A THEORETICAL AND EXPERIMENTAL STUDY OF FACTORS GOVERNING THE OPERATION OF CONTINUOUS FLOW CULTURES WITH FEEDBACK

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by

William A. Sack

AN ABSTRACT OF A THESIS

Submitted to
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ABSTRACT

A THEORETICAL AND EXPERIMENTAL STUDY OF FACTORS GOVERNING THE OPERATION OF CONTINUOUS FLOW CULTURES WITH FEEDBACK

by William A. Sack

A continuous flow culture with feedback was operated under steady state conditions at growth rates ranging from 0.009 to 0.236. For comparison, the system was also operated without feedback under steady state conditions at three growth rates ranging from 0.079 to 0.292. An artificial sterile medium based on glucose served as the feed. Substrate concentrations of 200, 400 and 1000 mg/l were used. The majority of the runs were made at a concentration of 1000 mg/l. A mixed culture of microorganisms was used in the system.

A mathematical model based on equations developed by Herbert (1961) was presented to describe the operation of the system. It was found that the experimental steady state cell concentration $\bar{x}$ was consistently greater than that predicted by the model. From a material balance around the system and from direct measurements, it was found that cells were being retained in the reactor longer than

predicted by the equation for complete mixing conditions. This was found to be due to evaporation, adhesion of cells to the vessel walls and partial blockage of the effluent port. When the model was modified to include the increased retention of solids, good agreement was obtained between measured and computed steady state reactor cell concentrations. The correction of the model was expressed as a retention factor L which varied from 0.727 to 0.999 during the runs.

The reactor cell concentration was largely dependent on a parameter called the feedback factor A''. A'' is in turn a function of the recycle ratio α, the cell concentration factor C and L. The value of C was found to decrease from 2.20 to 1.28 when α was increased from 0.36 to 3.37.

Daily fluctuations in $\overline{x}$ during a given run were due to differences between the amount of cells produced and the amount of cells discharged from the system. The daily fluctuations were predicted reasonably well using a transient condition analysis instead of a steady state type of analysis.

The yield coefficient $\overline{Y}$ was found to increase as the rate of growth was increased. For runs made at S' = 1000 mg/l, $\overline{Y}$ increased from 0.413 to 0.556 as k_1 increased from 0.01 to 0.29. The variation in $\overline{Y}$ for all runs was from 0.252 to 0.556.

The steady state reactor substrate concentration $\bar{S}$ was found to increase as k_1 increased. The relationship between the two parameters was correctly expressed by a first order equation first suggested by Teissier (1936) as:

$$k_1 = k_m(1-e^{-c\bar{S}})$$

where k_m = maximum growth rate = 0.87 and c = 0.02. When the substrate consumption rate k_c was plotted against k_1, a linear relation was obtained. However, the curve showed that 15 mg glucose per gram cell weight per hour were being consumed at a k_1 of zero. Hence a certain rate of substrate uptake was necessary to sustain cell metabolism without producing growth.

The settling properties of the mixed culture were quite variable. However flocculent growth was present during all the runs with feedback.

Mixing studies conducted using the reaction chamber showed that a dye solution exhibited perfect mixing while various suspensions showed slight departures from theoretical complete mixing. The departure from complete mixing was greatly influenced by the specific gravity of the particles in suspension.

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I. INTRODUCTION

The activated sludge process has been used as a method of sewage treatment in this country for nearly half a century. During this time the process has been refined and modified until today it is the most popular method of waste treatment for cities of over 10,000 population. However, the design of the process is based largely on empirical relationships which are known to have been successfully applied in the past. In the conventional plant, the untreated waste and return sludge enter at one end of the aeration tank and spend a theoretical detention time of about 6 hours flowing through the tank. A bacterium moving along with a given element of waste may initially find adequate food for growth. However, by the time the element of waste reaches the outlet end of the tank its substrate concentration is reduced to a low level and the bacterium may be approaching the endogenous metabolism level. Because of the variation in growth rate and the gradients in substrate and cell concentration, this type of system does not lend itself well to theoretical analysis. However, studies have shown (Kehr, 1936; Sawyer and Rohlich, 1943) that the hydraulic flow pattern of many of the conventional plants is close to complete mixing. The completely mixed process has found increased popularity in recent years in the treatment of high concentration industrial wastes and in the small so-called "package" plants. In these plants there are no appreciable substrate or solids concentration gradients in the aeration tank.

A number of different experimental approaches have been used to study the activated sludge process. They range from operation of a laboratory-scale reactor to that of a full-scale plant or pilot plant. These units may be operated as batch or continuous culture systems.

The term "batch system" will be used for a system to which materials are added and reaction products withdrawn at relatively large time intervals. Herbert (1961) points out that the term "continuous culture" is applied to almost any process involving passage of fluids through a reactor system in which they are exposed to microbiological action. The classification of continuous culture systems may be made according to the nature of the overall metabolic reaction taking place or the type of reactor operation. Because a system of given operational type will behave much in the same way regardless of its metabolic classification, the reactor operation designation system is the most widely accepted and will be used in this paper.

Continuous culture systems may then be divided into open and closed systems which are further subdivided into homogeneous and heterogeneous systems with reference to mixing characteristics. The terms open and closed refer respectively to those systems where cells are discharged continuously in the effluent or wholly retained within the system. The homogeneous designation refers to the type of reactor where no gradients exist with respect to cell or substrate concentration. This is also referred to as a completely mixed system.

Ideal heterogeneous reactors are characterized by "piston" or "plug" flow through the reactor with no inter-mixing of the contents. All systems can further be subdivided into those with and without feedback and into single and multi-stage systems. With regard to the type of control, the systems can be classified as those subject to internal and those subject to external control. In the internally controlled type, nutrients are supplied in excess at all times. The organisms thus grow at their maximum growth rate with the steady state concentration of organisms being regulated by the nutrient feed rate. In the externally controlled unit the nutrient supply is limited and thus determines growth rate. These are the conditions most similar to those of the activated sludge process and hence this type of control is almost exclusively used to study this process.

The two system types pertinent to this investigation may be described as (1) the homogeneous, single stage, open externally controlled continuous flow system and (2) the homogeneous, single stage, open externally controlled continuous flow system with feedback. For the sake of expediency, (1) and (2) will be referred to respectively as the continuous flow system without feedback and the continuous flow feedback system. A third general type often used is the continuous flow system with aeration and concentration combined in the same unit.

The completely mixed activated sludge plant may be considered to be a continuous flow feedback system. This system has recently

been analyzed and mathematical equations have been developed which may be used to describe its operation. It is the object of this thesis to test the validity of these formulations by operation of a laboratory-scale unit under controlled conditions. Two factors which are extremely important to the operation of the process are the recycle ratio α and the solids concentration factor C. The latter is the ratio of the cell concentration in the feedback to the reactor cell concentration. The experimental program was especially designed to investigate the inter-relation between C, α and the reactor cell concentration. Very few data are available in the literature relating these parameters under controlled conditions.

II. LITERATURE REVIEW

Batch fed units require relatively little attention and instrumentation and have therefore been extensively used to study the bio-oxidation process and to establish design criteria for full-scale continuous flow plants (Busch et al., 1960; Weston and Stack, 1960; Heukelekian et al., 1951; Busch, 1962; Quirk, 1959; Eckenfelder, 1956; Symons and Mckinney, 1958; Dryden et al., 1956; Nemerow and Ray, 1956; Gellman and Heukelekian, 1955; Gaudy et al., 1963; Weston, 1961; Sawyer et al., 1955; Zablatzky et al., 1959).

Although batch study is useful for determining the maximum attainable growth rate or basic susceptibility to oxidation of a given waste, it cannot duplicate steady state conditions produced by continuous organic and hydraulic loading and continuous solids wastage. Busch et al., 1960 found significant differences in solids production and settling characteristics of companion batch and continuous units fed the same number of grams of glucose daily.

The single stage open system without feedback is the simplest type of continuous flow system and probably has been the most widely used constant flow unit to study bio-systems. As the name implies, substrate is continuously fed at a given rate to a reactor containing a stirred culture. The reactor volume is kept constant by a fixed overflow line through which the reaction products leave the reactor.

Much of the basic theory concerning growth and substrate removal has been derived using this type of unit (Monod, 1942, 1950; Novick and Szilard, 1950; Herbert, 1956; Schulze and Lipe, 1963). Since the system does not provide for concentration of the culture as in the activated sludge process, it has been little used by sanitary engineers. However, the growth of mixed cultures and BOD removal rates at several different temperatures were studied by Garrett and Sawyer (1952) with this type of system.

The continuous flow feedback system most closely simulates the activated sludge process. It differs from the system described above in that the cells in the reactor effluent are concentrated in a separate unit and partly recycled to the reactor. Laboratory units with separate concentration and cell return facilities obviously present operational difficulties and require close supervision. This is especially true at the low flow rates used in small scale operation. However, this type of apparatus allows study of the recycle ratio and cell concentration factor. Both of these parameters vitally influence the operation of the system.

The use of continuous culture units with feedback has been reported by a number of workers. Gaudy et al. (1960) employed a system featuring an air lift sludge return to study the efficiency of removal of glucose at various organic loading rates. Kountz and Fourney (1959) investigated sludge production by a metabolic energy

balance around a multiple unit total oxidation system. Pasveer (1955) determined the feasibility of activated sludge treatment of citrus wastes. Schulze (1963) developed a mathematical model for the continuous flow feedback system and applied it to operational data from full-scale activated sludge plants.

Continuous flow culture systems with combined aeration and concentration in the same vessel are designed to maintain a higher level of suspended solids than would be possible with the normal aeration unit. This is accomplished by the use of baffles which effectively divide the unit into an aeration chamber and a sedimentation chamber. Laboratory-scale plants of this type have been used for basic study of the bio-oxidation phenomena as well as for investigation and design of specific municipal and industrial waste problems (Coe, 1952; Busch and Myrick, 1960; Busch, 1954, 1959; Ludzack, 1960; Stack and Conway, 1959; Orford et al., 1960; Hatfield and Strong, 1954; Washington and Symons, 1962).

The performance of plant-scale activated sludge units has been widely studied both in pilot and full-scale investigations. However, a listing of the studies probably filling several pages would seem to fulfill no purpose in connection with this dissertation. It is difficult to obtain fundamental information from plant-scale studies because of wide fluctuation in organic and hydraulic loading. In addition, the waste itself is usually subject to continuous variation in its composition and temperature.

III. THEORETICAL CONSIDERATIONS

The purpose of this investigation is to formulate and experimentally test a mathematical model of the open type, single stage continuous flow feedback system. The parameters on which the model is based should be fundamental and readily measurable. Various approaches for this purpose have been suggested by a number of authors (Busch, 1959, 1960a, b, 1962; Doshi, 1961; Eckenfelder, 1959, 1961; Eckenfelder and Porges, 1957; Garrett, 1958; Garrett and Sawyer, 1952; Gaudy et al., 1960; Herbert et al., 1956, Herbert, 1958a, b, 1960; McCabe, 1960; McKinney, 1962; Rich, 1963; Stack and Conway, 1959; Washington et al., 1963; Weston, 1961; Weston and Stack, 1960). Each approach is based on some type of material balance around all or part of the system, and a mathematical statement of sludge growth-substrate removal relationships.

A model representing any continuous flow culture system must specify (1) the flow pattern expected to exist in the aerator, and (2) a formulation relating cell growth to substrate concentration or the kinetics of the growth process. These topics are considered below.

A. Flow or mixing pattern in aerator

Chemical engineering literature (Danckwerts, 1953; Greenhalgh et al., 1959; Levenspiel, 1962) lists two general types of steady state continuous flow reactors. The ideal piston or plug flow type is

characterized by an orderly flow of fluid through the vessel with no element of fluid overtaking any other element. This type of flow would be approximated in a long tank having a relatively small cross section. Each particle of fluid would have the same theoretical residence time. The other type is called the backmix or completely mixed reactor. The increments of the influent intermix immediately with the vessel contents and lose their identity in every respect. Thus the exit stream from the reactor has the same composition as that of the reactor fluid. Each element of fluid has an equal chance of being withdrawn from the reactor at any instant and the residence time of any individual element has little significance.

The standard vigorously aerated activated sludge tank obviously does not completely conform to either the ideal piston flow or ideal backmix flow model. However, studies by Kehr (1936) showed that in a short mixing tank (6 feet wide by 15 feet long by 3 3/4 feet deep) the detention or washout rate was reasonably close to that predicted by complete mixing theory. In a full-scale aeration tank, Sawyer and Rohlich (1943) found that the back-flow was very significant when practicing stage addition of activated sludge along the tank. The existence of backflow suggests the tank contents were well mixed. Obviously, to ascertain the degree of departure from complete mixing would require specific studies in each case. However, it is felt that the vigorous mixing provided in modern plants by either diffused air

or by mechanical aerators produces a flow pattern more closely approximating backmix than plug flow.

The contents of the reactor used in this investigation were continuously mixed by both a magnetic stirrer and by the air flow used to aerate the culture. The incoming feed and the recycled flow appeared to be quickly dispersed and intermixed throughout the vessel. In order to determine how closely the flow pattern of the experimental reactor conformed to the complete mixing model, a series of wash-out experiments were conducted as will be reported in Section V.

B. Growth kinetics

Symbols used in the following section are defined as follows:

x = cell concentration, mg dry wt/liter, at time t

x_o = initial cell concentration

t = time, hr

t_d = average doubling time, that is, the time required for the concentration of organisms to double

S = substrate concentration in reactor, mg/l

S' = concentration of influent substrate, mg/l

S_n = saturation constant numerically equal to the substrate concentration at which $k_l = 1/2 k_m$

D = dilution rate = F_i/v = number of complete reactor volume changes per unit time

F_i = rate of flow of feed solution into the reactor

v = volume of reactor

Y = yield factor = net unit weight of cells formed per unit weight of substrate used

k_1 = specific growth rate = $\frac{1}{x}\frac{dx}{dt}$, hr^{-1}

k_m = maximum growth rate for a given set of conditions

$\frac{dx}{dt}$ = overall growth rate.

Discussions of bacterial growth usually start with the familiar relation:

$$dx/dt = k_1 x \qquad 1$$

or

$$x = x_o e^{k_1 t} \qquad 2$$

The specific growth rate may also be expressed in terms of the time required for the concentration of organisms to double, t_d as:

$$k_1 = \frac{\ln 2}{t_d} \qquad 3$$

The relationship between the specific growth rate and the concentration of a substrate required for growth was first suggested by Monod (1942) as:

$$k_1 = k_m \left(\frac{S}{S_n + S}\right) \qquad 4$$

k_m is the maximum growth rate obtained when substrate is present in excess and S_n is a constant numerically equal to the substrate

concentration when k_1 equals $k_m/2$. According to equation 4, the relation between k_1 and S is hyperbolic with k_1 reaching k_m only at infinity. The formulation also predicts that exponential growth can occur at any value of k_1 between zero and k_m if the substrate concentration is held constant at its corresponding value. Equation 4 has the form of an adsorption isotherm and is essentially the same as the Michaelis-Menten equation used in enzyme kinetics. The bacterial cell is thus considered as an enzyme molecule reacting with the substrate at the reaction velocity k_1.

Schulze and Lipe (1963), working with E. coli in continuous flow culture, found a different type of equation also gave close agreement between actual and theoretical reactor substrate concentration:

$$dk/dS = c(k_m - k_1) \qquad 5$$

In integrated form equation 5 becomes

$$k_1 = k_m(1 - e^{-cS}) \qquad 6$$

where c is a constant computed from a series of experimental data. This first order equation was first proposed by Teissier (1936) but seems to have been largely neglected in recent years. Equation 6 predicts that the growth rate will approach its maximum value asymptotically as S increases.

As first shown by Monod (1942), there is a direct relation between cell growth and substrate consumption. For a medium containing but a single organic substrate, the growth rate is directly

proportional to the organic substrate metabolized as expressed by the equation:

$$\frac{dx}{dt} = -Y \frac{dS}{dt} \qquad 7$$

The parameter Y is known as the economic or yield coefficient.

$$Y = \frac{\text{net weight of cell material formed}}{\text{unit weight of substrate used}}$$

Using equation 7 and either equation 4 or 6 growth may be described in batch or continuous culture if the appropriate constants can be determined.

C. Effect of basal metabolism

In recent years, work by a number of authors has suggested that, in addition to anabolic metabolism, organisms also exhibit a basal or endogenous metabolism. Herbert (1958) found that the yield coefficient decreased at low growth rates when working with Torula utilis growing on a glucose-NH_3 medium and A. aerogenes growing on a glycerol-NH_3 medium. These results were explained by postulating a constant endogenous metabolism resulting in the oxidation of cell substance. More direct evidence is provided from studies carried out by Herbert (1959) on cell respiration at different growth rates. Using A. aerogenes, the respiratory carbon dioxide production was found to be a linear function of growth rate. However, when extrapolated back to zero growth rate, a positive respiration rate was

noted, indicating the presence of a continuous endogenous respiration in addition to the anabolic respiration. Using E. coli in continuous culture with glucose as the limiting carbon source, Schulze and Lipe (1963) also found evidence for a basal rate of metabolism. Their work again revealed a decrease in the yield constant as the growth rate decreased and a positive respiration rate at a theoretical growth rate of zero. In addition, when the rate of substrate uptake was plotted against the growth rate, the intercept at zero growth rate showed a substrate uptake rate of 55 mg glucose per gram cell weight per hour. Hence both growth and substrate uptake was influenced by basal metabolism.

D. Mathematical model of the continuous flow system

This dissertation deals primarily with the continuous flow feedback system. However, several short-duration runs were made without feedback, and hence this system is also discussed. Assume the growth chamber contains a culture of volume v, with cell concentration X and substrate concentration S. Culture medium of substrate concentration S' is fed at a steady flow rate F_i. The dilution rate is $F_i/v = D$.

The following assumptions are made as a basis for development of the model:

1. The contents of the reactor are completely mixed.
2. The influent contains a single organic substrate which

is the sole growth-limiting factor. All other substances required for growth, including oxygen, are present in excess.

Equations relating cell and substrate concentration in the reactor at steady state have been developed by Monod (1950), Novick and Szilard (1950) and Herbert et al. (1956). However, the following derivation of the fundamental equations describing continuous culture behavior is based on that of Herbert et al. (1956). The material balance equations around the reactor are as follows:

Cell balance

$$\text{Increase} = \text{growth} - \text{outflow} \qquad 8$$

$$\frac{dX}{dt} = k_1 X - DX$$

Substrate balance

$$\text{Increase} = \text{input} - \text{outflow} - \text{consumption} \qquad 9$$

$$\frac{dS}{dt} = DS' - DS - \frac{k_1 X}{Y}$$

In equations 8 and 9, the terms $k_1 X$ and $k_1 X/Y$ representing cell growth and substrate consumption respectively include both the anabolism and catabolism processes. Since both of these processes are functions of the cell concentration, **X**, the growth rate k_1, may be thought of as consisting of a term k_1' representing cell formation and k_1'' representing cell loss due to endogenous respiration. The yield coefficient Y should also be considered a net factor representing the result of growth and loss of cell material due to endogenous respiration.

Steady state conditions

As noted in equation 8, organisms in the reactor are growing at a net rate k_1X and being washed out of the vessel at a rate DX. Obviously, if k_1 is greater than D, dX/dt is positive, and the reactor cell concentration will increase. Conversely, if the dilution rate is greater than k_1, cell washout reduces the value of **X**. At a critical dilution rate, D_c, the cells are growing at their maximum rate. Above D_c, complete washout occurs.

However, at D values below D_c, the continuous flow system has a built-in self-adjusting property which tends to influence k_1 to adjust itself to any given D value. Powell (1956) has shown that the steady state is the only stable state of the system. For example, in a system which has just been inoculated, **X** is small, S is nearly equal to S', and k_1 is greater than D. However, as the cell concentration begins to increase, the value of S' decreases, resulting in a decreasing k_1 until the specific growth rate is equal to the dilution rate. At this point the combined rates of substrate consumption and outflow just balance the rate of inflow and there is no further tendency toward change. Hence small fluctuations from steady state set up opposing reactions and equilibrium is restored.

At steady state dX/dt and ds/dt equal zero, and equations 8 and 9 may be written respectively as:

$$k_1 = D \qquad 10$$

and

$$\bar{X} = \frac{YD}{k_1}(S' - \bar{S}) \qquad 11$$

or

$$\bar{X} = Y(S' - \bar{S}) \qquad 12$$

Combining equations 4 and 10 gives a relation for steady state substrate concentration as:

$$\bar{S} = \frac{S_n D}{k_m - D} \qquad 13$$

or from 6 and 10:

$$\bar{S} = \frac{\ln\left(\frac{k_m}{k_m - D}\right)}{c} \qquad 14$$

The parameters $\bar{S}$ and $\bar{X}$ refer to steady state substrate and cell concentrations. The above equations describe the steady state operation of the system for any value of D below D_c and of the substrate concentration S'.

Schulze and Lipe (1963) have pointed out that there is also a minimum dilution rate D_m, below which washout will occur. Apparently below D_m there is not sufficient substrate supply to allow cell reproduction to keep pace with the dilution rate.

Transient conditions

Equations 8 and 9 may be solved simultaneously over a finite period of time to give:

$$\frac{\Delta X}{\Delta t} = Y(S' - S)D - DX - \frac{Y\Delta S}{\Delta t} \qquad 15$$

The term $Y(S' - S)D$ is the actual rate of cell yield and DX is the rate of loss of cells from the system.

