SOME KINETIC PROPERTIES OF TRANSIENT-STATES IN CONTINUOUS CULTURES OF NATURAL MICROBIAL POPULATIONS INDUCED BY INCREASING THE FEED CONCENTRATION OF THE GROWTH-LIMITING NUTRIENT

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CHAPTER I

INTRODUCTION

The continuous culture of microorganisms is the simplest form of many continuous microbiological rate processes, and the activated sludge process used for the biological treatment of industrial and domestic wastes is a form of continuous culture.

Transient-states caused by variations in the influent stream are a common problem in the activated sludge process. They cause reductions in the quality of the effluent and therefore contribute to the pollution of the receiving stream. The continuous culture was used in the present study to examine one type of transient-state which occurs in the activated sludge process. The primary difference between this continuous culture and the completely mixed activated sludge process is the lack of biological solids recycle.

Variations in the influent stream which cause a reduction in the quality of the effluent of the activated sludge process are termed shock loads (1).

The shock loads may be qualitative, consisting of a change in the chemical structure of the carbon source(s) of the waste, or they may be quantitative, consisting of a change in the amount of a constituent of the waste, or hydraulic, consisting of a change in the influent flow rate (1). Only quantitative shock loads were examined here. The transient-states were induced by increasing the concentration of the growth-limiting nutrient in the feed.

Techniques used for prediction of transient response of continuous cultures have been based on functional relationships derived for steady-state systems (2) (3) (4). The Monod equation for specific growth rate - substrate concentration dependence (5) is used in these techniques. It is customary to make assumptions which simplify the Monod equation to allow closed solutions of the rate equations which give the rate of change of biological solids and substrate concentration and to assume that the yield, biological solids produced per unit of substrate consumed, is constant.

Krishnan observed several systems analogous to the systems studied here (6). Predictions using the Monod equation without any simplifying assumptions were made for the observations of Krishnan and for the early observations made in this study using a numerical integration technique. The parameters for the Monod equation and the yield were arbitrarily selected to obtain the best fit of the observed data.

The best curves calculated in this way did not provide satisfactory predictions of the response of the systems. It then became evident that an examination of the kinetic properties would be useful for understanding the peculiar properties of the transient-state and that the use of equations which describe cultures of bacteria in the steady-state may not be valid for transient-states.

The kinetic properties selected for examination during the transient were the specific growth rate and the yield. These properties were selected because the difficulty with the prediction techniques appeared to lie in the assumption that the steady-state specific growth rate - substrate concentration relationship can be extended to the transient-state and the assumption that the yield is constant.

CHAPTER II

LITERATURE REVIEW

Methods used for the characterization of specific growth rate and yield and reports of observations of bacterial systems in the transient-state are reviewed below. Kinetic models which have been proposed for the transient-state are also reviewed.

A. Characterization of Specific Growth Rate and Yield

1. Specific Growth Rate

The rate of growth of bacterial populations is usually described by the first order equation,

$$\frac{dX}{dt} = \mu \cdot X, \tag{1}$$

where X is the concentration of biological solids or the viable count of microorganisms and μ is the specific growth rate. For bacterial populations growing as a continuous culture, this equation must be changed to account for the washout of the bacterial population. The equation becomes:

$$\frac{dX}{dt} = \mu \cdot X - D \cdot X \tag{2}$$

where D is the dilution rate, the reciprocal of the detention time (7). The specific growth rate for continuous cultures becomes:

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt} + D \ . \qquad (3)$$

2. The Master Reaction

Blackman proposed that it is possible to work out relationships for biological systems between a function and a single one of the various factors which control it (8).

A functional relationship between the specific growth rate and the growth-limiting nutrient concentration is an example of Blackman's proposal. A functional relationship between the yield and the specific growth rate would be another.

Such a functional relationship has been called a "Master Reaction". Burton discussed this term and said it is derived from the assumption that a single slowest reaction of a complex system controls the overall rate of reaction (9).

3. The Equations of Monod and of Moser

The equations of Monod and of Moser are "Master Reaction" equations which give a functional relationship between the specific growth rate and the concentration of the growth-limiting nutrient.

The equation proposed by Monod is:

$$\mu = \mu_m \frac{S}{K_s + S} \qquad (4)$$

where μ_m is the maximum value of the specific growth rate, S is the concentration of the growth-limiting nutrient in the aerator, and K_S is a constant (5)

The equation proposed by Moser is:

$$\mu = \mu_m \frac{S^{\lambda}}{K_S^{\lambda} + S^{\lambda}} \qquad (5)$$

where λ is a system-dependent parameter (10).

Both equations give curves of the same general shape and have been shown to be adequate for many bacterial systems (11).

4. Growth-Limiting Nutrient

The concentrations of the nutrients required for growth can be adjusted to make any of the principle nutrients growth-limiting. The carbon source appears most commonly as the growth-limiting nutrient. In all systems discussed here the carbon source is the growth-limiting nutrient.

In continuous cultures of bacteria, except possibly those in which the dilution rate is approaching the maximum specific growth rate, the carbon source in the reactor is composed of several different compounds including, although not necessarily in abundance, the compound or compounds found in the feed. In a transient-state intermediate compounds produced during the metabolism of the constituent supplied in the feed frequently compose the bulk of the carbon source available for growth.

Since all these constituents can affect the specific growth rate, a common measurement of the carbon source is useful. Such a common measurement of the carbon source is of practical importance in biological waste treatment where the substrate is very complex. From a practical standpoint it is important to note that in biological waste treatment inorganic compounds seldom appear as the growth-limiting nutrients. When they do the composition of the waste is supplemented before treatment by addition to the influent stream to make the carbon source growth-limiting. Ramanathan has shown that the carbon source measured as chemical oxygen demand gives curves which can be described by the Monod equation (11).

5. Time Independence

Equations such as those proposed by Monod and by Moser can be used.to describe the performance of a bacterial system when growing at steady-state. The determination of the parameters is best done by observing a continuous culture at several steady-states. However, it is more frequently accomplished by observing batch systems in the region where they approximate time independence; that is, when the bacteria can be considered as an expanding biological phase.

Batch systems approximate steady-state when the bacteria are in exponential growth and the constituents of the biological mass per unit of total biological mass are constant for some time increment and where the concentration of the growth-limiting nutrient has not been significantly changed by the growth of the biological mass (12).

Both Monod and Moser determined the parameters under the proper conditions of steady-state or approximate steady-state. Since Monod's equation produces the general form of most substrate - specific growth rate curves, and since it contains one less parameter to evaluate, it would seem to be the more useful of the two. This was concluded by Gaudy, Ramanathan, and Rao (13).

Monod suggested the use of a continuous culture for determining the properties of a bacterial population. It is important to note that, in addition to the use of the continuous culture for determining the relationship between the specific growth rate and the concentration of the growth-limiting nutrient, he also suggested that it be used for determining the effect of specific growth rate on the yield of biological solids per unit of substrate consumed (14). However, the yield is usually assumed to be independent of the specific growth rate.

B. Transient-States in Continuous Cultures Caused by a Change in Growth-Limiting Nutrient Concentration

1. Observations of Transient-States

Experimental observations of transient-states in continuous cultures of bacteria caused by increasing the concentration of growth-limiting nutrient have been made by Krishnan (6). The carbon source, glucose, was the growth-limiting nutrient. He concluded that the performance of the system is dependent upon the detention time, the original steady-state concentration of the growth-limiting nutrient, the magnitude of the shock, and whether biological solids were separated from the effluent and returned to the aeration vessel.

Eckhoff and Jenkins have also observed transient-states in the activated sludge process (4). Their experimental data showed agreement with the conclusions of Krishnan.

2. Methods for Predicting Performance During the Transient-State

The rate of change of concentration of biological solids in the continuous culture is:

$$\frac{dX}{dt} = \mu \cdot X - D \cdot X \quad . \qquad (6)$$

The rate of change of concentration of growth-limiting nutrient, as given by Monod, is:

$$\frac{dS}{dt} = D \cdot S_i - D \cdot S - \frac{\mu \cdot X}{Y} \qquad (7)$$

where S_i is the concentration of substrate in the feed and Y is the yield of biological solids (14).

Moser has combined equations (6) and (7) to give a functional relationship between the solids concentration, the substrate concentration, and the time. The solution, which assumes constant yield, is:

$$(S_i - S) \cdot Y - X = [(S_i - S_o) \cdot Y - X_o] \cdot e^{-D \cdot t} \qquad (8)$$

where S_o and X_o correspond to the conditions where time, t, is equal to zero (10).

Both Moser (2) and Eckhoff and Jenkins (4) have solved the differential equations for the time dependent state by assuming that the system operated according to the equation of Monod in the area where the specific growth rate is approximately directly proportional to the substrate concentration, where S is much smaller than K_s, and by assuming the yield to be constant.

The operation of continuous cultures at steady-state in the area where the specific growth rate is approximately directly proportional to the substrate concentration is common and it is emphasized that this assumption made by Moser refers only to variations about these steady-states (2). However, Eckhoff and Jenkins have used this assumption for transient substrate concentrations up to one-half of the value of K_s (4). At S equal to one-half K_s, the value of μ calculated by this simplification of the Monod equation is 51 per cent higher than the value of μ calculated by the hyperbolic Monod equation. Eckhoff and Jenkins found it necessary to deviate widely from their initial kinetic model in order to explain their observed experimental data; this has been discussed by Gaudy (15).

Luedeking and Piret considered the yield to be constant and the system to operate in the area where the specific growth rate is independent of substrate concentration, i.e., at or near μ_m. This occurs as S becomes much larger than K_s. Luedeking and Piret found that this method allows good prediction when the substrate concentration is high (3).

Although for short segments of time substrate concentrations during the transient may be high enough to allow the achievement of μ_m, the method of Luedeking and Piret is not sufficient to describe a large portion of the transient-state. The relatively high values of K_s, ranging from 59 to 178 mg/l COD, reported by Gaudy, Ramanathan, and Rao (13) for the activated sludge process show that only a very short time segment would be expected where the culture could grow at μ_m during the transient-state.

3. Time Dependence

The parameters of the equations of Monod and of Moser are properly determined by observation of steady-states. These equations can not be legitimately extended to all transient-states because a biological system in a steady-state of expansion (a steady-state of a nature which can be characterized by an equation which ascribes to that biological system a unique property corresponding to some imposed environmental condition), when subjected to a sudden change in its environment requires some finite interval of time before a new steady-state characteristic of the new imposed condition can be reached. Perret (16) has used a conceptual model of a bacterial population to show how changes in substrate concentration might affect the performance of the system. He considers two containers of media both containing the same substrate, but at different concentrations. A transfer of organisms at exponential balanced growth from the weaker to the stronger medium would cause a period of increasing specific growth rate unless the organisms were already growing at the maximum specific growth rate. Similarly transfer from the strong to the weak medium would cause a period of decreasing specific growth rate. If culture conditions allow, the systems will eventually reach a new specific growth rate characteristic of balanced growth at the corresponding substrate concentration.

The general form of this phenomenon, called "growth rate hysteresis" by Perret, is shown in Fig. 1 for a system described by the Monod equation.

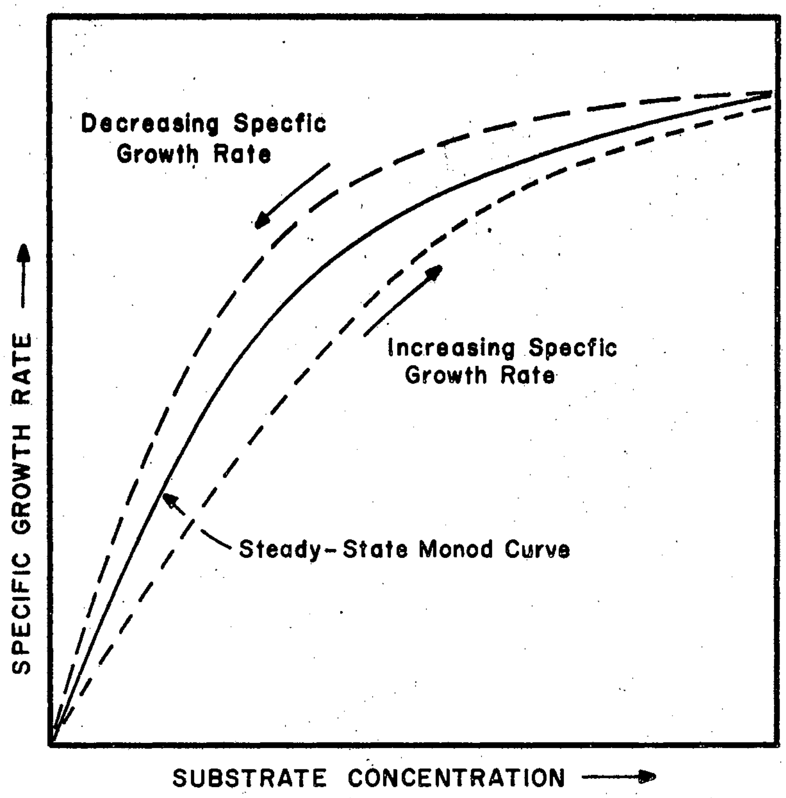

Figure 1 - Effect of Changing Substrate Concentration on Specific Growth Rate

CHAPTER III

MATERIALS AND METHODS

A. General

The continuous culture was maintained in a completely mixed reactor for observation of transient-states.

The growth-limiting nutrient in the feed was glucose. Glucose was used because a considerable amount of work has been done with it as a sole carbon source, in particular the work of Krishnan (6) in observing transient-states analogous to those reported on here. The concentration of glucose in the feed for all systems was initially approximately 500 mg/l and finally approximately 1500 mg/l. The transient-state was induced by changing from 500 mg/l feed concentration to 1500 mg/l feed concentration.

The detention times, constant for each system, varied from 4 to 5 hours for the six systems observed. This variation in detention times from one system to another is not significant in the present studies; the primary consideration was to accurately determine the detention time and to assure identical times both before and after the initiation of the transient to avoid hydraulic shock loading.

Biological solids concentration, filtrate COD, and filtrate glucose or carbohydrate concentration were determined prior to, throughout, and after the transient-state.

During some of the continuous flow experiments cells were harvested and used in batch experiments conducted in shaker flasks. Batch growth rate experiments were made using cells harvested before and during the transient imposed on the continuous flow systems. These studies were made to determine the effect of these two types of environmental conditions on the growth pattern of the organisms.

Natural populations of bacteria were developed from fresh settled sewage seed obtained from the primary clarifier of the Stillwater municipal sewage treatment plant.

Five hundred ml of sewage were used, with the remainder of the reactor volume filled with growth medium to develop the bacterial population. The system was operated as a batch system for 24 hrs before continuous operation was begun. The system was then operated continuously for 72 hrs or until steady-state was reached, before the transient-state was induced.

B. Equipment

The reactor volume was approximately 2.5 liters, maintained constant by an over-flow weir. The reactor configuration was that used by Ramanathan, the result of his examination of various completely mixed constant volume configurations (11). Agitation and mixing were provided by diffused air at 1600 cc/min/l of reactor volume. The temperature of the reactor liquor was maintained at room temperature, 25° ± 3° C.

Feed was pumped from pre-sterilized glass carboys to the reactor by reciprocating pumps manufactured by Milton Roy Company ("mini Pump").

The transient was begun by changing feed lines to the reactor. The feed lines were connected to identical pumps previously adjusted to equal flow rates.

C. Analytical Procedure

Biological solids were measured using a membrane filter (Millipore Filter Corp., HA, 0.45μ), by filtering centrifuged samples through dried and desiccated tared filters which were then oven-dried at 103° C for 2 hours and desiccated before weighing. The biological solids were measured at steady-state and during transient-states by this technique and correlated with optical density which allowed the approximation of solids concentration at more frequent intervals than would otherwise be possible. The solids - optical density correlation showed that the solids concentration was directly proportional to optical density in the range employed in these studies, which is in agreement with the observations of Rao (17).

Optical density of the reactor effluent was measured at 540 mμ by a spectrophotometer manufactured by Coleman Instruments, Inc. (Junior Spectrophotometer, Model 6D).

Filtrate COD was determined by the "Standard Methods" technique (18).

Filtrate glucose concentration was determined using an enzymatic reagent of the Worthington Biochemical Corp. ("Glucostat"). Filtrate carbohydrate was measured using the anthrone carbohydrate test (19).

Batch growth rate studies were made concurrently with the observations of four continuous flow systems. The batch systems were run in shaker-flasks at 100 oscillations per min. Five ml of freshly collected reactor effluent were used to seed the 60 ml of growth medium. Glucose concentrations of 500 and 1000 mg/l were used.

D. Growth Medium

The medium composition used was such that glucose was the growth-limiting nutrient. The medium was buffered to pH 7.0. The composition of the medium is given in Table I for 1000 mg/l glucose concentration. For other glucose concentrations, the concentrations of the nutrients were directly proportional to the glucose concentration.

TABLE I

COMPOSITION OF GROWTH MEDIUM FOR 1000 mg/l GLUCOSE AS GROWTH-LIMITING SUBSTRATE

Constituent	Concentration	
Glucose	1000	mg/l
Ammonium sulfate, $(NH_4)_2SO_4$	500	mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	100	mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.50	mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	10.00	mg/l
Calcium chloride, $CaCl_2$	7.50	mg/l
KH_2PO_4	527.0	mg/l
K_2HPO_4	1070.0	mg/l
Tap water	100	ml/l

CHAPTER IV

CALCULATION PROCEDURE

The calculation procedure discussed here was used for determining the kinetic properties of the transient-state.

The kinetic properties of interest are the specific growth rate and the yield of biological solids. In order to determine these properties during the transient-state they were assumed to be linear functions (to facilitate computation) of time for segments of the transient. The response was then calculated and compared to the observed response of biological solids and substrate concentration.

A. General and Steady-State Yield

Monod showed that the rate of substrate removal is:

$$\left(\frac{dS}{dt}\right)_g = \frac{1}{Y} \cdot \left(\frac{dX}{dt}\right)_g \qquad (9)$$

where $\left(\frac{dX}{dt}\right)_g$ is the rate of growth. The yield is therefore the rate of growth divided by the rate at which substrate is removed by growth. This is incorporated in Monod's equation for the rate of change in substrate concentration in the continuous culture (14).

Moser's general solution of the rate equations for substrate and biological solids concentration, equation (8), can be rearranged to give the general expression for yield:

$$Y = \frac{X - X_o \cdot e^{-D \cdot t}}{S_i - S - (S_i - S_o) \cdot e^{-D \cdot t}} \qquad (10)$$

The special case solution for steady-state,

$$Y = \frac{X}{S_i - S} \qquad (11)$$

can be obtained by setting X equal to X_o and S equal to S_o in Moser's equation. Both steady-state yield and transient yield were calculated based on substrate as the total carbon source, i.e., COD.

B. Dilute-In of Additional COD

It was useful to contrast the observed shock load response with the result which would be expected if no additional biological activity took place. In order to do this, the rate of substrate removal by growth is assumed to be constant and equal to the level prior to shock loading. The rate of substrate removal by growth is then:

$$\left(\frac{dS}{dt}\right)_g = D\ (S_{i_o} - S_o) = \frac{\mu \cdot X}{Y} \qquad (12)$$

where S_{i_o} is the concentration of substrate in the feed prior to shock and S_o is the concentration of substrate in the reactor prior to shock. The equation for the rate of change of substrate concentration due to additional substrate is:

$$\frac{dS}{dt} = D \cdot S_i - D \cdot S - D(S_{i_o} - S_o) \qquad (13)$$

Integrating equation (13) with S_o as the value of S at time zero and solving for S, the result is the equation for dilute-in of additional substrate:

$$S = (S_i - S_{i_o} - S_o) - (S_i - S_o) \cdot e^{-D \cdot t} \qquad (14)$$

If the dilute-in curve calculated in this way gives greater values for substrate concentration than are observed, the rate of substrate removal by growth has increased following the start of the shock load.

If the dilute-in curve gives values of substrate concentration equal to the observed, the rate of substrate removal by growth has remained the same. And if the observed values exceed the dilute-in the rate of substrate removal by growth has decreased from its original level.

C. Specific Growth Rate and Yield as a Function of Time

In order to determine what linear functions to assume for yield and specific growth rate, a curve was drawn through the observed response and divided into small segments, i.e., X_n to X_{n+1} and S_n to S_{n+1}. For the segment, Δt, the specific growth rate and the yield were assumed to be constant. From the values of X and S at the initial and final points of the interval, the values of μ and Y were calculated from equations (15) and (16) which are the result of integration of the differential rate equations (6) and (7) for the system with constant μ and Y.

$$\mu = \frac{\ln\,(X_{n+1}/X_n)}{t} + D \tag{15}$$

$$Y = \frac{(X_{n+1} - X_n \cdot e^{-D \cdot t})}{S_i - S_{n+1} - (S_i - S_n) \cdot e^{-D \cdot t}} \tag{16}$$

The value of μ during the transient was then approximated for an interval of time during the transient by an equation of the form:

$$\mu = a + b \cdot (t - f) \tag{17}$$

where a is the value of μ when t is equal to f and b is the slope of the curve approximating transient μ. The time of beginning of the transient μ is designated as f. Linear approximations of the transient specific growth rate were selected because the assumption of the parameters would have been difficult if other approximation curves were used. It was felt that the transient approximations obtained in this way sufficiently approximated the true performance; the true performance may vary slightly from the results obtained but the techniques used did not allow sufficiently accurate observations to reveal discrepancies. The determination of transient μ as a function of time is a method of observation of the transient kinetic properties, not a method of prediction of transient response.

