Unit	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
11	Xylose and Mannose (Mannose acclimated cells)	Mannose Control	0.0339	23	0.337	198	485	0.117	0.167	0.168	70.1	32.5	34.0	36.1
		Xylose Control	0.0315	22	0.349	205	505	0.125	0.155	0.165	80.6	33.8	34.2	35.9
		Combined	0.0269	18	0.634	383	975	0.136	0.173	km = 0.174 kx = 0.214	78.6	34.8	36.2	37.5
12	Xylose and Fructose (Xylose acclimated cells	Xylose Control	0.0693	42	0.620	370	525	0.177	0.357	0.362	49.6	60.8	60.8	62.5
		Fructose Control	0.0718	45	0.520	302	435	0.148	0.337	0.357	44.0	54.2	54.2	59.1
		Combined	0.0693	42	0.912	740	1035	0.196	0.333	kx = 0.332 kf = 0.288	58.8	65.2	65.2	67.4
13	Xylose and Fructose (Fructose acclimated cells)	Fructose Control	0.0339	23	0.527	307	410	0.180	0.343	0.371	52.5	53.6	53.6	69.3
		Xylose Control	0.0292	19	0.491	286	505	0.146	0.179	0.192	81.6	50.4	50.4	52.3
		Combined	0.0281	18	0.721	469	895	0.198 0.019	0.280 0.036	kf = 0.300 kx *	70.7 53.9	42.6	42.6	50.4
										*linear removal, rate is zero-order				
14	Xylose and Sorbose (Xylose acclimated cells)	Xylose Control	0.0901	55	0.530	310	530	0.113	0.191	0.224	59.2	46.4	46.4	48.2
		Sorbose Control	0.0901	55	0.551	321	525	0.58	0.120	0.161	48.3	50.2	50.2	50.7
		Combined	0.0915	56	0.846	631	1010	0.114 0.045	0.195 0.067	ks = 0.092 kx = 0.283	58.5 67.2	53.2	53.2	56.9
		Sorbose Shocked	0.0875	53	0.810	580	1020	0.109 0.028	0.247 0.041	kx = 0.302 ks = 0.109	44.2 68.3	48.8	48.8	51.6
15	Xylose and Sorbose (Sorbose acclimated cells)	Xylose Control	0.0327	22	0.2273	135	450	0.101	0.118	0.132	85.6	22.0	22.0	25.1
		Sorbose Control	0.0351	23	0.2403	142	510	0.223	0.262	0.262	85.1	22.5	22.5	23.4
		Combined	0.0351	23	0.417	244	945	0.224 0.031	0.382 0.028	ks = 0.221 kx = 0.204	58.7 110.8	17.7	17.7	23.4

TABLE VI (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Exp. No.	System	Unit	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
16	Xylose and Maltose (Xylose acclimated cells)	Xylose Control	0.0809	50	0.602	355	500	0.135	0.226	0.280	59.8	56.4	56.4	61.0
		Maltose Control	0.0796	49	0.2676	157	125	0.092	0.165	0.173	55.7	77.2	77.2	86.3
		Combined	0.0796	49	0.810	580	795	0.155	0.187	kx = 0.200 km = 0.428	82.9	62.9	62.9	66.8
17	Xylose and Maltose (Maltose acclimated cells)	Maltose Control	0.047	30	0.374	220	490	0.144	0.198	0.199	72.7	36.6	36.6	38.8
		Xylose Control	0.041	26	0.445	260	515	0.114	0.206	0.207	55.4	42.9	42.9	45.5
		Combined	0.0446	27	0.752	505	980	0.168	0.218	km = 0.178 kx = 0.231	77.1	45.6	45.6	48.8
18	Xylose and Lactose (Xylose acclimated cells)	Xylose Control	0.0374	23	0.409	240	525	0.241	0.475	0.482	50.7	41.0	41.0	41.3
		Lactose Control	0.0386	23	0.469	272	535	0.308	0.254	0.254	121.2	46.2	46.2	46.6
		Combined	0.0398	24	0.704	450	1065	0.243	0.302	kx = 0.361 kl = 0.345	90.3	40.0	40.0	40.0
19	Xylose and Lactose (Lactose acclimated cells)	Lactose Control	0.0505	31	0.495	289	460	0.215	0.499	0.502	43.2	52.1	52.1	56.1
		Xylose Control	0.0505	31	0.367	214	380	0.169	0.279	0.315	60.6	41.1	41.1	48.2
		Combined	0.0543	34	0.694	440	910	0.315 0.062	0.467 0.108	kl = 0.366 kx = 0.330	67.4 57.4	41.8	41.8	44.7
20	Xylose and Sucrose (Xylose acclimated cells)	Xylose Control	0.0505	31	0.565	330	490	0.205	0.317	0.314	64.6	59.8	59.8	61.0
		Sucrose Control	0.0494	30	0.678	421	510	0.203	0.336	0.338	60.4	63.0	72.4	76.7
		Combined	0.494	30	0.912	740	915	0.231	0.329	kx = 0.349 ks = 0.376	70.2	72.1	72.1	77.6
21	Xylose and Sucrose (Sucrose acclimated cells)	Sucrose Control	0.0568	34	0.469	331	430	0.214	0.261	0.277	82.0	54.2	55.0	69.1
		Xylose Control	0.0568	34	0.444	260	515	0.057	0.074	0.078	77.0	42.7	42.7	43.9
		Combined	0.0555	33	0.763	519	940	0.194 0.011	0.195 0.022	ks = 0.205 kx = 0.094	99.5 50.0	26.2	29.5	51.7