E. Mathematical model of the continuous flow feedback system

The following development of a mathematical model of the continuous flow feedback system is based on that of Herbert (1961). Figure 3 shows a schematic diagram of the continuous flow feedback system. Substrate of concentration S' enters the aerator containing culture of volume v at a flow rate F_i. The concentrations of cells and substrate in the vessel are respectively x and S. The culture leaves the aerator at a flow rate $(1 + \alpha) F_i$ and is concentrated in a settling tank. The concentrate is continuously returned to the reactor at a flow rate αF_i, where α is the ratio of the feedback flow to the influent substrate flow and is called the recycle ratio. The cell concentration of the recycled flow is Cx. The parameter C is defined as the ratio of the feedback cell concentration to the reactor cell concentration and is thus called the cell concentration factor. The clarified supernatant of the settling tank flows to waste at a rate equal to F_i. The cell concentration of the final effluent is x_e. The dilution rate for the reactor is

$$D_1 = (1 + \alpha) F_i/v \qquad 16$$

whereas the dilution rate for the whole system is

$$D = F_i/v \qquad 17$$

The balance equations around the reactor are then:

Cell balance

Increase = feedback - outflow + growth

$$dx/dt = D\alpha x C - xD(1 + \alpha) + xk_1 \qquad 18$$

Substrate balance

Increase = input + feedback - outflow - consumption

$$dS/dt = DS' + \alpha DS - (1 + \alpha) DS - k_1 x/Y \qquad 19$$

Steady state conditions

It was pointed out that for a system without feedback operating at a given substrate concentration and dilution rate, the growth rate would tend to adjust itself to the dilution rate fostering steady state conditions or $k_1 = D$. However, when equation 18 is solved for the equilibrium condition of $dx/dt = 0$, the following formulation is obtained:

$$k_1 = (1 + \alpha - \alpha C) D = AD \qquad 20$$

where

$$A = (1 + \alpha - \alpha C) \qquad 21$$

The quantity $(1 + \alpha - \alpha C)$ is called the feedback factor. The equilibrium growth rate is now seen to be a function of the recycle ratio and cell concentration factor as well as the dilution rate.

The theoretical inter-relations between the parameters A, C, and α are depicted in Figure 1. The curves are plotted for values of A ranging from 1.0 to -1.0, and for values of C and α from 0 to 11.0 and 0 to 3.2 respectively. In actual practice C seldom exceeds about

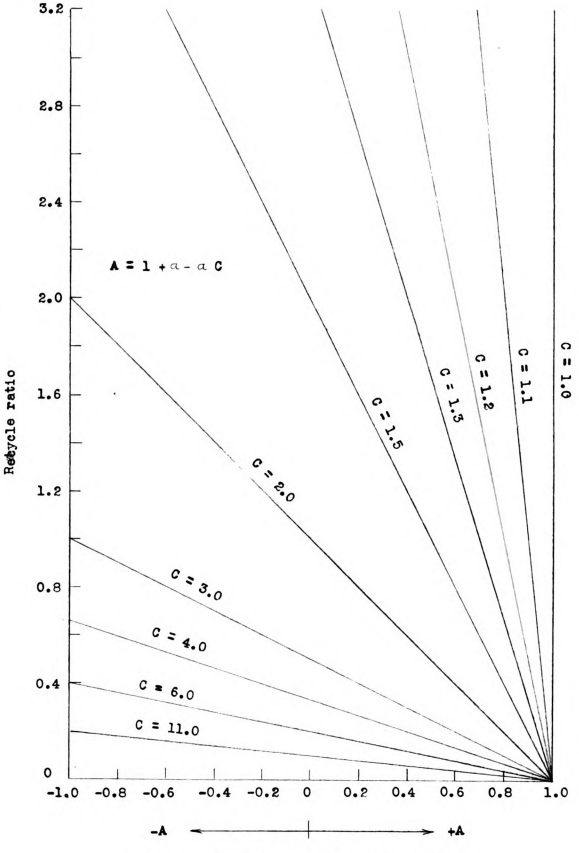


Figure 1. The inter-relation of A, C and α .

5 or 6 and α is usually less than 1.0. Theoretically, A may have any value from 1.0 to minus infinity. However, negative feedback factors are only transient and cannot exist for any significant length of time as will be shown later.

Generally, Figure 1 shows that for large values of α, the feedback factor will tend to decrease and that for small values of C it will increase. However, α and C are not strictly independent of one another. Hence a large change in α may bring about only a slight modification in A due to an adjustment in C. Since the value of A is usually less than unity, higher dilution rates may be used without washout as may be seen from equation 20. Theoretically, D may approach infinity as A approaches 0 without the product AD exceeding the maximum growth rate of the culture and without resulting in complete washout of the cells. In practice limitations in the concentration factor and in the oxygen transfer rates impose practical restrictions on the magnitude of D.

Solving equation 19 for $dS/dt = 0$, and combining with equation 20 gives an expression for the steady state cell concentration:

$$\bar{x} = \frac{Y}{A}(S' - \bar{S}) \qquad 22$$

From a consideration of the cell concentration and flow rates in and out of the settling tank at equilibrium, the steady state system effluent solids concentration, $\bar{x}_e$ may be found as:

Reactor solids effluent - system effluent = solids in feedback

$$Dx\,(1 + \alpha) - Dx_e = D\,\alpha\,xC$$

or

$$\bar{x}_e = (1 + \alpha - \alpha C)\,\bar{x} = A\bar{x} \qquad 23$$

From this relation the feedback or A factor is seen to be simply the ratio of the system effluent cell concentration and the reactor solids concentration, $\bar{x}_e/\bar{x} = A$.

Using equations 4 and 20, the steady state reactor substrate concentration becomes:

$$\bar{S} = \frac{S_n\,AD}{k_m - AD} \qquad 24$$

or from equations 6 and 20

$$\bar{S} = \frac{\ln\left(\dfrac{k_m}{k_m - DA}\right)}{c} \qquad 25$$

The above formulations allow prediction of the steady state cell and substrate concentrations of the system if values for the constants k_m, S_n and c are known.

Transient conditions

Equations 18 and 19 may be solved simultaneously over a finite period of time to give:

$$\frac{\Delta x}{\Delta t} = Y\,(S' - S)\,D - ADx - Y\,\frac{\Delta S}{\Delta t} \qquad 26$$

Under most operating conditions the term $Y \frac{\Delta S}{\Delta t}$ approaches zero and equation 26 may be rewritten as

$$\frac{\Delta x}{\Delta t} = Y (S' - S) D - AxD \qquad 27$$

The term $Y (S' - S) D$ is the actual rate of yield of cells and AxD is the rate of loss of cells from the system.

IV. EXPERIMENTAL APPARATUS, CULTURE AND METHODS

A. Description of the apparatus

The primary components of the apparatus were the reaction chamber and the settling tank. Feed solution was pumped into the reactor at a constant rate from storage bottles. The culture in the constant volume reactor was aerated and mechanically mixed. Reactor effluent was settled and the concentrate continuously recycled to the reactor. The supernatant effluent from the settling tank was stored in 20 liter bottles for analysis. A photograph of the apparatus is shown in Figure 2, and a schematic diagram of the flow system is presented in Figure 3.

1. Substrate feed system

Feed solution was made up and sterilized in batches of 20 liters in pyrex bottles. The feed bottle was vented to the atmosphere and protected against contamination by a cotton plug. A sigmamotor model T-65 pump was used to pump the feed to the reactor through tygon lines. The feed entered the reactor through a Kjeldahl bulb which afforded an air break between the sterile feed and the aerosol which existed in the reactor. Several sets of feed lines were held in abeyance at all times in the event contamination developed in the feed lines or bottle. When contamination was detected in the lines, a set was

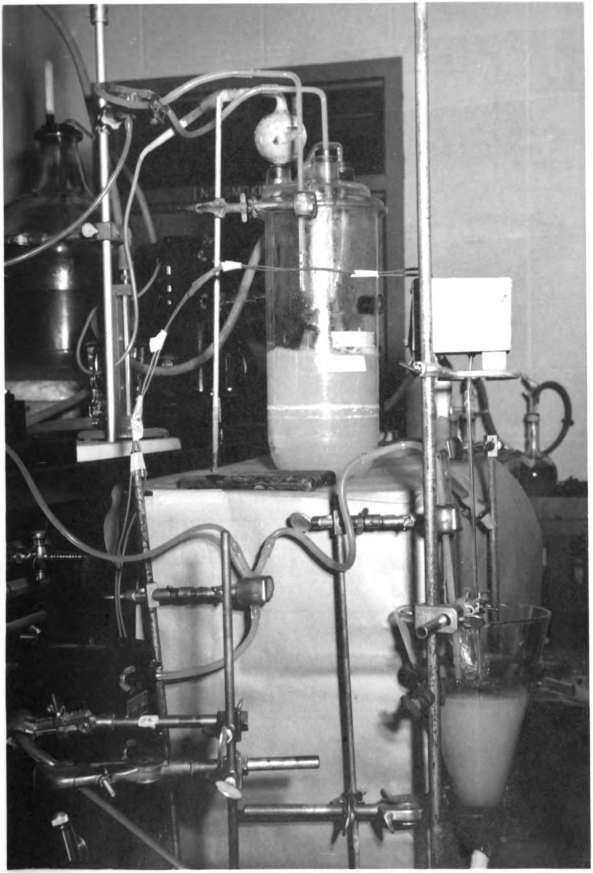


Figure 2. Continuous flow feedback apparatus.

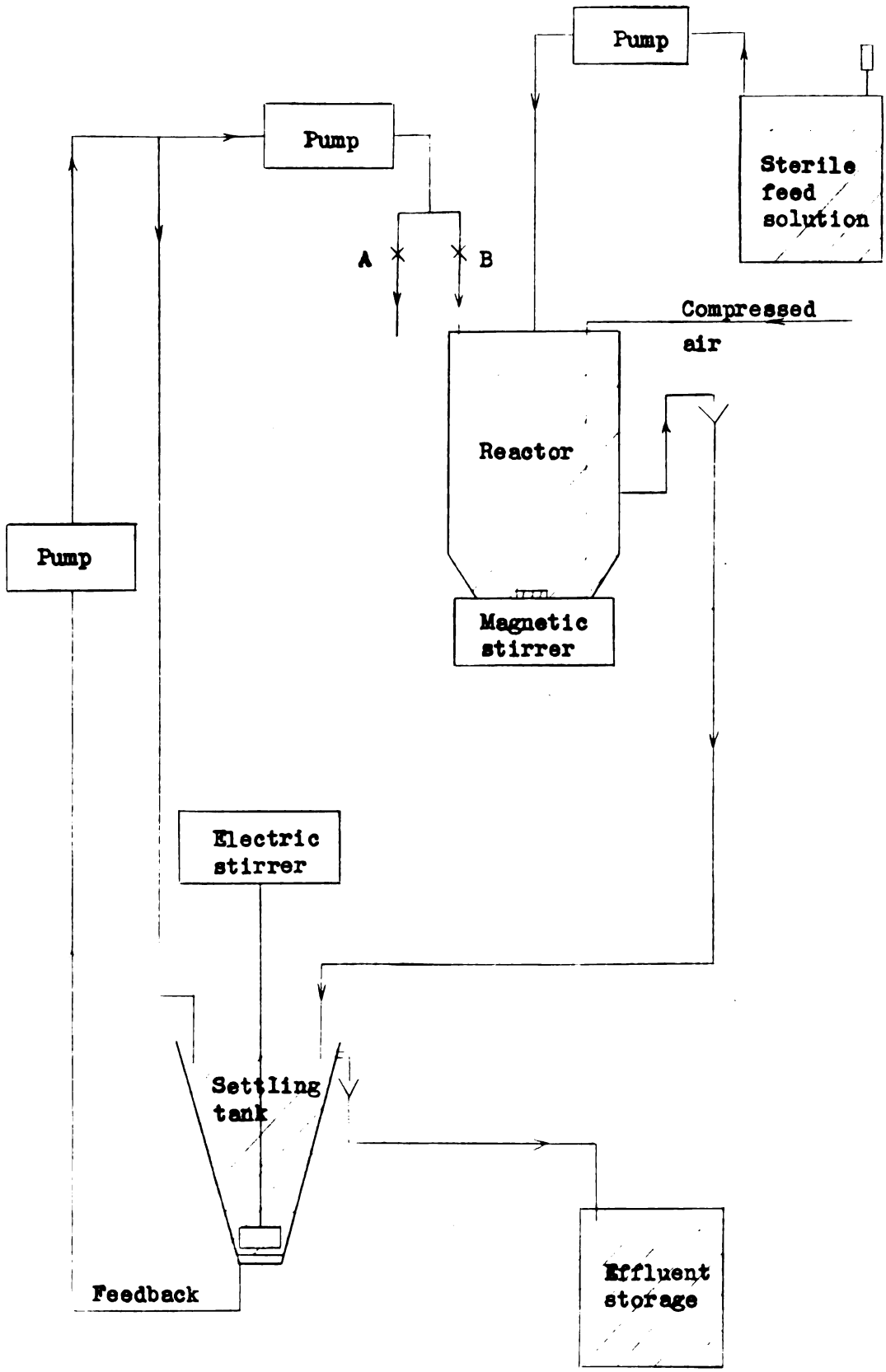


Figure 3. Schematic diagram of continous flow feedback apparatus

sterilized and aseptically installed in the system. If organisms had developed in the lines and the feed bottle, both were replaced.

2. Reactor and reactor overflow

The reaction chamber was a pyrex glass container 12. 5 cm in diameter and 32 cm deep. As may be seen in Figure 2, the bottom of the vessel was shaped like an inverted dome so that almost no stagnant pockets could develop. The chamber was capped with a removable pyrex cover having three 2.5 cm openings positioned around a 3. 5 cm opening.

Overflow ports were located in the side of the vessel so that culture volume could be maintained at one or two liters. However, it was found that pieces of heavy floc collected around the port, seeming to block the outlet. It was also felt that a top overflow might favor the retention of the heavier flocs in the reactor. To minimize the above effects, the effluent port was submerged to about the mid-depth of the vessel by means of the "S" shaped effluent tube shown schematically in Figure 3.

Mixing in the reactor was accomplished by vigorous aeration of the culture and by a magnetic stirrer. The stirrer was mounted directly under the reactor and separated from it by a 3/16" glass plate built into the bottom of the constant temperature bath tank. The reactor was immersed in a water-filled galvanized tank for temperature control (not shown in Figure 2). The temperature of the bath was maintained

at the desired temperature ± 0.5° C by means of a Fenwal thermo-regulator and heater.

3. Settling tank

Continuous concentration of the sludge in the settling tank and its return to the reactor proved to be the most difficult problem encountered in operation of the laboratory-scale apparatus. Even if the organisms were flocculating and settling well, great difficulty was encountered in continuously removing the concentrated sludge from the settling tank at the low flows used. It was also found that the floc would "bridge" the opening of the sludge return line. Since no high specific gravity inert suspended solids were mixed with the floc as in full-scale plants, the floc usually settled quite slowly and often clung to the sides of the settling tank. To overcome these difficulties, many different sizes and shapes were tried for the settling tank. Various types of stirrers and scrapers were installed in an attempt to stop the bridging and still promote sedimentation. One unsuccessful innovation was an apparatus to shake the whole settling tank periodically to dislodge the floc clinging to the walls of the tank.

The settling tank which finally evolved was an inverted two liter erlenmeyer flask with the bottom removed (see Figure 2). The clarified effluent overflowed through a 3/4 inch port into a 20 liter bottle, thus maintaining the liquid volume of the settling vessel at 0.60 liters. Sludge was drawn off at the bottom of the tank at a rate

of approximately 20 ml per minute by a model T-65 sigmamotor pump. The sludge then passed through a glass "Y" which split the flow, channeling most of it back to the settling tank. The remainder of the sludge flow was fed back to the reaction chamber by another sigmamotor pump. The main objective of the recirculation around the settling tank was to create a flow high enough to keep in motion the settled sludge. This also served to help eliminate stagnant sludge pockets which tended to become septic. A thin plastic strip shaped to follow the contour of the bottom and side of the settling tank served as a scraping mechanism. It was attached to a vertical shaft which rotated at one rpm driven by a small electric motor.

The return sludge went through a "T" connection just before entering the reactor. By opening clamp B and closing A (see Figure 3) samples for measuring the return flow rate and sludge concentration were obtained.

The detention time in the settling tank (based on flow from the reactor) varied from 0.3 to 3.0 hours during the experiments.

4. Aeration system

A schematic diagram of the culture aeration system is given in Figure 4. Air from the compressed air line was passed through pressure reducing and needle valves. The air pressure to the reactor was maintained at approximately 4 psi and the rate of air flow varied between 1.0 and 3.7 liters per minute during the runs. The air was

filtered through a cotton plug to remove any oil or other impurities from the air line. Finally the rate of flow was measured through a Rotameter and the air saturated with water by passage through a water filled gas saturator. The saturator (not used in runs 1 through 4) was installed to replace moisture constantly escaping from the reactor in the air stream. The air passed into the reaction chamber through one or more fritted glass diffusers. At the highest k_1 values, three diffusers were used to maintain dissolved oxygen in the reactor.

5. Method of sludge wasting

As noted previously, the supernatant from the settling tank was collected in a 20 liter bottle. For most of the runs cells were allowed to build up in the settling tank until the sludge blanket was slightly below the overflow. The overflow thus carried out a concentration of cells approximately equal to that formed in the reactor. However, due to fluctuation in the settling properties of the organisms, the effluent cell concentration varied significantly as will be discussed in Section V. Because of the significant variation in effluent cell concentration, an attempt was made to adjust the cell loss from the system by manually wasting sludge from the settling tank. This procedure was practiced only in runs 2b through 6. In these runs, in addition to the automatic wasting of cells in the effluent, cells were withdrawn by pipette 4 to 6 times each day from the settling tank. The quantity of cell material wasted was determined by calculations based

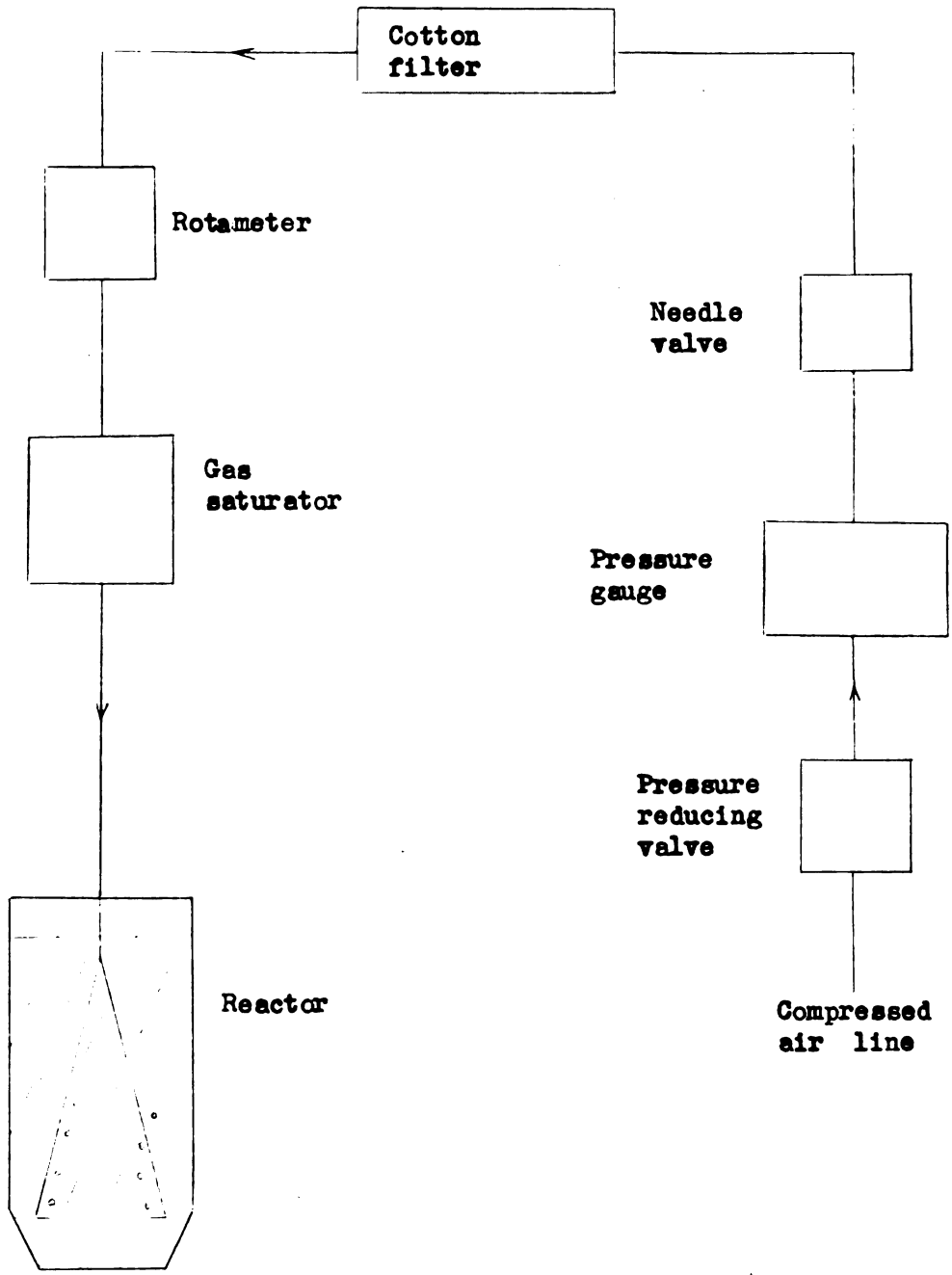


Figure 4. Culture aeration system.

on cell production and cell loss in the system effluent. However, because of the difficulty of correctly estimating the quantity of cell material to waste, the manual wasting procedure was abandoned after run 6.

B. Sterilization of the apparatus

The only part of the apparatus routinely sterilized (in addition to the media) was the substrate feed system. The glass and rubber parts of this system were placed in a gas autoclave for at least 15 minutes at 15 pounds pressure. The tygon part of the system which passed through the sigmamotor pump was sterilized using 4 per cent per-acetic acid for at least one hour. Chemical sterilization of the tygon was practiced since heat was found to deform and eventually harden the tygon. It was usually necessary to sterilize a new set of lines to replace a contaminated set every three or four days. Growth in the lines was thought to originate from cells in the aerosol of the reactor entering through the Kjeldahl bulb and growing back through the lines. Line contamination may also have originated from accidental contamination occurring during assembly of the sterilized lines. It was necessary to sterilize the entire apparatus several times as a result of the appearance of excessive populations of rotifers or protozoa developing in the reactor. In these cases all components (except the substrate feed lines) were sterilized in a gas autoclave for at least 30 minutes at a pressure of at least 15 psi.