If the transient approximation of μ is substituted in equation (6) the equation for solids response can be found from the time dependent solution. The value of X at time t, X_t, is then:

$$X_t = X_f \cdot e^{[(a - b \cdot f - D) \cdot (t - f)] + [b \cdot (t^2 - f^2)/2]} \qquad (18)$$

where X_f is the value of X at time f. By proper selection of the linear segments the solids response can be calculated and a good approximation of μ as a function of time obtained.

For the transient values of the yield a similar equation was used:

$$Y = a' + b' \cdot (t - f) \qquad (19)$$

where a' is the value of Y at time t equal to f.

In order to determine if the Y as a function of time is correct, the differential rate equation for the rate of change of substrate concentration containing the value of X as a function of time must be integrated. This equation is obtained by substituting the transient approximation of μ and Y and the time dependent solution for X, equation (18), into the rate equation for substrate concentration, equation (7). The rate of change of substrate concentration is:

$$\begin{aligned}\frac{dS}{dt} = D\cdot S_i - D\cdot S - \frac{(a + b\cdot(t - f))}{(a' + b'\cdot(t - f))}\cdot X_f\cdot \\ e^{[(a - b\cdot f - D)\cdot(t - f)] + [b\cdot(t^2 - f^2)/2]}\end{aligned} \qquad (20)$$

The integration of this equation is easily accomplished by a suitable numerical method. If the assumed Y as a function of time predicts the transient substrate concentration response, it approximates the proper function.

D. Transient Specific Growth Rate vs. Substrate Concentration

It is useful to contrast the properties of the time independent state with those of the time dependent state by use of the Monod equation for substrate - specific growth rate interdependence. In the present study the following approach was employed. On a plot of the Monod equation the values of μ from the assumed linear segments were plotted against the corresponding observed substrate concentration to compare the time dependent state with the time independent state.

E. Time Dependent Solution to the Monod Equation

Since it is popular to extend the Monod equation to the prediction of transient response the use of the differential rate equation containing the Monod equation was examined. The equation for the rate of change of solids concentration is:

$$\frac{dX}{dt} = \mu_m \cdot \frac{S}{K_s + S} \cdot X - D \cdot X \qquad (21)$$

The equation for the rate of change of substrate concentration is:

$$\frac{dS}{dt} = D \cdot S_i - D \cdot S - \mu_m \cdot \frac{S}{K_s + S} \cdot X \cdot \frac{1}{Y} \qquad (22)$$

The yield is assumed to be constant when the transient is predicted in this way. The common solution of these equations is obtained by numerical integration.

The numerical integration of the equation for substrate response where Y and μ are functions of time and of the rate equations incorporating the Monod equation was performed using Taylor's series with differentials computed to the third derivative (20). The calculations were performed on a digital computer (I.B.M., Model 7040) at the Oklahoma State University Computer Center.

CHAPTER V

RESULTS

The observed transient responses of the six systems are given below. Also the kinetic properties determined by the interpretation of the observed responses and the predicted response from the solution of the rate equations incorporating the Monod equation are given.

A. Steady-State

The steady-state parameters for the six systems are given in Table II. The yield changes somewhat for all systems except System No. 2. The initial and final substrate concentrations are relatively close for Systems Nos. 2, 3, and 6. In this respect Systems Nos. 1, 4, and 5 do not show good agreement with the Monod equation which requires equal concentrations for any one dilution rate.

B. Observed Transient Response and Dilute-In of Additional COD

The observed response of the six systems: Nos. 1, 2, 3, 4, 5, and 6 are given in Figs. 2, 3, 4, 5, 6, and 7 respectively. The responses of biological solids, filtrate

TABLE II

STEADY-STATE PARAMETERS

System	Dilution Rate Hr^{-1}	S_i, COD mg/l		S, COD mg/l		X mg/l		Y	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
No. 1	0.203	527	1528	170	35	150	762	0.42	0.51
No. 2	0.222	541	1622	20	26	248	750	0.48	0.47
No. 3	0.244	445	1455	88	88	228	750	0.64	0.55
No. 4	0.254	534	1600	250	550	132	432	0.47	0.41
No. 5	0.253	538	1613	250	75	175	800	0.51	0.52
No. 6	0.257	516	1586	32	49	265	750	0.55	0.49

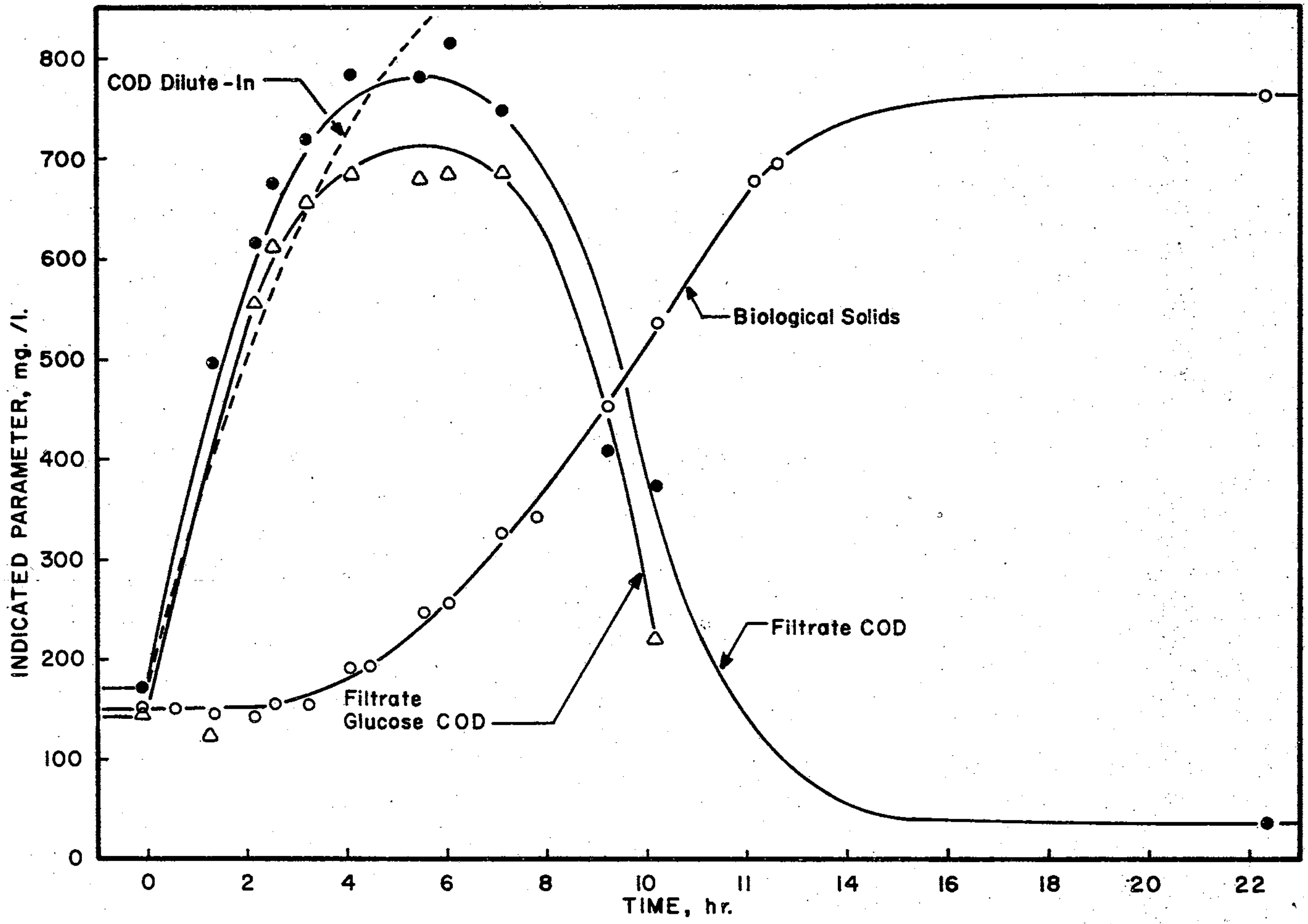

Figure 2 - Observed Response of Biological Solids and Substrate to Change in the Growth-Limiting Nutrient Concentration in the feed and Calculated Dilute-In of Additional Substrate of System No. 1

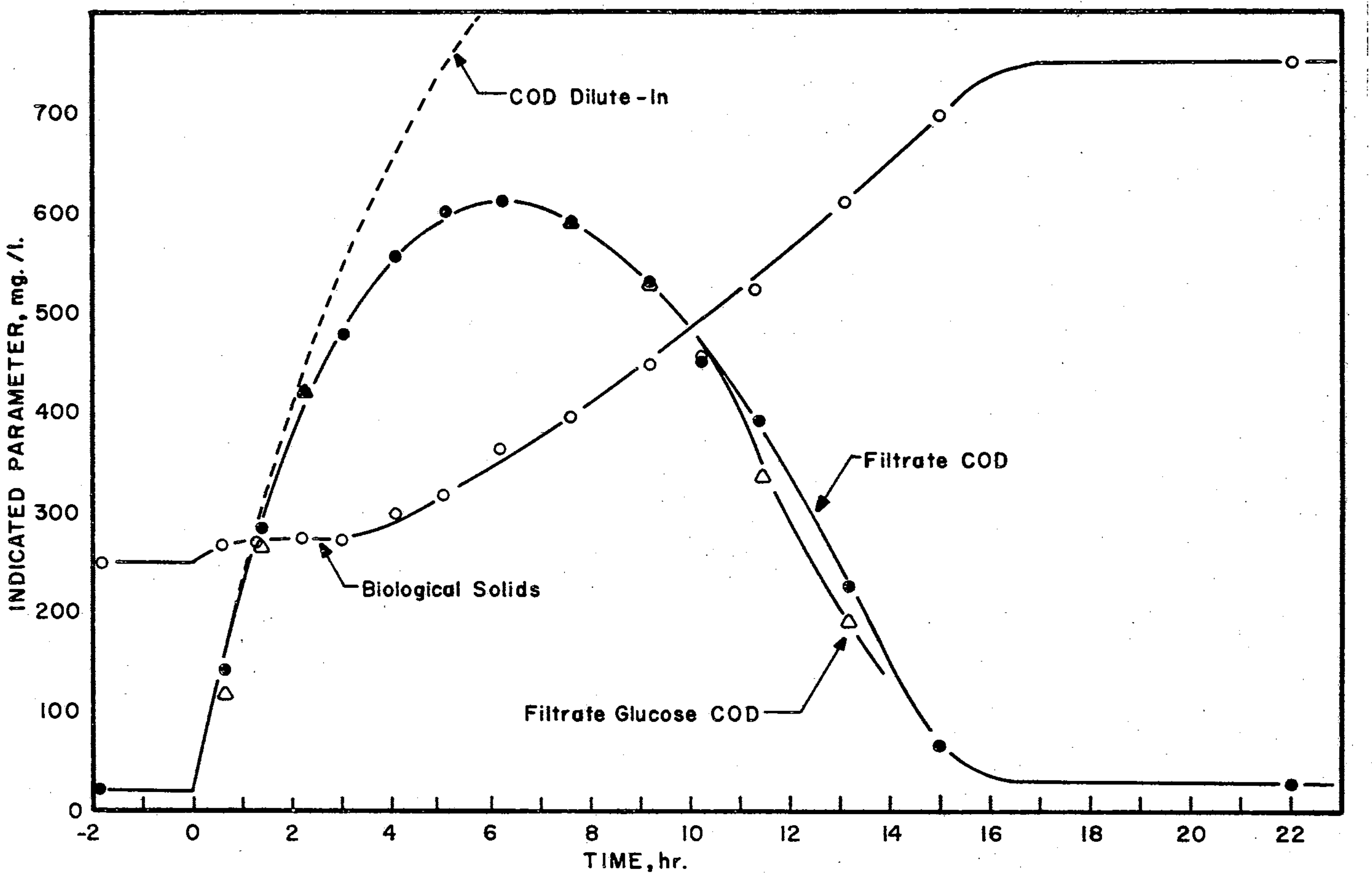

Figure 3 - Observed Response of Biological Solids and Substrate to Change in the Growth-Limiting Nutrient Concentration in the Feed and Calculated Dilute-In of Additional Substrate of System No. 2

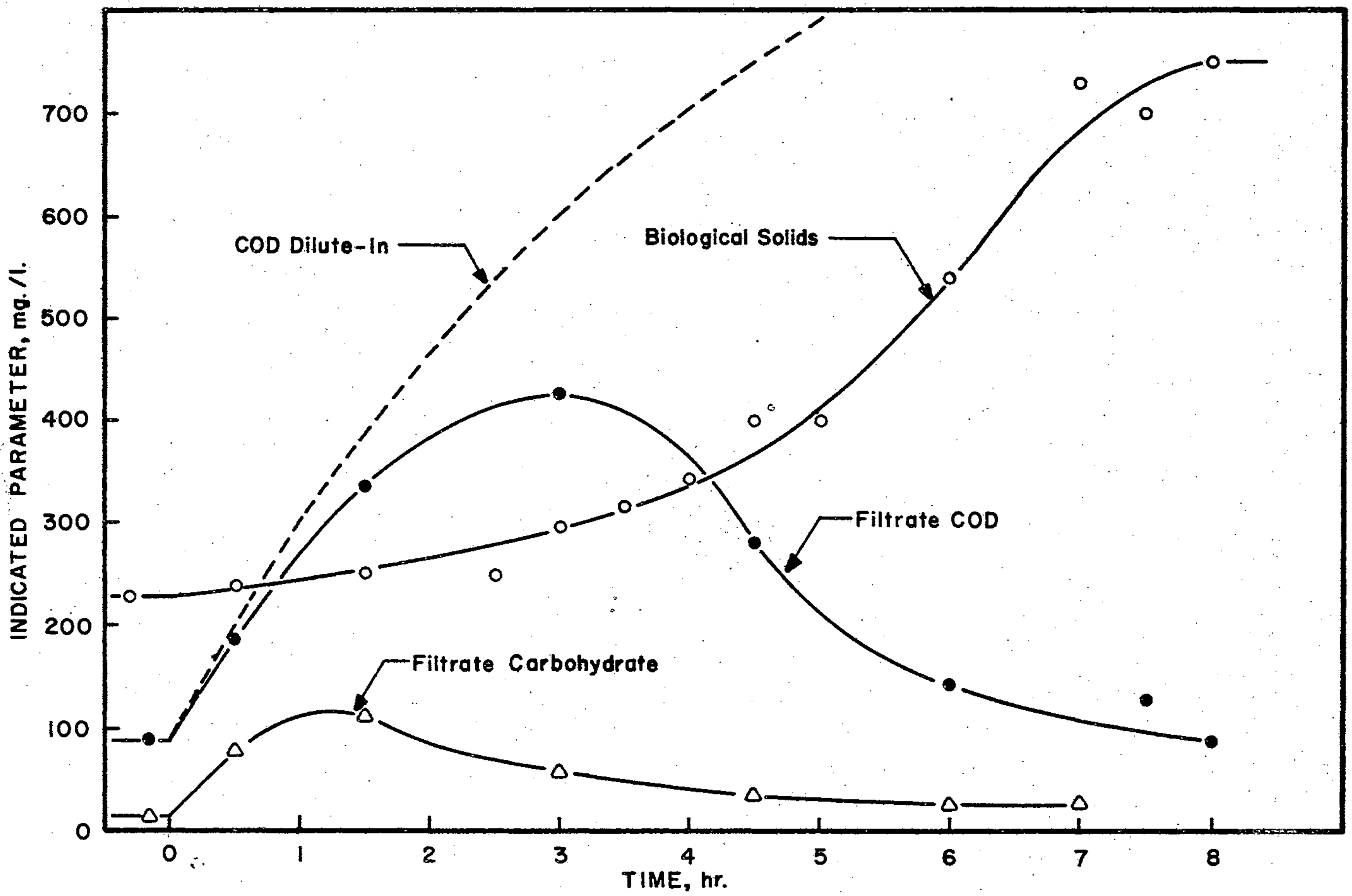

Figure 4 - Observed Response of Biological Solids and Substrate to Change in the Growth-Limiting Nutrient Concentration in the Feed and Calculated Dilute-In of Additional Substrate of System No. 3

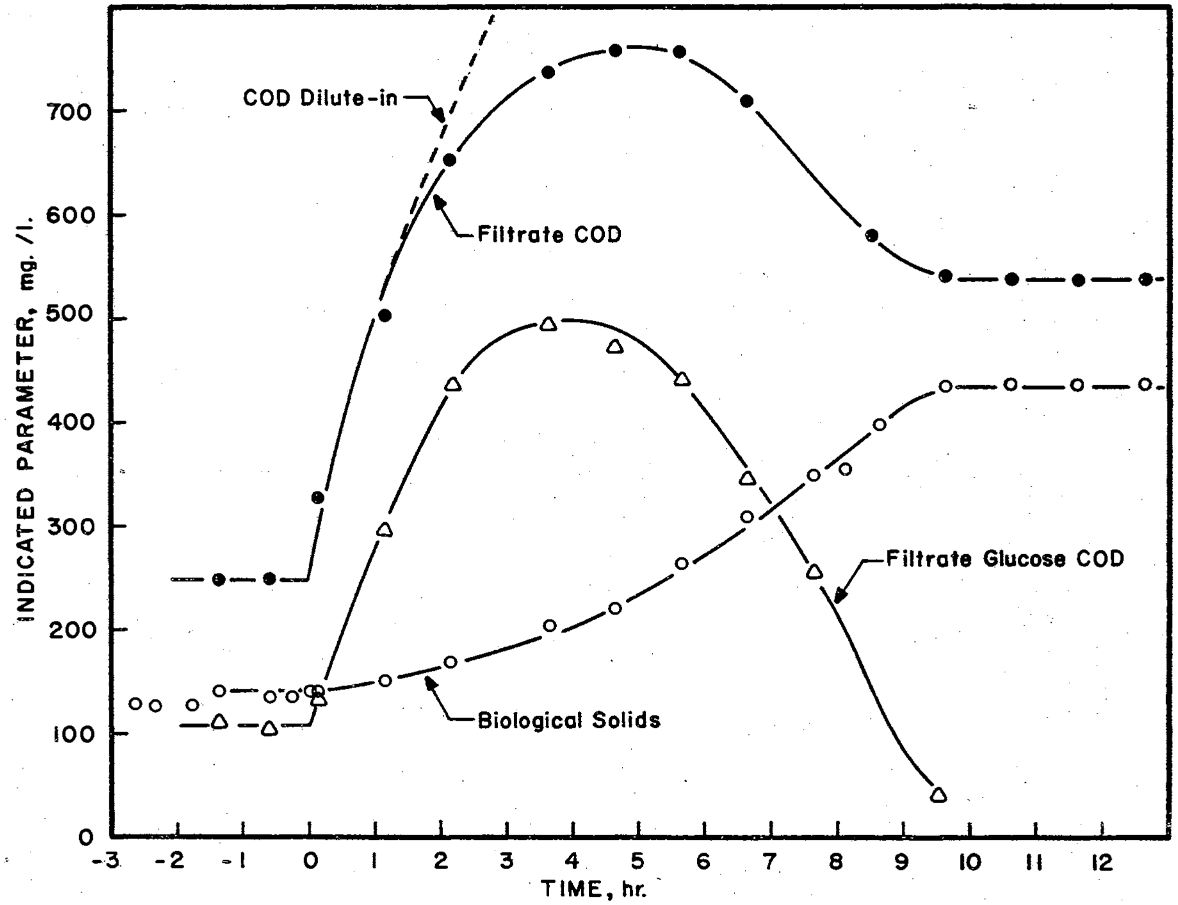

Figure 5 - Observed Response of Biological Solids and Substrate to Change in the Growth-Limiting Nutrient Concentration in the Feed and Calculated Dilute-In of Additional Substrate of System No. 4