TABLE VI (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Exp. No.	System	Unit	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
22	Xylose and Cellobiose (Xylose acclimated cells)	Xylose Control	0.0458	28	0.557	325	475	0.193	0.286	0.293	67.4	55.0	55.0	62.5
		Cellobiose Control	0.0410	27	0.493	291	485	0.182	0.272	0.273	66.8	52.8	52.8	54.4
		Combined	0.0446	28	0.854	645	990	0.204	0.258	kx = 0.259 kc = 0.491	69.1	59.9	59.9	62.4
23	Xylose and Cellobiose (Cellobiose acclimated cells)	Cellobiose Control	0.0235	15	0.530	310	485	0.143	0.248	0.257	57.7	56.7	56.7	60.8
		Xylose Control	0.0315	22	0.485	283	445	0.108	0.154	0.152	70.1	49.2	49.2	58.7
		Combined	0.0269	18	0.643	390	1000	0.180	0.296	kc = 0.244 kx = 0.150	60.8	33.5	37.2	37.2
24	Lyxose and Glucose	There were no organisms capable of utilizing lyxose as substrate when lyxose was present alone or when lyxose was present in combination with glucose.												
25	Sorbose and Glucose (Sorbose acclimated cells)	Sorbose Control	0.1192	71	0.549	320	570	0.165	0.372	0.373	44.4	45.3	45.3	43.7
		Glucose Control	0.1192	71	0.585	342	490	0.198	0.282	0.414	70.2	32.6	49.3	44.3
		Combined	0.1192	71	0.886	695	1040	0.209 0.164	0.292 0.180	ks = 0.532 kg = 0.672	71.6 91.2	55.8	56.7	59.9
		Glucose Shocked	0.1163	70	0.854	645	1000	0.215	0.274	ks = 0.152 kg = 0.550	78.5	49.1	52.3	57.5
26	Sorbose and Galactose (Sorbose acclimated cells)	Sorbose Control	0.1855	111	0.751	501	550	0.169	0.387	0.409	43.7	68.4	68.4	71.0
		Galactose Control	0.1838	110	0.739	489	550	0.210	0.467	0.476	45.0	68.3	68.3	68.9
		Combined	0.1956	115	1.000	905	1045	0.175	0.447	ks = 0.328 kg = 0.445	39.2	70.6	70.6	75.6
		Galactose Shocked	0.1838	110	1.000	905	1020	0.186	0.367	ks_1 = 0.410 ks_2 = 0.134 kg = 0.618	50.6	62.9	71.0	78.0
27	Sorbose and Galactose (Galactose acclimated cells)	Galactose Control	0.0982	60	0.634	382	480	0.181	0.333	0.352	60.3	48.4	57.5	67.1
		Sorbose Control	0.0888	54	0.678	421	500	0.149	0.284	0.289	52.4	66.1	66.1	73.4
		Combined	0.0875	53	0.886	695	970	0.186	0.210	kg = 0.312 ks = 0.197	88.6	56.5	58.4	66.2
		Sorbose Shocked	0.0862	52	0.886	695	920	0.219 0.084	0.402 0.180	kg = 0.403 ks = 0.509	54.5 46.7	58.4	58.4	69.9

TABLE VI (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Exp. No.	System	Unit	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
28	Sorbose and Mannose (Sorbose acclimated cells)	Sorbose Control	0.0862	52	0.653	400	495	0.283	0.450	0.452	62.9	65.0	65.0	70.3
		Mannose Control	0.0835	51	0.648	393	485	0.198	0.330	0.343	60.0	64.3	65.7	70.5
		Combined	0.0862	52	0.959	822	1020	0.229	0.354	ks = 0.378 km = 0.773	64.7	74.8	74.8	75.5
		Mannose Shocked	0.0862	52	0.959	822	990	0.214	0.321	ks = 0.360 km = 0.702	66.7	74.1	74.4	77.8
29	Sorbose and Mannose (Mannose acclimated cells)	Mannose Control	0.1412	84	0.545	318	475	0.129	0.342	0.342	37.8	48.2	48.2	49.3
		Sorbose Control	0.1427	85	0.585	341	465	0.121	0.318	0.318	38.1	47.4	47.4	55.1
		Combined	0.1427	85	0.878	680	995	0.141	0.210	km = 0.423 ks = 0.192	67.1	52.1	59.8	59.8
		Sorbose Shocked	0.1367	81	0.886	695	990	0.163	0.282	km = 0.283 ks = 0.367	57.8	56.2	60.2	62.0
30	Sorbose and Maltose (Sorbose acclimated cells)	Sorbose Control	0.1367	81	0.643	390	555	0.250	0.481	0.475	51.9	50.7	54.6	56.1
		Maltose Control	0.1337	80	0.527	307	535	0.075	0.212	0.222	35.4	41.3	41.3	42.4
		Combined	0.1337	80	0.817	590	1125	0.242 0.041	0.409 0.048	ks = 0.502 km = 0.197	59.1 84.3	44.7	44.7	45.3
		Maltose Shocked	0.1367	81	0.870	670	1055	0.266 0.043	0.413 0.098	ks = 0.452 km = 0.545	63.6 43.9	53.1	54.5	55.8
31	Sorbose and Maltose (Maltose acclimated cells)	Maltose Control	0.1643	98	0.598	352	505	0.155	0.356	0.356	43.6	48.4	48.4	50.2
		Sorbose Control	0.1565	94	0.435	253	405	0.116	0.160	0.185	72.4	31.8	31.8	39.3
		Combined	0.1675	100	0.886	695	1025	0.161	0.258	km = 0.189 ks = 0.256	62.4	55.6	55.6	58.1
		Sorbose Shocked	0.1612	95	0.870	670	1040	0.246 0.048	0.442 0.070	km = 0.412 ks = 0.299	55.7 68.6	51.4	55.3	55.3
32	Sorbose and Lactose (Sorbose acclimated cells)	Sorbose Control	0.1675	100	0.698	443	540	0.135	0.432	0.437	31.4	58.1	62.4	63.5
		Lactose Control	0.1723	102	0.602	354	455	0.077	0.232	0.229	33.2	51.4	51.4	55.4
		Combined	0.1612	95	1.034	985	925	0.101 0.014	0.266 0.022	ks = 0.418 kl = 0.107	38.0 63.6	87.3	87.3	97.2
		Lactose Shocked	0.1612	95	1.034	985	1005	0.132 0.02	0.345 0.066	ks = 0.375 kl = 0.180	38.3 30.4	83.1	85.6	88.6