C. Culture

Organisms to be used in a feedback system employing gravity separation must obviously settle and to some degree concentrate within some given basin detention time. Most bacteria are just slightly larger than the upper limit of the colloidal range and possess specific gravities close to that of water. Hence the settling velocity of a single bacterium is usually too low for gravity separation. However, some or perhaps all species agglomerate or flocculate under certain conditions. Flocculation greatly increases settling velocity as may be seen from Stoke's law

$$V = \frac{g}{18}(s - 1)\frac{d^2}{z}$$

In the above equation

V = settling velocity of the particle

z = the kinematic viscosity of water

g = the acceleration due to gravity

d = particle diameter

s = specific gravity of the particle

In order to obtain flocculent species of bacteria, a 100 ml sample of mixed liquor from the East Lansing Sewage Treatment Plant was inoculated into one liter of the 200 mg/l feed solution in the reactor and aerated for at least 24 hours to allow the culture to establish itself. Feeding was then begun on a continuous basis and the system operated

in an attempt to build up the reactor solids concentration. This procedure was tried several times without success. After about 36 hours the floc which had been in the original inoculum was washed out of the system and the remaining growth consisted of only dispersed organisms. Failure of the above procedure to produce flocculent growth may have been due in part to organic overload of the culture. McKinney and Weichlein (1953) found that floc formation is correlated with metabolic activity. Using both sewage and a synthetic medium based on glucose, they observed that no floc was formed as long as the organisms were actively metabolizing and reproducing. However, out of 72 isolates from activated sludge, all cultures had formed flocs within 17 days after inoculation into unaerated liquid media. Twenty-six of the 72 isolates were found to flocculate within 48 hours in an aerated culture. The authors concluded that floc formation was the result of complete metabolism of the organic substrate and not caused by special zooglea-producing bacteria.

Assuming that the organic load on the system had been too high to allow the development of flocs, the system was again inoculated and the feed rate reduced to load the reactor at about 0.25 lbs of BOD_5 per pound of suspended solids per day (300 mg/l glucose equals approximately 224 mg/l BOD_5). This is a common load for small activated sludge plants. The effluent from the settling tank was settled for 24 hours and floc which had formed during that time returned to the reactor.

Using these latter procedures a flocculent culture was developed from a sewage inoculation. The above method for obtaining flocculent growth was used successfully again during the course of the runs when it was found necessary to develop a new culture from a small inoculum of the reactor culture which had been stored in the refrigerator. Four or five days were required to build up a significant cell concentration in this manner because of the low organic load which could be placed on the system.

It should be noted that no attempt was made to maintain a pure culture because of the difficulty to operate a feedback system in a sterile condition. As might be expected, a heterogeneous population developed in the system. Microscopic examination showed the culture to consist mainly of bacteria intermixed with varying numbers of vorticella, rotifers and other protozoa. The majority of the bacteria were present in flocs. However, a certain amount of non-flocculent bacteria was always in evidence. Several different types of flocs were present at all times. Microscopic examination showed the predominant type during most of the runs was a dense floc composed of bacilli and giving a characteristic "folded" appearance. The yellowish floc was found to be composed of a coliform organism or organisms.

Several times during runs the protozoa became quite numerous and forced termination of the run. It was found that by running for several days with a low or zero concentration of dissolved oxygen in the

reactor, the protozoa population would be reduced to an insignificant level and a new run could be started. However, when this procedure was not effective, the apparatus was sterilized, and the reactor inoculated with either cells which had been stored in a refrigerator from earlier runs or from the stock culture of coliform organisms.

1. Identification of bacteria

During most of the runs a yellowish floc composed of baccilli was predominant. A small piece of this floc was washed 9 times to remove other organisms by vigorously shaking it in 100 ml bottles of sterile buffered tap water. The washed piece of floc was then streaked out on a plate containing in a solidified form the glucose based media described in part D of this section. One of the typical isolated colonies which formed was restreaked in order to obtain a pure culture of the organism. The organism thus isolated was found to be a small Gram negative rod, occurring singly or in groups. The colonies were mucoid, smooth, convex, regular, and white to cream.

The organism was also tested by the Michigan State University Veterinary Diagnostic Laboratory and found to have the following fermentation reactions:

1. Glucose	Acid and gas
2. Lactose	Acid and gas
3. Hydrogen sulfide	Negative

4.	Indole production	Positive
5.	Methyl red test	Positive
6.	Voges-Proskauer test	Positive
7.	Citric acid or salts utilized	Positive

The diagnostic laboratory identified the organism as a member of the coliform group. Since no single member of this group ordinarily shows positive reactions in all four tests of the IMViC Series (Nos. 4-7 above) it was assumed that the culture contained more than one coliform organism. For instance, most varieties of E. coli would give a positive reaction for tests 4 and 5 and most varieties of A. aerogenes would produce positive reactions for tests 6 and 7. In an attempt to separate the organisms, they were plated out in tryptone-glucose extract media using the pour plate technique. The organisms were vigorously shaken in each dilution before plating. However, again all colonies which grew appeared to be the same morphologically and a repeat of tests 4 through 7 using cells from 7 separate colonies again gave all positives. The pour plate procedure was repeated two more times and tests 4 through 7 run once more with identical results.

The standard coliform test was also run on the organism as given in Standard Methods (Standard Methods for the Examination of Water and Waste-water, 11th edition, 1960). The test showed the organism to be a member of the coliform group.

On the basis of the above tests, it would seem that two or more members of the coliform group were growing so intimately together as to preclude separation. It is, of course, also possible that a genetic transformation had taken place, allowing one organism to give positive results for tests 4 to 7.

It should be noted that when the isolated coliform organism was later used for the k_m tests, the cells flocculated after several days aeration. The floc thus formed was of the same yellowish folded type present in the reactor.

D. Nutrient medium

The medium used was designed to contain amounts of nitrogen, phosphorus, magnesium, iron, calcium, trace elements (tap water), and nutriles (yeast extract) in excess of actual growth requirements. Glucose was used as the carbon source and as the limiting substance for growth.

A list of the components and their concentrations are given below for the case when the glucose concentration was 1000 mg/l. For runs where the glucose concentration was 400 or 200 ppm, the concentration of the remaining components was reduced respectively to 2/5 and 1/5 of that shown below. One liter of tap water was added per 20 liters of feed solution.

Component	Concentration mg/l
Glucose	1000
$(NH_4)_2SO_4$	283
KH_2PO_4	140
$FeSO_4$	10
$CaCl_2$	10
$MgSO_4$	75
Yeast extract	50

In addition to the above constituents, varying amounts of a phosphate buffer (pH 7.2) and a potassium hydroxide solution were added to the feed for pH control. The amounts of base and buffer added as well as the resulting feed pH after sterilization are shown below.

Glucose concentration mg/l	Buffer ml	KOH Concentration (%)	KOH Volume ml	Feed pH
200	0	0	0	7.0 - 7.2
400	25	10	10	7.6 - 7.9
1000	30	20	20	9.4 - 9.8

The components of the phosphate buffer used are given in Standard Methods (Standard Methods for the Examination of Water and Wastewater, 11th edition, p. 319).

1. Preparation

Feed solution was prepared in batches of 20 liters. To prevent precipitation of the salts and carmelization of the glucose, the sugar, base and buffer were sterilized in separate flasks in a gas heated autoclave for at least 20 minutes at 15 psi. Three ml of 0. 5 N sulfuric acid were added to the glucose solution to prevent carmelization during sterilization. The remaining constituents of the feed solution were sterilized in 20 liter pyrex bottles in the autoclave for at least one hour at 15 psi. The glucose, buffer, and base were added to the 20 liter bottle containing the sterilized nutrients and salts just prior to use.

E. Analytical techniques

1. Measurement of cell concentration

Samples were taken each day for measurement of the cell concentration of the reactor, the return flow and the plant effluent. The reactor effluent was also sampled during runs 10 through 12 and 20 through 22. The applicability of photometric, turbidimetric and gravimetric techniques was investigated for cell concentration measurement. The first two proved unsuitable due to the non-homogeneity of the flocculated cell suspension. Some success was attained by homogenizing cells in a Waring Blender for 10 minutes prior to photometric examination. However, the method did not give consistently reproducible results and was abandoned.

All the cell concentration determinations were made using a modification of the membrane filter technique as suggested by Engelbrecht and Mckinney, 1956.

The procedure used is as follows:

1. The membrane was dried for one hour at 103° C to drive off substances volatile at this temperature (mainly glycerol).

2. After cooling for 10 minutes in a desiccator, the membrane was weighed to the nearest tenth of a milligram.

3. The membrane was mounted in a Gelman Filter Apparatus and a sample of between 10 to 30 ml put through the filter under vacuum.

4. The membrane was dried for one hour at 103° C and weighed as in step 2.

2. Glucose determination

Samples were taken for determination of glucose concentration in the reactor and of the influent media at least once during each run. The method used for determination of glucose concentration was a modification of that used by Scott and Melvin (1953). In the presence of glucose the anthrone reagent develops a blue-green color which is proportional to the glucose concentration. The optical density of the sample was read against a de-ionized water blank at 625 mμ using a Bausch and Lomb Spectronic 20 colorimeter. The anthrone reagent was made in the proportion of 0. 2 gm anthrone to 100 ml of concentrated sulfuric acid. The reagent darkens with age and was used only up to

eight days after preparation. A 20 mg/l glucose standard was included with each set of samples for standardization.

In order to determine whether the blue-green color developed would obey the Lambert-Beer law, the reagent was tested against a series of known glucose concentrations using the procedure given below. The following results were obtained.

Sample No.	Glucose concentration mg/l	Optical density
blank	0	0
2	2	0.021
3	10	0.160
4	15	0.252
5	22	0.398

The above data are plotted in Figure 5 showing that the color development followed the Lambert-Beer law when measured at 625 mμ.

The procedure used was as follows:

1. The sample was taken and immediately filtered through a membrane filter to remove bacteria. Approximately the first 5 ml of the filtrate were discarded.

2. Five ml of each sample, a blank (de-ionized water), and a glucose standard (20 ppm) were pipetted into test tubes which were then immersed into a rapidly circulating cold water bath (approximately $6^{\circ}C$ and allowed to attain the temperature of the bath.

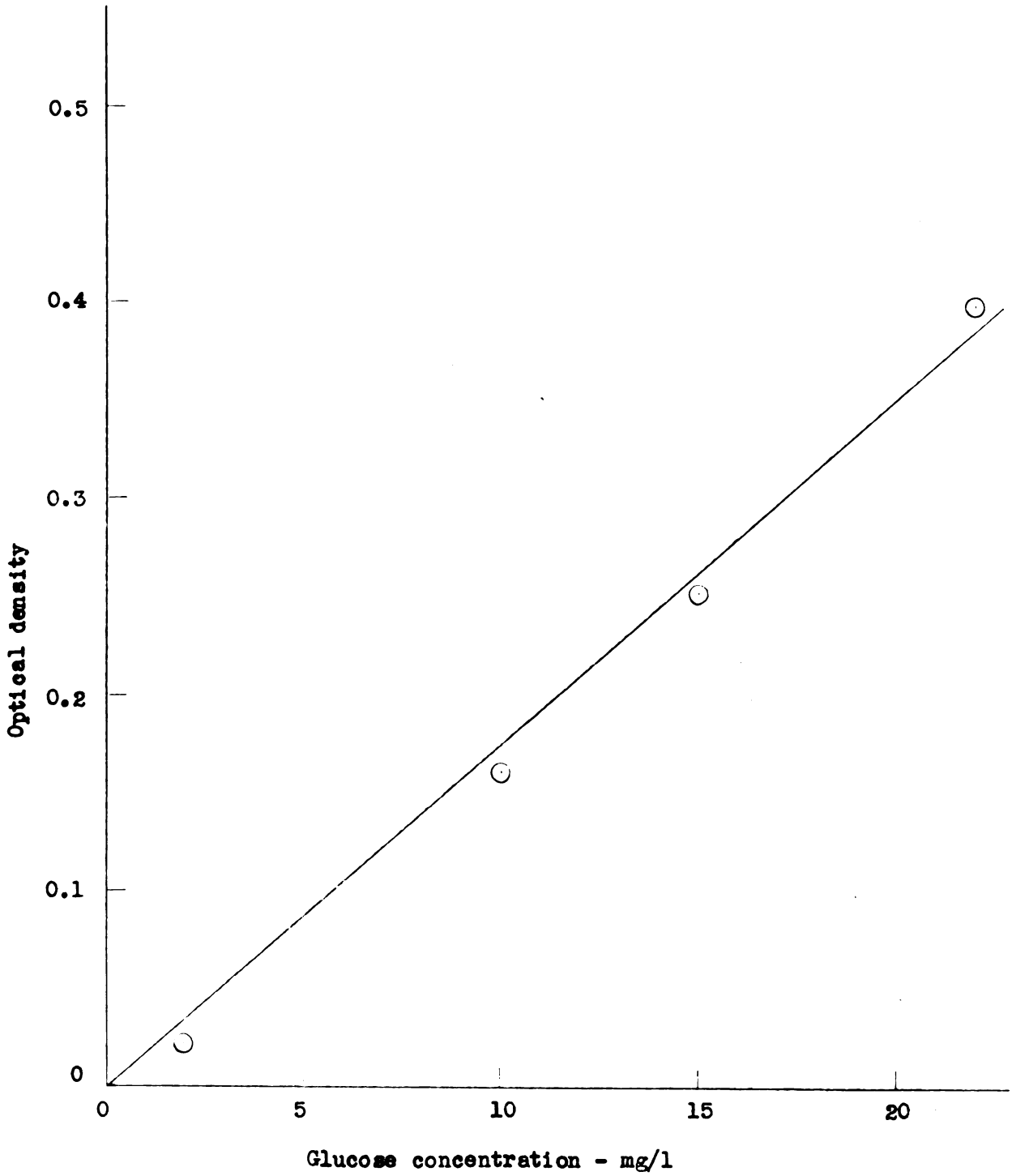


Figure 5. Standard glucose curve.

3. With the test tubes still in the bath, ten ml of cooled anthrone reagent (approximately 6°C) were vigorously syringed into the tubes, using a 10 cc syringe, with such force as to immediately mix the sample and anthrone reagent.

4. The tubes were loosely covered with aluminum foil and placed into a rapidly circulating 90° C water bath for 16 minutes to allow color development.

5. Next the samples were cooled to approximately room temperature for one minute in the 6° C bath and immediately transferred to matched cuvettes (1 cm in diameter). The optical density was measured at 625 mμ on the spectrophotometer against the deionized water blank.

3. Flow rate measurement

The rates of influent and return flow were each checked several times daily. The liquid collected in a graduate cylinder or volumetric flask was read over a given period of time to determine the rate. The influent was sampled and measured from the Kjeldahl bulb which was first moved to a ringstand beside the reactor. The elevation of the bulb was not changed during the measurement so as not to alter the head on the influent pump. The return rate was sampled by closing clamp B and opening clamp A (see Figure 3). The head on the return pump was also maintained at a constant level during measurement.

4. Measurement and control of pH

The pH of the reactor liquor was measured at least once daily using a Beckman Model D-2 pH meter. Due to the production of acid by the organisms, the pH of the reactor would drop if not adjusted. The inclusion of base and buffer in the feed reduced significant pH variation. However, pH adjustment was usually required once or twice a day (depending upon the growth rate) and was made when the pH fell below 6.8 (except for run No. 1) by addition of from 1 to 25 ml of a 1 per cent KOH solution to the reactor. The pH was maintained between 6.6 and 7.3 except for run number 1. In run No. 1, no buffer or base was added to the feed solution and the pH in the reactor ranged as low as 6.3. The average pH for each run is given in column 18 of Tables 17 and 18.

5. Measurement of dissolved oxygen

It was desired to keep the dissolved oxygen level in the reactor between 0.5 and 3.5 ppm at all times in order to favor the growth of aerobic organisms. The dissolved oxygen level was checked several times each day upon initiation of a new run and approximately once a day during the run. If the level of dissolved oxygen was below 0.5 or above 3.5 ppm, the air rate was increased or decreased respectively. The Alsterberg (Azide) Modification of the Winkler Method was used to measure dissolved oxygen as given in Standard Methods (Standard Methods for the Examination of Water and Wastewater, 11th edition,

1960). The procedure was slightly modified in that a 50 ml sample was used instead of the 300 ml suggested and quantities of reagents added to the sample reduced to 0. 5 ml instead of 2. 0 ml. The level of dissolved oxygen varied between 0. 2 and 4. 2 ppm during the runs. The average concentration of dissolved oxygen for each run is recorded in column 19 of Tables 17 and 18.

6. Microscopic examination

Samples were taken approximately once every two days from the reactor and examined microscopically to determine the general types and relative numbers of organisms present. The culture was examined more often during periods of high plankton population. If the population of organisms other than bacteria (rotifers, protozoa, etc.) was found to be increasing, the run was terminated and the rate of aeration greatly reduced in order to obtain a zero value of the dissolved oxygen in the reactor. It was found that running the system for a day under these conditions was often effective in greatly reducing the population of the intruders without materially harming the bacterial floc present. If the lack of dissolved oxygen did not significantly reduce the percentage of intruders, the system was shut down, sterilized, and reinoculated as previously noted.

V. RESULTS

The data presented herein were taken over a two year period of almost continuous operation of the system. The majority of the runs (sixteen) were carried out with feedback of the cells to the reactor. They were designed to test the validity of the formulations proposed in Section III (equations 18 to 27) to analyze the operation of a continuous flow feedback system. Three runs were also made without feedback in order to compare results obtained from the two systems. Elimination of the return flow allowed attainment of a higher growth rate at a relatively lower dilution rate than could be obtained with the feedback system.

A. The yield factor

The yield factor Y must first be determined before the reactor cell concentration can be computed. Since both substrate consumed and cell weight produced were measured, the yield factor could be computed for each day's run as:

$$Y = \frac{\text{net weight of cell material produced per unit time}}{\text{weight of substrate used per unit time}}$$

The substrate consumed was determined from the measured influent and steady state reactor substrate concentrations which are designated as S' and $\bar{S}$ respectively. $\bar{S}$ is assumed to be equal to the substrate concentration in the system effluent. The average values of S' and

$\bar{S}$ for a given run were used in the computation of Y and are recorded in columns 4 and 16 respectively of Tables 17 and 18. The net weight of cell material formed includes all cell material which left the system in the effluent and that which was mechanically wasted in some runs. In addition, it includes cell weight lost from the system due to sampling. The net weight was also adjusted for any increase or decrease in the reactor solids concentration since the last measurement.

The average yield factor $\bar{Y}$ for each feedback run is given in column 13 of Tables 17 and 18. $\bar{Y}$ is seen to vary from 0.252 to 0.556. For the runs using $S' = 1000$ mg/l, $\bar{Y}$ varies from 0.413 to 0.556. Each average value is based on a series of daily measurements and is felt to give the true yield factor under the conditions of the run. The variation in $\bar{Y}$ from run to run will be related to growth rate in a later section.

The daily yield factors Y are listed in column 12 of Tables 1 to 16. The value of Y is seen to vary by as much as 0.1 in many of the runs and in the neighborhood of 0.2 in two runs. Some fluctuation in Y within a given run is to be expected because of the changing population of organisms. However, it is felt that most of the variation is due to fluctuation in the mass of cell material contained in the settling tank. The settling properties of the floc fluctuated daily, resulting in alternate periods of buildup and excessive loss of cells from the tank. The effects of this buildup and excessive loss on system operation are discussed in a later section.

For the runs without feedback, the substrate consumed was again determined from measurement of S' and $\bar{S}$. The average values of S' and $\bar{S}$ are given in columns 3 and 12 respectively of Table 20. The net weight of cell material formed was computed from measurements of the reactor effluent solids concentration R_e. As will be discussed in more detail later, R_e decreased if the reactor was allowed to operate without scraping the cells from the walls. Hence only those samples of R_e taken at least three hours after scraping were used in the computation. The values of R_e and $\bar{Y}$ for each run are shown in columns 7 and 9 respectively of Table 20. The average yield factor may be seen to vary from 0.483 to 0.559.

B. The reactor cell concentration

Using equations 20 and 22, the steady state reactor cell concentration $\bar{x}'$ was computed for each day for the feedback runs as shown in column 7 of Tables 1 to 16. The daily measured values of the steady state cell concentration $\bar{x}$ are given in column 6 for comparison. The average values for $\bar{x}'$ and $\bar{x}$ for each run are presented in columns 8 and 7 respectively of Tables 17 and 18. It may be seen that the computed cell concentrations are well below the actual measured values in almost every case. The same result was found for the runs without feedback as may be seen in columns 4 and 5 of Table 20.

1. Solids balance around settling tank

In an attempt to ascertain the reason for the above noted discrepancy, a solids balance was established around the settling tank.

Rate of cells entering from reactor = Rate of cells returning to reactor + Rate of cells leaving in effluent

$$(F_i + F_r)x = x_r F_i \alpha + x_e F_i \qquad 28$$

Equation 28 relates the principal flow of cell mass in and out of the settling tank. However, several additional terms are necessary to include all the factors influencing the operation of the laboratory-scale unit used in this investigation.

First, as will be discussed below, some evaporation occurred in the system, reducing the hydraulic waste flow from the system to some value less than F_i. The actual rate of cell mass leaving in the effluent was determined by measurement of the concentration and volume of the effluent over a given period of time. The total cell mass in the effluent W_n divided by the elapsed time over which the sample was taken Δt gives the desired rate of cell mass leaving in the effluent.

Another modification of equation 28 is necessary to account for the various samples taken which were not returned to the system. Samples taken from the reactor or reactor effluent are designated as W_r and those from the feedback flow as W_f.

As noted in the preceding section, there were alternate

periods of cell buildup and excessive cell loss from the settling tank. This was reflected by variation in the yield factor. In order to make a material balance around the settling tank, it is necessary to determine any changes in the weight of cells contained in the settling tank over the given period of time. This may be done using the average yield factor obtained over the entire length of the run. Using the average yield factor $\bar{Y}$ and the amount of substrate consumed, the average actual weight of cells that must have been formed may be found. When the average weight of cells formed found in this way is greater than the daily measured weight of cells formed, the difference W_c divided by the elapsed time is added to the right side of equation 28. When the daily weight is greater than the average weight the value $W_c/\Delta t$ is subtracted from the right side of equation 28.