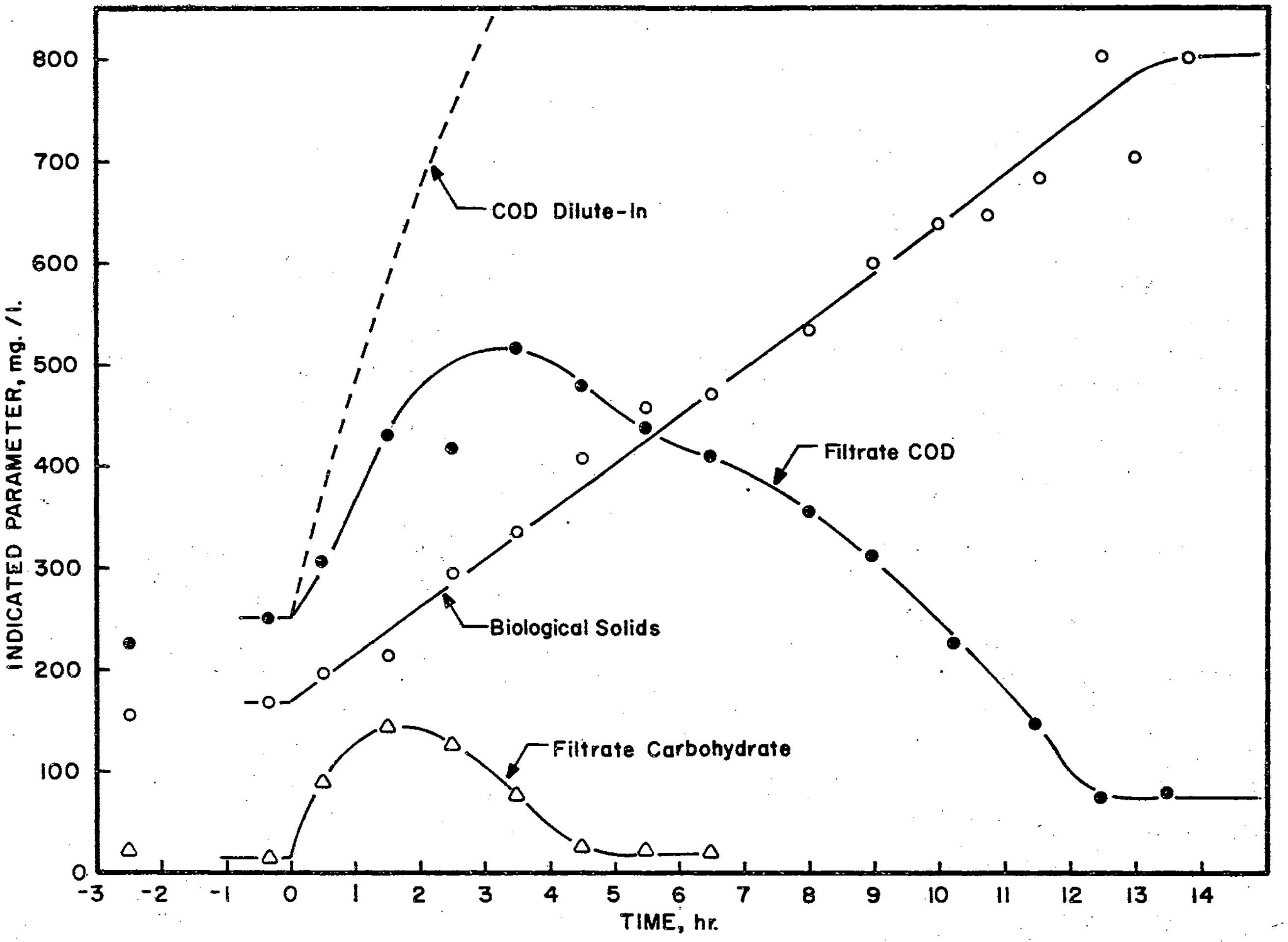

Figure 6 - Observed Response of Biological Solids and Substrate to Change in the Growth-Limiting Nutrient Concentration in the Feed and Calculated Dilute-In of Additional Substrate of System No. 5

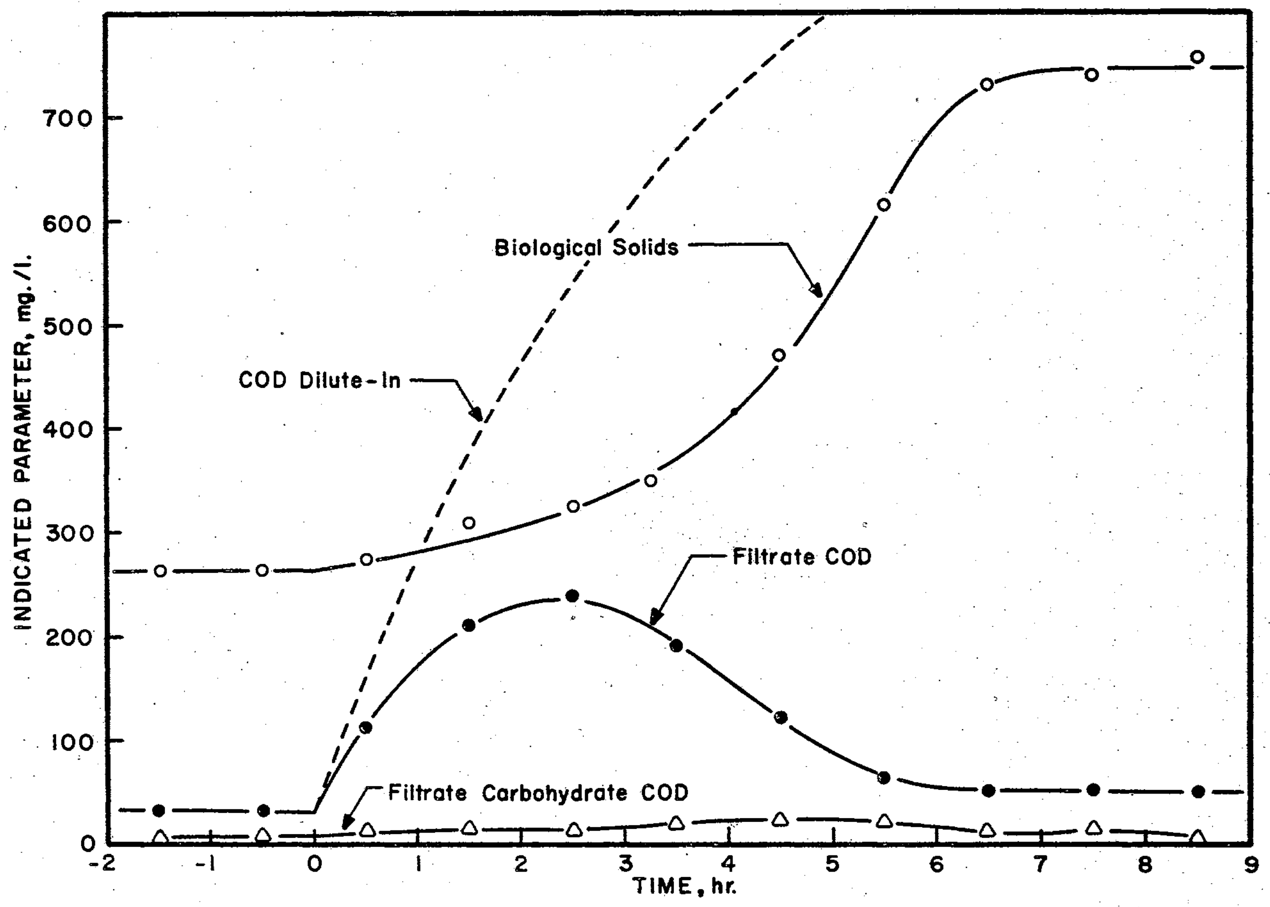

Figure 7 - Observed Response of Biological Solids and Substrate to Change in the Growth-Limiting Nutrient Concentration in the Feed and Calculated Dilute-In of Additional Substrate of System No. 6

COD and glucose or carbohydrate are shown.

The observed responses show a great amount of variety with 7 to 17 hrs. being required before a new steady-state was reached. Intermediates present at the point of maximum substrate concentration varied from none to more than 90 per cent of the COD.

The dilute-in of additional COD is shown on the figures. The rate of substrate removal was assumed to be equal to the initial steady-state rate of substrate removal by growth. All systems except No. 1 showed COD concentrations equal to or lower than the calculated dilute-in values immediately following the beginning of the transient-state. This indicates that the substrate removal rate immediately following the beginning of the transient was equal to or greater than the initial steady-state removal rate. System No. 1 showed higher observed response than the calculated COD dilute-in for the first 4.5 hrs. indicating that one effect of the shock load was to reduce the substrate removal rate.

C. Transient Specific Growth Rate and Yield

The curves plotted for the response of biological solids and substrate in Figs. 8 through 19 are the result of the solutions of the rate equations (18) and (20) incorporating μ and Y as functions of time.

The transient values of μ and Y were approximated by assuming linear functions with time for short intervals. The assumed transient values are not unique; slight

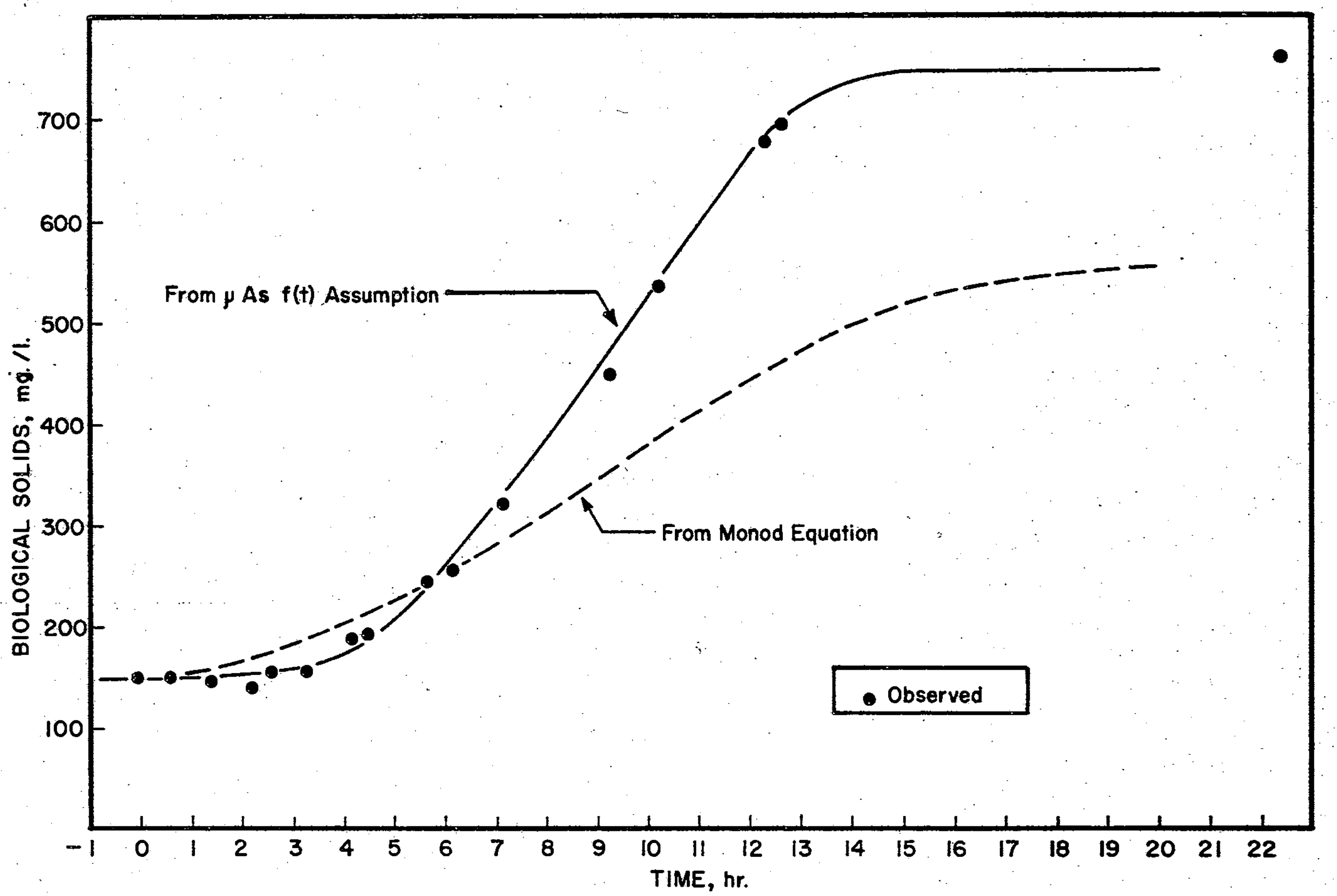

Figure 8 - Observed and Calculated Response of Biological Solids of System No. 1 to Change in Growth-Limiting Nutrient Concentration in the Feed

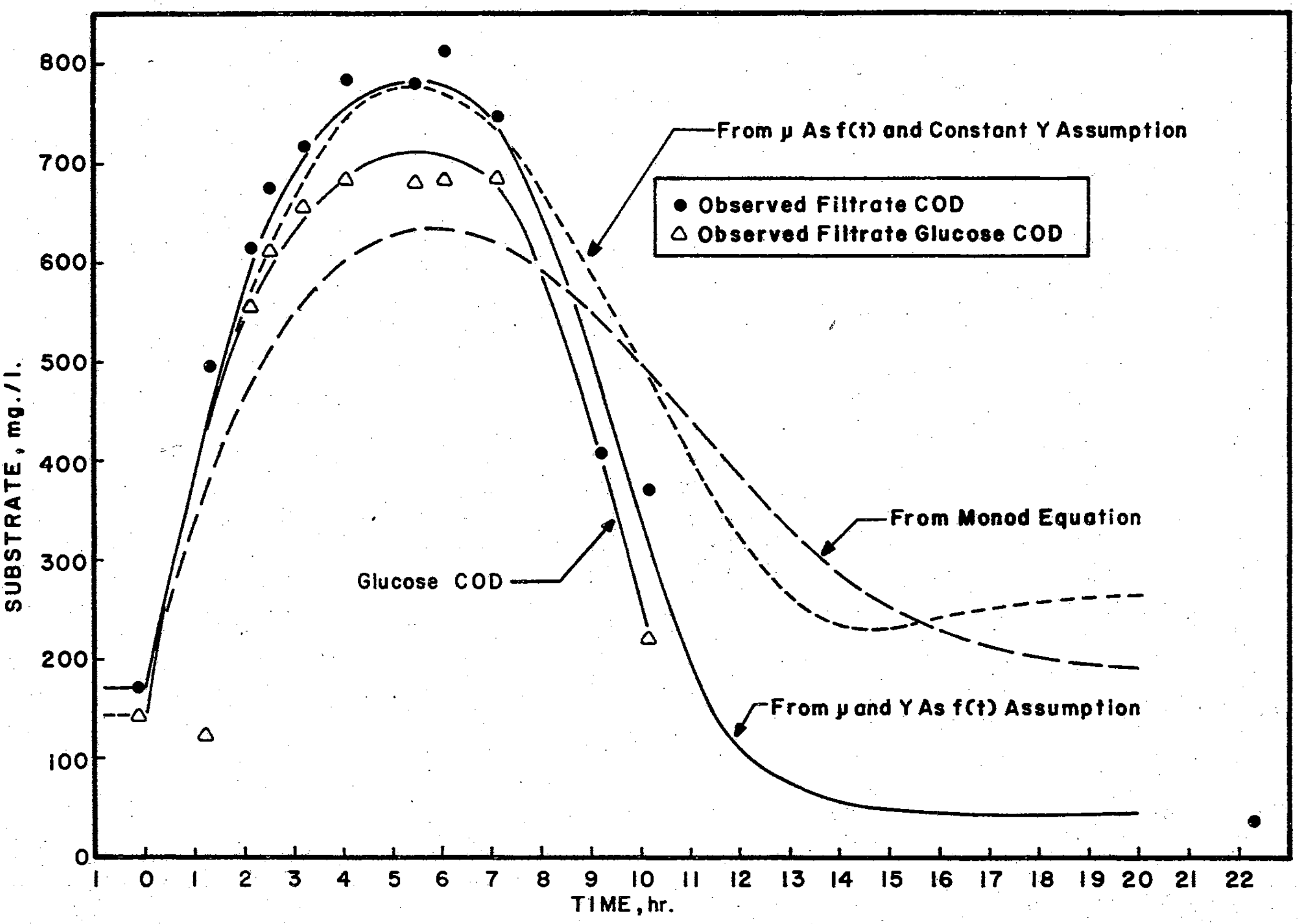

Figure 9 - Observed and Calculated Response of Substrate of System No. 1 to Change in Growth-Limiting Nutrient Concentration in the Feed

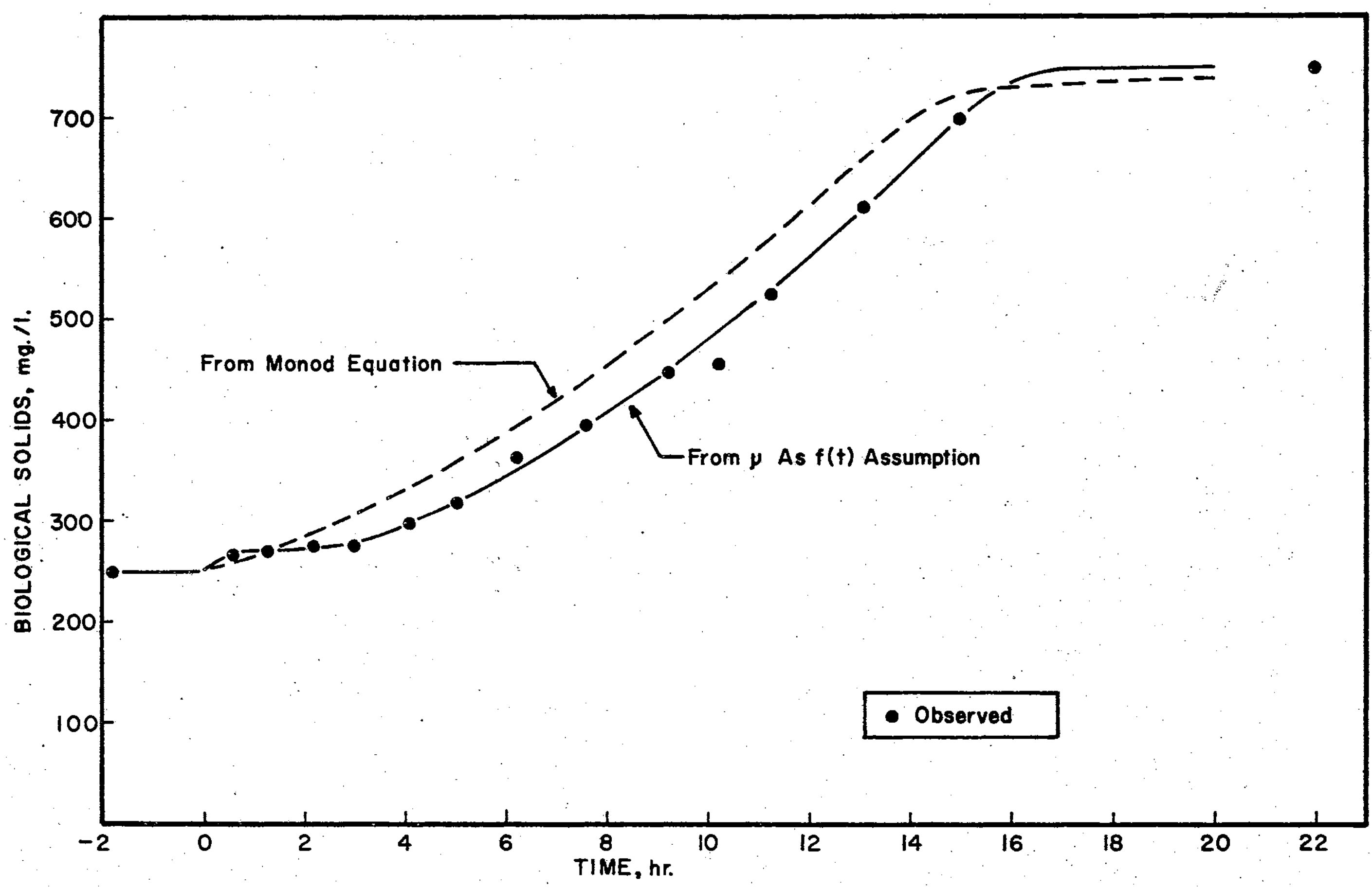

Figure 10 - Observed and Calculated Response of Biological Solids of System No. 2 to Change in Growth-Limiting Nutrient Concentration in the Feed