TABLE VI (Continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Exp. No.	System	Unit	Inital Optical Density	Inital Biolog. Solids (mg/l)	Peak Optical Density	Peak Biolog. Solids (mg/l)	Total COD Removed (mg/l)	Specific Growth Rate (hr^{-1})	Specific Total-COD Removal Rate (hr^{-1})	Specific Carbohydrate COD Removal Rate (hr^{-1})	Yield (%)	Yield (%)	Yield (%)	Yield (%)
33	Sorbose and Lactose (Lactose acclimated cells)	Lactose Control	0.1427	85	0.602	354	480	0.177	0.434	0.435	40.8	51.8	51.8	56.0
		Sorbose Control	0.1367	81	0.721	469	400	0.072	0.157	0.231	45.8	73.2	73.2	97.0
		Combined	0.1442	86	0.846	630	1010	0.171	0.231	kl = 0.308 ks = 0.376	74.0	53.8	53.8	53.8
		Sorbose Shocked	0.1397	82	0.921	752	990	0.150 0.045	0.327 0.079	kl = 0.318 ks = 0.231	45.9 57.0	64.8	65.1	68.3
34	Sorbose and Cellobiose (Sorbose acclimated cells)	Sorbose Control	0.1135	70	0.295	175	400	0.052	0.093	0.093	56.0	22.4	22.4	25.1
		Cellobiose Control	0.1107	67	0.372	220	450	0.082	0.218	0.224	37.6	33.4	33.4	34.0
		Combined	0.1121	69	01634	382	970	0.091	0.183	ks = 0.187 kc = 0.132	49.7	31.3	31.3	32.3
35	Sorbose and Cellobiose (Cellobiose acclimated cells)	Cellobiose Control	0.0593	37	0.498	291	490	0.132	0.273	0.294	48.4	49.8	49.8	51.8
		Sorbose▲ Control	0.0593	37	0.523	305	500	0.142	0.261	0.283	54.4	53.1	53.1	53.6
		Combined	0.0605	37	0.602	354	975	0.112 0.024	0.186 0.049	kc = 0.161 ks = 0.139	60.2 49.0	31.4	31.4	32.5
		Sorbose Shocked	0.0568	35	0.581	340	970	0.146 0.037	0.184 0.044	kc = 0.218 ks = 0.165	79.4 84.1	27.5	29.6	31.5

▲ Very few organisms grew in the sorbose control unit during the 37 hour operation. Another Sorbose Control unit was run separately. The experimental parameters of this repeated unit are shown in the table.

Explanation of Columns in Table VI:

1. Column 1 shows the number assigned to a specific group of experiments.
2. Column 2 shows the substrates and cells used.
3. Column 3 indicates the substrate employed for specific aeration units in each system.
4. Column 4 shows the initial optical density in each unit.
5. Column 5 shows the corresponding initial biological solids concentration in mg/l, converted from the optical density of Column 4 by using the relationship of Figure 8.
6. Column 6 shows the peak optical density obtained in each unit.
7. Column 7 shows the maximum or peak biological solids concentration in mg/l obtained in each unit, converted from Column 6 by using the relationship of Figure 8.
8. Column 8 shows the total COD which was removed at the time of peak solids production (mg/l).
9. Column 9 shows the specific rate of growth during the log phase (hr^{-1}).
10. Column 10 shows the specific rate of total COD removal during the log phase (hr^{-1}).
11. Column 11 shows the specific rate of substrate COD removal during the log phase (hr^{-1}).
12. Column 12 shows the sludge yield in percent calculated as specific growth rate divided by specific rate of total COD removal; i.e., Column 9 divided by Column 10.
13. Column 13 shows the sludge yield in percent calculated as increase in biological solids divided by carbohydrate COD removal.
14. Column 14 shows sludge yield in percent calculated as increase in biological solids at the time of peak solids production divided by carbohydrate COD removed.
15. Column 15 shows the sludge yield in percent calculated as increase in biological solids divided by total COD removed at the time of peak sludge production; i.e., (Column 7 - Column 5) / Column 8.

ing which the bacterial cells are increasing at an exponential rate.