The cell balance around the settling tank may be rewritten incorporating the above modifications as:

Rate of cells entering from reactor	-	Rate of cells sampled from reactor flow	=	Rate of cells returned to reactor	+	Rate of cells leaving in effluent	-	Rate of cells sampled from feedback flow	±	Rate of storage or excess loss of cells from settling tank
$(F_i+F_r)x$	-	$\frac{W_r}{\Delta t}$	=	$x_r F_r$	+	$\frac{W_n}{\Delta t}$	-	$\frac{W_f}{\Delta t}$	±	$\frac{W_c}{\Delta t}$

or rearranging

$$(F_i + F_r)x = x_r F_r + \frac{W_n + W_r - W_f \pm W_c}{\Delta t} \qquad 29$$

By substitution of data from Tables 1 to 16 into equation 29, it was found that the left hand side of the equation was larger than the right hand side in almost every case. Each parameter in equation 29 is an experimentally measured quantity except W_c, which evolves directly from the data as shown above. However, the balance assumes that the cell concentration $\bar{x}$ leaving the reactor is equal to that in the reactor. It also assumes that the flow entering the reactor $(F_i + F_r)$ is the same as that leaving (no evaporation).

Since all quantities on the right hand side of equation 29 are measured, it was concluded that either the cell concentration in the reactor effluent was less than $\bar{x}$, or the effluent rate from the reactor was less than $F_i + F_r$. The existence of either or both of the above conditions would result in making the actual weight of cells in the reactor effluent per unit time less than that as given by $(F_i + F_r)(\bar{x})$. For purposes of comparison in later computations, the ratio of the experimentally determined rate of cell mass leaving the reactor (mg/min) to that given by $(F_i + F_r)(\bar{x})$ will be designated as L. All the cell mass actually leaving the reactor went to the settling tank and is accounted for by the terms of the right side of equation 29. The L or retention factor may then be computed as:

$$L = \frac{x_r F_r + \frac{W_n + W_r - W_f \pm W_c}{\Delta t}}{(F_i + F_r)\,\bar{x}} \qquad 30$$

The L values have been computed in this manner for each day of each run and are presented in column 10 of Tables 1 to 16. The average L for each run is presented in column 11 of Tables 17 and 18. The value of L is seen to vary from 0.727 to 0.999 when computed using the average data of each run. Several of the daily L values slightly exceed unity. It is felt that these cases represent small errors in the data.

2. Evaporation

The volume of effluent from the system was measured routinely for determination of the cell yield. By comparing this figure with the volume of substrate used, evaporation for the system was found to average about 200 ml per day.

In order to determine whether evaporation from the reactor itself was sufficient to cause the imbalance noted above, the rate of evaporation from the reactor was both measured and computed. Four evaporation experiments were made with tap water in the reactor using rates of aeration comparable to those used in actual growth experiments. The data, which are presented in Table 21, show an average evaporation rate of 0.0165 ml/min at 28° C and 0.016 ml/min at 24° C per liter of air per minute (column 5). During the runs, the average air rate

varied from a low of 1.0 l/min to a high value of 3.7 l/min. Taking a median value of about 2.5 l/min, and using the rate of 0.016 ml/min per liter per minute of air obtained above, the evaporation rate per day would be about 58 ml. This is much less than the measured value of about 200 ml/day and shows that most of the evaporation occurred in the settling and effluent systems which were not covered.

To determine what the maximum evaporation rate due to aeration might be, computation was made based on the assumption that the influent air was dry and that it became saturated in passing through the reactor. The method of computation is shown in Appendix 1. At an aeration rate of 1.0 l/min, one atmosphere pressure, and 24° C, the rate of evaporation is predicted to be 0.0234 ml per minute per liter of air. This is, of course, higher than the measured rates noted above, since the influent air actually contained some moisture, a factor which reduced evaporation.

As noted in a previous section, the ratio of the actual weight of cells in the reactor effluent per unit time to that given by $(F_i + F_r)(\bar{x})$ is denoted as L. Assuming that evaporation is the only factor causing L to be less than unity, equation 29 may be solved for $(F_i + F_r)$. The value thus obtained, $(F_i + F_r)'$, should be the actual effluent flow from the reactor at a cell concentration of $\bar{x}$ mg/l. The average values of $(F_i + F_r)'$ have been computed for runs 1 through 4 and are presented in Table 22 along with those of $(F_i + F_r)$. The difference between the two

quantities has been expressed as an evaporation rate for comparison with the evaporation rate of 0.016 ml/minute per liter per minute of air as obtained by experiment. From Table 22 it may be noted that all evaporation rates thus computed are considerably larger than 0.016. Hence, evaporation was not the only factor affecting L.

3. Reactor effect

In order to eliminate the evaporation effect, a gas saturator was installed for runs 4a to 22. The influent air was saturated with water so that there would be no evaporation loss. Since the L value did not approach unity upon introduction of this correcting factor, it was concluded that some other factor was tending to hold back cells in the reactor. As noted previously, vigorous mixing in the reactor was accomplished by at least two air diffusers and a magnetic stirrer. The influent substrate and return cells appeared to be almost immediately distributed throughout the vessel by the vertical and horizontal currents developed. However, it was noticed that significant quantities of cells built up on the reactor walls and effluent tube as the run progressed. If the effluent tube was not cleaned at least twice a day, the cells tended to build up around its mouth and would sometimes completely shut off the effluent until the head inside the reactor had increased to 4 or 5 inches and had forced the plug of cells through the effluent line. One or two runs had to be prematurely terminated due to such sudden discharges of cells, which disrupted the equilibrium of the system.

In order to determine whether the cells adhering to the reactor walls and the partial blockage of the effluent tube could cause a significant differential between the reactor cell concentration and the reactor effluent cell concentration R_e, both quantities were measured in runs 10 through 22. Normally the cell walls were scraped and any plug formed in the effluent tube removed about 5 times daily. However, since the buildup of cells in the reactor was observed to increase as the run progressed, samples were taken as follows:

1. The system was allowed to run various amounts of time without scraping.
2. Next either procedure (a) or (b) was followed.
   (a) The reactor effluent sample was taken first and the reactor sample was taken after scraping the walls of the reactor and the effluent tube.
   (b) The reactor and effluent tube were first scraped and then the samples taken.

The data are presented in Table 23. The heading "Hours after scraping" refers to the time elapsed between the previous scraping of the reactor and effluent tube and sampling as in procedure 2a or 2b. The data show that R_e was consistently lower than the reactor cell concentration and hence the ratio of $R_e/\bar{x}$ was less than unity whereas the theory of complete mixing assumes that both concentrations would be

equal. The ratio $R_e/\bar{x}$ is then a measure of L since

$$L = \frac{\text{actual rate of cell mass leaving reactor}}{(F_i + F_r)\bar{x}} = \frac{(F_i + F_r)\ R_e}{(F_i + F_r)\ \bar{x}}$$

where $\bar{R}_e$ is the average reactor effluent cell concentration. $R_e/\bar{x}$ is termed L' to differentiate it from the L computed using equation 30. The values of L' are given in column 5 of Table 23. It may be noted that they generally decrease as the length of time after scraping increases indicating a buildup of cells in the reactor with time. Since the value of L' is dependent upon when the reactor was last scraped, the L computed using equation 30 is thought to give a better estimate of the actual retention factor and will be used in subsequent calculations.

To theoretically estimate how many milligrams of cells might adhere to the reactor walls, an analysis was made assuming a layer of cells 0.05 mm thick on the walls of the reactor when the vessel contained 2 liters of mixed liquor. A typical bacterium was assumed to weigh (Lamanna and Mallette, 1959) 1.6×10^{-12} gms, occupy 1.5 cubic microns, possess a 75 per cent water content, and a density of 1.10 gms/cc.

Using the preceding values, it was found that a layer of cells 0.05 mm thick would weigh approximately 935 mg and hence if completely scraped from the wall could increase the cell concentration in the 2 liter reactor by 467 mg/l. The complete computation is given in Appendix 2. The value of 467 mg/l assumes that the film covers the

entire surface of the reactor and that it is composed solely of cells. Since there will obviously be void space between the cells filled with water, the value is probably at least 50 per cent too high. However, it would seem that a layer of cells clinging to the reactor wall could significantly raise the actual concentration of cells in the reactor.

4. Modification of equations to include evaporation and reactor effect for runs with feedback

It has been shown above that the weight of cells leaving the reactor per unit time is actually smaller than would be given by $(F_i + F_r)(\bar{x})$. It is therefore necessary to rewrite equations 18 to 27 taking this factor into account. This may be done most conveniently by including the L factor in the derivation.

Cell balance

Rate of increase	=	Rate of feedback	-	Rate of outflow	+	Rate of growth

$$\frac{dx}{dt} = \alpha DCx - DLx(1+\alpha) + xk_1 \qquad 31$$

At equilibrium $\frac{dx}{dt} = 0$ and

$$k_1 = D(L + L\alpha - \alpha C) = A''D \qquad 32$$

where

$$(L + L\alpha - \alpha C) = A'' \qquad 33$$

The feedback factor has thus been modified to include L. The original feedback factor was defined as:

$$(1 + \alpha - \alpha C) = A \qquad 21$$

It may be seen that equations 21 and 33 are identical when L = 1.0. In order to portray the effect of L on the feedback factor, α, C, and A have been related again in Figure 6 (see figures at end of this section) as in Figure 1 for three values of C using equation 21. To see what happens when L is reduced below 1.0, the same curves were plotted using equation 33 with L = 0.9. It may be seen that the effect is to shift all the curves to the left. The value of A'' at α = 0 is now 0.9 instead of 1.0. However, the same basic relations are seen to exist between the parameters for A'' as for A.

The substrate balance given by equation 19 remains unchanged. Combining equations 32 and 19 under steady state conditions gives:

$$\bar{x}'' = \frac{\bar{Y}}{A''} (S' - \bar{S}) \qquad 34$$

The formulations for the steady state cell concentration in the system effluent and the steady state reactor substrate concentration become respectively:

$$\bar{x}_e'' = (L + L\alpha - \alpha C) x'' = A'' \bar{x}'' \qquad 35$$

and

$$\bar{S} = \frac{S_n A'' D}{k_m - A'' D} \qquad 36$$

or

$$\bar{S} = \frac{\ln \left(\frac{k_m}{k_m - A'' D} \right)}{c} \qquad 37$$

The above equations are identical to equations 18 to 25 except for the revised feedback factor. The formulations hold regardless of whether correction is being made for the evaporation or reactor effect or both. The value of L is computed using equation 30 as described in a previous section.

5. Modification of equations to include evaporation and reactor effect for runs without feedback

Equations 8 to 15 for runs without feedback may also be modified to include the retention of solids in the reactor using the computed L. If the terms representing feedback are dropped from equation 30, L may be computed as:

$$L = \frac{\frac{W_n + W_r}{\Delta t}}{F_i \bar{X}} \qquad 38$$

Cell balance

Increase = growth - outflow

$$\frac{dX}{dt} = k_1 X - LDX \qquad 39$$

At equilibrium $dX/dt = 0$ and

$$k_1 = LD \qquad 40$$

Combining equation 11 and 40 gives:

$$\bar{X}'' = \frac{\bar{Y}(S' - \bar{S})}{L} \qquad 41$$

The effluent cell concentration is no longer equal to the reactor cell concentration, but now becomes:

$$\bar{X}_e'' = L\,\bar{X}''$$

The steady state substrate concentration becomes:

$$\bar{S} = \frac{S_n LD}{k_m - LD} \qquad 43$$

or

$$\bar{S} = \frac{\ln\left(\frac{k_m}{k_m - LD}\right)}{c} \qquad 44$$

C. Relationship between growth rate and reactor substrate concentration

The average measured values of the steady state reactor substrate concentration for the runs with feedback $\bar{S}$ are recorded in column 16 of Tables 17 and 18. The average values of $\bar{S}$ for the runs without feedback are listed in column 12 of Table 20. Since the reactor is assumed to be completely mixed, $\bar{S}$ is also the effluent substrate concentration. A plot showing the relationship between growth rate and reactor substrate concentration is given in Figure 7. The data shown represent the average measured $\bar{S}$ values from the continuous flow runs with and without feedback. The growth rates were computed using equations 32 and 40. Figure 7 shows that the growth rate increases with increasing substrate concentration.

As noted in Section III, the Michaelis-Menten equation relates growth rate to $\bar{S}$ as:

$$k_1 = k_m \left(\frac{\bar{S}}{\bar{S} + S_n} \right) \qquad 4$$

Dixon and Webb (1958) suggest three methods of treating the data to obtain the constants k_m and S_n. However, when tried, none of the methods gave values of the constants which would yield a curve similar to the experimental curve of Figure 7.

The Teissier equation (Schulze and Lipe, 1963) also allows k_1 to be expressed in terms of $\bar{S}$ as:

$$k_1 = k_m (1 - e^{-c\bar{S}}) \qquad 6$$

Here again there is the problem of evaluating the constants k_m and c. Reed and Theriault (1931) suggested a rather cumbersome method for calculating the constants by relating the data in simultaneous equations. Using this method, the values of k_m and c were found to be respectively 0.869 and 0.0201. Equation 6 would then be:

$$k_1 = 0.869 (I - e^{0.0201\bar{S}}) \qquad 45$$

The curve predicted by equation 45 is plotted in Figure 7 showing a good fit to the experimental data. However, the data extend to only about 35 per cent of the computed maximum k_1 value. The feedback runs were not made at higher growth rates because of the mechanical

difficulty of supplying the required large amounts of sterilized media. Higher rates would also have made it difficult to maintain a sufficient level of dissolved oxygen in the reactor.

The values of k_m and c as obtained from the preceding analysis may be used to compute $\bar{S}$ by substitution in equation 37 to give for the runs with feedback:

$$\bar{S} = \frac{\ln\left(\frac{0.869}{0.869 - A'' D}\right)}{0.0201} \qquad 46$$

Values of $\bar{S}$ have been computed using the above equation both for each day as given in column 17 of Tables 1 through 16 and average values for each run as presented in column 17 of Tables 17 and 18. The computed values agree closely with the measured ones as would be expected.

For runs without feedback, equation 44 becomes:

$$\bar{S} = \frac{\ln\left(\frac{0.869}{0.869 - LD}\right)}{0.0201} \qquad 47$$

Values of $\bar{S}$ computed from equation 47 are given in column 13 of Table 20. They are in fair agreement with those measured experimentally.

1. Experimental determination of k_m

In order to check the value of k_m obtained above using the Reed-Theriault analysis, the maximum growth rate was determined from batch tests. As noted previously, a mixed culture was used during the continuous flow runs. Microscopic examination showed that many

species of bacteria and protozoa were present during the runs. It was felt that if a sample of this heterogeneous culture taken from a substrate limiting environment was allowed to grow under conditions of unlimited substrate, the population would shift radically to favor those organisms capable of the highest growth rate under the new conditions. The value of k_m thus obtained would probably be meaningless.

It was found, however, that one type of yellowish "folded" floc made up a significant proportion of the culture during most of the runs. The floc was found to consist of a member or members of the coliform group. It was felt that determination of the k_m of this isolated organism or organisms would give a value more representative of the actual population than by using a sample of the mixed culture.

Two batch determinations of k_m were made using the isolated coliform organisms. In each case, several loopfuls of cells were inoculated into 2.5 liters of sterile media. The growth of organisms was followed by aseptically withdrawing samples for analysis of cell concentration until logarithmic growth had ceased. The pH and dissolved oxygen of the culture were checked at intervals and adjusted when necessary. The substrate concentration was determined initially and near the end of the test in order to compute the yield factor. A summary of the data is given in Table 24. Figure 8 shows the resulting growth curves. The slope of the straight line portion of the curve of the logarithm of the organism concentration versus time gives the

desired value of k_m. The values of k_m obtained in tests 1 and 2 were 0.454 and 0.457. The respective values of Y were 0.523 and 0.556 which are in the same range as the yield coefficients obtained during the continuous flow runs. In both tests 1 and 2, the cells grew in a dispersed manner at their maximum rates of growth. However, after gently aerating the cultures for 4 or 5 days, partial flocculation occurred. Microscopically, the floc was found to be the same yellow "folded" type which was present during most of the runs.

The average k_m of 0.456 does not agree with the value of 0.869 obtained by the Reed-Theriault analysis of the continuous flow data. This is not too surprising when one considers the continuously changing heterogeneous group of organisms cultured in the reactor. The continuous flow data actually reflect the composite effect of all organisms growing in the unit in 19 runs over a two year period. Another factor contributing to the difference between the two maximum growth rates is the comparatively small range of k_1 values covered by the data. The highest growth rate attained using the continuous flow system was 0.29, which is approximately only 1/3 of the k_m obtained from the Reed-Theriault analysis. As may be seen from Figure 7 the relation between k_1 and $\bar{S}$ at this point was still close to a straight line and the value of 0.869 may therefore be somewhat in error. However, since $k_1 = 0.869$ was obtained using data from the continuous flow runs, it is felt to be the best estimate of the maximum growth rate available.

D. Comparison of actual and predicted reactor cell concentrations

1. Runs with feedback

Equations 34 and 37 may be combined to allow computation of the steady state reactor solids concentration as:

$$\bar{x}'' = \frac{\bar{Y}}{A''}\left[\frac{S' - \ln\left(\frac{k_m}{k_m - A''D}\right)}{c}\right] \qquad 48$$

Using equation 48, the daily values of the reactor solids concentration $\bar{x}''$ have been computed and are presented in column 8 of Tables 1 to 16. The daily values of $\bar{x}''$ are plotted in Figures 9 to 24. The measured values of the reactor cell concentration $\bar{x}$ are also plotted for comparison. The agreement is seen to be good in the majority of cases.

Each feedback factor shown was computed using the average values of x and x_r found from two consecutive measurements. Each feedback factor thus reflects two sets of data and gives a better estimate of the actual trend of conditions between the two measurements. The same may be said for the computed cell concentrations and k_1 values, since both parameters are computed using the feedback factor.

The average values of $\bar{x}''$ have been computed for each run and are given in column 9 of Tables 17 and 18. The overall values of $\bar{x}''$ are plotted against $\bar{x}$ in Figure 25 for comparison. If $\bar{x}$ and $\bar{x}''$ agreed perfectly, a 45 degree line would be formed. The computed and measured values are seen to follow the 45 degree line quite well except for

runs 2a and 2b. It was to be expected that these two runs might yield poor results, as only two values of x_r were measured in each case. No measurements of x_r were made for run 3. However, the overall value of x_r has been estimated using the plot of α vs C given in Figure 27. Parameters computed using this estimated x_r are marked with an asterisk in Table 17.

2. Runs without feedback

Substitution of equation 47 into equation 41 gives an expression which may be used to compute the steady state reactor solids concentration as:

$$\bar{X}'' = \frac{\bar{Y}}{L}\left[\frac{S' - \ln\left(\frac{k_m}{k_m - LD}\right)}{c}\right] \qquad 49$$

Using the above formulation, the average cell concentration for each run was computed and is presented in column 6 of Table 20. The computed and measured concentrations show close agreement. This is to be expected, since $\bar{Y}$ was calculated using the same data and only a difference between the computed and measured values of the substrate concentration causes a difference between $\bar{X}$ and $\bar{X}''$.

The yield factor was computed from the concentration of cells in the reactor effluent, R_e. Since R_e decreased with the length of time after scraping the reactor, only values of R_e taken 3 hours or more after scraping were used in computing $\bar{Y}$ or L.

E. Comparison of actual and predicted final effluent cell concentrations

1. Runs with feedback

The steady state final effluent solids concentration $\bar{x}_e''$ may be computed from equation 35 as:

$$\bar{x}_e'' = A''\bar{x}''$$

Daily values of $\bar{x}_e''$ are given in column 14 of Tables 1 through 16 and are plotted in Figures 9 through 24. In addition, the average values for each run are presented in column 15 of Tables 17 and 18. The computed effluent cell concentration is seen to exhibit almost no variation within a given run. This is to be expected since from equation 34

$$\bar{x}''A'' = \bar{Y}(S' - \bar{S})$$

Thus the steady state final effluent cell concentration is also equal to the rate of cell production $\bar{Y}(S' - \bar{S})$. Since $\bar{Y}$ and S' are constants, and the computed $\bar{S}$ values vary only slightly within a given run, $\bar{x}_e''$ will show little variation. The actual daily system effluent concentration x_e is presented in column 13 of Tables 1 through 16. The average value of x_e for each run is also given in column 14 of Tables 17 and 18. x_e is the concentration of all cells lost from the system, including those lost through sampling. The value of x_e varies significantly from day to day and is sometimes greater and sometimes less than $\bar{x}_e''$. Hence for some periods more cells were being lost from the system than were being produced (x_e greater than $\bar{x}_e''$) and during other periods more

cells were being produced than were lost from the system ($\bar{x}_e$" greater than x_e). As will be discussed in Section VI, it is this fluctuation in x_e which was largely responsible for the daily variation in the reactor cell concentration.

2. Runs without feedback

The equilibrium effluent cell concentration is given by equation 42 as:

$$\bar{X}_e'' = L\bar{X}''$$

The values of $\bar{X}_e$" have been computed for the three runs and are recorded in column 11 of Table 20. $\bar{X}_e$" is seen to agree with the measured value R_e,(column 7). This is to be expected since the computations used to calculate $\bar{X}_e$" were dependent upon R_e.

F. Relationship between feedback factor and reactor cell concentration

One of the most important parameters influencing the operation of the feedback system is the feedback factor A". The solids concentration in the aerator depends directly upon A" as may be seen from equation 34. A given value of A", producing a certain reactor cell concentration, may result from an almost infinite combination of C, α, and L values, as may be seen from Figure 6. Hence if equation 34 holds, a plot of the computed A" values and the respective measured cell concentrations should show a definite correlation with $\bar{x}$ increasing as A" decreases. In Figure 26, A" has been plotted versus $\bar{x}$. For comparison,

a curve has been drawn through the computed values of the reactor cell concentration $\bar{x}''$ which are also plotted. The measured values of $\bar{x}$ are seen to follow the curve closely, showing the expected correlation with A''. The cell concentration increases with decreasing A'' and approaches infinity as A'' approaches zero. For the runs without feedback, the A'' factor is equal to L since α and C are zero. The points representing the three runs without feedback are shown as squares on the figure.