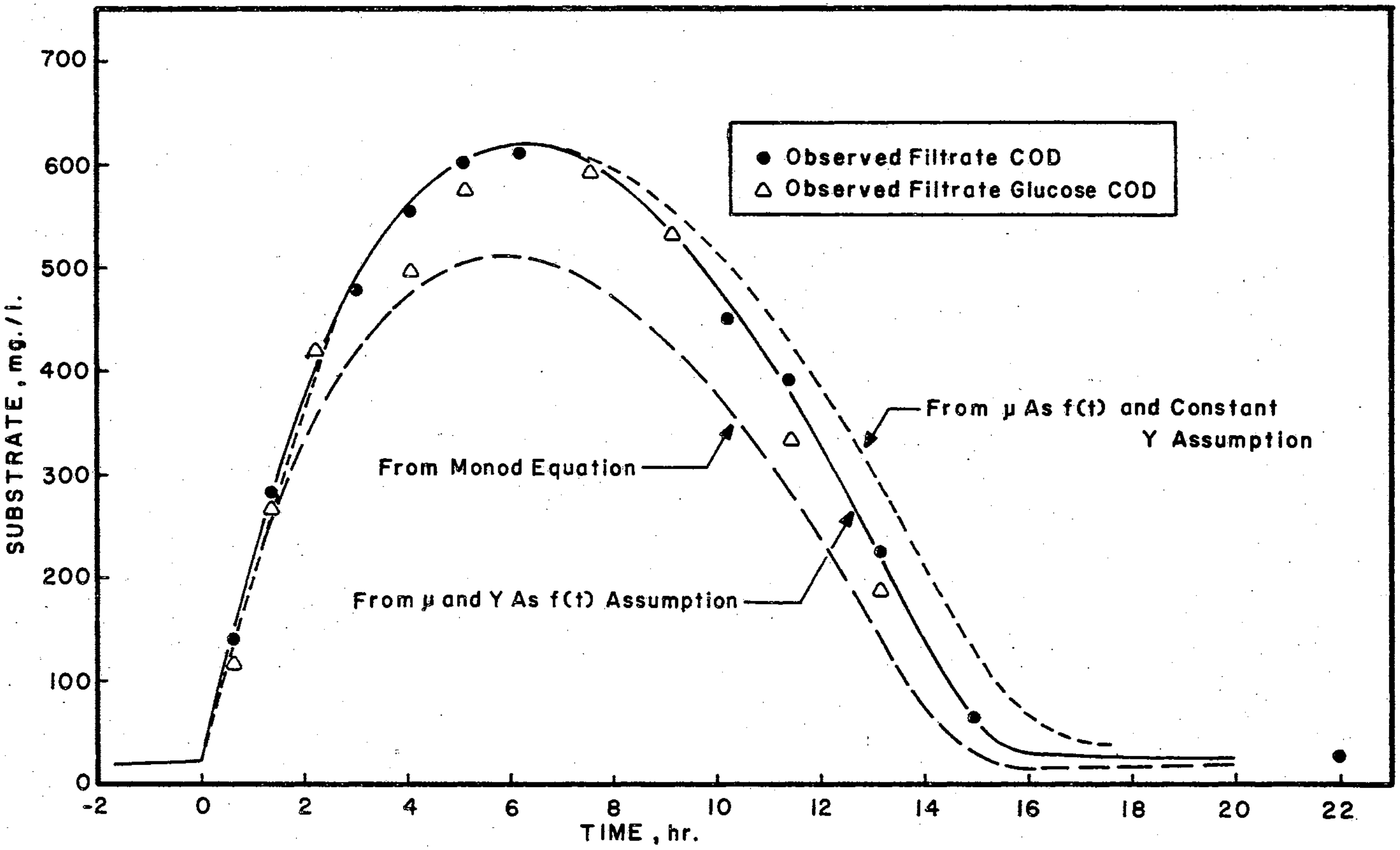

Figure 11 - Observed and Calculated Response of Substrate of System No. 2 to Change in Growth-Limiting Nutrient Concentration in the Feed

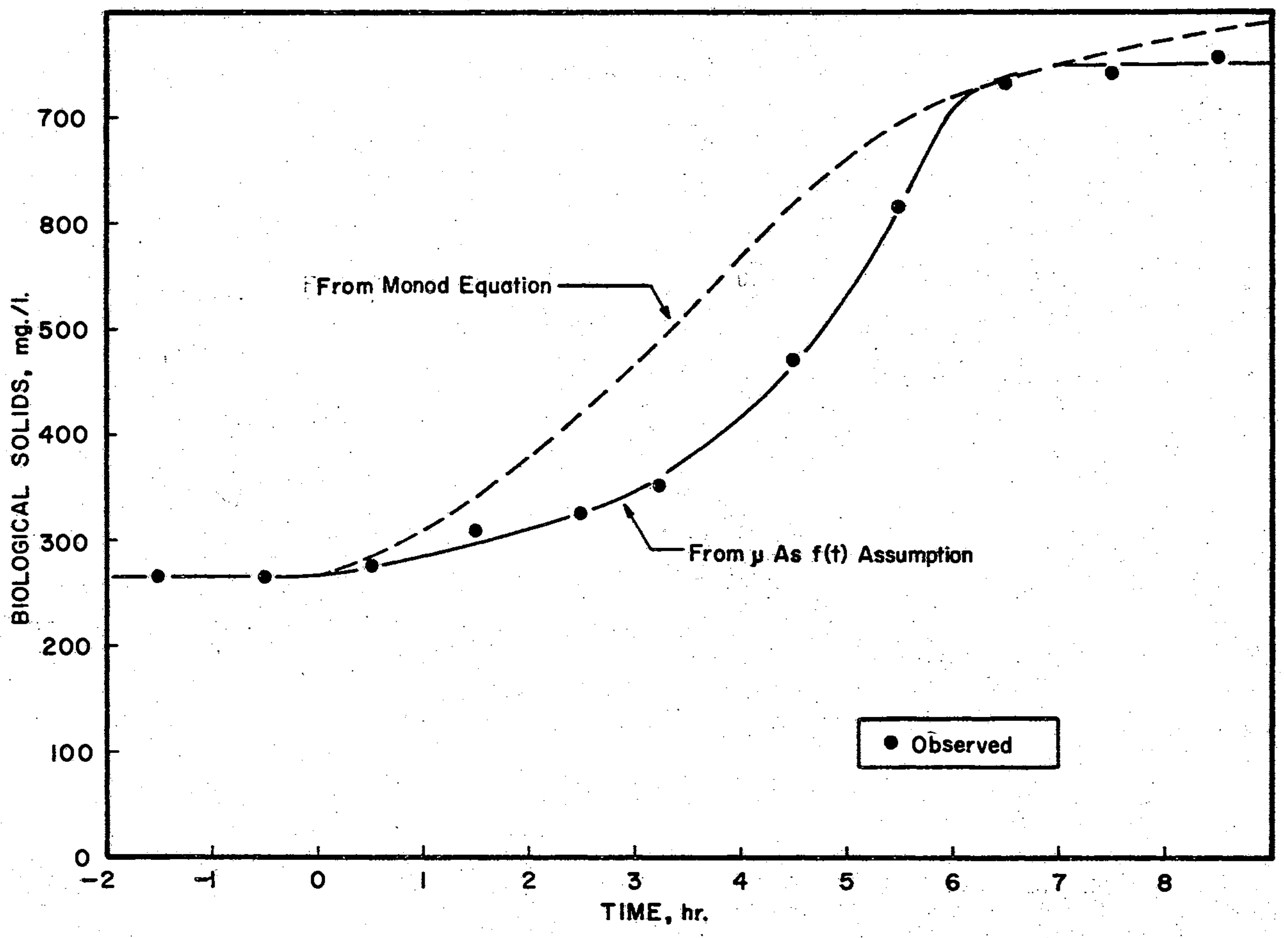

Figure 12 - Observed and Calculated Response of Biological Solids of System No. 3 to Change in Growth-Limiting Nutrient Concentration in the Feed

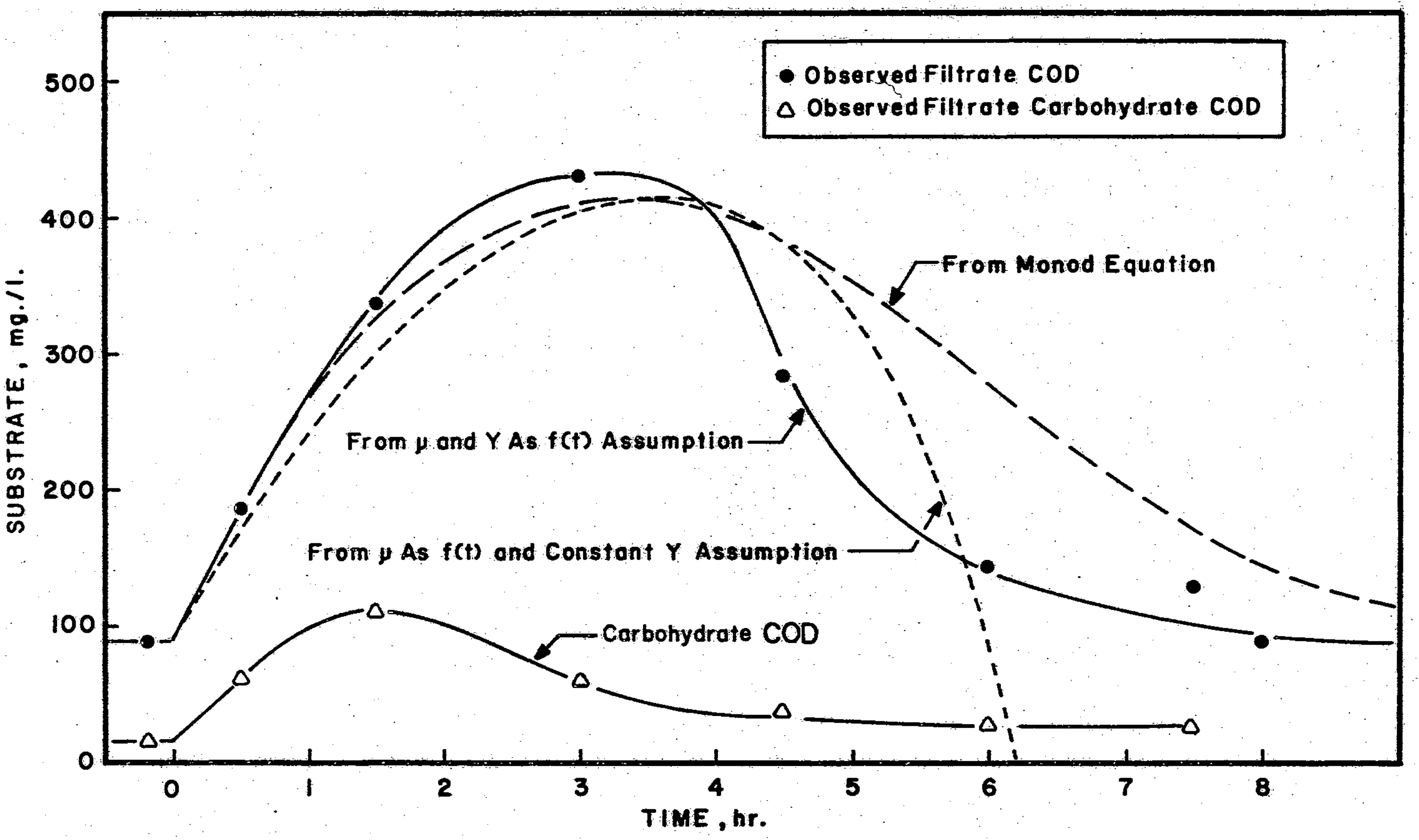

Figure 13 - Observed and Calculated Response of Substrate of System No. 3 to Change in Growth-Limiting Nutrient Concentration in Feed

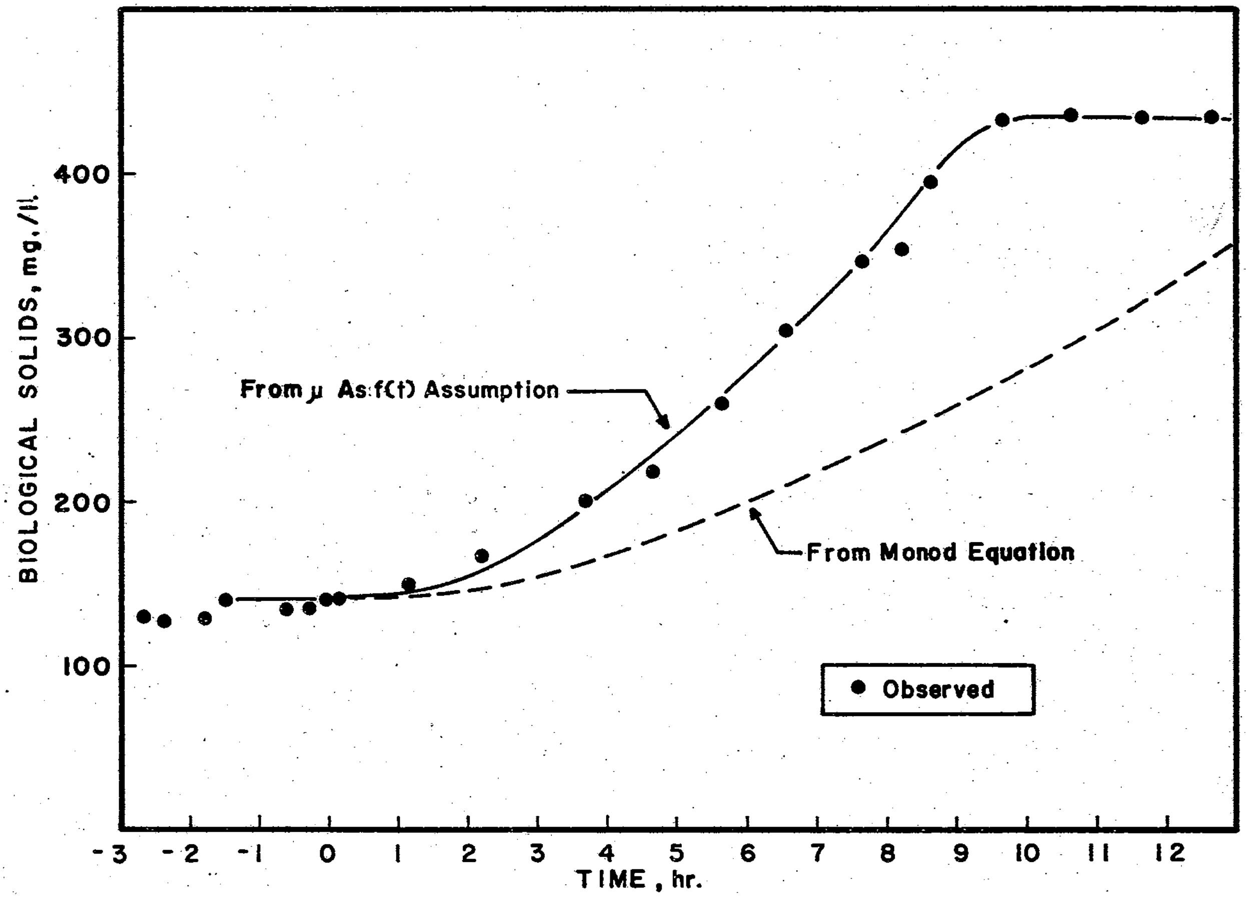

Figure 14 - Observed and Calculated Response of Biological Solids of System No. 4 to Change in Growth-Limiting Nutrient Concentration in the Feed

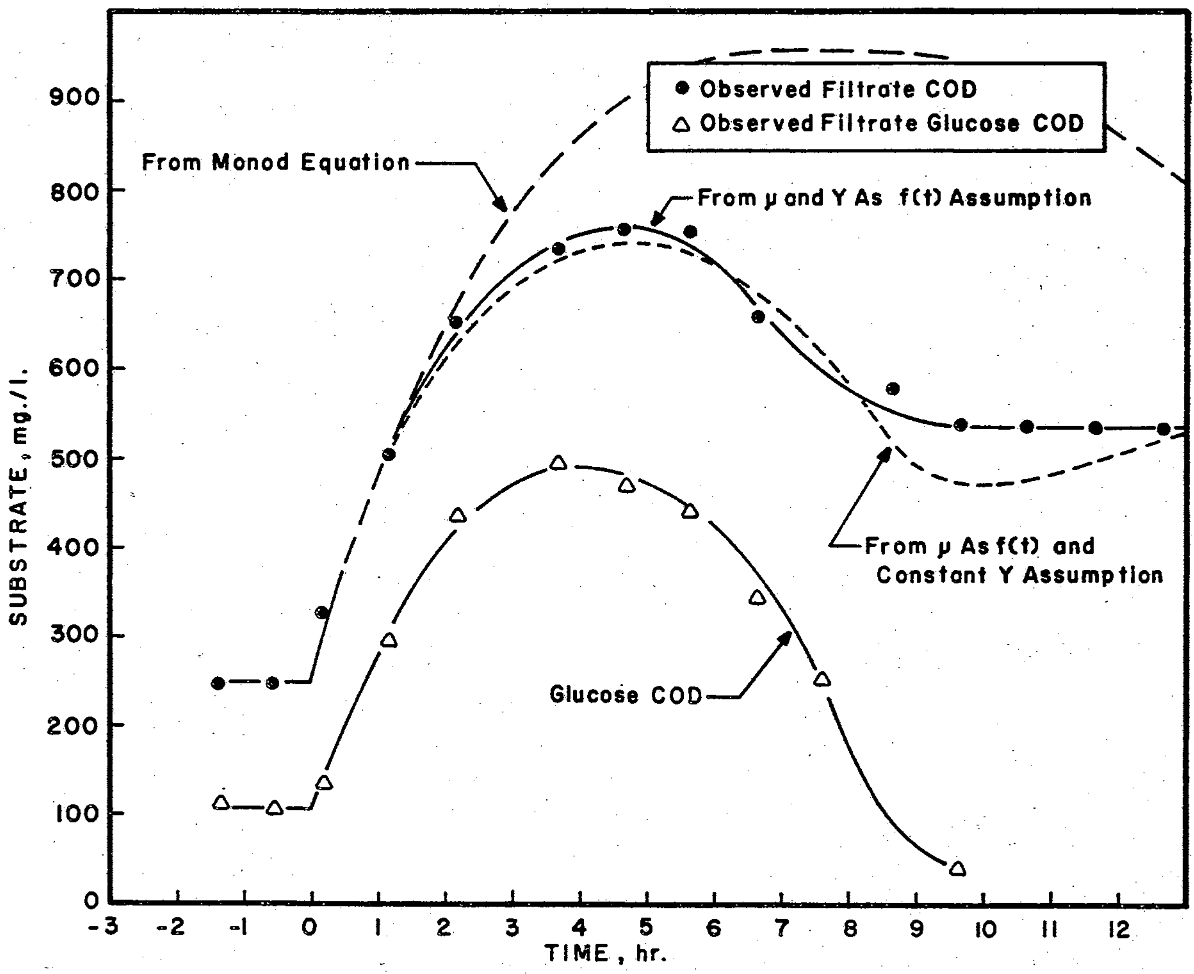

Figure 15 - Observed and Calculated Response of Substrate of System No. 4 to Change in Growth-Limiting Nutrient Concentration in Feed

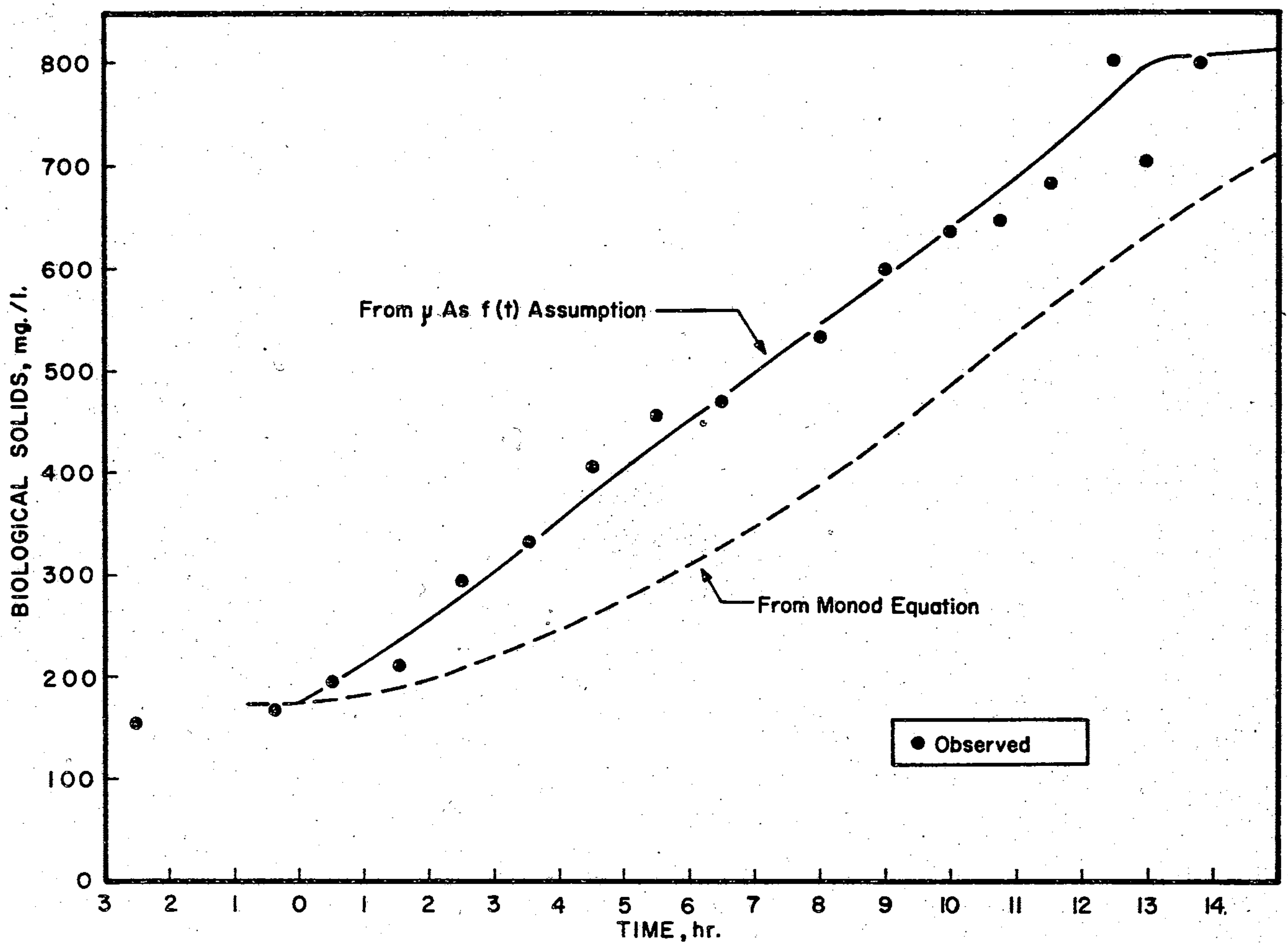

Figure 16 - Observed and Calculated Response of Biological Solids of System No. 5 to Change in Growth-Limiting Nutrient Concentration in the Feed