It is found that the specific rate of substrate COD removal was larger than the specific rate of total COD removal. The difference between these two parameters is due to the excretion and accumulation of metabolic intermediates into the medium during log phase. Thus, the greater the amount of intermediates which accumulated in the medium, the greater the difference between these two specific rates.

C. Study of Carbohydrate Utilization by Sludge Acclimated to Various Compounds

Since xylose and sorbose were used quite frequently in combination with other carbohydrates in this study, the experimental parameters for the various systems in which these two components were used are tabulated in Tables VII and VIII. Table VII shows that the growth rate and the total COD and xylose COD removal rates for xylose-acclimated sludges were usually higher than those of the comparable sludges acclimated to other carbohydrates. The trend of the results given in Table VIII are similar to Table VII. These two tables also show that the sludge yield on a particular carbohydrate varied appreciably. A change in sludge yield for systems metabolizing different carbohydrates is to be expected. However, it is also quite apparent that the sludge yields are not the same even in systems using the same substrate to which they were thoroughly

TABLE VII

XYLOSE UTILIZATION BY DIFFERENT ACCLIMATED SLUDGES

Exp. No.	Activated Sludge	Initial Biolog. Solids (mg/l)	Specific Growth Rate (hr^{-1})	Specific Xylose-COD Removal Rate (hr^{-1})	Specific Total COD Removal Rate (hr^{-1})	(1) Yield (%)	(2) Yield (%)
6		41	0.130	0.405	0.256	50.8	56.3
8		61	0.102	0.317	0.305	34.6	51.0
10	Xylose	33	0.173	0.274	0.272	63.1	38.6
12		42	0.177	0.362	0.357	49.6	62.5
14	Acclimated	55	0.113	0.224	0.191	59.2	48.2
16		50	0.135	0.280	0.226	59.8	61.0
18	Sludge	23	0.241	0.482	0.475	50.7	41.3
20		31	0.205	0.314	0.317	64.6	61.0
22		28	0.193	0.293	0.286	67.4	62.5
7	Glucose Acclimated Sludge	82	0.058	0.457	0.156	39.4	71.2
9	Galactose Acclimated Sludge	67	0.076	0.175	0.160	47.6	45.6
11	Mannose Acclimated Sludge	22	0.125	0.165	0.155	80.6	35.9
13	Fructose Acclimated Sludge	19	0.146	0.192	0.179	89.6	52.8
15	Sorbose Acclimated Sludge	22	0.101	0.132	0.118	85.6	25.1
17	Maltose Acclimated Sludge	26	0.114	0.207	0.206	55.4	45.5
19	Lactose Acclimated Sludge	31	0.169	0.315	0.279	60.6	48.2
21	Sucrose Acclimated Sludge	34	0.057	0.078	0.074	77.0	43.9
23	Cellobiose Acclimated Sludge	22	0.108	0.152	0.154	70.1	58.7

(1) Yield during the log phase, Column 12 of Table VI.

(2) Yield at the time of peak sludge production, Column 15 of Table VI.

TABLE VIII

SORBOSE UTILIZATION BY DIFFERENT ACCLIMATED SLUDGES

Exp. No.	Activated Sludge	Initial Biolog. Solids (mg/l)	Specific Growth Rate (hr^{-1})	Specific Sorbose COD Removal Rate (hr^{-1})	Specific Total COD Removal Rate (hr^{-1})	(1) (Yield) (%)	(2) (Yield) (%)
15	Sorbose Acclimated Sludge	23	0.223	0.262	0.262	85.1	23.4
25	"	71	0.165	0.373	0.372	44.4	43.7
26	"	111	0.169	0.409	0.387	43.7	71.0
28	"	52	0.283	0.452	0.450	62.9	70.3
30	"	81	0.250	0.481	0.475	51.9	56.1
32	"	100	0.135	0.437	0.432	31.4	63.5
34	"	70	0.052	0.093	0.093	56.0	25.1
14	Xylose Acclimated Sludge	55	0.058	0.161	0.120	48.3	50.7
27	Galactose Acclimated Sludge	54	0.149	0.289	0.284	52.4	73.4
29	Mannose Acclimated Sludge	85	0.121	0.317	0.318	38.1	55.1
31	Maltose Acclimated Sludge	94	0.116	0.185	0.160	72.4	39.3
33	Lactose Acclimated Sludge	81	0.072	0.231	0.157	45.8	97.0
35	Cellobiose Acclimated Sludge	37	0.142	0.283	0.261	54.4	53.6

(1) Yield during the log phase, Column 12 of Table VI.

(2) Yield at the time of peak sludge production, Column 15 of Table VI.

acclimated. Rao and Gaudy (15) and Gaudy and Gaudy (48) have observed the same result, and the difference in sludge yield on the same substrate may be attributed to change in predominance of bacterial species.