G. Relationship between recirculation ratio and cell concentration factor

The recirculation ratio and the cell concentration factors are readily measurable basic system parameters. In this investigation, the value of α could be easily manipulated by changing F_i or F_r. However, the value of C was largely non-controllable. The feedback factor is, of course, dependent upon both parameters.

In order to determine if α and C were inter-related, the two parameters have been plotted against one another in Figure 27. It may be seen that the data show C and α to be inversely related. This inverse relation might be expected since as α is increased the detention time in the final tank is decreased (assuming F_i remains constant) and C will thus be reduced. The detention time in the settling tank varied from 0.3 to 3.0 hours during the experiments. The theoretical curves in Figure 6 also suggest C will decrease with increasing α. For any given value of A'', if α is increased, C will decrease. However, it

may be argued that A'' will also change to maintain the same value of C or even result in a larger C. The inter-relation between C and α will be discussed more fully in Section VI.

H. Effect of a negative feedback factor on system operation

As pointed out in a previous section, it is theoretically possible to have a negative feedback factor A where $A = 1 + \alpha - \alpha C$. It may be seen from Figure 1 that A will become negative if α is increased and C does not decrease. However, the experimental curve presented in Figure 27 indicates that C will decrease if α is increased.

Run No. 12 was designed to investigate what effect a negative A might have on the system. After completion of run No. 11, run 12 was begun by increasing the return rate, and data were taken immediately without allowing the system to adjust itself to a new equilibrium. Just prior to increasing the return rate, C, α, and A were 1.732, 0.538 and 0.605 respectively. The variation in α, C and A during run 12 is plotted in Figure 28. Upon increasing the return rate and producing an α of 2.130, the A factor decreased to -0.560. However, it may be seen that one hour later A had become +0.590, due to a decrease in C from 1.732 to 1.193. The average value of C for run 12 is 1.28 as compared to an average C of 1.72 for run 11. The only condition changed was the increase of the return rate, causing α to change from an average of 0.549 in run 11 to 2.163 in run 12. The average A for run 12 is 0.393. As

may be seen from Figure 28, the feedback factor did become slightly negative a second time during run 12. However, it is felt that the relatively high C value which caused the negative A was not representative of actual conditions. No other negative values of A were found during any of the runs except these two in run 12.

The above discussion has considered the effect of a negative A on system operation. It is also theoretically possible to obtain a negative value of the modified feedback factor A'' where $A'' = L + L\alpha - \alpha C$. It may be seen from Figure 6 that A'' will become negative if α is increased and C does not decrease. However, the computation of A'' is based on L, which in turn is based on a calculation assuming an equilibrium between cell mass flowing in and out of the settling tank as shown in equation 30. In run 12, when the return rate was suddenly increased, the settling tank was losing cells at a much higher rate than it was receiving them from the reactor. This imbalance was soon resolved by the decrease in the return concentration which quickly occurred as shown in Figure 28. The almost immediate increase in reactor cell concentration as shown in Table 16 (column 6) also helped to resolve the imbalance by increasing the concentration of cells going into the settling tank. Immediately after a sudden increase of the return rate, the system needs time to adjust itself. Since the balance around the final tank assumes equilibrium, it predicts a higher reactor effluent cell concentration, making the L value greater than 1.0 and thus not allowing A'' to

become negative. For example, in run 12, the L value increased to 1.305 immediately upon increasing α, resulting in a positive A'' of 0.395.

I. Relationship between specific growth rate and specific substrate consumption rate

The specific substrate consumption rate k_c (mg glucose consumed per gram cell weight per hour) may be calculated as:

$$k_c = \frac{D(S' - \bar{S})}{\bar{x}} \qquad 50$$

The consumption rates for each run are given in column 4 of Table 25. The specific growth rates as computed from equations 32 and 40 are included for comparison in column 5. k_c is plotted against the growth rate in Figure 29. The value of k_c is seen to increase directly with k_1 as:

$$k_c = n + hk_1 \qquad 51$$

where n is the ordinate intercept representing the consumption rate when k_1 equals zero and h is a factor giving the milligrams of glucose consumed per gram of cell weight formed. The method of least squares was used to evaluate n and h giving:

$$k_c = 15 + 1764\, k_1 \qquad 52$$

From equation 52 it is seen that a substrate consumption rate of 15 mg glucose per gram cell weight per hour is occurring under conditions of no growth.

Schulze and Lipe (1963), using E. coli and a glucose media, have shown that their data follow equation 51. They found a value of n equal to 55 mg glucose per gram cell weight per hour. The authors termed n the maintenance supply since it is just sufficient to sustain cell metabolism but not enough to allow growth and reproduction.

The parameter h is by definition the inverse of the yield factor, Y. Using the value of h obtained above, expressed as grams of glucose consumed per gram of cell weight formed, the yield factor is computed to be $1/1.764 = 0.567$. The value of 0.567 is slightly higher than the highest value of Y measured during the continuous flow runs of 0.556. This would be expected since 1/h gives a corrected yield factor which does not include the substrate consumed just to maintain the cell metabolism.

J. Relationship between growth rate and yield coefficient

The average yield coefficient for each run is given in column 13 of Tables 17 and 18 and column 9 of Table 20. $\bar{Y}$ is seen to vary from 0.252 to 0.559. When considering only the runs with feedback which used an S' of 1000 mg/l, the variation is reduced to from 0.413 to 0.556. In Figure 30, $\bar{Y}$ is related to the growth rate. It may be seen that $\bar{Y}$ increases sharply with k_1 up to a growth rate of approximately 0.06. As k_1 increases above 0.06, $\bar{Y}$ increases much more slowly and probably tends toward a maximum value of around 0.56. The

organisms were therefore converting substrate into cell material at a much higher efficiency at the higher k_1 values. A similar relationship has been noted by other workers (Herbert, 1958; Lipe, 1961; Orford et al., 1960). The reason for the sharp dropoff of $\bar{Y}$ at low k_1 values is probably due to the influence of endogenous metabolism, as was discussed in Section III.

K. Organic load on system

The organic loading rate is an important parameter to the operation of an activated plant. It is usually expressed as the unit weight of substrate applied to the aeration tank per unit weight of suspended solids per unit time. The average organic loading rate expressed in terms of glucose and BOD_5 for each run is given in column 3 of Table 25. The BOD values have been calculated assuming that 1 mg/l of glucose equals 0.746 mg/l of BOD_5 (Standard Methods, 11th Edition, 1960. The consumption rate (column 4) and efficiency (column 6) are also listed. The loading rate varies from 32.0 to 532.0 mg glucose per gram cell weight per hour. Efficiency of substrate removal is above 98 per cent in all runs.

The loading rate expressed in terms of BOD is seen to vary from 0.57 to 9.54 lbs BOD_5 per lb of reactor cell material per day. These loading rates are considerably higher than those used in practice and yet did not impair the efficiency of BOD removal. The design organic

loading used in the conventional activated sludge process seldom exceeds 0.5 lbs per lb of suspended solids per day. One purpose of keeping the loading rate at this range is to promote the growth of flocculent organisms as pointed out by Garrett and Sawyer (1952). Pilot plant studies by Logan and Budd (1955) suggest that BOD loading rates over 0.38 or under 0.22 lbs per lb of suspended solids per day tend to increase the Sludge Volume Index and hence reduce the floc settling properties. During the feedback runs flocculent growth was obtained even at the highest loading rate (7.96 lbs BOD_5/lb cell material/day). However, there was also a high concentration (approximately 100 to 150 mg/l) of non-flocculent organisms in the effluent which would not have been an acceptable effluent from a full-scale plant.

L. Transient condition analysis

The analysis of the data has thus far been based on equations which were derived assuming steady state operation of the system. These equations assume that there is no change in cell or substrate concentration in the reactor. It has been shown that the steady state equations predict the operation of the system quite well considering the number of variables involved.

However, as may be seen from Tables 1 through 16, the reactor cell concentration did vary from day to day for a given run.

In Section III, equations 26 and 27 were derived to describe operation of the system under non-steady state or transient conditions. These equations are given below using the modified feedback factor.

$$\frac{\Delta x}{\Delta t} = \bar{Y} D (S' - S) - A'' Dx - \bar{Y} \left(\frac{\Delta S}{\Delta t}\right) \qquad 53$$

and

$$\frac{\Delta x}{\Delta t} = \bar{Y} D (S' - \bar{S}) - A'' Dx \qquad 54$$

or

$$\Delta x = \Delta t \left[\bar{Y} D (S' - \bar{S}) - A'' Dx\right] \qquad 55$$

Equations 54 and 55 are written assuming the term $\bar{Y}\left(\frac{\Delta S}{\Delta t}\right)$ is close to zero. This seems a reasonable assumption since the maximum variation in S between two consecutive days in a given run is about 3 mg/l. The maximum value of the term $\bar{Y}\left|\frac{\Delta S}{\Delta t}\right|$ would then be only about one per cent of the computed variation in $\Delta x/\Delta t$. If large variations in S occurred over short time intervals the term could not be neglected.

Using equation 55, Δx has been computed for each day's data and is recorded in column 5 of Tables 26 through 28. The actual Δx as found from the measured initial (x_I) and final (x_F) cell concentrations is also given for each day in column 4. It may be seen that the computed and measured values of Δx agree reasonably well in most cases. However, in some instances they are widely divergent. In computing

Δx, the average value of $\bar{x}$ between two measurements has been used. Using the measured values of x_I and the predicted Δx values, the final cell concentrations (x_F') have been computed. These are given in column 7 of Tables 26 through 28 and are plotted in Figures 9 through 24 for comparison with the measured final cell concentrations. The computed variation in cell concentration as given by $\Delta x'$ follows the actual variation in the majority of cases. That is, if the actual cell concentration has increased during a given day, the computed variation follows this trend. In those few cases where the predicted and measured variation are widely divergent, it is felt that the disagreement is due to the influence of fluctuations in the cell mass in the settling tank on the system.

M. Mixing studies

The development of the equations used in this investigation assumes that the contents of the reactor are completely mixed at all times. In a completely mixed reactor the increments of the influent completely intermix with the vessel contents. The concentration of any reaction product in the exit flow from the reactor thus has the same value as that of the reactor fluid. The washout rate of a suspension of concentration, Q, from a completely mixed continuous flow unit may thus be expressed as:

$$\frac{-dQ}{dt} = DQ \qquad 56$$

Equation 56 may be integrated to yield an expression for the concentration at any time as:

$$Q = Q_o e^{-Dt} \qquad 57$$

where Q_o is the initial concentration at t = 0 and t is the time elapsed since initiation of the flow. Since D equals F_i/v and is actually the reciprocal of the nominal detention time, t_o, the term Dt may also be written t/t_o.

It has already been shown that the cell concentration in the reactor was different from that of the reactor effluent in the experiments performed during this investigation. Thus one of the basic characteristics of the complete mixing model described above did not hold for the laboratory unit. To correct for this departure from the completely mixed model, the theoretical analysis was modified and a correlation factor L introduced as shown earlier.

In order to get a better understanding of the mixing characteristics of the reactor, a series of washout tests were conducted. Tests were made using a dye solution, mixed liquor from the East Lansing Sewage Plant, cells from the reactor and Fuller's earth. The tests were carried out in the same reaction vessel and under the same mixing conditions used during the investigations already described. The reactor was filled with a known concentration of one of the above substances and tap water pumped in at a steady dilution rate. The decrease in concentration of the substance in the reactor was checked periodically until it

became too small to be measured accurately. In order to determine if the position of the overflow tube had any measurable effect on the mixing pattern, runs were made with the overflow tube at mid-depth and at the top of the liquid level.

If perfect mixing conditions prevailed in the reactor, the concentration would follow the negative exponential curve predicted by equation 57. In chemical engineering literature, the curve represented by equation 57 is usually presented in reduced or dimensionless units. A plot of Q/Q_o vs Dt is called the "F" curve. This curve depicts the residence time distribution of labeled elements in the reactor. Figure 31-1 shows the shape of the F curve for some representative types of mixing systems after Danckwerts (1953). The piston flow and the complete mixing systems represent the ideal types of flow.

Danckwerts (1953) pointed out that the F diagram may be used to estimate the degree of departure of the system from complete mixing. If the F diagrams of a completely mixed and an incompletely mixed system are superimposed on each other, a result such as that shown in Figure 31-2 may be obtained. The size of the shaded area $(A_1 + A_2)$ shows the departure from complete mixing.

1. Washout pattern of a dye solution (Run W-1)

In order to investigate the flow pattern of a solution through the reactor without the complicating effects of material sticking to the walls of the reactor and the effluent tube, a dye was chosen. The washout of

the dye was followed with a Coleman Jr. Spectrophotometer set at a wavelength for maximum absorption of the incident light. A calibration curve of per cent transmittance vs concentration was made to allow determination of the dye concentration in the reactor at any time.

A mixing experiment was first conducted using methylene blue. However, about two hours after the start of the run, it was noted that the dye was coming out of solution and forming particles visible to the naked eye. Since the calibration curve would no longer hold, the run was discarded.

After unsuccessfully experimenting with several other dyes, picric acid was found to remain in solution for prolonged periods despite vigorous mixing. Using a dilution rate of 0.271, a solution of picric acid was washed out of the reactor and its concentration followed as noted above. The pertinent data are given in Table 29 and the theoretical and actual F curves are presented in Figure 32. It may be seen that the two curves are essentially the same, showing that the contents of the reactor were completely mixed.

2. Washout patterns of mixed liquor and reactor cells (Runs W-2, W-3 and W-4)

Two washout runs were carried out using mixed liquor from the East Lansing Sewage Treatment Plant. The organisms were first killed by addition of 300 mg/l of free chlorine in the form of sodium hypochlorite. A side overflow was used for run W-3 and a top overflow for run W-2.

Run W-4 was carried out with a side overflow using cells from the continuous flow experiments. The cells which had been stored in the refrigerator for several weeks were first killed with 3 mg/l free chlorine. Reactor solids concentration for the above runs was determined by the membrane filter technique. Since solids were necessarily removed from the reactor for each sample, the measured concentration has been corrected to account for this removal in each case.

The data for runs W-2 through W-4 are given in Tables 30 to 32. The respective F diagrams are presented in Figures 33 to 35. The curves show that the mixing conditions were close to perfect during the three experiments. In run W-2 using mixed liquor and a top overflow the solids were initially washed out of the vessel slightly faster than predicted. However, toward the end of the run the measured reactor concentration was slightly higher than theoretical and solids were retained in the vessel. Mixed liquor was also used in run W-3, but the solids left the vessel from a side overflow. Run W-3 exhibited perfect mixing during most of the run. However, the data show that solids were retained in the vessel toward the end of the run as in W-2. The data for run W-4 show that the washout was close to theoretical. In contrast during the continuous flow experiments, it was found that the reactor effluent cell concentration was regularly lower than the cell concentration in the reactor, indicating retention of cells in the vessel. It was expected that the washout experiments might exhibit the same

tendency by showing a slower cell washout than was predicted by complete mixing theory. The reason for the fact that runs W-2 to W-4 did not show the expected retention is probably that the washout experiments were conducted over much shorter periods of time than the actual growth runs. Hence the cells did not build up on the reactor or the effluent tube which was a major cause of solids retention in the continuous flow runs. Killing the floc with chlorine was also observed to alter its appearance and may have affected its washout. Finally, the washout runs were different from the growth runs in that cells were not fed back to the reactor. The return of a high cell concentration to the unit may have had an influence on its overall mixing characteristics.

3. Washout patterns of Fuller's earth (Runs W-5 and W-6)

Fuller, s earth was used in two runs in order to determine what effect a completely inert high specific gravity material might have on mixing. Run W-5 was made using a side overflow and W-6 a top overflow. The data are given respectively in Tables 33 and 34. The F diagram for run W-5 is presented in Figure 36 showing a faster rate of washout than predicted over the entire curve. This would be expected since the relatively heavy solids would tend to settle despite mixing and thus be washed out faster due to the low position of the effluent port. It would also be expected that such a suspension would wash out more slowly than predicted if the overflow were at the top of the vessel.

This proved to be the case for run W-6 as shown in Figure 37. Using a top overflow, the experimental washout curve lagged well behind the complete mixing curve. Hence mixing of a suspension is greatly influenced by the specific gravity of the solids.

Table 1. Measured and Computed data - Run No. 1

v = 2.530 l S' = 210 mg/l T = 27° C

Length of run hours	D	α	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0	0.123	0.721	2.350	996	424											
13.50	0.123	0.721	2.190	1008	460	614	1150	0.046	0.976	0.006	0.298	39.2	52.7	29.7	1.2	0.3
37.50	0.128	0.739	1.765	720	408	196	365	0.144	0.929	0.019	0.268	72.6	52.5	69.4	1.5	0.1
61.50	0.133	0.713	2.490	1024	412	263	449	0.117	0.952	0.019	0.215	43.6	52.5	51.4		0.9

Table 2. Measured and computed data - Run No. 2

v = 2.340 l S' = 419 mg/l T = 27° C

Length of run hours	D	α	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	$\bar{S}$ mg/l	$\bar{S}$ mg/l
0					650											
10.50	0.078	3.303	1.462	948	648						0.314					
26.00	0.074	3.490	0.922	540	586	586	598	0.274	0.999	0.020	0.314	163	164	196	0.8	1.1
48.25	0.079	3.250	1.062	684	644	160	782	0.210	0.808	0.017	0.295	90	164	131		0.9
58.75	0.083	3.080	1.210	740	612	282	588	0.279	0.024	0.023	0.405	191	164	187		1.2
73.00	0.085	3.030	1.294	772	596	701	582	0.282	1.010	0.024	0.405	191	164	187		1.3
84.25	0.085	3.030	1.280	692	540	1260	537	0.306	1.043	0.026	0.509	237	164	188		1.4
98.50	0.085	3.030	1.118	608	544	421	534	0.307	0.980	0.026	0.509	237	164	188		1.4
109.25	0.067	3.850			432											
129.00	0.067	3.850	1.489	828	556											
179.75	0.074	3.450			612											
192.50	0.074	3.450	1.186	688	580	348	533	0.308	0.964	0.023	0.489	238	164	198		1.2
207.75	0.073	3.510			564											
224.00	0.072	3.575			588											
231.00	0.072	3.575			536											

Table 3. Measured and computed data - Run No. 2a

v = 2.340 l S' = 410 mg/l T = 27° C

Length of run hours	D	α	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0	0.050	0.682	2.520	1517	602											
6.00	0.050	0.682	1.440	893	620	397	2995	0.046	0.827	0.002	0.632	198	138	77		0.5
25.50	0.051	0.680			706						0.509	122	138	52	2.0	
36.00	0.050	0.706			574						0.338	131	138	131		
53.75	0.050	0.716			714						0.338	131	138	131		
71.50	0.049	0.728			598						0.032	146	138	267		

Table 4. Measured and computed data - Run No. 2b

v = 2.315 l S' = 1020 T = 27° C

Length of run hours	D	α	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	V	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0	0.078	1.26			1935											
7.50	0.078	1.26			1997						0.394	307	420	328		
18.00	0.073	1.32	1.583	3259	2059											
29.50	0.079	1.22			2071											
42.00	0.077	1.26	1.620	3543	2187	1912	2919	0.144	0.967	0.011	0.395	334	420	353	2.1	0.6
56.00	0.075	1.30			2115						0.438	516	420	490		
66.00	0.078	1.26			2047						0.438	516	420	490		
78.00	0.079	1.24			1967						0.419	433	420	427		
90.00	0.079	1.24			2032						0.419	433	420	427		
105.00	0.071	1.37			2036											

Table 5. Measured and computed data - Run No. 3

v = 2.315 l S' = 1019 T = 27° C

Length of run hours	D	α	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0	0.127	0.759			2425											
23.50	0.130	0.742			2665						0.406	335	427	348		
51.00	0.124	0.771			2652						0.449	460	427	431	3.5	
74.25	0.126	0.767			2800						0.435	391	427	349		
98.50	0.132	0.729			2355						0.387	521	427	552		
124.25	0.122	0.798			2348						0.398	406	427	429		
154.25	0.127	0.755			2620						0.427	367	427	359		
169.17	0.124	0.778			2640						0.478	488	427	430		

Table 6. Measured and computed data - Run No. 4

$\forall$ = 2.315 l S' = 1018 mg/l T = 27° C

Length of run hours	D	A	C	x_r	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0	0.128	1.430	1.498	2940	1965	1653										
15.25	0.126	1.450	1.200	2535	2110	958	2260	0.212	0.797	0.027	0.446	440	480	467		1.6
42.75	0.121	1.500	1.491	3037	2035	995	2135	0.225	0.896	0.027	0.446	440	480	467		1.6
66.75	0.116	1.500	1.379	2750	1995	1387	1951	0.246	0.960	0.028	0.473	497	480	496		1.7
88.25	0.126	1.425	1.630	3170	1945	1696	1986	0.242	0.982	0.030	0.473	497	480	496		1.7
120.25	0.123	1.445	1.637	3247	1985	5650	2000	0.240	1.064	0.030	0.468	492	480	497		1.7
133.00	0.126	1.402	1.637	3022	1850	4360	1903	0.252	1.058	0.032	0.468	492	480	497		1.8
162.25	0.126	1.409	1.453	3065	2110	1975	2623	0.183	0.973	0.023	0.537	475	480	409	1.8	1.3
186.75	0.124	1.423	1.507	3260	2165	1514	2263	0.212	0.955	0.026	0.430	410	480	453		1.5
213.75	0.121	1.465	1.434	3270	2280	1534	2363	0.203	0.955	0.025	0.430	410	480	453		1.3
233.00	0.128	1.399	1.509	3730	2475	1412	2858	0.168	0.928	0.021	0.501	429	480	400		1.2
253.75	0.126	1.418			2355						0.538	591	480	525		