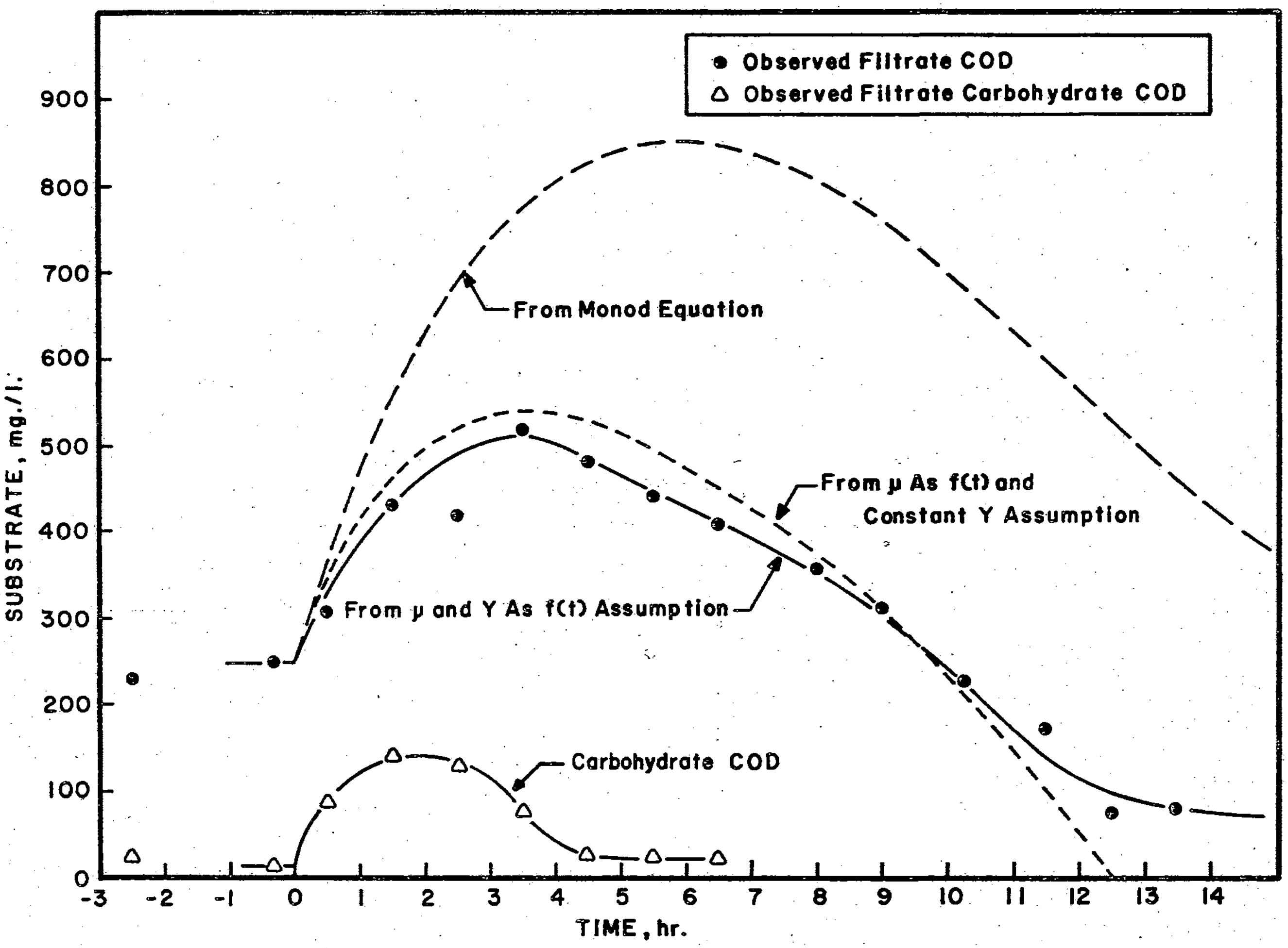

Figure 17 - Observed and Calculated Response of Substrate of System No. 5 to Change in Growth-Limiting Nutrient Concentration in the Feed

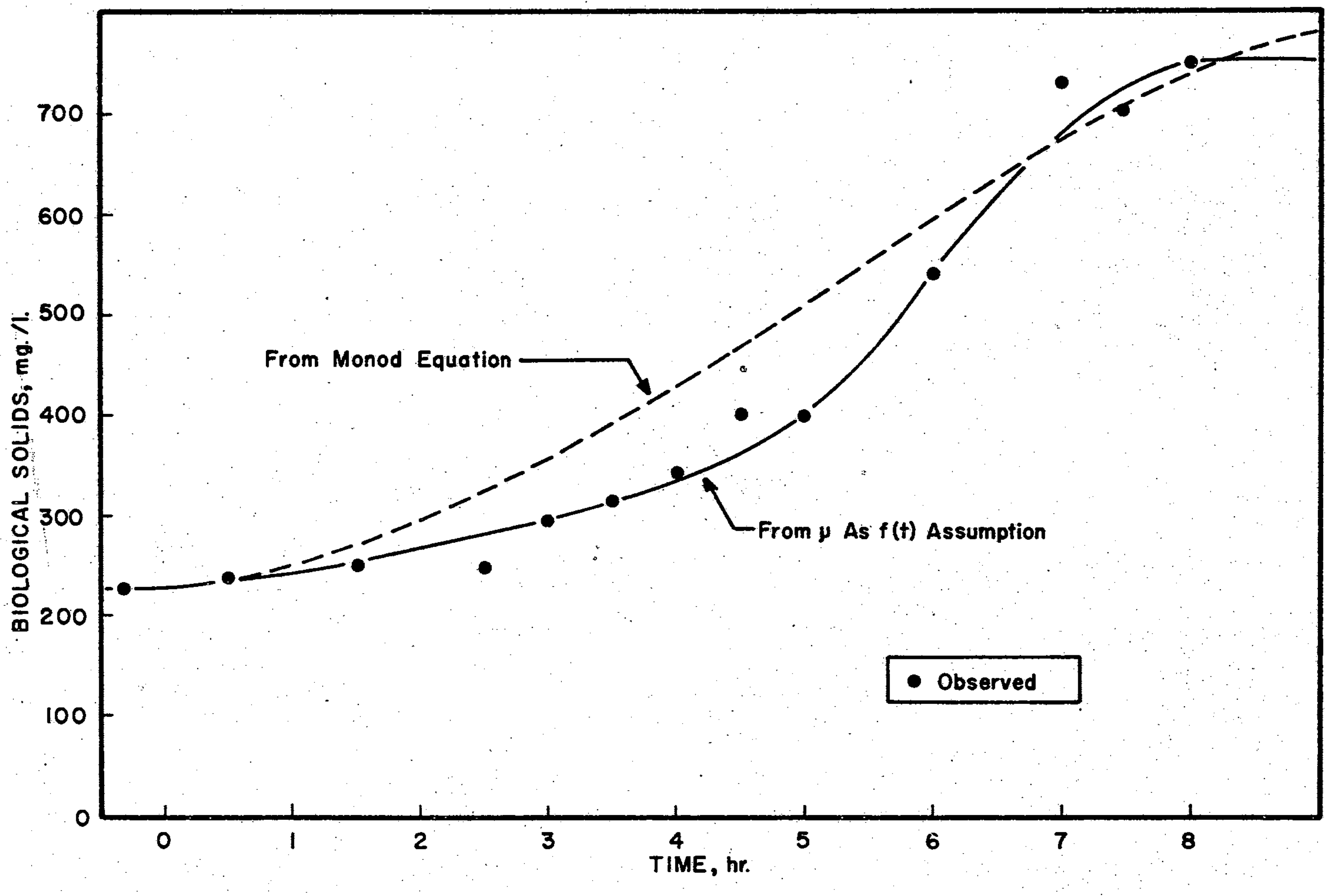

Figure 18 - Observed and Calculated Response of Biological Solids of System No. 6 to Change in Growth-Limiting Nutrient Concentration in the Feed

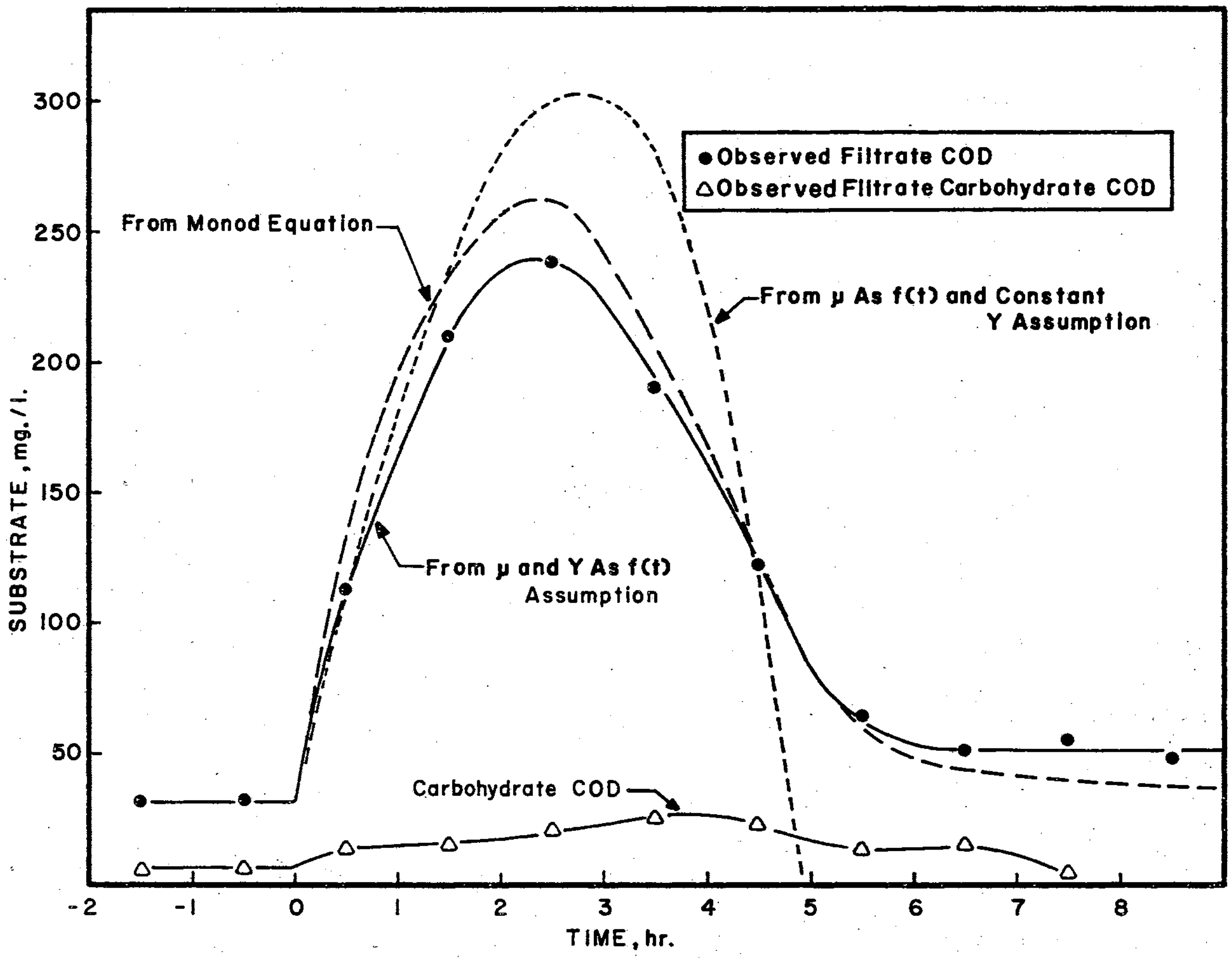

Figure 19 – Observed and Calculated Response of Substrate of System No. 6 to Change in Growth-Limiting Nutrient Concentration in Feed

deviations from the functions will not yield significant discrepancies in the curves for biological solids and substrate response. The transient specific growth rate and yield are given for the six systems in Figs. 20 through 25. A sample calculation of the biological solids and substrate response for μ and Y as functions of time using the technique outlined in Chapter IV is given in Appendix B.

Since a common assumption used in predicting transient behavior is that the yield is constant, calculation of substrate response was performed using the transient function for μ and an assumed constant Y. The value of Y used was that which gave the best agreement with the observed response. The substrate response calculated in this way was inaccurate and the value of Y did not show any agreement with the initial steady-state Y with the exception of System No. 2. The best constant Y values are given in Table III.

The plots of transient Y show an initially high yield with the exception of System No. 5. Since this result can not be considered a unique interpretation of the observations a different method was used to illustrate this phenomenon. Average yields were calculated over an interval of time following the initiation of the transient-state. The length of the interval was selected to correspond with the time required for the decrease of the initially high yield to occur. These time intervals ranged fron one-half to three hours. The average yield calculated is not a