D. Study of Residual COD and Intermediate Release from Carbohydrate Metabolism

An attempt was made to determine if the metabolic intermediate production resulting from the metabolism of one particular carbohydrate was constant or variable, and to see the effect of the acclimation of cells on the production of intermediates. The maximum intermediate production during growth and the residual COD for glucose, xylose, and sorbose in the various systems are tabulated in Tables IX, X, and XI. It may be seen that in most cases there was some evidence for accumulation of metabolic intermediates and/or end products during substrate removal. However, in no case was this production severe and there does not appear to be any specific relationship between intermediate production and the immediate past acclimated history of the sludge. A comparison of the residual COD values in these tables reveals that the residual COD was comparatively low and more or less of the same magnitude when the cells were supplied with the substrate to which they have been previously acclimated. However, when cells were fed substrate different from the one to which they were acclimated, the residual COD values were generally higher than those obtained with acclimated substrates. It should be noted

TABLE IX

PRODUCTION OF METABOLIC INTERMEDIATES DURING METABOLISM OF GLUCOSE BY SLUDGES ACCLIMATED TO VARIOUS COMPOUNDS

Expt. No.	Activated Sludge	Maximum* Intermediates (mg/l)	Residual COD (mg/l)
2	Glucose Acclimated Sludge	160	120
7	Glucose Acclimated Sludge	150	60
1	Galactose Acclimated Sludge	170	105
3	Galactose Acclimated Sludge	140	50
6	Xylose Acclimated Sludge	330	40
25	Sorbose Acclimated Sludge	260	90

*Maximum arithmetical difference between xylose COD curve and total COD curve during growth.

that most of the experiments were run for a long time period to allow total substrate removal.

It is of particular interest to note that the production of maximum metabolic intermediates was generally higher for glucose metabolism (Table IX) than for metabolism of either xylose (Table X) or sorbose (Table XI).

TABLE X

PRODUCTION OF METABOLIC INTERMEDIATES DURING METABOLISM OF XYLOSE BY SLUDGES ACCLIMATED TO VARIOUS COMPOUNDS

Exp. No.	Activated Sludge	Maximum* Intermediates (mg/l)	Residual COD (mg/l)	Exp. No.	Activated Sludge	Maximum* Intermediates (mg/l)	Residual COD (mg/l)
6	Xylose Acclimated Sludge	230	30	7	Glucose Acclimated Sludge	330	140
8	"	45	40	9	Galactose Acclimated Sludge	100	90
10	"	40	40	11	Mannose Acclimated Sludge	90	45
12	"	20	35	13	Fructose Acclimated Sludge	105	35
14	"	140	40	15	Sorbose Acclimated Sludge	85	75
16	"	100	50	17	Maltose Acclimated Sludge	50	40
18	"	30	30	19	Lactose Acclimated Sludge	90	90
20	"	35	-	21	Sucrose Acclimated Sludge	185	40
22	"	20	70	23	Cellobiose Acclimated Sludge	105	90

*Maximum arithmetical difference between xylose-COD curve and total COD curve during growth.

TABLE XI

PRODUCTION OF METABOLIC INTERMEDIATES DURING METABOLISM OF SORBOSE BY SLUDGES ACCLIMATED TO VARIOUS COMPOUNDS

Exp. No.	Activated Sludge	Maximum* Intermediates (mg/l)	Residual COD (mg/l)	Exp. No.	Activated Sludge	Maximum* Intermediates (mg/l)	Residual COD (mg/l)
15	Sorbose Acclimated Sludge	40	35	14	Xylose Acclimated Sludge	150	50
25	"	135	20	27	Galactose Acclimated Sludge	100	60
26	"	100	30	29	Mannose Acclimated Sludge	60	110
28	"	50	50	31	Maltose Acclimated Sludge	70	70
30	"	35	35	33	Lactose Acclimated Sludge	50	170
32	"	70	30	35	Cellobiose Acclimated Sludge	40	40
34	"	80	-				

*Maximum arithmetical difference between sorbose-COD curve and total COD curve during growth.

CHAPTER V

DISCUSSION

A. Carbohydrate Metabolism and Substrate Interaction in Heterogeneous Populations

In order to discuss the interaction of carbohydrates in a heterogeneous population more clearly, it is appropriate and necessary to depict the metabolism of carbohydrates used in this study. Figure 43 shows a general metabolic flow chart for these carbohydrates. It is seen that glucose, galactose, mannose, fructose, and sorbose are metabolized generally via similar routes. These hexoses are phosphorylated to individual hexose-6-phosphate by a "kinase system" and enter the oxidative pathway (50). In these studies it has been found that sludge acclimated to one type of hexoses (e.g., galactose, sorbose, glucose, etc.) can grow on other hexoses without a lag period. This result may be attributed to the fact that all of these hexoses are metabolized by similar routes. The disaccharides may first be hydrolyzed into the component hexoses, which then penetrate into the cells and are phosphorylated. After phosphorylation, the hexoses are metabolized via closely related pathways which may yield common intermediary metabolites.