Table 7. Measured and computed data - Run No. 4a

v = 2.38 l S' = 1041 mg/l T - 27° C

Length of run hours	D	α	C	x_r	$\bar{x}$	$\bar{x}'$	$\bar{x}''$	A"	L	k_1	Y	x_e	$\bar{x}_e''$	x_{er}	Meas $\bar{S}$	Calc $\bar{S}$
					mg/l	mg/l	mg/l			hr^{-1}		mg/l	mg/l	mg/l	mg/l	mg/l
0			0.946	2125	2250											
15.50	1.237	1.151	1.089	2520	2315											
23.50	0.133	1.178	1.638	3825	2335	948	2530	0.215	0.835	0.029	0.586	589	544	524		1.7
40.50	0.119	1.352	1.417	3125	2205	1253	1347	0.404	0.988	0.048	0.502	593	544	615	2.7	2.8
47.50	0.133	1.228	1.417	3175	2240	1110	2590	0.210	0.879	0.028	0.564	548	544	505		1.7
67.00	0.120	1.382	1.519	3140	2065											
91.00	0.119	1.408	1.473	3215	2130	1777	2295	0.237	0.972	0.028	0.480	457	544	503		1.7
121.00	0.115	1.464	1.553	3240	2085											
146.00	0.116	1.460	1.521	3600	2365	2461	2958	0.184	0.985	0.021	0.565	414	544	371		1.2

Table 8. Measured and computed data - Run No. 5

v = 2.38 l S' = 1040 mg/l T = 24° C

Length of run hours	D	α	C	x_r	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	$\bar{x}er$ mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0	0.131		1.618	2825	2365	2230										
20.17	0.129	1.289	1.536	3800	2475	2139	2697	0.206	0.976	0.021	0.520	497	555	548		1.1
49.75	0.133	1.263	1.498	3740	2500	1591	2532	0.219	0.943	0.029	0.523	537	554	569		1.8
73.50	0.133	1.254	1.540	3780	2455	1582	2471	0.225	0.945	0.030	0.511	545	554	553		1.8
44.25	0.131	1.292	1.565	3850	2460	1938	2523	0.220	0.971	0.029	0.554	574	554	577	2.2	1.8
118.00	0.129	1.311	1.589	3800	2395	2260	2360	0.235	0.995	0.030	0.553	595	554	512		1.7

Table 9. Measured and computed data - Run No. 6

v = 2.38 l S' = 1040 mg/l T = 24° C

Length of run hours	D	α	C	x_r	$\bar{x}$	$\bar{x}'$	$\bar{x}''$	A"	L	k_1	Y	x_e	$\bar{x}_e''$	x_{er}	Meas $\bar{S}$	Calc $\bar{S}$
					mg/l	mg/l	mg/l			hr^{-1}		mg/l	mg/l	mg/l	mg/l	mg/l
0	0.200	1.296	1.563	4755	3040											
23.25	0.120	1.300	1.565	4640	2965	2011	2962	0.180	0.963	0.036	0.462	488	533	550		2.0
47.25	0.200	1.292	1.550	4505	2910	1904	2885	0.185	0.957	0.037	0.555	476	533	543		2.2
73.75	0.201	1.288	1.619	4730	2925	2132	2917	0.181	0.970	0.036	0.502	510	533	530		2.1
98.00	0.203	1.272	1.700	4835	2842	3270	2852	0.167	1.010	0.038	0.572	602	533	551		2.2
122.25	0.206	1.248	1.550	4350	2810	2423	2822	0.189	0.985	0.039	0.483	500	533	540		2.3
145.00	0.204	1.264	1.540	4375	2840	1708	2910	0.184	0.943	0.037	0.559	565	533	527	3.5	2.2
170.50	0.203	1.268	1.683	4965	2955	2379	3059	0.175	0.979	0.036	0.482	470	533	511		2.0
142.00	0.204	1.267	1.772	4865	2765	658	2703	0.197	1.051	0.040	0.522	581	533	581		2.4

Table 10. Measured and computed data - Run No. 7

v = 2.38 l S' = 1038 mg/l T = 24°C

Length of run hours	D	α	C	x_r	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0			1.498	4050	2705											
15.00	0.199	1.088	1.663	4375	2630	1338	2603	0.190	0.914	0.038	0.505	547	494	518		2.2
42.50	0.201	1.078	1.683	3860	2295	1790	2225	0.222	0.973	0.045	0.549	628	494	554		2.6
74.00					2525											
86.50	0.199	1.086	1.679	4510	2685	2386	2906	0.170	0.982	0.034	0.445	396	494	430		1.9
97.00	0.200	1.078			2610	1727	2730	0.181	0.950	0.036	0.393	404	494	491	3.1	2.0
110.00	0.202	1.071	1.653	4455	2695	1727	2730	0.181	0.950	0.036	0.393	404	494	491		2.0
123.50	0.203	1.067			2660											

Table 11. Measured and computed data - Run No. 8

v = 1.26 l S' = 1040 mg/l T = 24° C

Length of run hours	D	α	C	x_r	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e$ mg/l	x_{er} mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l
0					2970											
13.00	0.385	1.019	1.725	5480	3180											
25.00	0.383	1.040	1.700	5560	3270						0.662					
38.50	0.382	1.058	1.380	4460	3230	1130	3225	0.150	0.865	0.057	0.662	694	483	518		3.4
65.25	0.377	1.049	1.307	6010	4600	748	5410	0.190	0.728	0.034	0.427	307	484	529		2.0
71.00	0.377	1.049			4510										3.5	
84.75	0.386	1.019	1.540	6710	4350	852	4250	0.114	0.777	0.044	0.388	436	484	433		2.6
108.75	0.379	1.033	1.687	6660	3950	1315	3843	0.126	0.881	0.048	0.466	527	484	492		2.7

Table 12. Measured and computed data - Ru n No. 8a

v = 1.31 l S' = 1029 mg/l T = 25° C

Length of run hours	D	α	C	x_r	$\bar{x}$	$\bar{x}'$	$\bar{x}''$	A"	L	k_1	Y	x_e	$\bar{x}_e''$	x_{er}	Meas $\bar{S}$	Calc $\bar{S}$
					mg/l	mg/l	mg/l			hr^{-1}		mg/l	mg/l	mg/l	mg/l	mg/l
0			1.560	1880	1205											
22.00	0.289	0.520	1.667	2475	1410	872	1715	0.331	0.787	0.096	0.498	485	569	530	6.2	5.6
45.00	0.296	0.511	1.841	1810	920	999	1037	0.546	0.983	0.161	0.589	679	567	644		9.9
57.00	0.298	0.510	1.900	2315	1255	1036	1342	0.423	0.922	0.126	0.568	488	568	476		7.5

Table 13. Measured and computed data - Run No. 9

v = 1.31 l S' = 1026 mg/l T = 25° C

Length of run hours	D	α	C	x_r	$\bar{x}$	$\bar{x}'$	$\bar{x}''$	A''	L	k_1	Y	x_e	$\bar{x}_e''$	x_{er}	Meas $\bar{S}$	Calc $\bar{S}$
					mg/l	mg/l	mg/l			hr^{-1}		mg/l	mg/l	mg/l	mg/l	mg/l
0	0.458	0.508	1.810	2615	1445											
12.75	0.456	0.517	1.545	2345	1520	817	1528	0.348	0.800	0.159	0.419	411	532	518		9.9
24.25	0.480	0.498	1.981	3230	1630	864	1650	0.321	0.803	0.154	0.557	548	532	515		9.5
35.75	0.474	0.493	2.268	2885	1715	1197	1749	0.305	0.906	0.144	0.557	548	532	515		8.6
48.75	0.468	0.493	2.161	3415	1580	1334	1594	0.334	0.955	0.156	0.536	575	532	564		9.8
61.25	0.464	0.500	2.151	2915	1355	1268	1412	0.377	0.971	0.175	0.536	575	532	564	10.0	11.2
71.75	0.456	0.512	1.710	2265	1348	1325	1347	0.395	0.916	0.180	0.545	566	532	545		11.5
86.25	0.459	0.501	1.852	2250	1215	820	1262	0.422	0.871	0.193	0.545	566	531	545		12.5
96.75	0.454	0.509	1.978	2070	1095	956	1179	0.451	0.930	0.204	0.555	564	531	532		13.4
113.25	0.460	0.499	2.250	2035	1305	1157	1250	0.425	0.979	0.196	0.555	564	531	532		12.6
120.25	0.454	0.509	2.506	4080	1630	1834	1612	0.330	1.028	0.150	0.469	453	532	508		9.2
134.5	0.460	0.500	2.583	3785	1465	2323	1680	0.317	1.058	0.146	0.469	453	532	508		8.7

Table 14. Measured and computed data. - Run No. 10

v = 1.0 l S' = 1000 mg/l T = 25° C

Length of run hours	D	α	C	x_r	$\bar{x}$	$\bar{x}'$	$\bar{x}''$	A"	L	k_1	Y	x_e	$\bar{x}_e''$	x_{er}	Meas $\bar{S}$	Calc $\bar{S}$
					mg/l	mg/l	mg/l			hr^{-1}		mg/l	mg/l	mg/l	mg/l	mg/l
0					1615											
21.00	0.834	0.362	1.463	3190	2180	724	2020	0.270	0.695	0.225	0.528	286	545	511		15.0
39.00	0.830	0.363	2.463	3755	1525	799	1721	0.316	0.731	0.262	0.516	553	544	589		17.8
58.67	0.821	0.369	1.930	3850	1995	951	1887	0.289	0.793	0.237	0.615	567	545	515	15.1	15.8
68,67	0.827	0.366	2.524	5315	2105											

Table 15. Measured and computed data - Run No. 11

v = 1.95 l S' = 1000 mg/l T = 25° C

Length of run hours	D	α	C	x_r	$\bar{x}$	$\bar{x}'$	$\bar{x}''$	A"	L	k_1	Y	x_e	$\bar{x}_e''$	x_{er}	Meas $\bar{S}$	Calc $\bar{S}$
					mg/l	mg/l	mg/l			hr^{-1}		mg/l	mg/l	mg/l	mg/l	mg/l
0			1.640	2320	1415											
15.50	0.332	0.551	1.628	2065	1270	822	1255	0.425	0.854	0.141	0.566	623	534	595		8.4
23.50	0.331	0.559	1.745	2585	1480	873	1459	0.367	0.842	0.122	0.577	547	535	510	5.9	7.5
37.00	0.341	0.545	1.850	2785	1505	948	1608	0.333	0.850	0.113	0.577	547	535	510		6.8
48.50	0.334	0.555	1.816	2360	1300	996	1351	0.395	0.908	0.132	0.543	565	534	559		7.9
66.25	0.331	0.560	1.545	1990	1290	863	1270	0.421	0.874	0.139	0.543	565	534	559		8.4
81.75	0.347	0.538	1.820	2400	1320	845	1324	0.403	0.850	0.140	0.420	414	534	533	5.3	8.4

Table 16. Measured and computed data - Run No. 12

v = 1.95 l S' = 1002 mg/l T = 25°C

Length of run hours	D	α	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	x_{er}	Meas. $\bar{S}$ mg/l	Calc. $\bar{S}$ mg/l
0	0.340	2.130	1.732	2340	1350											
1.00	0.340	2.130	1.193	1945	1630						0.528	558		580		
8.00	0.340	2.170	0.980	1160	1185						0.528	558		580		
14.50	0.334	2.183	1.006	1630	1620						0.528	558		580		
31.50	0.324	2.220	1.532	1955	1275	864	1410	0.388	0.925	0.128	0.528	558	547	580	7.8	7.8
55.50	0.339	2.159	1.460	1730	1185		1205	0.453	1.167	0.154	0.552	559	547	558		9.5
80.50	0.328	2.202	1.162	2000	1720	1471	1680	0.326	0.985	0.110	0.574	504	547	482		6.5
87.50	0.338	2.138	1.246	1675	1345	950	1205	0.454	0.961	0.153	0.527	678	547	704	8.4	9.5

Table 17. Average values of measured and computed data - Run Nos. 1 to 5

Run No.	D	α	S'	C	x_r mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	Y	x_e mg/l	$\bar{x}_e''$ mg/l	Meas $\bar{S}$ mg/l	Calc $\bar{S}$ mg/l	pH	D.O. mg/l
1	0.202	1.279	210	2.200	927	426	392	438	0.120	0.992	0.015	0.252	54.0	52.6	1.4	0.9	6.4	4.1
2	0.078	3.370	419	1.209	706	584	195	556	0.294	0.999	0.023	0.392	181	164	0.8	1.4	6.9	3.6
2a	0.050	0.705	410	1.877	1205	642	397	775	0.178	0.879	0.009	0.337	138	138	2.0	0.5	6.9	1.3
2b	0.077	1.275	1020	1.655	3401	2054	2556	2716	0.155	0.996	0.012	0.413	404	421	2.1	0.7	7.2	0.8
3	0.128	1.757	1019	1.700	4365	2568		2570	0.166	0.826	0.021	0.420	415	427	3.4	1.3	7.1	2.4
4	0.124	1.441	1018	1.490	3093	2105	1610	2104	0.288	0.974	0.027	0.472	469	480	1.8	1.7	7.0	1.8
4a	0.120	1.367	1041	1.453	3189	2196	1432	2370	0.229	0.935	0.027	0.524	508	544	2.7	1.6	6.9	3.0
5	0.131	1.290	1040	1.553	3802	2449	1950	2463	0.225	0.974	0.037	0.534	550	554	2.2	1.8	7.1	1.6

Table 18. Average values of measured and computed data- Run Nos. 6 to 12

Run No.	D	α	S'	C	$\bar{x}_T$ mg/l	$\bar{x}$ mg/l	$\bar{x}'$ mg/l	$\bar{x}''$ mg/l	A"	L	k_1 hr^{-1}	$\bar{Y}$	x_e mg/l	$\bar{x}_e''$ mg/l	Meas. $\bar{S}$ mg/l	Calc. $\bar{S}$ mg/l	pH	D.O. mg/l
6	0.202	1.279	1025	1.619	4469	2892	2553	2952	0.181	0.986	0.037	0.522	535	533	3.5	2.2	7.0	1.3
7	0.202	1.074	1038	1.682	4361	2592	1864	2602	0.190	0.963	0.038	0.477	443	494	3.1	2.3	7.0	0.6
8	0.382	1.039	1040	1.551	5937	3822	1130	3881	0.125	0.850	0.048	0.467	463	484	3.5	2.9	6.8	1.1
8a	0.294	0.513	1029	1.777	2137	1202	951	1182	0.480	0.920	0.140	0.556	566	568	6.2	8.7	7.1	2.3
9	0.463	0.502	1026	2.098	2987	1423	1180	1476	0.361	0.940	0.167	0.524	532	532	10.4	10.0	7.0	0.9
10	0.830	0.364	1000	1.939	3614	1866	830	1910	0.285	0.727	0.236	0.553	537	545	15.1	15.7	7.0	1.6
11	0.338	0.549	1000	1.729	2337	1361	886	1346	0.397	0.866	0.134	0.538	553	534	5.6	8.3	7.1	3.1
12	0.331	2.163	1002	1.280	1773	1389	1390	1392	0.393	0.999	0.130	0.551	553	547	8.1	8.1	7.0	2.7

Table 19. Data obtained from Run Nos. 20 to 22.

Run No.	Length of run	D hr^{-1}	$\bar{X}$ mg/l	R_e mg/l	Hours after scraping	Meas. $\bar{S}$ mg/l	Settled floc vol. ml
20							
	24.00	0.105	612				
	37.25	0.105	644	480	6		600
	49.25	0.105	652	588	0	6.2	800
	59.75	0.105	520*	424*	0		370
21							
	19.25	0.259	664	524	3	9.4	200
22							
	12.00	0.360					45
	29.00	0.357	668	600	0	19.3	8
	35.50	0.357	680	640	0		
	72.50	0.345	664	548	5	42.6*	1

* Data not used in calculations

Table 20. Average values of measured and computed data - Run Nos. 20 to 22.

Run No.	D hr^{-1}	S' mg/l	$\bar{X}$ mg/l	$\bar{X}'$ mg/l	$\bar{X}'$ mg/l	R_e mg/l	L	$\bar{Y}$	k_1 hr^{-1}	$\bar{X}_e''$ mg/l	Meas. $\bar{S}$ mg/l	Calc. $\bar{S}$ mg/l	pH	D.O. mg/l
20	0.105	1000	636	480	636	480	0.754	0.483	0.079	480	6.2	4.8	7.0	2.4
21	0.259	996	664	524	660	524	0.790	0.531	0.205	524	9.4	13.4	7.1	3.5
22	0.354	998	667	548	665	548	0.822	0.559	0.292	548	19.3	20.4	7.0	2.3

Table 21. Data obtained from evaporation experiments.

Evaporation loss ml	Elapsed time min	Rate of evap. ml/min	Air rate l/min	Rate of evap. $\frac{ml}{min}/\frac{l}{min\ air}$	Temp. °C
43	886	0.0485	2.80	0.017	24.0
29	690	0.0420	2.80	0.015	24.0
20	936	0.0216	1.40	0.015	28.0
32	952	0.0340	1.90	0.018	28.0

Table 22. Computed rate of evaporation assuming no reactor effect.

Run No.	Actual $(F_i + F_r)$ ml/min	Computed $(F_i + F_r)'$ ml/min	Air rate l/min	Computed rate of evaporation $\frac{(F_i + F_r) - (F_i + F_r)}{\text{Air rate}}$
1	9.37	9.30	1.0	0.070
2	12.97	12.95	1.0	0.020
2a	3.32	2.92	1.1	0.364
2b	6.71	6.68	1.1	0.031
3	8.64	7.14	1.8	0.833
4	11.74	11.43	1.7	0.194

Table 23. Comparison of measured reactor effluent concentration and reactor cell concentration

Run. No.	Length of run hours	Hours after scraping	Measured R_e (mg/l)	L'	Reactor cell conc. mg/l
10	21.00	6	1050	0.482	2180
	68.67	5	1395	0.664	2105
11	0	7	1225	0.867	1415
	15.50	0	1205	0.950	1270
	23.50	5	1345	0.910	1480
	37.00	0	1410	0.938	1505
	48.50	0	1200	0.924	1300
	66.25	0	1155	0.895	1290
12	31.50	0	1075	0.844	1275
	55.50	4	1060	0.895	1185
	80.50	0	1665	0.969	1720
	87.50	0	1330	0.990	1345
20	37.25	6	480	0.747	644
	47.25	0	588	0.902	652
	57.75	0	424	0.815	520
21	19.25	3	524	0.790	664
22	29.00	0	600	0.926	668
	35.50	0	640	0.942	620
	72.50	5	548	0.824	644

Table 24. Summary of data for batch determination of k_m.

	Test No. 1	Test No. 2
k_m	0.454	0.457
Generation time - hours	1.528	1.518
Y	0.523	0.556
Initial substrate conc.	1040	1092
Temperature	25°C	25°C

Table 25. Data concerning rates of substrate supplied and consumed.

Run. No.	Substrate supplied mg glu/gm cell wt / hour	Substrate supplied lbs BOD/lb cell / day	k_c Substrate consumed mg glu/gm cell wt / hour	k_1 hr^{-1}	Efficiency of removal %
2a	32.0	0.57	31.9	0.007	99.6
2b	38.0	0.68	37.9	0.012	99.8
1	63.7	1.14	63.3	0.015	99.4
2	54.7	0.98	54.6	0.023	99.7
4a	57.0	1.02	56.8	0.027	99.8
4	60.2	1.08	60.1	0.028	99.7
5	55.5	1.00	55.4	0.029	99.8
6	71.6	1.28	71.5	0.037	99.8
7	80.6	1.44	80.4	0.038	99.7
8	103.2	1.85	103.0	0.048	99.7
20	164.5	2.94	163.5	0.079	99.4
12	239.8	4.29	238.0	0.130	99.3
11	248.0	4.44	246.2	0.134	99.4
8	250.5	4.49	249.0	0.140	99.5
9	333.6	5.97	330.3	0.167	99.1
21	388.2	6.96	384.9	0.205	99.1
10	444.1	7.96	437.0	0.236	98.5
22	532.0	9.54	522.0	0.292	98.1

Table 26. Comparison of experimental and computed variation in reactor cell concentration. - Runs 1 to 5.

Run No.	Hours of Run	Δt	Actual Δx	Computed Δx	Actual x_F	Computed x_F'
1	13.50	13.50	+36	-54	460	478
	37.50	24.00	-52	-31	408	429
	61.50	24.00	+ 4	-14	412	422
2	26.00	15.50	-62	- 6	586	642
	48.25	22.25	+58	+62	644	648
	73.00	14.25	-16	- 8	596	604
	84.25	11.25	-56	-10	540	586
	98.50	14.25	+ 4	- 2	544	538
	192.50	12.75	-32	-19	580	593
4	15.25	15.25	+145	+63	2110	2028
	42.75	27.50	-75	+43	2035	2153
	66.75	24.00	-40	-45	1995	1990
	88.25	21.50	-50	+ 8	1945	2003
	120.25	32.00	+40	+ 3	1985	1948
	133.00	12.75	-135	-16	1850	1969
	162.25	29.25	+260	+431	2110	2281
	186.75	24.50	+55	+82	2165	2192
	213.75	27.00	+115	+95	2280	2260
	233.00	19.25	+195	+199	2475	2479
4a	23.50	8.00	+20	+47	2335	2362
	40.50	17.00	-130	-743	2205	1592
	47.50	7.00	+35	+73	2240	2278
	91.00	24.00	+115	+116	2180	2181
	146.00	25.00	+280	+387	2365	2472
5	20.17	20.17	+110	+143	2475	2508
	49.75	29.58	+25	+35	2500	2510
	73.50	23.75	-45	- 9	2455	2491
	94.25	20.75	+ 5	+43	2460	2498
	118.00	23.75	-65	-49	2395	2411

Table 27. Comparison of experimental and computed variation in reactor cell concentration - Runs 6 to 9.