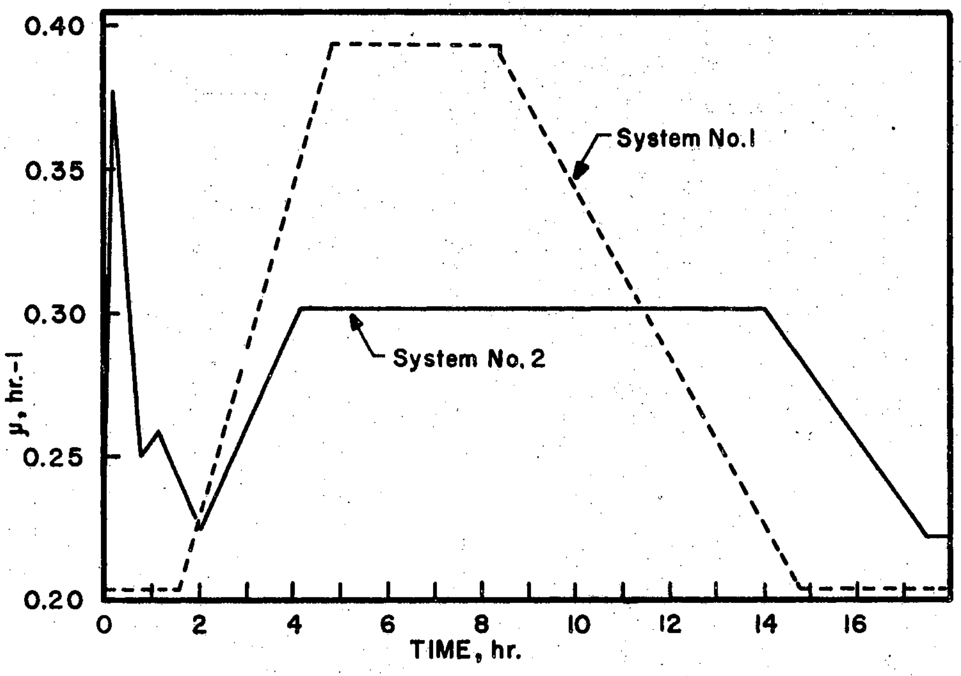

Figure 20 - Transient Specific Growth Rate For Systems Nos. 1 and 2

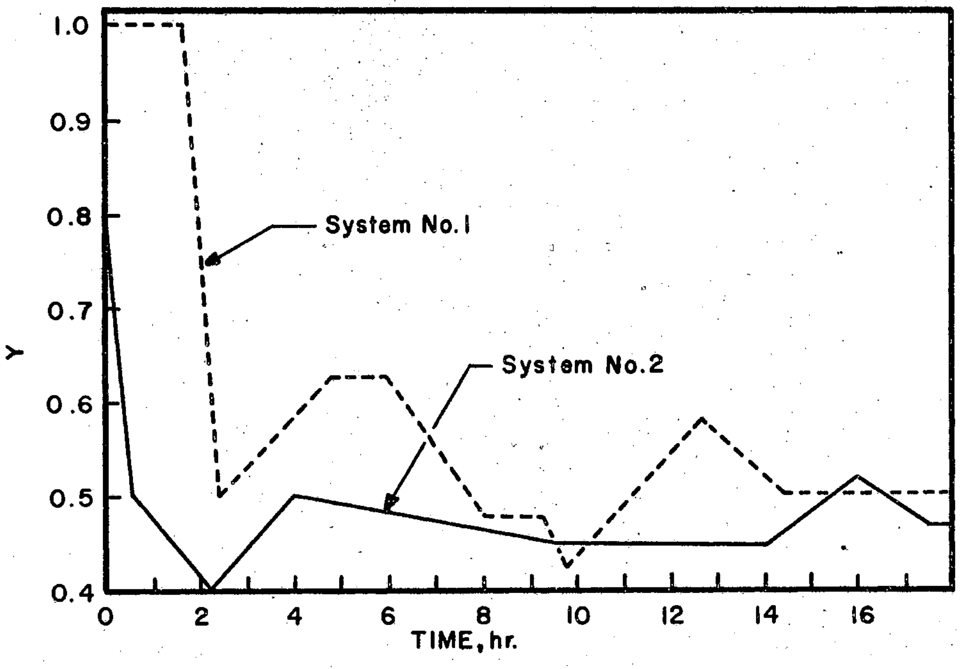

Figure 21 - Transient Yield for Systems Nos. 1 and 2

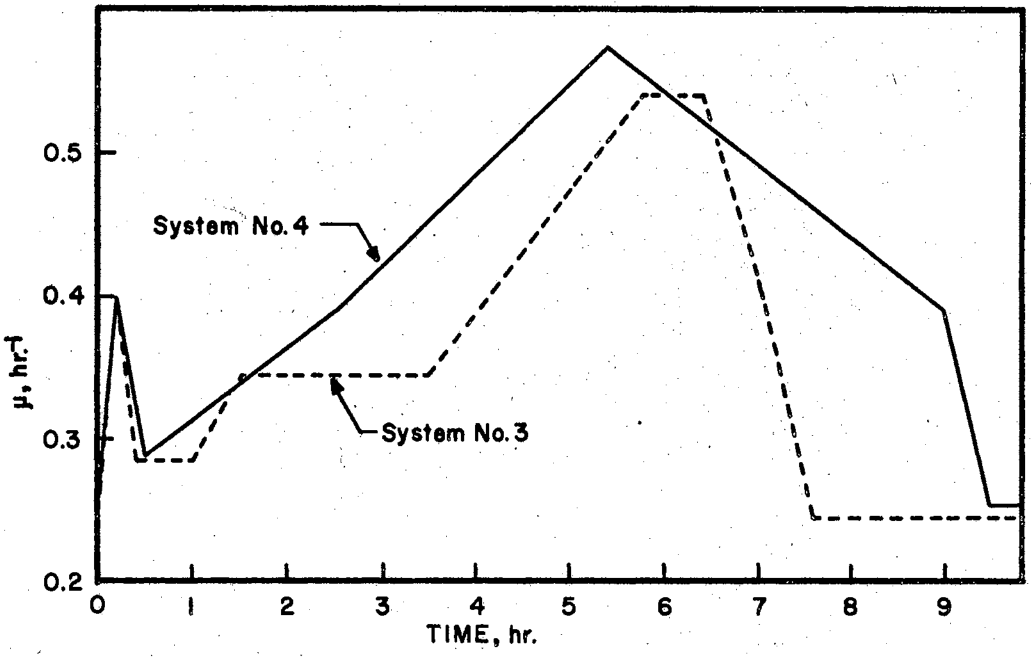

Figure 22 - Transient Specific Growth Rate for Systems Nos. 3 and 4

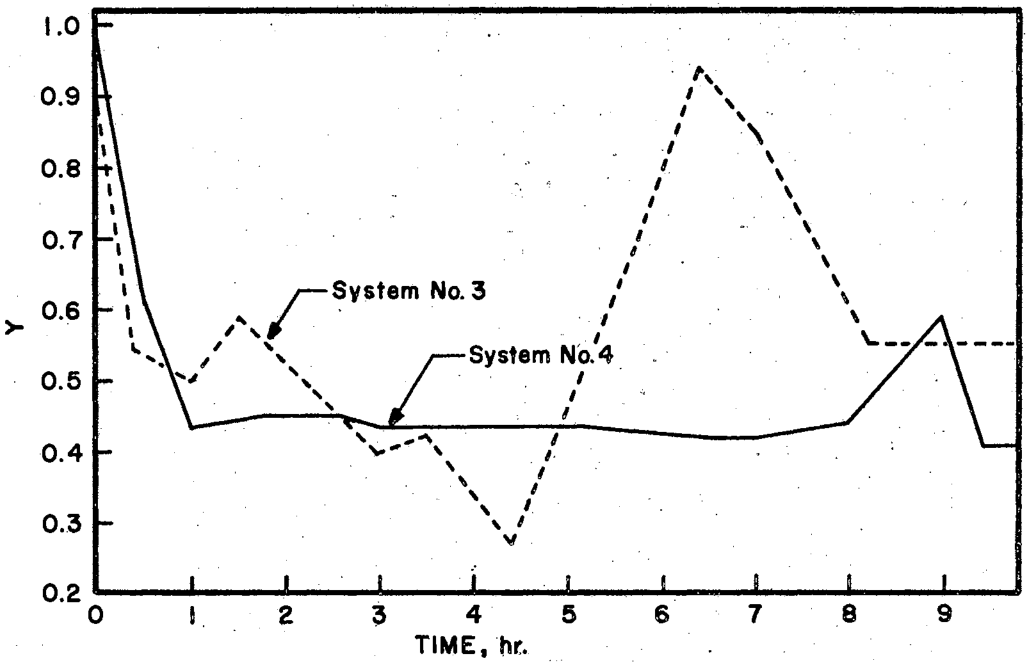

Figure 23 - Transient Yield for Systems Nos. 3 and 4

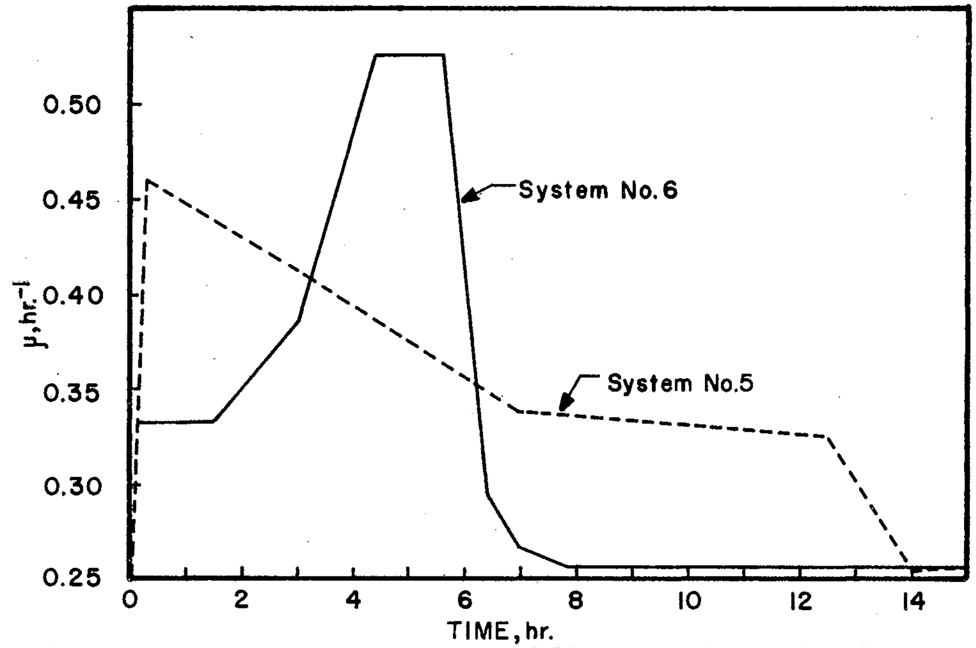

Figure 24 - Transient Specific Growth Rate for Systems Nos. 5 and 6

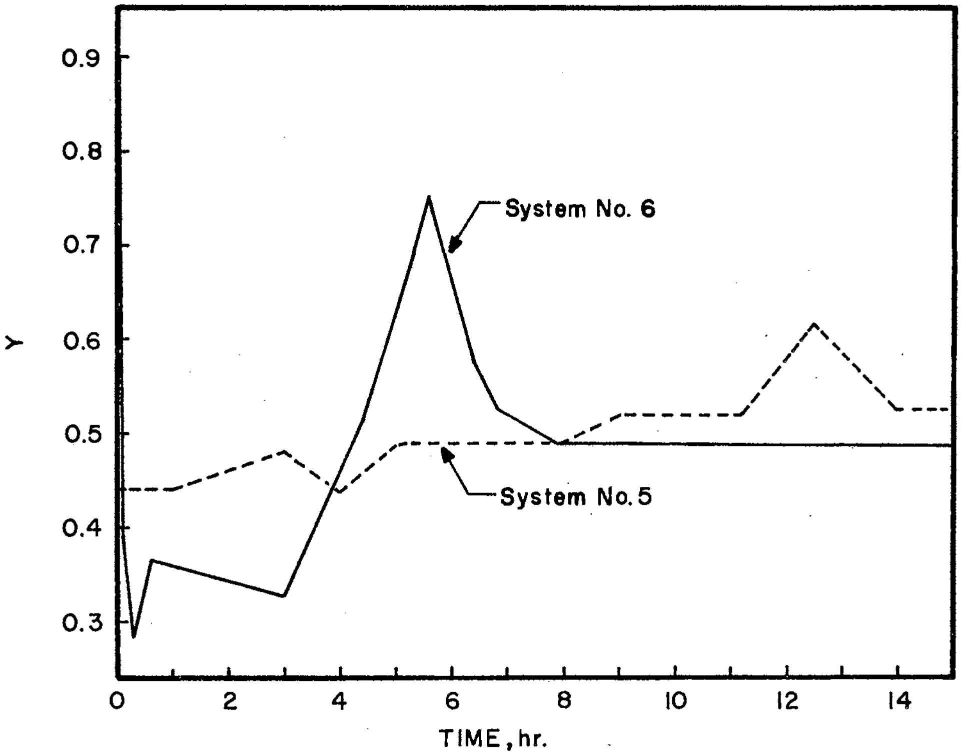

Figure 25 - Transient Yield for Systems Nos. 5 and 6

TABLE III

CALCULATED PARAMETERS

System	μ_m, hr.$^{-1}$ From μ As f(t) Assumption	K_s, COD mg/l From μ_m And Initial S	Y For Best Substrate Prediction from μ As f(t) Assumption
No. 1	0.394	170.0	0.600
No. 2	0.304	7.4	0.480
No. 3	0.540	106.0	0.460
No. 4	0.390	139.2	0.415
No. 5	0.460	205.0	0.490
No. 6	0.528	33.6	0.480

time average of Y but rather what Y would have to be for the substrate and biological solids concentration to reach the values observed at the end of the time interval starting from the observed values at the initiation of the transient-state. These values of Y were calculated from the solution of the rate equations provided by Moser, equation (10).

D. Prediction by the Monod Equation

The maximum transient value of the specific growth rates were used as μ_m in the Monod equation. The maximum specific growth rate occurred at and after the point of maximum transient substrate concentration for Systems Nos. 1 and 2; it occurred after the point of maximum substrate concentration for Systems Nos. 3, 4, and 6; it occurred before the point of maximum substrate concentration for System No. 5. The value of K_s is calculated from μ_m, D, and the initial steady-state substrate concentration, S_o, and the rearranged Monod equation:

$$K_s = \frac{S\cdot(\mu_m - D)}{D} \tag{23}$$

These Monod parameters are given in Table III.

Maximum specific growth rates were measured in batch experiments for Systems Nos. 3, 4, 5, and 6. The maximum batch specific growth rate for System No. 3 was approximately equal to the maximum transient value. For Systems Nos. 4, 5, and 6 the maximum batch specific growth rate was

TABLE IV

SPECIFIC GROWTH RATES

System	Maximum Transient Specific Growth Rate Hr.$^{-1}$	Batch Maximum Specific Growth Rates, Hr.$^{-1}$		
		Inoculation Time, Hr.	500 mg/l Glucose	1000 mg/l Glucose
No. 3	0.5400	0.0	0.54	0.54
		5.5	0.50	0.57
No. 4	0.3900	0.0	0.56	0.59
		2.0	0.63	0.63
		5.0	0.65	0.65
No. 5	0.4600	0.0	0.59	0.61
		2.0	0.58	0.59
		5.0	0.50	0.53
No. 6	0.5275	0.0	0.61	0.61
		2.0	0.75	0.75
		5.0	0.69	0.74

greater than the maximum transient value. The inoculation time given in Table IV corresponds to the time after initiation of the transient when the reactor initiation of the transient effluent was collected for observation of batch specific growth rates. The corresponding growth rates are also given in Table IV.

The maximum transient specific growth rate was used in the Monod equation rather than the batch maximum because plots of observed substrate concentration vs. transient specific growth rate which are discussed below were in agreement with Monod curves based on the maximum transient specific growth rate. It was not within the scope of this research to determine the possible differences in the Monod parameters derived from batch and continuous cultures because of the differences in concentration of biological solids. This has been briefly discussed by Ramanathan (11).

The initial steady-state values of Y given in Table II were used in obtaining predictions by application of the Monod equation. In all techniques using the Monod equation in prediction of transient response, the initial steady-state value is used and is assumed to be constant.

The predictions of biological solids and substrate response using the Monod equations for the six systems were given in Figs. 8 through 19. The predicted response using the Monod equation does not accurately represent the observed transient response. As noted previously, Monod parameters selected for best fit which do not necessarily represent any experimental observation do not produce satisfactory predictions of transient response.

The numerical technique discussed above was used to obtain the solution of the rate equations employing the Monod equation. A sample calculation is given in Appendix C.

E. Transient Specific Growth Rate - Substrate Concentration Observation

The techniques which employ the Monod equation for prediction of transient response imply by its use that a single unique specific growth rate exists for any one substrate concentration. While this is true for many systems growing at steady-state it has not been established experimentally for transient states. It should be noted that Monod did not claim that his empirical expression could be used in describing the transient-state following a change in external environment in a completely mixed continuous flow reactor.

Reactor effluent from Systems Nos. 3, 4, 5, and 6 was used to inoculate batch cultures at various times, immediately prior to initiation of the transient, and during the transient. High substrate concentrations in the batch cultures, 500 and 1000 mg/l glucose, insured specific growth rates approaching μ_m.

The growth responses of Systems Nos. 3 and 4 are shown in Figs. 26 and 27. When inoculated with steady-state reactor effluent, these systems show a lag before μ_m is reached. When inoculated with reactor effluent during the transient and after the population had been exposed to higher substrate concentrations in the reactor, no lag was detectable.

Systems Nos. 5 and 6 did not show a lag upon inoculation with steady-state reactor effluent. While this does not allow any general conclusions about similar conditions, the lag observed in Systems Nos. 3 and 4 indicates that the use of the Monod equation in some cases may not be valid for transient conditions depending upon the magnitude of the change in substrate concentration. In the present study it is emphasized that the change was quite high.

On plots of the Monod equation derived from parameters given in Table III the transient specific growth rate, determined by the approximation of μ by linear functions with time, was plotted vs. the corresponding observed substrate concentration for Systems Nos. 1, 2, 3, and 6, shown in Figs. 28, 29, 30, and 31 respectively.

This plot was not made for Systems Nos. 4 and 5 because their initial and final steady-states could not be approximated by the Monod equation and therefore any analogy with the Monod equation would be unproductive.

The plot of transient specific growth rate vs. substrate concentration for System No. 1, Fig. 28, shows the transient performance leaving the Monod curve at the ini-

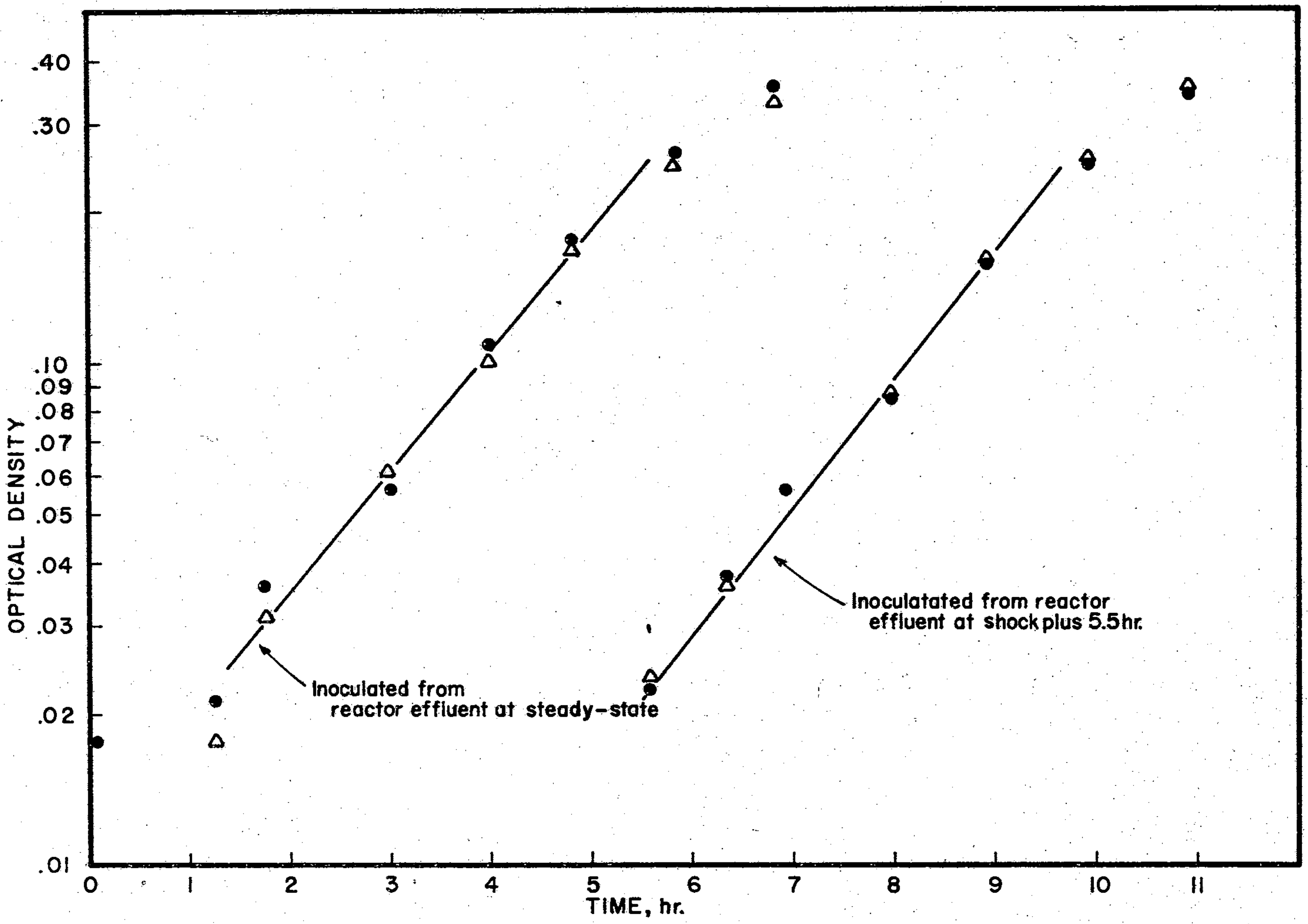

Figure 26 - Batch Systems of 1000 mg/l Glucose Medium Inoculated at Different Times During Observation of System No. 3

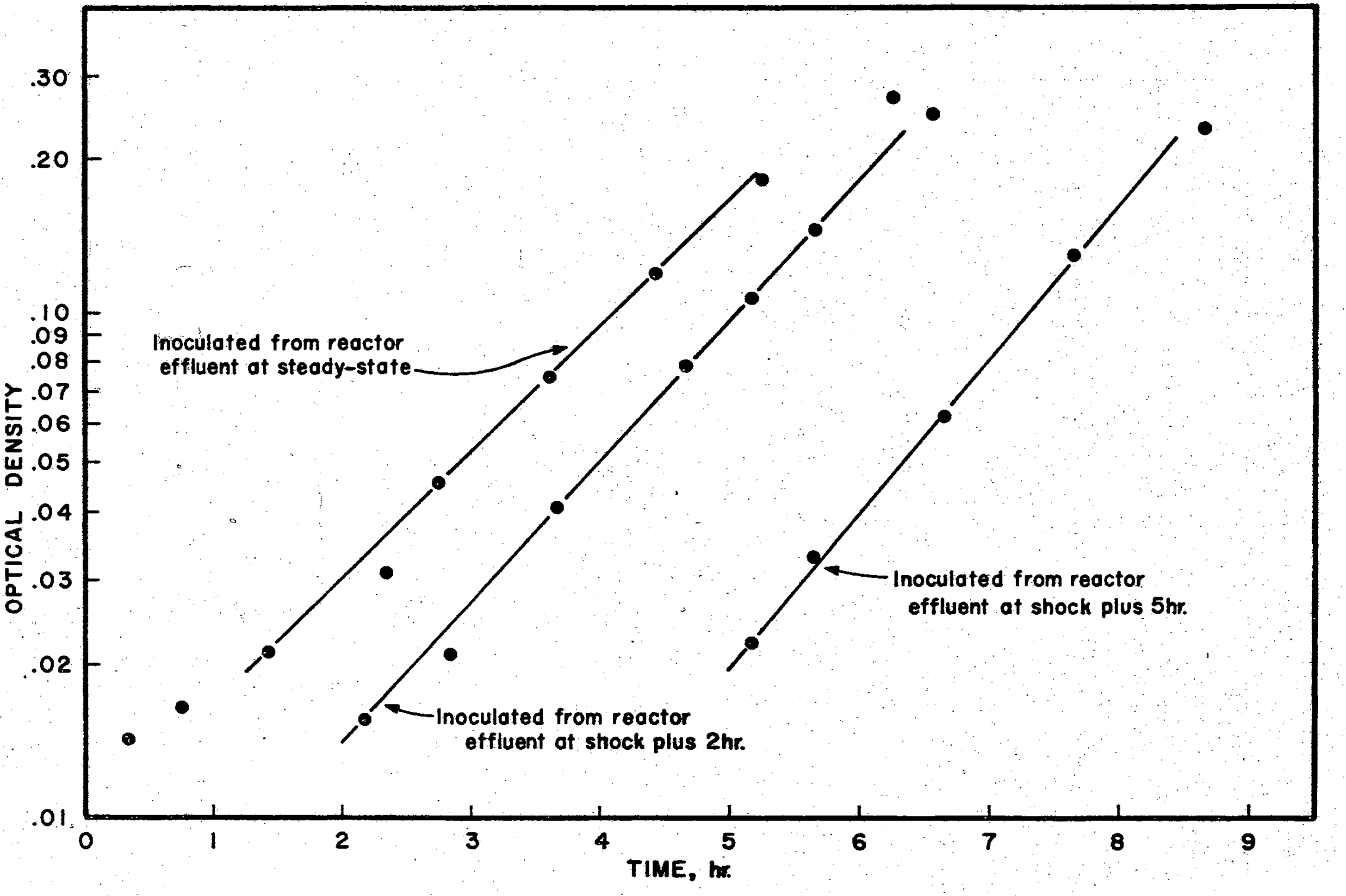

Figure 27 - Batch Systems of 1000 mg/l Glucose Medium Inoculated at Different Times During Observation of System No. 4