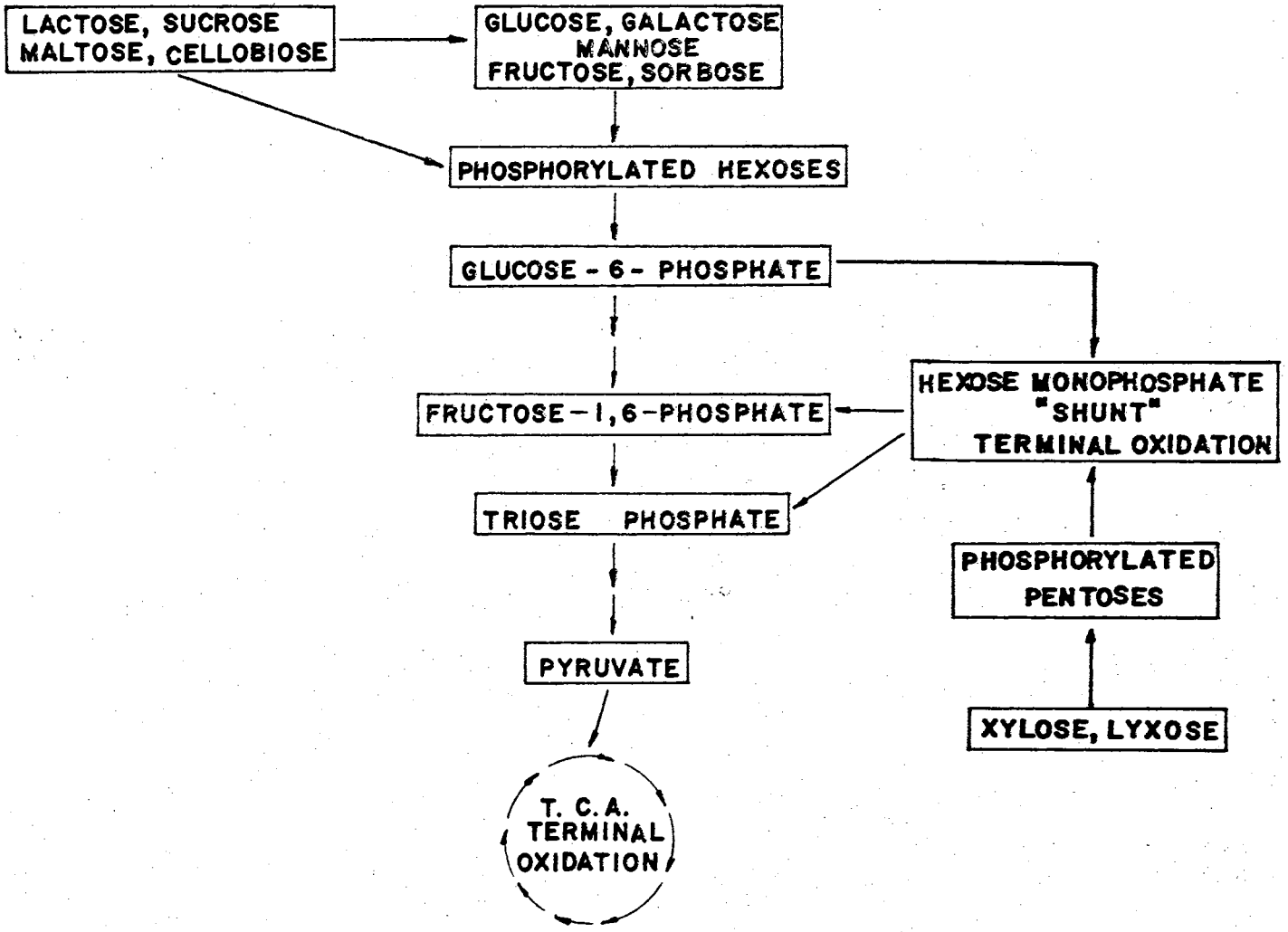


Fig. 43 - GENERALIZED METABOLIC FLOW CHART FOR VARIOUS CARBOHYDRATES UNDER STUDY.

Normally, pentoses are metabolized via the hexose monophosphate pathway, while hexoses may be metabolized through either the hexose monophosphate or glycolytic pathways. The pentoses are converted to D-xylulose-5-phosphate and then enter the hexose monophosphate pathway (50). Xylose is commonly found in nature, whereas lyxose rarely appears in the natural environment. Mortlock and Wood (51, 52) have indicated that growth of A. aerogenes on xylose occurs without a lag, i.e., in one half to one day, and that there was no evidence for selection of mutants, but growth on lyxose occurs with a lag of from two days to two weeks and in their studies there was evidence for selection of mutants during the lag period. In the present study the enzymes responsible for xylose degradation appeared to be constitutive in cells grown on glucose and various other carbon sources, and could be easily induced in others, while organisms pregrown on glucose took more than forty hours to induce lyxose-catabolizing enzymes. These observations seem to be in agreement with those found by Mortlock and Wood.

Although all of the hexoses used in this study are closely related in their mode of metabolism, the effects of these hexoses on metabolism of xylose were quite different. Glucose and fructose exerted a potent repressive effect, mannose posed a lesser repressive effect, whereas galactose exerted no effect. This phenomenon can possibly be explained on the basis of the rate of metabolism of these compounds, hence the rate of buildup of a common intermediate. It is

interesting to note that xylose-acclimated cells used glucose and fructose at rates of 0.426 and 0.357 hr^{-1} respectively, whereas mannose was used at a rate of 0.299 hr^{-1} and galactose at a rate of 0.277 hr^{-1}. A comparison of the rate of xylose removed and removal of glucose, fructose, and mannose (Table VI) indicates that they were all used at approximately the same rates in the control units; however, galactose was used at a significantly slower rate than xylose. This observation is actually in agreement with previous reports by Neidhart and Magasanik (53) in their studies with Aerobactor aerogenes, from which they stated that the degree of repression exerted by a compound depends upon the rate at which that compound supported growth.