Run. No.	Hours of Run	Δt	Actual Δx	Computed Δx	Actual x_F	Computed x_F'
6	23.25	23.25	-75	-28	2965	3012
	47.25	24.00	-55	-48	2910	2917
	73.75	26.50	+15	+29	2925	2939
	98.00	24.25	-83	-30	2842	2895
	122.25	24.25	-32	- 5	2810	2837
	145.00	22.25	+30	+59	2840	2869
	170.50	25.50	+115	+145	2955	2985
	192.00	21.50	-210	-127	2745	2828
7	15.00	15.00	-75	-39	2630	2666
	42.50	27.50	-335	-293	2295	2337
	86.50	12.50	+160	+127	2685	2652
	110.00	13.00	+85	-33	2695	2577
8	38.50	13.50	-40	-21	3230	3249
	65.25	26.75	+1370	+1350	4600	4580
	84.75	13.75	-250	-203	4350	4397
	108.75	24.00	-400	-355	3950	3995
8a	22.00	22.00	+205	+865	1410	2070
	45.00	23.00	-490	-453	920	957
	57.00	12.00	+355	+386	1255	1306
9	12.75	12.75	+75	+91	1520	1536
	24.25	11.50	+110	+143	1630	1663
	35.75	11.50	+85	+125	1715	1755
	48.75	13.00	-135	-110	1580	1605
	61.25	12.50	-225	-128	1355	1452
	71.25	10.05	-30	+10	1325	1365
	86.25	14.50	-110	-30	1215	1295
	96.75	10.50	-120	+47	1095	1262
	113.25	16.50	+210	+165	1305	1262
	120.25	7.00	-325	+156	1630	1461
	134.50	14.25	-165	+269	1465	1899

Table 28. Comparison of experimental and computed variation in reactor cell comparison - Runs 10 to 12.

Run. No.	Hours of Run	Δt	Actual Δx	Computed Δx	Actual x_F	Computed x_F'
10	21.00	21.00	+565	+578	2180	2193
	39.00	18.00	-655	-597	1525	1583
	58.67	19.67	+470	+580	1995	2105
11	15.50	15.50	-142	-180	1270	1235
	23.50	8.00	+210	+79	1480	1349
	37.00	13.50	+25	+174	1505	1654
	48.50	11.50	-205	-73	1300	1432
	66.25	17175	-10	-59	1290	1241
	81.75	15.50	+30	+43	1320	1333
12	31.50	31.50	-75	-89	1275	1261
	55.50	24.00	-90	-90	1185	1185
	80.50	25.00	+535	+594	1720	1779
	87.50	7.00	-375	-354	1345	1366

Table 29. Data from washout Run W-1.

Picric acid Side overflow v = 2.180 liters

D = 0.271 Air rate = 1.05 liters air/liter reactor vol./min

Dt	Measured conc. micrograms/l	Corrected conc. micrograms/l	Theoretical conc. micrograms/l	Q/Q_o
0	93.4		93.4	1.000
0.135	81.5		81.6	0.872
0.416	62.4		61.7	0.668
0.614	51.4		50.6	0.550
1.110	30.8		30.8	0.330
1.381	23.5		23.5	0.252
1.660	17.9		17.7	0.192
1.879	14.3		14.4	0.153
1.973	13.0		13.0	0.139
2.040	12.2		12.1	0.131
2.109	11.5		11.3	0.123

Table 30. Data from washout Run W-2.

Mixed liquor Top overflow v = 1.945 liters

D = 0.323 Air rate = 1.10 liters air/liter reactor vol./min

Dt	Measured conc. mg/l	Corrected conc. mg/l	Theoretical conc mg/l	Q/Q_o
0	9780	9730	9730	1.000
0.183	7525	7525	8080	0.773
0.595	4955	4987	5360	0.513
1.028	3190	3228	3480	0.332
1.324	2430	2465	2585	0.253
2.180	1185	1220	1098	0.125
2.763	665	685	613	0.071
3.051	545	560	457	0.057

Table 31. Data from washout Run W-3.

Mixed liquor Side overflow v = 2.038 liters

D = 0.297 Air rate = 1.08 liters air/liter reactor vol./min

Dt	Measured conc. mg/l	Corrected conc. mg/l	Theoretical conc. mg/l	Q/Q_o
0	7655	7580	7580	1.000
0.158	6320	6320	6326	0.834
0.470	4590	4619	4740	0.609
0.801	3400	3438	3398	0.452
0.994	2880	2917	2803	0.384
1.595	1565	1608	1530	0.212
2.080	985	1007	946	0.183
2.437	775	797	659	0.105

Table 32. Data from washout Run W-4.

Reactor cells Side overflow v = 2.095 liters

D = 0.377 Air rate = 1.43 liters air/liter reactor vol./min

Dt	Measured conc. mg/l	Corrected conc. mg/l	Theoretical conc. mg/l	Q/Q_o
0	885	877	877	1.000
0.344	610	610	621	0.695
0.722	415	419	425	0.478
1.444	168	173	208	0.197
2.262	83	87	91	0.099

Table 33. Data from washout Run W-5.

Fuller's earth Side overflow v = 2.310

D = 0.210 Air rate = 1.19 liters air/liter reactor vol./min

Dt	Measured conc. mg/l	Corrected conc. mg/l	Theoretical conc. mg/l	Q/Q_o
0	3430	3400	3400	1.000
0.203	2608	2608	2777	0.767
0.349	2220	2229	2399	0.658
0.751	1415	1434	1606	0.422
1.307	788	810	921	0.238
1.834	436	460	544	0.136
2.448	217	239	292	0.070

Table 34. Data from washout Run W-6.

Fuller's earth Top overflow v = 1.920

D = 0.244 Air rate = 1.25 liters air/liter reactor vol./min

Dt	Measured conc. mg/l	Corrected conc. mg/l	Theoretical conc. mg/l	Q/Q_o
0	3875	3835	3835	1.000
0.304	3095	3095	2830	0.807
0.567	2520	2544	2174	0.664
0.956	1723	1761	1471	0.459
1.300	1285	1323	1046	0.345
2.010	760	760	513	0.208
2.773	425	452	238	0.118

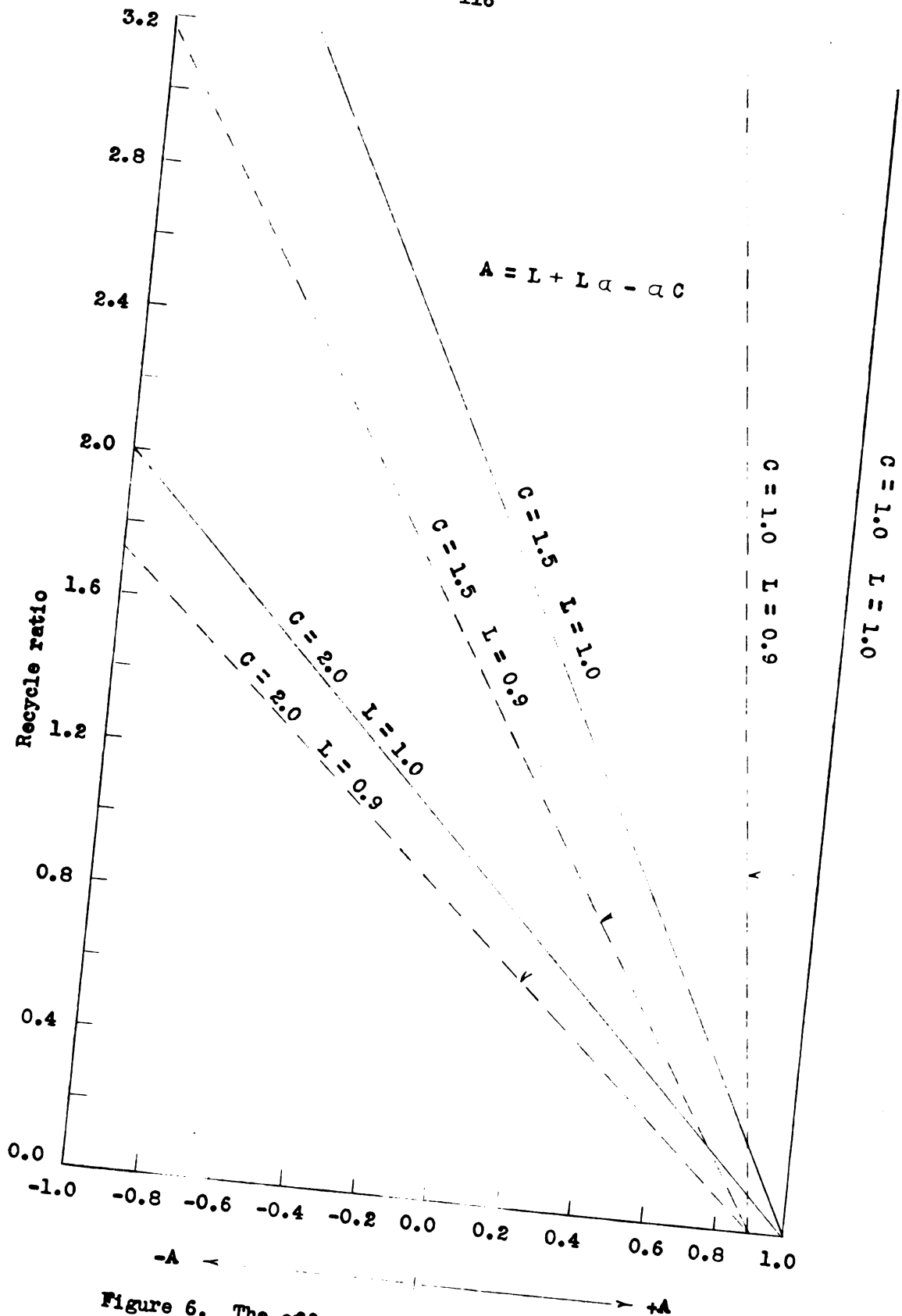


Figure 6. The effect of L on the feedback factor.

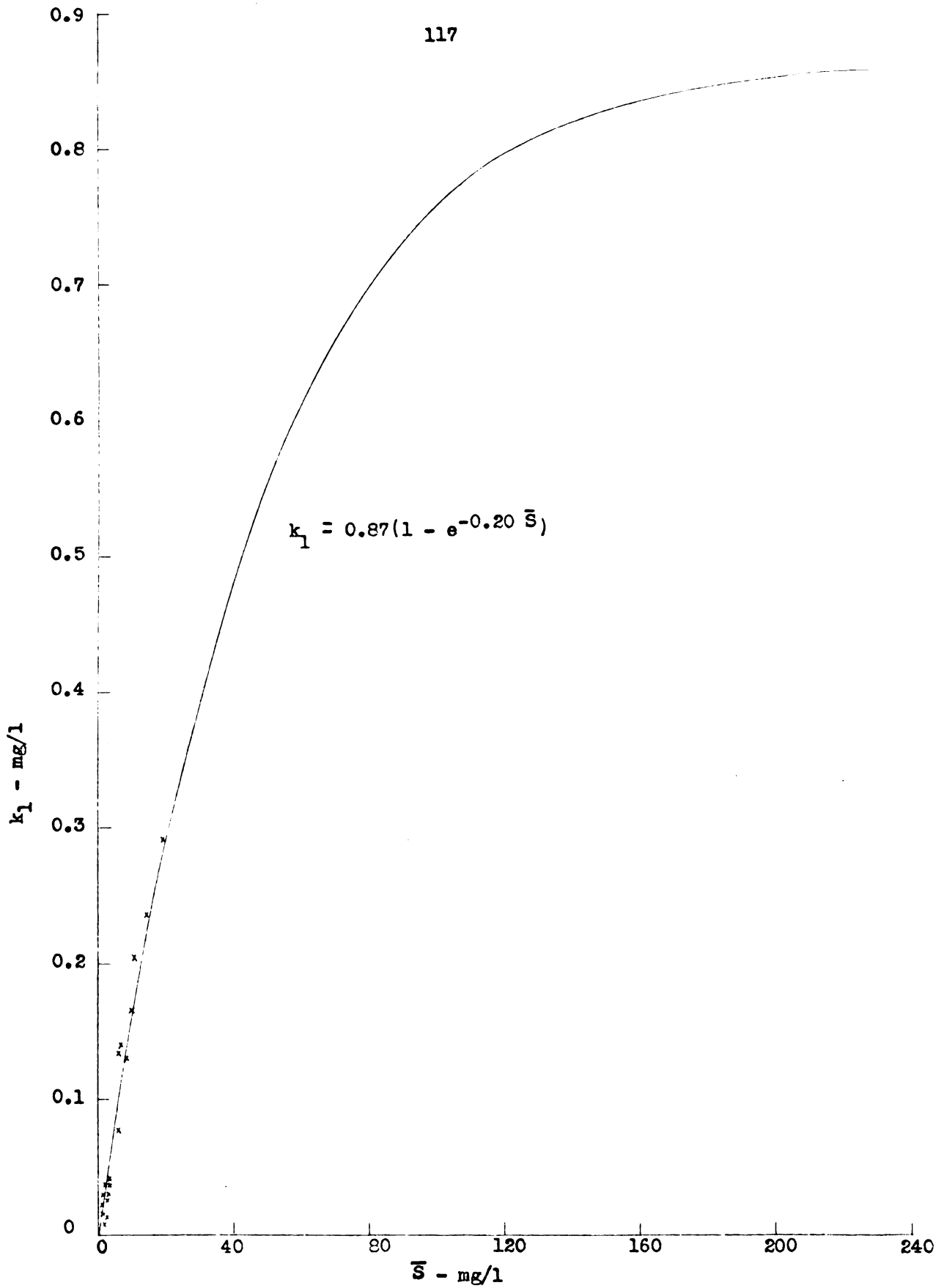


Figure 7. Relationship between k_1, experimental and calculated $\bar{S}$ values.

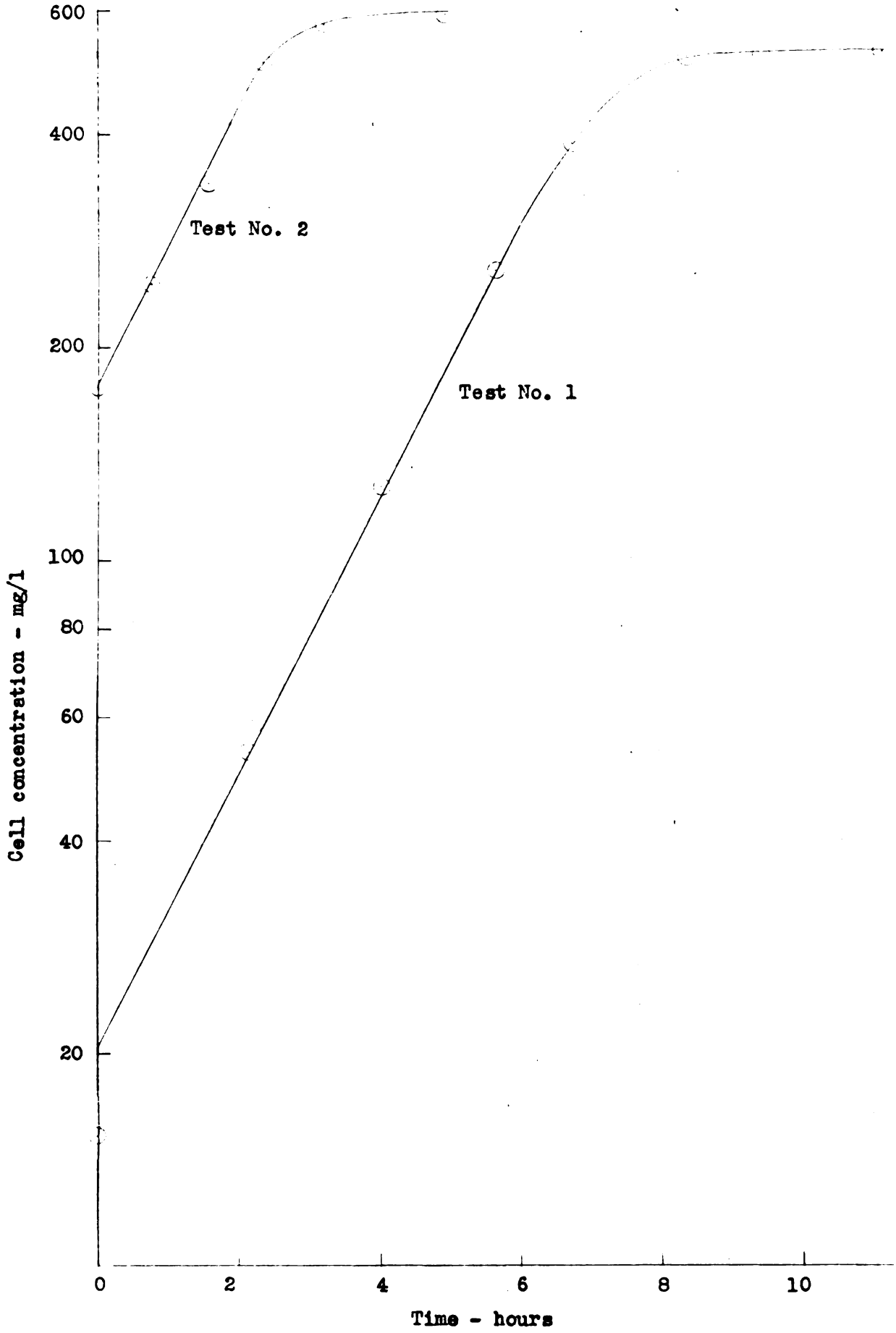


Figure 8. Determination of k_m.

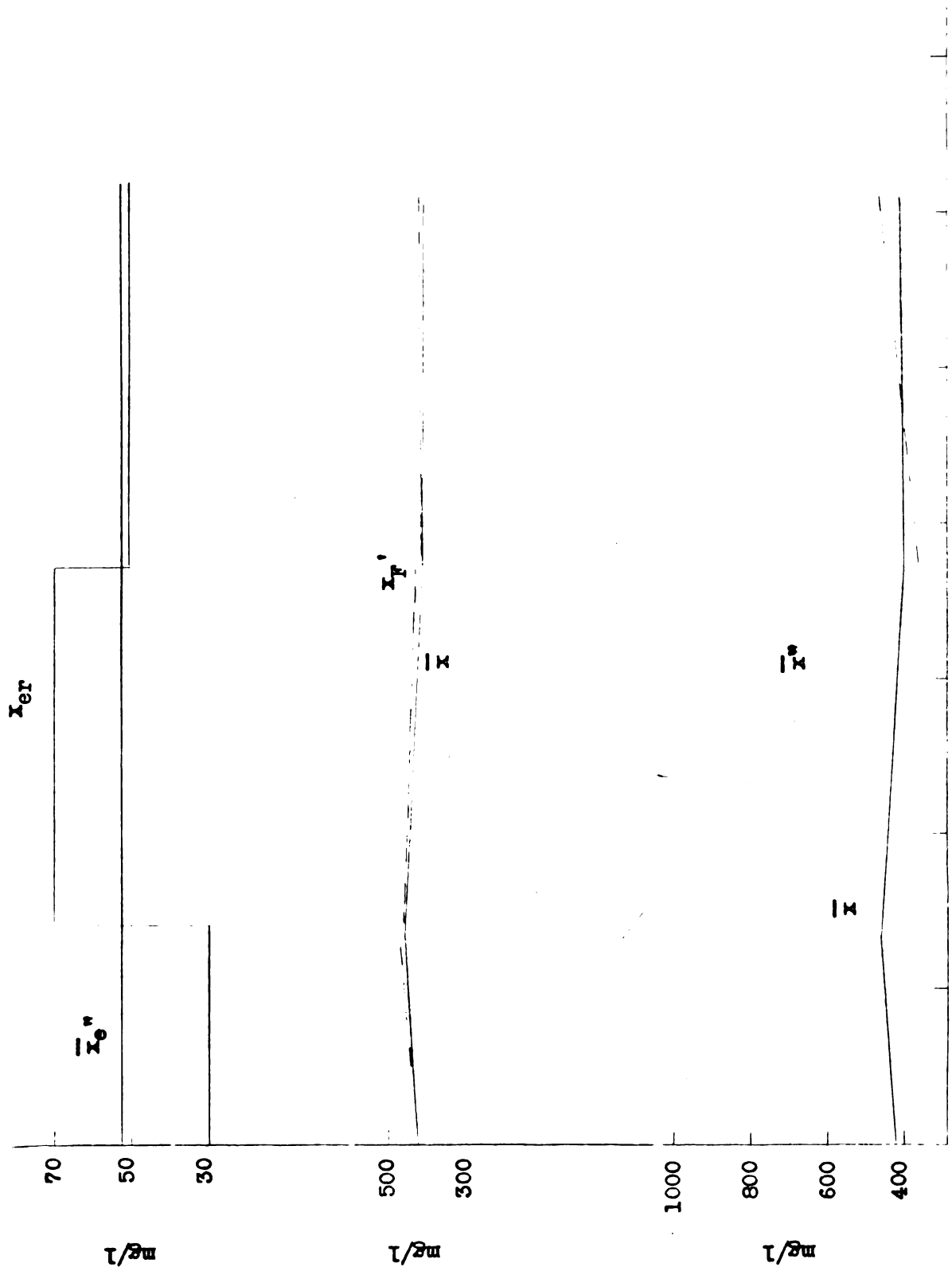


Figure 9. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 1.