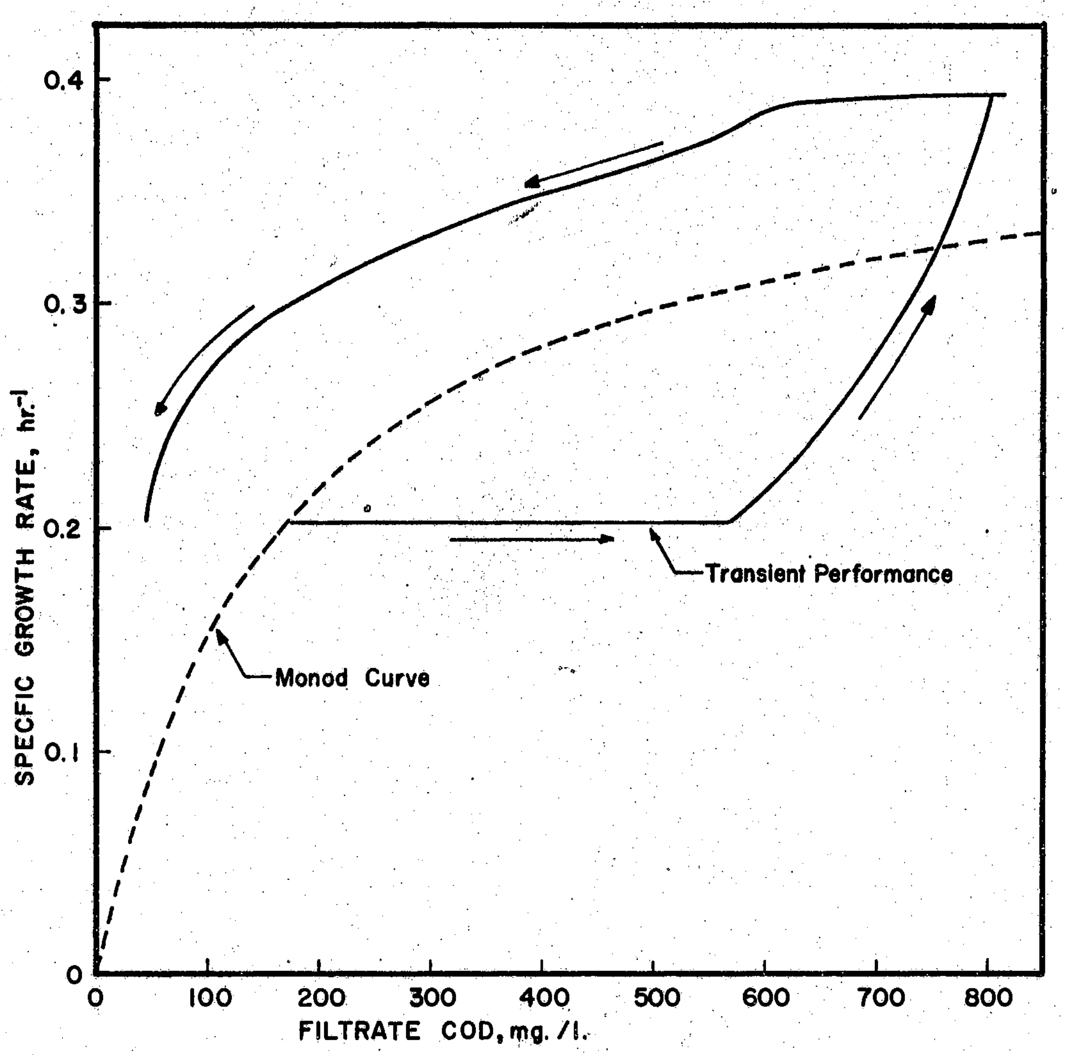

Figure 28 - Plot of Substrate Concentration vs. Specific Growth Rate Showing Transient Performance of System No. 1 and Steady-State Monod Curve

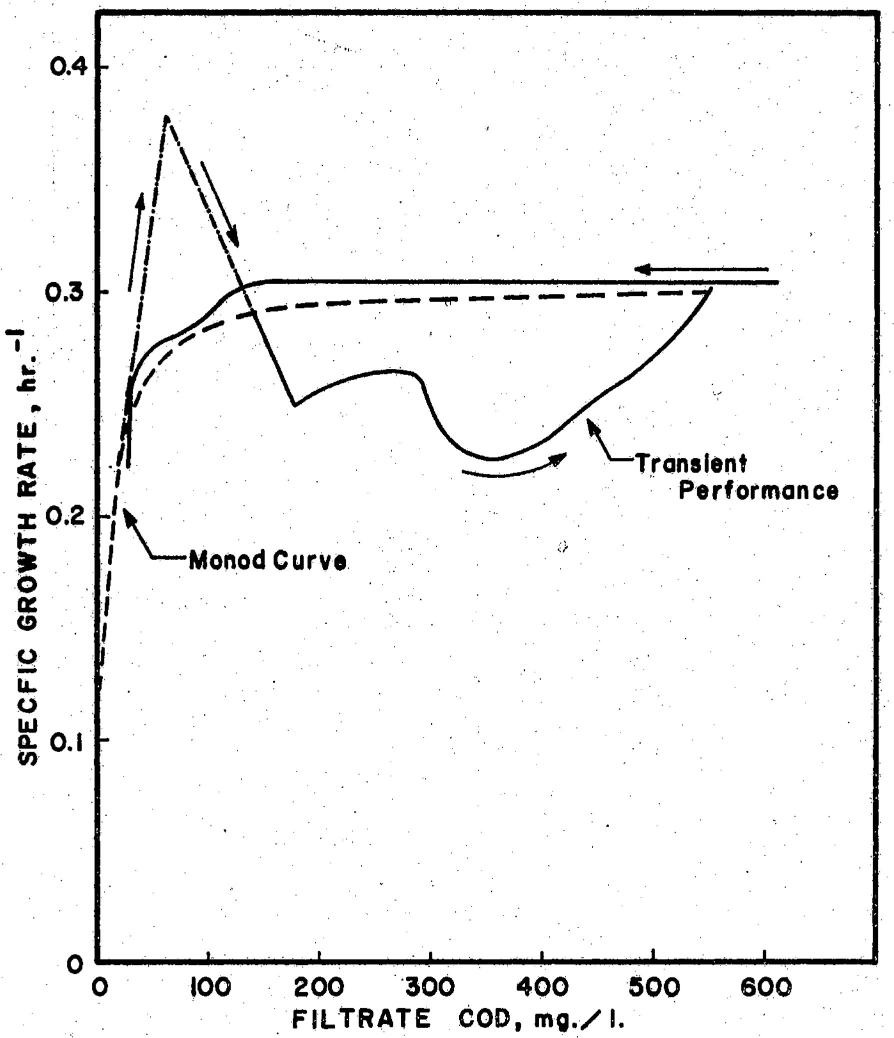

Figure 29 - Plot of Substrate Concentration vs. Specific Growth Rate Showing Transient Performance of System No. 2 and Steady-State Monod Curve

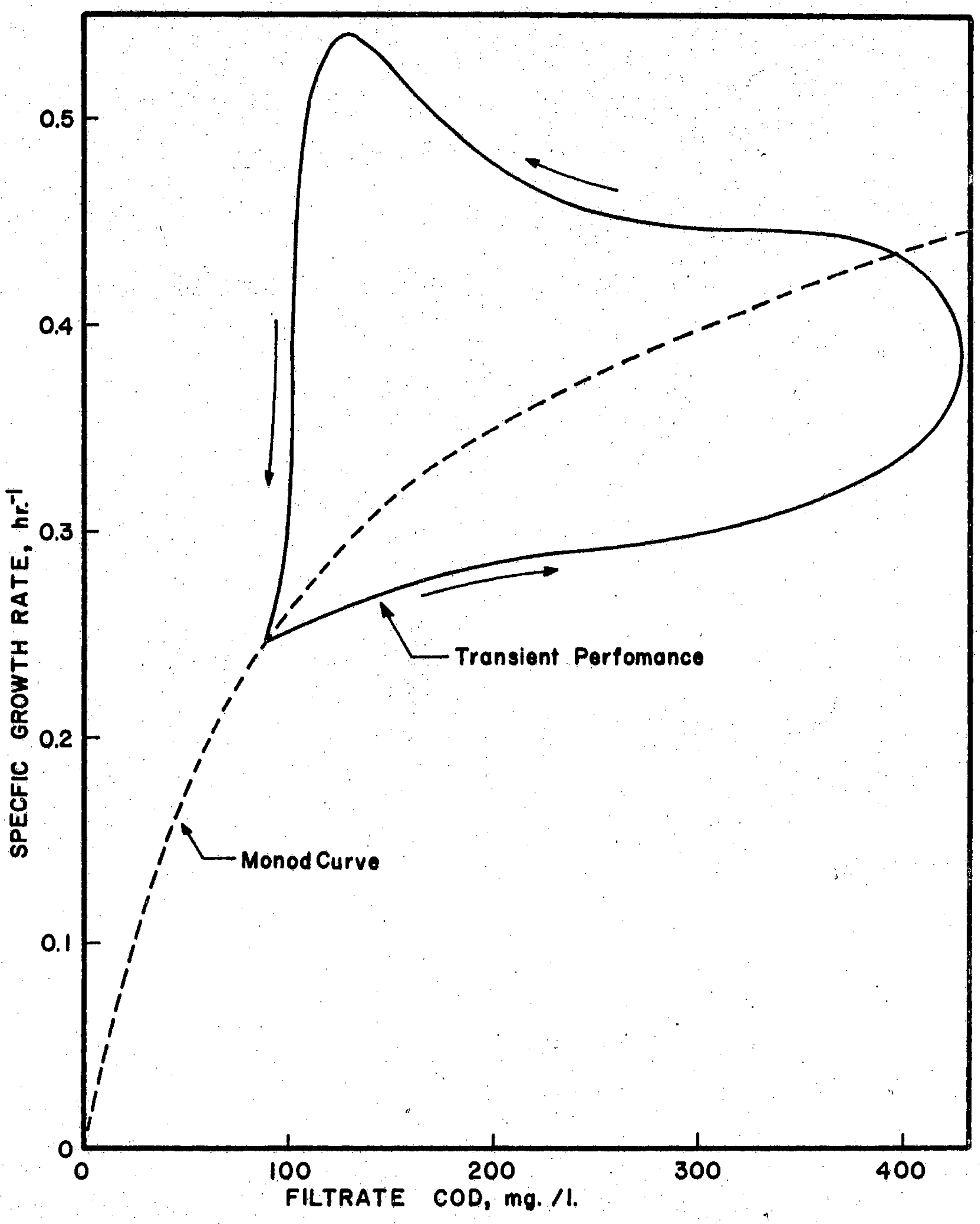

Figure 30 - Plot of Substrate Concentration vs. Specific Growth Rate Showing Transient Performance of System No. 3 and Steady-State Monod Curve

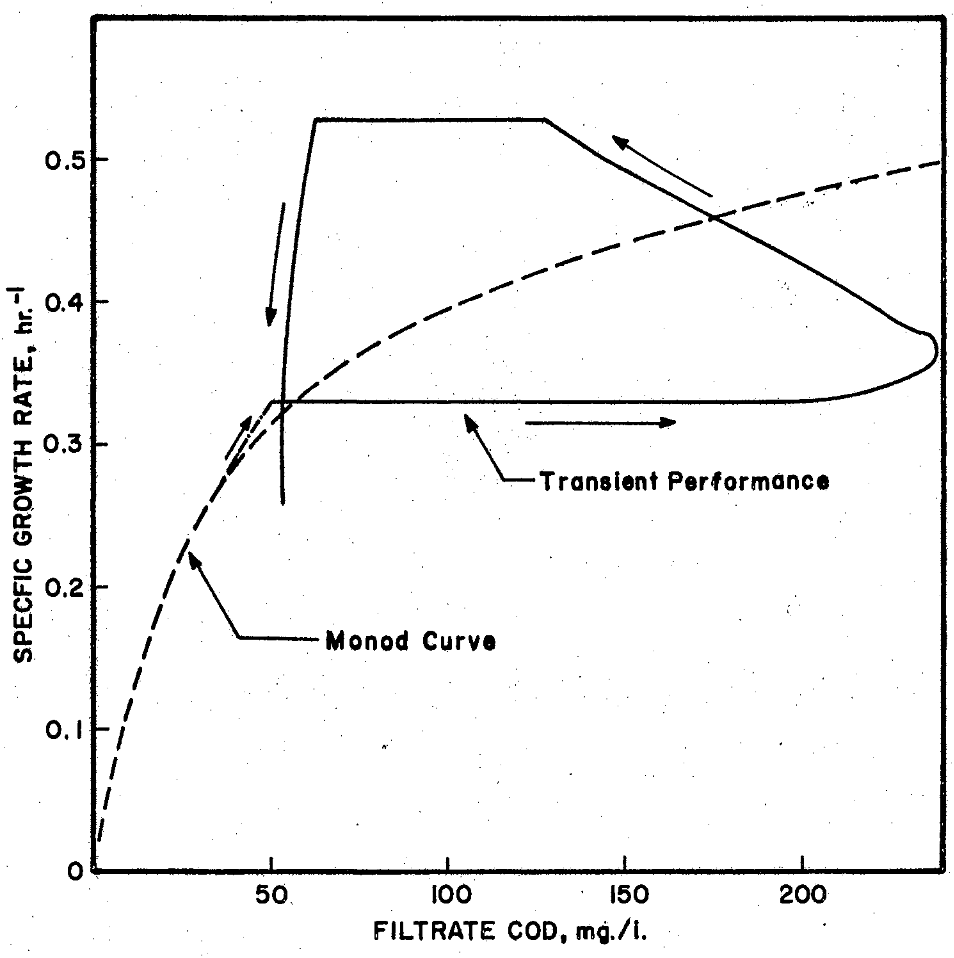

Figure 31 - Plot of Substrate Concentration vs. Specific Growth Rate Showing Transient Performance of System No. 6 and Steady-State Monod Curve

tiation of the transient. During the initial stages of the transient as the substrate concentration increased the transient specific growth rate was less than the steady-state specific growth rate. As the substrate concentration approached the maximum, the transient specific growth rate became greater than the steady-state specific growth rate and then decreased with decreasing substrate concentration while remaining greater than the steady-state specific growth rate. The transient performance terminated as a new steady-state was reached and the transient specific growth rate became equal to the dilution rate. The transient performance curve did not close at the point of its initiation indicating some deviation from the Monod equation theory.

The performance of System No. 2, shown in Fig. 29, was similar to that of System No. 1. However, as indicated by the dashed portion of the transient performance curve, immediately following initiation of the transient there was a rise in transient specific growth rate. This was the result of a rise in biological solids concentration of 20 mg/l during the first 0.8 hr. following initiation of the transient.

In the period of rising substrate concentration, the performance of System No. 3, shown in Fig. 30, was similar to that of Systems Nos. 1 and 2. However, as the substrate concentration began to decrease, the transient specific growth rate continued to rise before returning to the steady-state value.

System No. 6, shown in Fig. 31, like System No. 2 initially exceeded the steady-state specific growth rate, then continued below the Monod curve and finally crossed it to return to the steady-state condition.

These four systems show variety in their transient behavior when examined in this way but each has a common property. This property is the double-valued nature of the transient specific growth rate, lower than the steady-state value during periods of increasing substrate concentration and greater than the steady-state value during periods of decreasing substrate concentration.

CHAPTER VI

DISCUSSION OF RESULTS

The kinetic properties of transient-states have been examined by interpretation of observed transient-states. These kinetic properties, the transient yield and the transient specific growth rate - substrate concentration relationship, are discussed below. Also discussed are the extension of the Monod equation and constant yield assumption to transient-states and the effect of the production of intermediates on the transient behavior.

A. Transient Yield

The transient yield curves show that the yield is not constant and that a variety of responses can be expected even when the imposed system conditions are approximately the same. The only common characteristic of the systems is the high value of the yield immediately following initiation of the transient-state. This occurred in all cases except System No. 5.

In each of these five systems, the yield is shown to begin at the high value at time zero. The technique employed for the determination of transient yield is not sufficient to determine the kinetic properties of the transient-state in the small interval of time where the yield changes from its initial steady-state value to the high transient value.

The transient yield functions can not be considered unique; that is, slight deviations from the assumed functions will not result in significant variations in the predicted substrate response. Also, the approximation of Y as f(t) is not necessary in order to show that Y is not constant during the transient-state; this is illustrated from the substrate response predictions using constant Y. The observed transient responses shown here do not reveal the nature of Y during the transient-state without the aid of mathematical interpretation. The definition of Y used to obtain the kinetic nature of Y is the one provided by Monod, given in equation (9). Rearrangement of equation (9) gives:

$$Y = \left(\frac{dX}{dt}\right)_g \Big/ \left(\frac{dS}{dt}\right)_g \qquad (24)$$

It is not meant to be inferred that the transient value of Y as f(t) is the end to which kinetic interpretation should be taken. This is presented only as a tool of interpretation and observation; like other kinetic models of chemical systems, the end result should essentially be prediction of response based solely on initial conditions.

B. Transient Specific Growth Rate and Substrate Concentration

As does the transient yield, the transient specific growth rate for the six systems shows that a variety of responses can be expected for similar conditions.

Like the transient yield determination, Y as f(t), the determined transient specific growth rate is solely a method for prediction of transient responses. The plots for the four systems, Nos. 1, 2, 3, and 6, of the transient specific growth rate vs. the observed substrate concentration, in Figs. 28, 29, 30, and 31 respectively, show general agreement with the argument of Perret concerning growth rate hysteresis.

Systems Nos. 4 and 5 do not obey either master reaction equation since both Monod's and Moser's equations require that the steady-state substrate concentrations be the same for any one dilution rate regardless of the influent concentration, therefore an analysis of these systems for the growth rate hysteresis effect is not possible. This does not detract from the general kinetic property of growth rate hysteresis, only from the general applicability of equations of the type of Monod's or Moser's.

C. Transient Predictions Using the Monod Equation and Constant Yield

Transient predictions using the Monod equation and constant yield are inaccurate in theory and practice. The yield has been shown to vary considerably and the transient specific growth rate to be double-valued for a given change in substrate concentration. The method of transient prediction did not require any simplification of the Monod equation; this would make it, in terms of past use, the most general solution.

In theory, the methods which employ simplifications of the Monod equation were not supposed to give better results than the general solution. However, for particular conditions the simplified methods may give better results since the Monod equation is not a valid representation of transient performance.

It can be seen in Figs. 8 through 19 that to varying degrees the shape of the response is produced by the use of the Monod equation. This superficial agreement has probably contributed to the acceptance of the Monod equation for transient-states. The difficulty of obtaining solutions to the rate equations incorporating the Monod equation (a numerical integration technique must be used) has inhibited examinations which might have led to early corrections of the error.

D. Effect of The Production of Intermediates on Transient Behavior

The production of intermediates during the transient-state may be responsible for some of the unexplained transient behavior such as the considerable variation in the transient yield and the high transient specific growth rate of System No. 3 at relatively low substrate concentration. The balance of intermediates and the quality produced during some transient-states may not be comparable to any steady-state medium composition even though the concentration of the total carbon source as COD is the same. Since the specific growth rate and the yield are in many cases substrate-dependent, some of the transient behavior is attributable to changing substrate composition.

There is no contradiction between this argument and the measurement of growth-limiting nutrient concentration as COD for the description of steady-states of continuous cultures by the Monod equation. Generally for heterogeneous populations large accumulations of metabolic intermediates do not occur under steady-state operation (11). All that is required for the empirical equation of Monod to be valid for the prediction of steady-state behavior related to substrate measured as chemical oxygen demand is for the substrate complement to be reproducible for a given dilution rate.

E. Application of This Study to Activated Sludge Processes

It has been shown under the experimental conditions of the present study that transient-states can not be described adequately by use of the Monod equation. Although the transient performance of most activated sludge plants would be somewhat different because of the recycling of biological solids, the general kinetic property of the specific growth rate lagging the steady-state specific growth rate during the initial stages of the transient and leading it during the latter part would be expected to be exhibited. Solids recycle might lengthen the time for the system to respond to the environmental change, that is, lengthen the time for the specific growth rate to increase as a result of higher substrate concentrations, because cell recycle would permit cells to exist in the system which were "older" than those which would be present without cell recycle. However, loadings of equal magnitude would produce lower transient substrate concentrations where solids are returned to the reactor and such systems might be capable of assimilating greater shock loads than "once through" systems.

If the systems employed in the study (analogous to dispersed phase aeration) were returned to their original steady-state (if indeed they were capable of returning to their original steady-state), it is not possible to say, based on our present understanding of the transient response, whether these systems would respond in the same way to a change identical to the one which caused the observed transition.

Systems already in transient or in continual transient might show responses to further changes depending on their properties at the beginning of the additional alteration of their environment. However, the properties which affect their behavior, the complement of biochemical constituents (in the transport system, in the synthetic and degradative pathways, in the synthesis of enzymes, etc.) and their corresponding activities, would be expected to depend upon how the environment has been changing. The response immediately following deviation from the steady-state would be expected to be characteristic of the imposed change and the previous steady-state.

For the systems observed here, the environmental alteration which causes the transition, i.e., the substrate concentration, continually changes until the new steady-state is reached. The response at any time would be a function of how the substrate is and has been changing, what the response to these changes has been, and the previous steady-state.

Activated sludge processes, many of which are in continual transient, would be expected to exhibit response to shock loads dependent upon their recent history.

CHAPTER VII

CONCLUSIONS

The phenomenon of growth rate hysteresis theorized by Perret to be the result of the implicit kinetic structure of the bacterial system has been demonstrated experimentally. This behavior was similar for all systems whose steady-states were describable by the Monod equation.

The transient responses, which were in principle similar, show that prediction of transient kinetic behavior at best would be difficult.

Attempts to predict transient behavior by use of the Monod equation and constant yield should be abandoned.