B. Metabolic Control Mechanisms and Patterns of Substrate Removal and Growth

Much information is now becoming available concerning the mechanisms of substrate removal and the interactions which occur in systems with heterogeneous populations and multicomponent substrate. Although monophasic growth and concurrent substrate removal by heterogeneous populations have been found on many carbohydrate combinations (e.g., xylose and galactose), the results of this study show that the interaction of substrates does occur in heterogeneous populations for a great variety of carbohydrate combinations, and that these interactions often lead to discontinuities in the growth and carbon source removal. The pat-

terns of growth and kinetics of substrate removal for these combinations indicate that the diphasic growth and sequential removal are caused either by repression of enzymes formation (e.g., xylose and sucrose) or inhibition of enzymes activity (e.g., sorbose and glucose). However, sequential removal accompanied by monophasic growth and concurrent removal accompanied by diphasic growth are also found in some special cases. The pattern of monophasic growth and sequential removal occurs when the shift from growth on one substrate to growth on another substrate is so rapid that the change of growth rate becomes undetectible (e.g., galactose and glucose in Figures 10 and 11), while diphasic growth and concurrent removal (e.g., xylose and sorbose in Figures 26 and 27) are due to the inhibition of catabolites.

C. Significance of Acclimation

Several investigators have emphasized that a period of acclimation or adaption is necessary for optimum efficiency of biological systems (54, 55, 56, 57). It was seen from Tables VII and VIII that the rates of growth and substrate removal attained by the sludges supplied with substrate to which they were acclimated are generally faster than the rates attained by sludge supplied with carbohydrates to which they were not previously acclimated. This change in the rates of growth and substrate removal may be brought about in two ways:

1) increased enzymes production by the acclimated sludges;

2) a change in the predominant microorganisms, i.e., selection of species which were more efficient in utilizing the substrate. While selection of cells best suited to the particular environment (in this case the substrate) is always a prime factor for heterogeneous populations, both mechanisms of response in the present studies lead to production of a large amount of the enzymes required to metabolize the substrate.

From the standpoint of operation of activated sludge processes, it is evident that the various carbohydrate-acclimated sludges employed in these studies could acclimate to the other carbohydrate substrates and remove these substrates in a relatively short time. Thus, the microbial population stabilizing carbohydrate waste in a treatment plant (or in the receiving stream) would appear to possess the required genetic potential for adjustment to new incoming carbohydrates in the waste stream.

D. Sludge Yield of Carbohydrate Utilization

The results of the present study indicate that the sludge yields for carbohydrates (in percent calculated as increase in biological solids divided by COD removal (column 15, Table IV) over a range of 40 to 78 per cent for populations grown on a single carbohydrate or combinations of carbohydrates. The reasons why such a range of cell yields should be expected for heterogeneous populations have been thoroughly discussed by Gaudy and Gaudy (48), and need not be further delineated here. It is important to

note, however, that the range of sludge yields observed in the present studies agrees well with those reported by Rao and Gaudy (15) and provides further substantiation of their conclusion that for design and operation purposes, a range of sludge yield rather than a single figure should be employed.

E. Residual COD and Production of Metabolic Intermediates by Different Acclimated Sludges

The results shown in Tables IX, X, and XI reveal that when the cells are supplied with substrates different from those to which they were acclimated, the residual COD values were somewhat higher than those obtained for acclimated sludge. One possible explanation for this finding may be that newly acclimated cells may not yet be capable of metabolic utilization of all materials (i.e., metabolic intermediates and/or end products) excreted into the medium during metabolism on the new compound. On the other hand, it may be possible that upon prolonged acclimation, satellite populations which make use of residual materials of other cells can exist. It is interesting to note that Krishnan, Gaudy and Gaudy (58) have isolated species from a system thoroughly acclimated to sorbitol which could not grow on sorbitol as a sole source of carbon. Since the system did not give evidence for accumulation of substantial amounts of intermediates during sorbitol removal, these cells may have existed on the small residual carbon source provided to them by the sorbitol utilizers or on their

lysis products. Such an ecological situation might not be expected for a newly activated system. The above speculation could well be the subject of future research.

It is also noted that a considerable amount of metabolic intermediates and/or end products were released during substrate removal. The phenomenon of release of metabolic intermediates during the metabolism of glucose has been reported by Krishnan and Gaudy (59). The release of intermediates may come about because the rate at which their breakdown products can be channelled into synthesis of cellular materials such as proteins and nucleic acids may be much slower than the rate of breakdown of the original carbon source. Also, the fact that more intermediates accumulated during glucose removal than during metabolism of either xylose or sorbose may help explain the repressive effect of glucose on metabolism of these substrates, since it could be expected that the buildup of a "common intermediate" would result more rapidly for glucose metabolism.

F. Effects of Substrate Concentration on Substrate Interactions

Although all of the experiments of this study were accomplished with fairly high and constant initial concentrations of substrates, it is of significant interest to note that some dependence of substrate interaction on substrate concentration was observed for some substrate combinations (e.g., sorbose and glucose). It did appear that

the inhibitive capability of glucose on the metabolism of sorbose was detectible only when the glucose concentration was equal to or higher than a certain level. Since the structure of glucose closely resembles that of sorbose, and since the inhibitions did appear to be reversed by a relative increase in the concentration of sorbose, the inhibition glucose exerted on the metabolism of sorbose was of a competitive nature. This result would seem in keeping with the concept of inhibition whereby glucose inhibits via the buildup of a common intermediate since the rate of utilization of glucose (thus, the rate of buildup of the common intermediate) might be expected to be controlled in some degree by the substrate concentration.