Methods used for the prediction of transient response based upon the Monod equation have been shown to be incorrect in theory and insufficient for practical application; furthermore, they can not be modified by any rational method for accurate results. The characteristics of transient-states are best found by observation of a variety of systems followed by interpretation to delineate the general properties. It is evident from the variety of responses shown here that any method of prediction of transient response from initial and imposed conditions will require more extensive examination of the biochemical structure which affects the kinetic properties. However, for particular microbial rate processes such as the activated sludge process numerical characterization of responses based on monitored parameters and operating conditions may be found. These descriptions may be used for process control and consequent improvements of effluent quality.

CHAPTER VIII

SUGGESTIONS FOR FUTURE STUDY

As a result of this investigation, the following suggestions are made for future study of the kinetic properties of continuous cultures in the transient-state.

1. The parameters observed in this study did not reveal any method for predicting transient response. An intensive examination of the biochemical structure of the bacterial system before, during, and after the transient-state might provide a basis for a method of predicting transient response or, in any event, might illuminate the complexities which make prediction difficult or impractical.

2. As a corollary to this study the kinetic properties of the continuous culture in transient should be investigated for transient-states induced by decreasing the concentration of the limiting nutrient in the feed.

3. This study has led to the supposition that the kinetic properties of one transient-state may not be reproducible by returning the system to its original steady-state and imposing an environmental change identical to the first. This would be somewhat analogous to the problem of pulsating shock loads in the activated sludge process. This would be an important area of study from the stand-

point of theoretical bacterial kinetics and from the standpoint of application to pulsating shock loads in the activated sludge process. With respect to application to the activated sludge process, the pulsating time interval may not correspond to the time required for a new steady-state to be reached.

4. The method of interpreting the kinetic properties of the transient developed in this study is particularly amenable to the investigation of hydraulic shock loads. This is of particular importance because the growth rate hysteresis analogy should be valid for the hydraulic shock; it may not be valid in the case of qualitative shock loads.

5. The kinetic properties of the activated sludge process employing cell feedback should be investigated by the techniques outlined in this study. The addition of biological solids recycle will require modification of the differential rate equations but the general format of the computational procedure will remain the same. Such an investigation will require additional parameters such as solids recycle rate, concentration of solids in the recycle and the aeration vessel, reaeration time (if used), response to changes in the solids separation device, etc. The result of investigation of the kinetic properties of the activated sludge process should be the establishment of controls and changes in operational procedures coupled with the monitoring of environmental conditions to improve the treatment efficiency of plants subjected to shock loads.

Repeated observation of transient-states in continuous cultures employing biological solids recycle can do much to establish the general properties of the activated sludge process subjected to shock loads, e.g., characterization of system capacities and time required for the achievement of a new steady-state. This knowledge may find extensive use long before predicted response based on a theory of bacterial growth is achieved.

6. The investigation of the transient properties of pure cultures of microorganisms while not expected to produce appreciably different results than those given here would serve to allow the separation of effects caused by population dynamics. This would be of particular significance in systems subjected to persistent transients such as pulsating shock loads.

7. The amount of intermediates present during the transient varied considerable within the study. Some difference may exist between the composition of the nutrients found during the transient and that found at various dilution rates in steady-state continuous cultures. It is suggested that the nutrient composition during the transient-state be compared with that of several steady-states of varying dilution rates, particularly at dilution rates approaching the maximum specific growth rate. Differences in the nutrient composition may be reflected in differences in the maximum steady-state specific growth rate and the maximum transient specific growth rate.

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APPENDIX A

LIST OF SYMBOLS

a - constant from linear approximation of μ, hr^{-1}

a' - constant from linear approximation of Y

b - constant from linear approximation of μ, hr^{-2}

b' - constant from linear approximation of Y, hr^{-1}

D - dilution rate, hr^{-1}

f - time beginning linear approximation of μ or Y, hr.

K_s - system dependent constant from Monod equation, mg/l

S - substrate concentration in the reactor, mg/l COD

S_i - substrate concentration in the feed during transient-state, mg/l COD

S_{i_o} - substrate concentration in feed at initial steady-state, mg/l COD

S_n - substrate concentration at beginning of time interval, mg/l COD

S_{n+1} - substrate concentration at Δt after S_n, mg/l COD

S_o - substrate concentration at initiation of transient-state, mg/l COD

S' - first derivative of S

S'' - second derivative of S

S''' - third derivative of S

t - time after initiation of transient-state, hr

X - biological solids concentration, mg/l

X_f - biological solids concentration at time f, mg/l

X_n - biological solids concentration at beginning of time interval, mg/l

X_{n+1} - biological solids concentration at Δt after X_n, mg/l

X_t - biological solids concentration at time t, mg/l

X' - first derivative of X

X'' - second derivative of X

X''' - third derivative of X

Y - yield of biological solids per unit of substrate consumed

z - exponent for prediction of transient biological solids concentration from linear approximation of μ.

λ - system dependent constant from Moser equation

μ - specific growth rate, hr^{-1}

μ_m - maximum specific growth rate, hr^{-1}

COD - chemical oxygen demand

APPENDIX B

SAMPLE CALCULATION OF BIOLOGICAL SOLIDS AND SUBSTRATE RESPONSE FROM μ AND Y AS f(t) ASSUMPTION

Assumed μ and Y functions:

$$\mu = a - b(t-f) \tag{17}$$

$$Y = a' - b'(t-f) \tag{19}$$

Solids response may be calculated from the equation:

$$X_t = X_f \cdot e^z \tag{18}$$

where:

$$z = [(a-b\cdot f-D)\cdot(t-f)] + [b\cdot(t^2-f^2)/2]$$

Substrate response may be calculated from Taylor series expanded to the third derivative:

$$S_{n+1} = S_n + \Delta t\cdot S' + \Delta t^2\cdot S''/2! + \Delta t^3\cdot S'''/3!$$

The derivatives of S and X must be found.

First derivative of X:

$$X' = \mu\cdot X - D\cdot X \tag{6}$$

Second derivative of X:

$$X'' = (a+b \cdot t-D) \cdot X' + b \cdot X$$

First derivative of S:

$$S' = D \cdot S_i - D \cdot S - \mu \cdot X/Y \qquad (7)$$

Second derivative of S:

$$S'' = -D \cdot S - X' \cdot \mu/Y - X \cdot b/Y + X \cdot \mu \cdot b'/(Y^2)$$

Third derivative of S:

$$S''' = -D \cdot S'' - X'' \cdot \mu/Y - 2 \cdot X' \cdot b \cdot b'/(Y^2) - 2 \cdot X \cdot \mu \cdot b' \cdot b'/(Y^3)$$

From System No. 2:

From Table II:

$$D = 0.222 \text{ hr}^{-1}$$

$$S_i = 1622 \text{ mg/l COD}$$

From calculation result:

X @ 1 hr after initiation of transient-state =
267.9 mg/l

S @ 1 hr after initiation of transient-state =
216.7 mg/l COD

Using time increment, Δt, equal to 0.005 hr the calculation results of solids and substrate concentration for 1 to 1.20 hr after initiation of transient-state are given below.

CALCULATION RESULTS FOR BIOLOGICAL SOLIDS AND SUBSTRATE RESPONSE FROM
LINEAR APPROXIMATION OF μ AND Y FOR SYSTEM NO. 2
TIME: 1-1.20 HR.

t	X_n	S_n	Y	μ	X'	X''	S'	S''	S'''	z	e^z	X_{n+1}	S_{n+1}
1.000	267.900	216.700	0.47469	0.25881	9.863	-9.549	165.909	-40.560	14.767	0.00018	1.00018	267.949	217.529
1.005	267.949	217.529	0.47438	0.25863	9.815	-9.555	165.706	-40.486	14.753	0.00037	1.00037	267.999	218.357
1.010	267.999	218.357	0.47406	0.25844	9.767	-9.560	165.504	-40.412	14.740	0.00055	1.00055	268.048	219.184
1.015	268.048	219.184	0.47375	0.25826	9.719	-9.565	165.302	-40.338	14.727	0.00073	1.00073	268.096	220.010
1.020	268.096	220.010	0.47344	0.25807	9.672	-9.571	165.100	-40.265	14.714	0.00091	1.00091	268.145	220.835
1.025	268.145	220.835	0.47312	0.25789	9.624	-9.576	164.899	-40.191	14.701	0.00109	1.00109	268.193	221.659
1.030	268.193	221.659	0.47281	0.25770	9.576	-9.581	164.698	-40.118	14.687	0.00127	1.00127	268.241	222.482
1.035	268.241	222.482	0.47250	0.25752	9.528	-9.586	164.497	-40.004	14.674	0.00145	1.00145	268.289	223.304
1.040	268.289	223.304	0.47219	0.25733	9.480	-9.592	164.297	-39.971	14.661	0.00163	1.00163	268.336	224.125
1.045	268.336	224.125	0.47188	0.25715	9.432	-9.597	164.097	-39.898	14.648	0.00180	1.00181	268.384	224.945
1.050	268.384	224.945	0.47156	0.25696	9.384	-9.602	163.898	-39.824	14.634	0.00198	1.00198	268.431	225.764
1.055	268.431	225.764	0.47125	0.25678	9.336	-9.607	163.699	-39.751	14.621	0.00215	1.00216	268.478	226.582
1.060	268.478	226.582	0.47094	0.25659	9.288	-9.612	163.500	-39.678	14.608	0.00233	1.00233	268.524	227.399
1.065	268.524	227.399	0.47062	0.25641	9.240	-9.617	163.302	-39.605	14.595	0.00250	1.00250	268.570	228.215
1.070	268.570	228.215	0.47031	0.25622	9.192	-9.623	163.104	-39.532	14.582	0.00267	1.00267	268.617	229.030
1.075	268.617	229.030	0.47000	0.25604	9.144	-9.628	162.106	-39.459	14.568	0.00284	1.00285	268.662	229.844
1.080	268.662	229.844	0.46969	0.25585	9.096	-9.633	162.709	-39.386	14.555	0.00301	1.00302	268.708	230.657
1.085	268.708	230.657	0.46938	0.25567	9.047	-9.638	162.512	-39.314	14.542	0.00318	1.00319	268.753	231.469
1.090	268.753	231.469	0.46906	0.25548	8.999	-9.643	162.316	-39.241	14.529	0.00335	1.00335	268.798	232.280
1.095	268.798	232.280	0.46875	0.25530	8.951	-9.647	162.119	-39.168	14.515	0.00351	1.00352	268.843	233.090
1.100	268.843	233.090	0.46844	0.25511	8.903	-9.652	161.924	-39.096	14.502	0.00368	1.00369	268.888	233.899
1.105	268.888	233.899	0.46813	0.25493	8.854	-9.657	161.728	-39.023	14.489	0.00385	1.00385	268.932	234.708
1.110	268.932	234.708	0.46781	0.25474	8.806	-9.662	161.533	-38.951	14.476	0.00401	1.00402	268.977	235.515
1.115	268.977	235.515	0.46750	0.25456	8.758	-9.667	161.338	-38.878	14.463	0.00417	1.00418	269.020	236.321
1.120	269.020	236.321	0.46719	0.25437	8.710	-9.672	161.144	-38.806	14.449	0.00434	1.00435	269.064	237.126
1.125	269.064	237.126	0.46688	0.25419	8.661	-9.677	160.950	-38.734	14.436	0.00450	1.00451	269.108	237.931
1.130	269.108	237.931	0.46656	0.25400	8.613	-9.681	160.756	-38.662	14.423	0.00466	1.00467	269.151	238.734
1.135	269.151	238.734	0.46625	0.25382	8.564	-9.686	160.563	-38.589	14.410	0.00482	1.00483	269.194	239.536
1.140	269.194	239.536	0.46594	0.25363	8.516	-9.691	160.370	-38.517	14.396	0.00498	1.00499	269.236	240.338
1.145	269.236	240.338	0.46563	0.25345	8.467	-9.695	160.178	-38.445	14.383	0.00513	1.00515	269.279	241.138
1.150	269.279	241.138	0.46531	0.25326	8.419	-9.700	159.986	-38.374	14.370	0.00529	1.00530	269.321	241.937
1.155	269.321	241.937	0.46500	0.25308	8.370	-9.705	159.794	-38.302	14.357	0.00545	1.00546	269.363	242.736
1.160	269.363	242.736	0.46469	0.25289	8.322	-9.709	159.602	-38.230	14.344	0.00560	1.00562	269.405	243.533
1.165	269.405	243.533	0.46438	0.25271	8.273	-9.714	159.411	-38.158	14.330	0.00576	1.00577	269.446	244.330
1.170	269.446	244.330	0.46406	0.25252	8.225	-9.718	159.220	-38.087	14.317	0.00591	1.00593	269.488	245.126
1.175	269.488	245.126	0.46375	0.25234	8.176	-9.723	159.030	-38.015	14.304	0.00606	1.00608	269.529	245.920
1.180	269.529	245.920	0.46344	0.25215	8.128	-9.727	158.840	-37.943	14.291	0.00621	1.00623	269.569	246.714
1.185	269.569	246.714	0.46313	0.25197	8.079	-9.732	158.650	-37.872	14.278	0.00636	1.00638	269.610	247.507
1.190	269.610	247.507	0.46281	0.25178	8.030	-9.736	158.461	-37.801	14.264	0.00651	1.00653	269.650	248.299
1.195	269.650	248.299	0.46250	0.25160	7.982	-9.741	158.272	-37.729	14.251	0.00666	1.00668	269.690	249.089
1.200	269.690	249.089	0.46219	0.25141	7.933	-9.745	158.083	-37.658	14.238	0.00681	1.00683	269.730	249.879

APPENDIX C

SAMPLE CALCULATION OF BIOLOGICAL SOLIDS AND SUBSTRATE RESPONSE FROM RATE EQUATIONS INCORPORATING THE MONOD EQUATION

The equation for rate of change of biological solids concentration incorporating the Monod equation:

$$\frac{dX}{dt} = \mu_m \cdot \frac{S}{K_s+S} \cdot X - D \cdot X \qquad (21)$$

The equation for rate of change of substrate concentration incorporating the Monod equation:

$$\frac{dS}{dt} = D \cdot S_i - D \cdot S - \mu_m \cdot \frac{S}{K_s+S} \cdot X \cdot \frac{1}{Y} \qquad (22)$$

The solids response can be calculated from Taylor series expanded to the third derivative:

$$X_{n+1} = X_n + \Delta t \cdot X' + \Delta t^2 \cdot X''/2! + \Delta t^3 \cdot X'''/3!$$

The derivatives of X and S must be found.

Second derivative of X:

$$X'' = \mu_m \cdot [X' \cdot S + X \cdot S' - X \cdot S \cdot S'/(K_s + S)]/(K_s + S) - D \cdot X'$$

Second derivative of S:

$$S'' = -D \cdot S' - \mu_m \cdot [X' \cdot S + X \cdot S' + [X \cdot S \cdot S'/(K_s + S)]]/[Y \cdot (K_s+S)]$$

Third derivative of X:

$$X''' = \mu_m \cdot [X'' \cdot S + 2 \cdot X' \cdot S' + X \cdot S'' + (-X' \cdot S' \cdot S - X \cdot S' \cdot X' - X \cdot S \cdot S'' +$$

$$[X \cdot S \cdot S' \cdot S']/[K_s + S])/[K_s + S]]/(K_s + S) - \mu_m \cdot S' \cdot [X' \cdot S +$$

$$X \cdot S' - (X \cdot S \cdot S'/[K_s + S])]/(K_s + S)^2 - D \cdot X'$$

The substrate response calculated from the solution of the rate equations provided by Moser:

$$S = [(S_i \cdot Y) - X - ([(S_i - S_o) \cdot Y] - X_o) \cdot e^{-D \cdot t}]/Y \quad (14)$$

The values of X calculated from the Taylor series solution and the corresponding time must be used to calculate S.

From System No. 2:

From Table II:

$D = 0.222\ \text{hr}^{-1}$

$S_i = 1622$ mg/l COD

$X_o = 248$ mg/l

$S_o = 20$ mg/l COD

From Table III:

$K_s = 7.4$ mg/l COD

$\mu_m = 0.304\ \text{hr}^{-1}$

Using time increment, Δt, equal to 0.005 hr the calculation results for solids and substrate concentration for 0 to

0.20 hr after initiation of transient-state are given below.

CALCULATION RESULTS FOR BIOLOGICAL SOLIDS AND SUBSTRATE RESPONSE FROM SOLUTION OF THE RATE EQUATIONS INCORPORATING THE MONOD EQUATION FOR SYSTEM NO. 2

TIME: 0-0.20 HR.

t	X_n	S_n	X'	S'	X''	S''	X'''	X_{n+1}	S_{n+1}
0.000	248.000	20.000	-0.025	237.146	176.226	-432.105	2276.459	248.002	21.180
0.005	248.002	21.180	0.815	235.072	160.560	-398.312	2171.015	248.008	22.351
0.010	248.008	22.351	1.583	233.156	146.980	-369.012	2065.803	248.018	23.512
0.015	248.018	23.512	2.288	231.376	135.124	-343.423	1963.192	248.031	24.664
0.020	248.031	24.664	2.937	229.717	124.706	-320.932	1864.555	248.047	25.809
0.025	248.047	25.809	3.537	228.163	115.498	-301.046	1770.621	248.067	26.946
0.030	248.067	26.946	4.093	226.703	107.315	-283.369	1681.716	248.088	28.075
0.035	248.088	28.075	4.611	225.326	100.009	-267.578	1597.917	248.113	29.199
0.040	248.113	29.199	5.095	224.025	93.456	-253.408	1519.137	248.139	30.316
0.045	248.139	30.316	5.547	222.790	87.553	-240.640	1445.198	248.168	31.426
0.050	248.168	31.426	5.971	221.616	82.216	-229.090	1375.866	248.199	32.532
0.055	248.199	32.532	6.370	220.498	77.374	-218.606	1310.883	248.232	33.631
0.060	248.232	33.631	6.745	219.429	72.966	-209.056	1249.978	248.267	34.726
0.065	248.267	34.726	7.100	218.406	68.940	-200.330	1192.883	248.303	35.815
0.070	248.303	35.815	7.435	217.425	65.254	-192.334	1139.336	248.341	36.900
0.075	248.341	36.900	7.753	216.482	61.869	-184.988	1089.089	248.381	37.980
0.080	248.381	37.980	8.054	215.574	58.752	-178.220	1041.904	248.422	39.056
0.085	248.422	39.056	8.341	214.699	55.877	-171.970	997.564	248.464	40.127
0.090	248.464	40.127	8.613	213.853	53.217	-166.186	955.861	248.508	41.194
0.095	248.508	41.194	8.873	213.036	50.752	-160.820	915.607	248.553	42.257
0.100	248.553	42.257	9.121	212.245	48.463	-155.834	879.624	248.599	43.316
0.105	248.599	43.316	9.358	211.477	46.332	-151.190	844.752	248.647	44.372
0.110	248.647	44.372	9.585	210.732	44.347	-146.857	811.840	248.695	45.424
0.115	248.695	45.424	9.802	210.008	42.493	-142.808	780.750	248.745	46.472
0.120	248.745	46.472	10.010	209.304	40.759	-139.018	751.357	248.795	47.517
0.125	248.795	47.517	10.210	208.618	39.135	-135.463	723.542	248.847	48.558
0.130	248.847	48.558	10.401	207.949	37.611	-132.126	697.199	248.899	49.596

CALCULATION RESULTS (cont.)

t	X_n	S_n	X'	S'	X''	S''	X'''	X_{n+1}	S_{n+1}
0.135	248.899	49.596	10.586	207.296	36.180	-128.987	672.229	248.953	50.631
0.140	248.953	50.631	10.763	206.659	34.833	-126.031	648.541	249.007	51.663
0.145	249.007	51.663	10.934	206.036	33.565	-123.243	626.049	249.062	52.691
0.150	249.062	52.691	11.099	205.426	32.369	-120.611	604.677	249.118	53.717
0.155	249.118	53.717	11.258	204.829	31.240	-118.123	584.354	249.175	54.740
0.160	249.175	54.740	11.412	204.245	30.173	-115.768	565.013	249.232	55.759
0.165	249.232	55.759	11.560	203.671	29.162	-113.537	546.593	249.290	56.776
0.170	249.290	56.776	11.703	203.109	28.206	-111.420	529.037	249.349	57.790
0.175	249.349	57.790	11.842	202.557	27.298	-109.410	512.293	249.409	58.802
0.180	249.409	58.802	11.976	202.015	26.437	-107.500	496.312	249.469	59.811
0.185	249.469	59.811	12.106	201.482	25.619	-105.682	481.049	249.530	60.817
0.190	249.530	60.817	12.233	200.958	24.841	-103.950	466.463	249.591	61.820
0.195	249.591	61.820	12.355	200.442	24.100	-102.300	452.515	249.653	62.821
0.200	249.653	62.821	12.474	199.935	23.395	-100.725	439.167	249.716	63.819

VITA

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Master of Science

Thesis: SOME KINETIC PROPERTIES OF TRANSIENT-STATES IN CONTINUOUS CULTURES OF NATURAL MICROBIAL POPULATIONS INDUCED BY INCREASING THE FEED CONCENTRATION OF THE GROWTH-LIMITING NUTRIENT

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