Bhatla and Gaudy (60) have found that glucose can exert a repressive effect on sorbitol utilization at low concentration (0.03 mg/l) (both compounds were present in very low concentration BOD bottle studies). McQuillon and Halvorson (61) have observed that glucose at a low concentration showed a stimulatory effect on induction of enzyme synthesis in yeasts, whereas at high concentration it caused repression of enzyme synthesis. At present it seems that no definite conclusion concerning the effect of substrate concentration on substrate interactions can be drawn. Therefore, it appears desirable to extend this

type of study to a wide range of substrate concentrations for various combinations of carbon sources. Such studies would have significant ramifications in determining the need for concern over the kinetic aspects of wastewater purification for various waste components, i.e., it may be found that such discontinuities are not applicable for certain ratios of concentrations.

G. Study on the Interaction of Galactose and Glucose

It is of interest to compare the results of this study with phenomena observed by other investigators in studies with pure cultures or heterogeneous populations. In E. coli glucose has been found to repress adaptation to galactose (62) and to possess no effect on the activity of the galactose-catabolizing enzymes (63). In natural microbial communities, glucose has been found to inhibit the activity of galactose-catabolizing enzymes and to possess no repression on the formation of galactose enzymes by Stumm-Zollinger (27). This observation seems to contradict the finding herein reported. In both cases the young cells were acclimated to galactose, and about 360 mg/l of initial cells were used to feed the units. The figures given by Stumm-Zollinger show that a slight utilization of galactose took place in the presence of glucose, but the rate of galactose elimination from the medium was constant and in all probability the slight galactose removal was due to the concentration of galactose enzymes formed before the start of the experiment. This would indicate that glucose

repressed the formation of new galactose enzymes but did not inhibit the activity of the pre-formed enzymes.

Therefore, it can be concluded that the repression by glucose of the formation of galactose enzymes occurred both in heterogeneous populations and in pure cultures.

H. Significance of This Type of Study

From the standpoint of operation of biological treatment processes, studies of this type are extremely useful and necessary. Biological wastewater treatment facilities are unit operations which are subjected to severe environmental changes from time to time. So far, however, it has not proven to be practicable to select the wastewater or to control the operational conditions precisely, nor is it possible to control the species of organism purifying wastewater. Therefore, it is extremely important to gain a better insight into the possible interactions of substrates and to determine experimentally the types of environmental changes which cause severe disruption of treatment efficiency. Then, hopefully, we can seek economical ways to control the environment or seek economical remedial measures for unavoidable changes in environment.

CHAPTER VI

CONCLUSIONS

The results of this study support the following conclusions:

1. The enzyme systems responsible for degradation of many carbohydrates found in nature are constitutive in activated sludge systems or can be easily induced on populations grown on one carbohydrate.

2. Because of the ease of acclimation to other carbohydrates of most carbohydrate-acclimated sludges, the effect of shock loads of carbohydrates to activated sludge systems previously grown on carbohydrate waste would not appear to cause drastic upsets in the metabolic efficiency of the plant for extended periods of time.

3. Substrate interactions (suppressive and/or repressive effect) have been found for a great variety of carbohydrate combinations (shown in Table V). The results offer further proof of Gaudy's findings that substrate interactions may be frequent phenomena and can be observed with heterogeneous populations in multicomponent substrate media. These interactions have been explained on the basis of work previously reported from the Bioenvironmental Laboratories

of Oklahoma State University.

4. Four patterns of substrate removal and growth in a two-component medium by a heterogeneous population have been observed in this study:

1) concurrent removal accompanied by monophasic growth;

2) sequential removal accompanied by diphasic growth caused by time for inductions and by either repression of enzyme synthesis or inhibitions of enzyme activity;

3) sequential removal accompanied by monophasic growth caused by rapid shift from growth on one substrate to another;

4) concurrent removal accompanied by diphasic growth caused by inhibition of enzymes by metabolic intermediates.

The first two patterns are usual occurrences in this study and in previous work.

5. When the cells are supplied with substrate to which they are acclimated, the residual COD values are lower compared with those obtained from systems fed with a new substrate to which they become "newly" acclimated.

6. The sludge yield on carbohydrates is not constant even in the systems fed the same substrate. The sludge yield on all carbohydrates investigated in this study, measured as percent of substrate consumed, covered the wide range of 40 to 78 per cent for heterogeneous populations grown on a single carbohydrate or multicomponent carbohydrate medium.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

Based on the results of the present study, it is felt that the following research aspects would provide valuable topics for future research:

1. It would be interesting to determine the extent to which the relative concentration of the carbon sources determine the manifestation of sequential substrate removal.

2. It appears desirable to extend these studies to include different environmental conditions (e.g., temperature, pH, absence of nitrogen sources, cell age).

3. It would be of interest to compare the results of this study with those of similar studies using a variety of pure bacterial cultures.

4. The study should be extended further to determine the extent of substrate interactions in steady state continuous flow activated sludge processes. In this type of study the waste components which have been found to interact in the batch studies should be chosen.

5. Further investigations on the growth response and substrate interactions occurring in a tri-substrate or tetra-substrate medium would be of interest.

6. Since almost all of the results reported in this research work were obtained at relatively low initial solids concentrations, it would be of interest to study the effect of initial solids concentrations on substrate interactions.

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