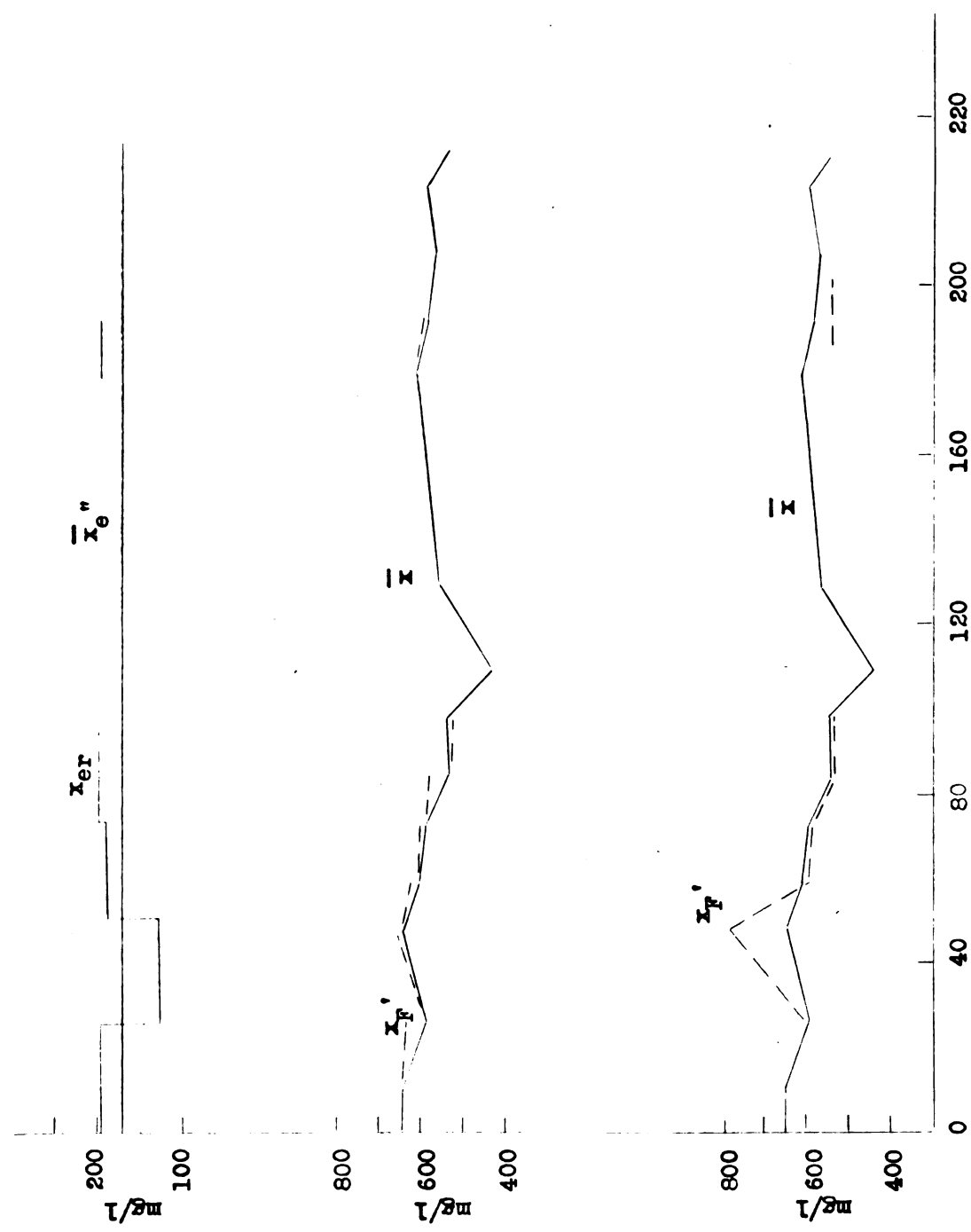


Figure 10. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 2.

Figure 11. Daily variation in $\bar{x}$; comparison of $\bar{x}_o$" and x_{or} - Run No. 2a.

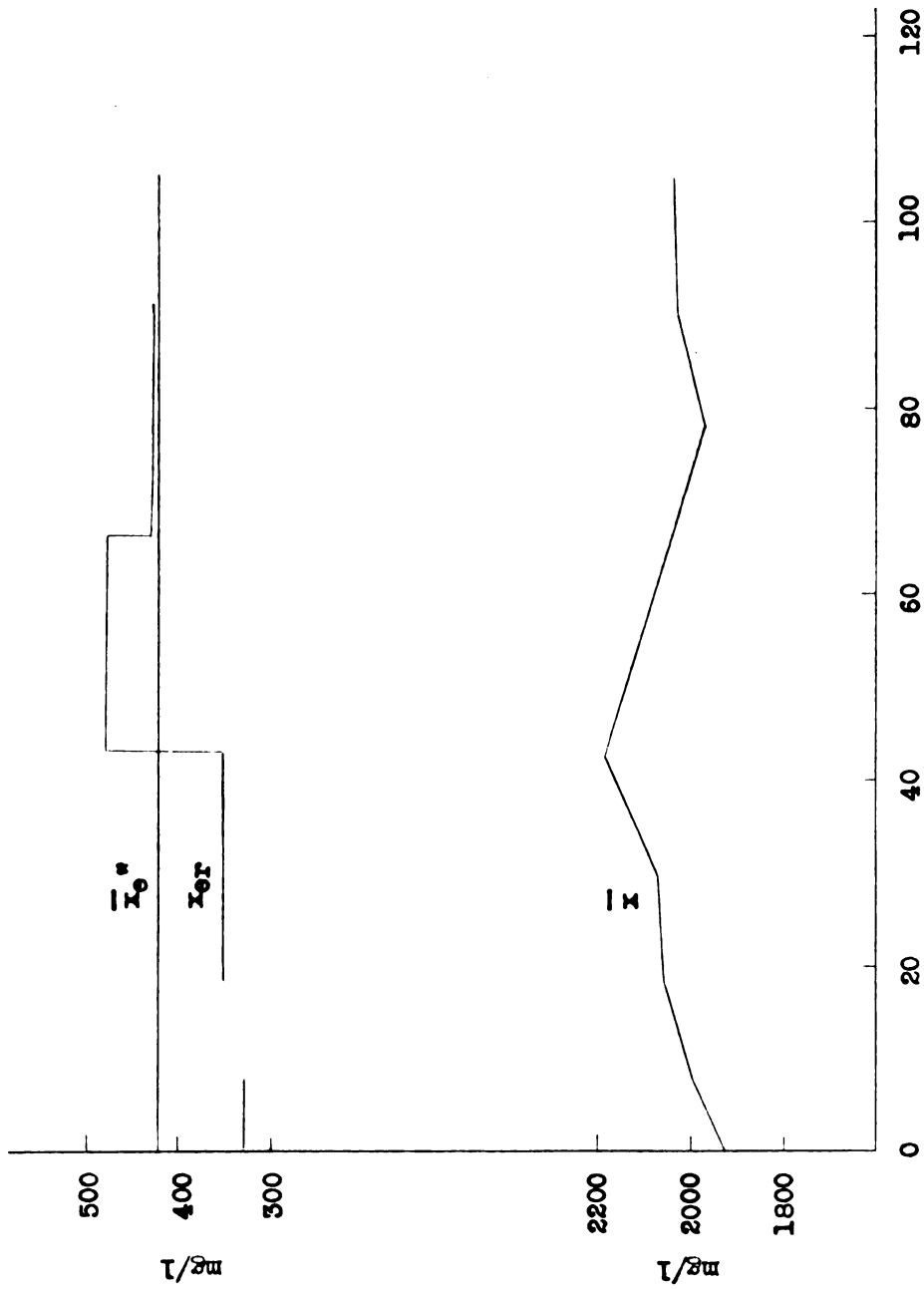


Figure 12. Daily variation in $\bar{x}$; comparison of $\bar{x}_e''$ and x_{er} - Run No. 2b.

Figure 13. Daily variation in $\bar{x}$; comparison of $\bar{x}_e$" and x_{er} - Run No. 3.

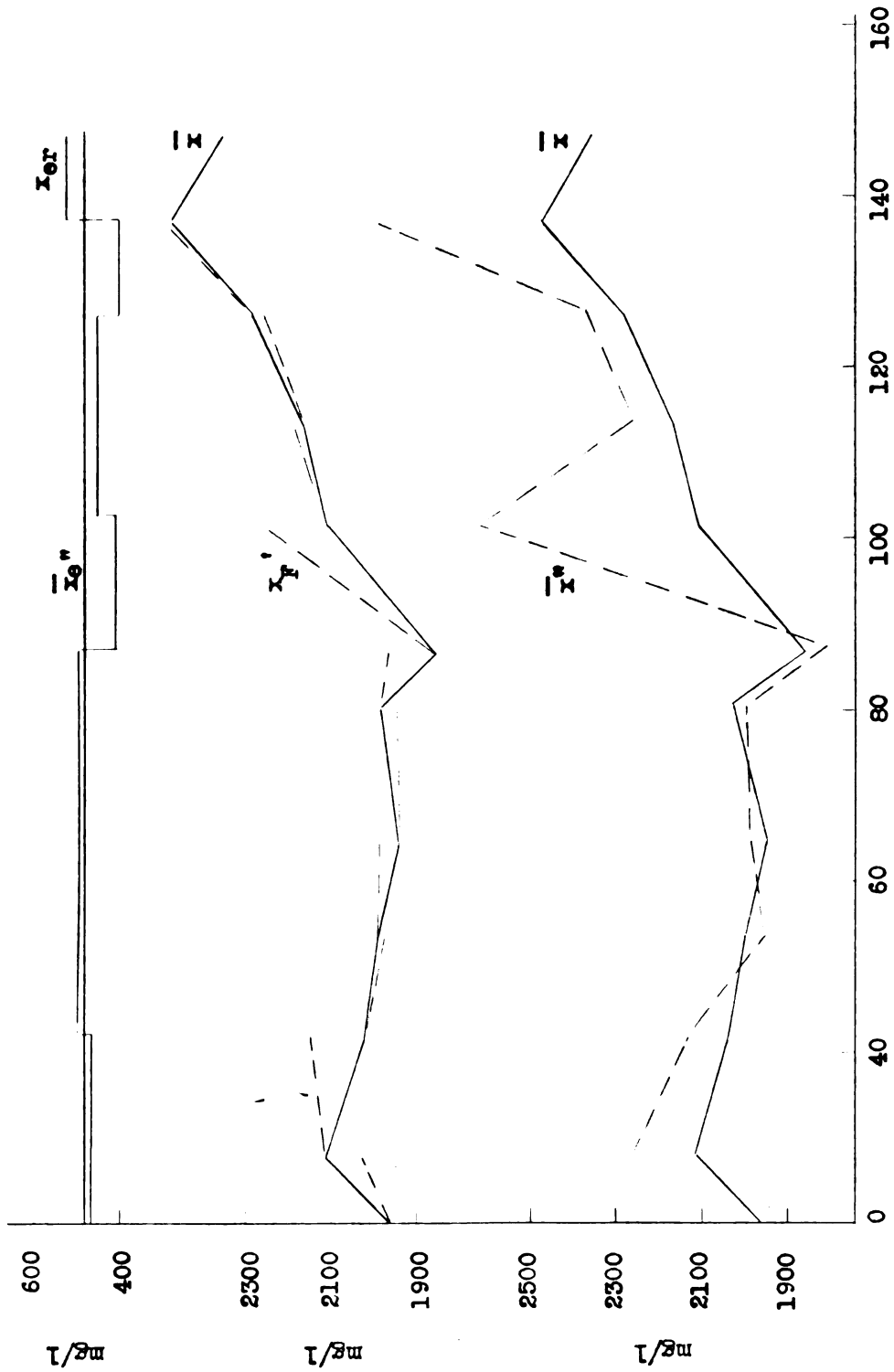


Figure 14. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 4.

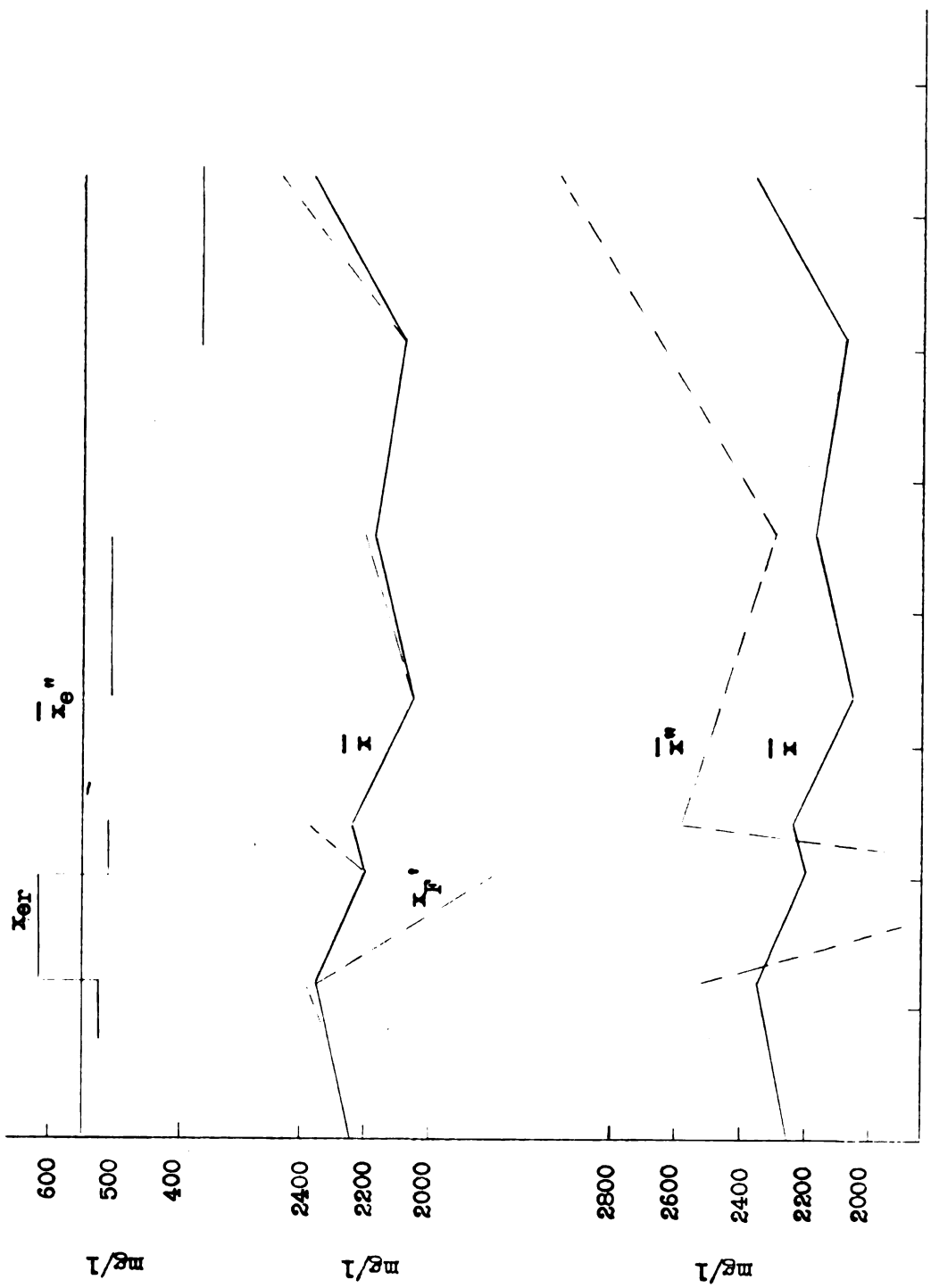


Figure 15. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 4a.

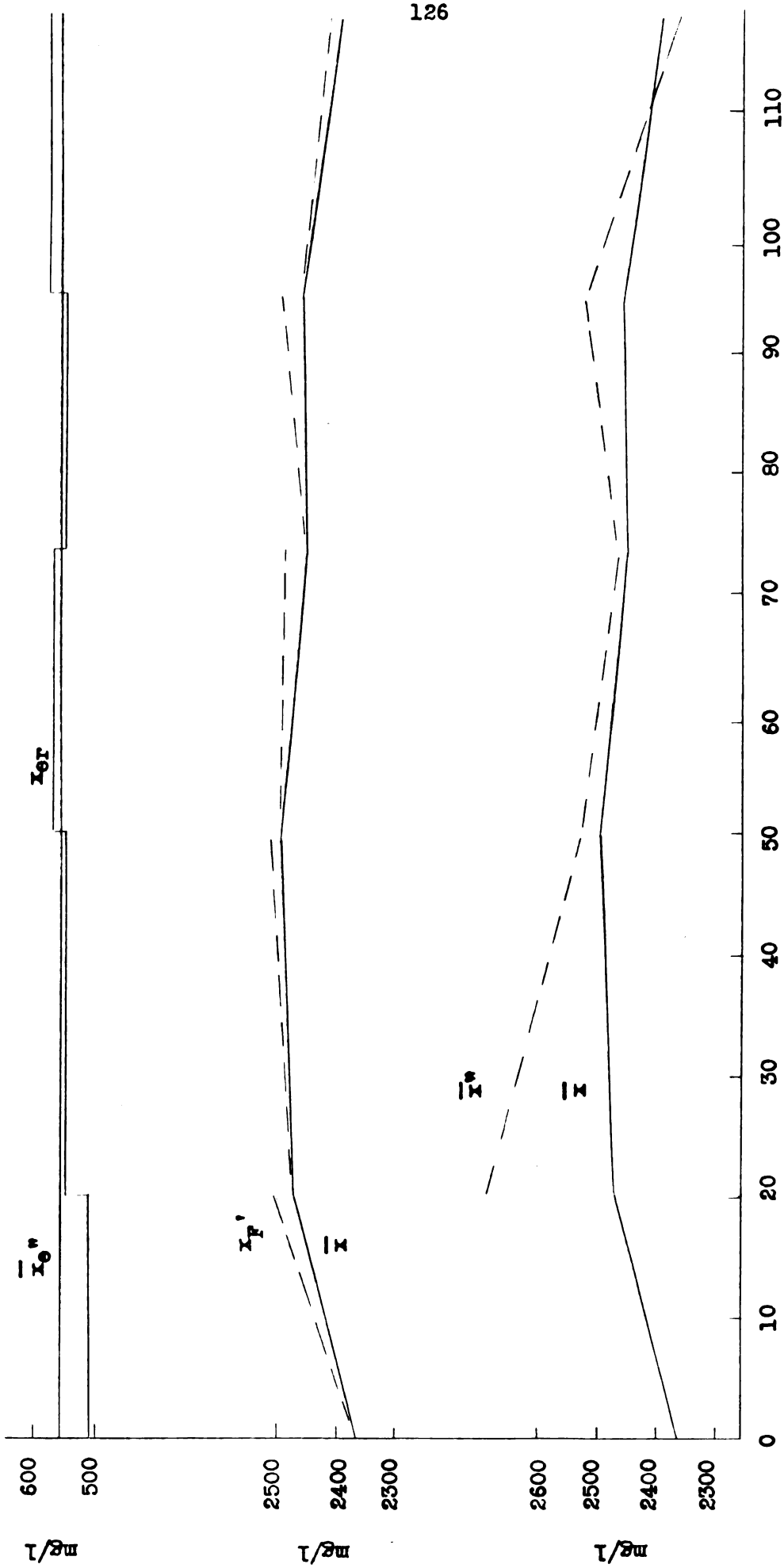


Figure 16. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 5.

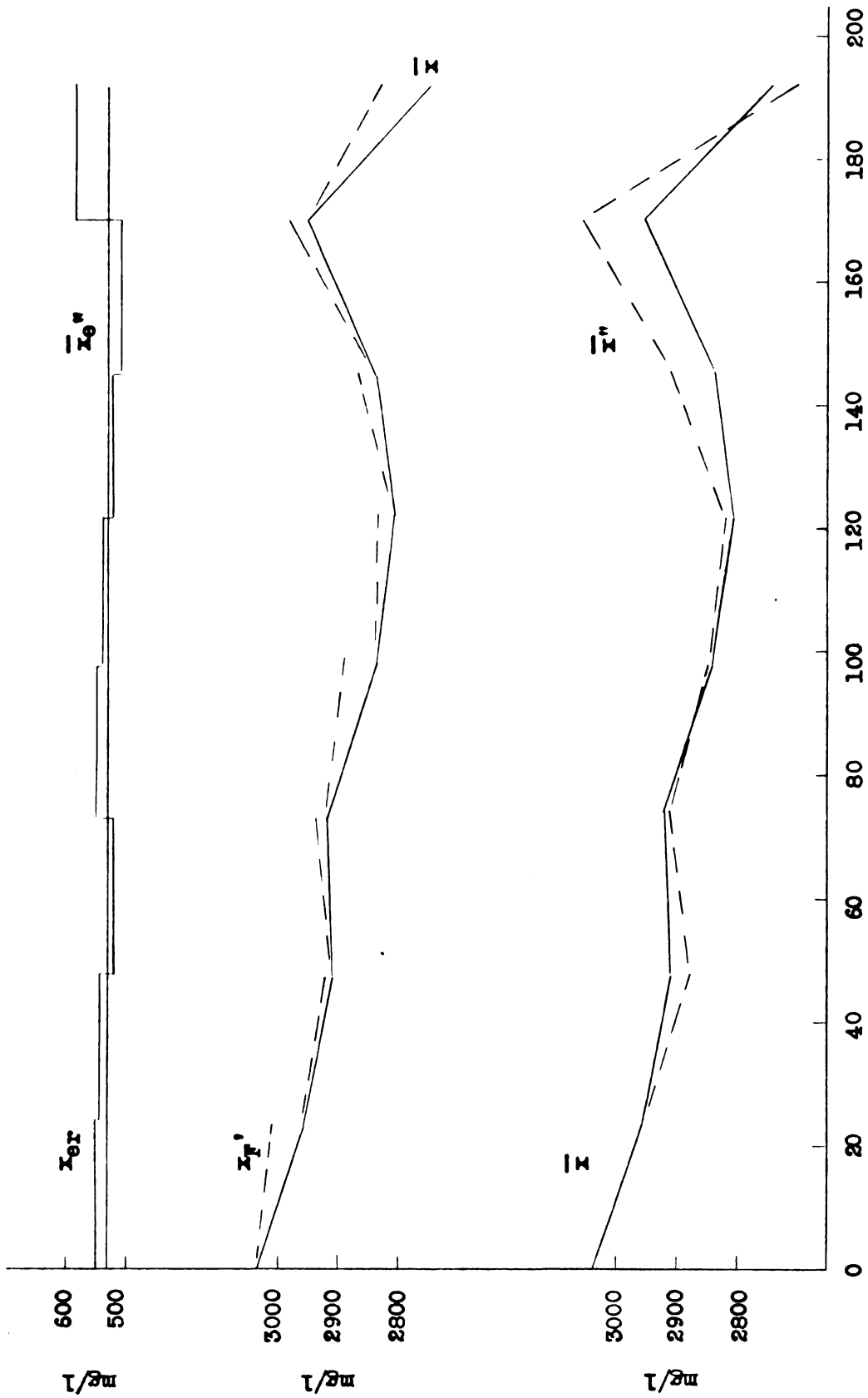


Figure 17. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 6

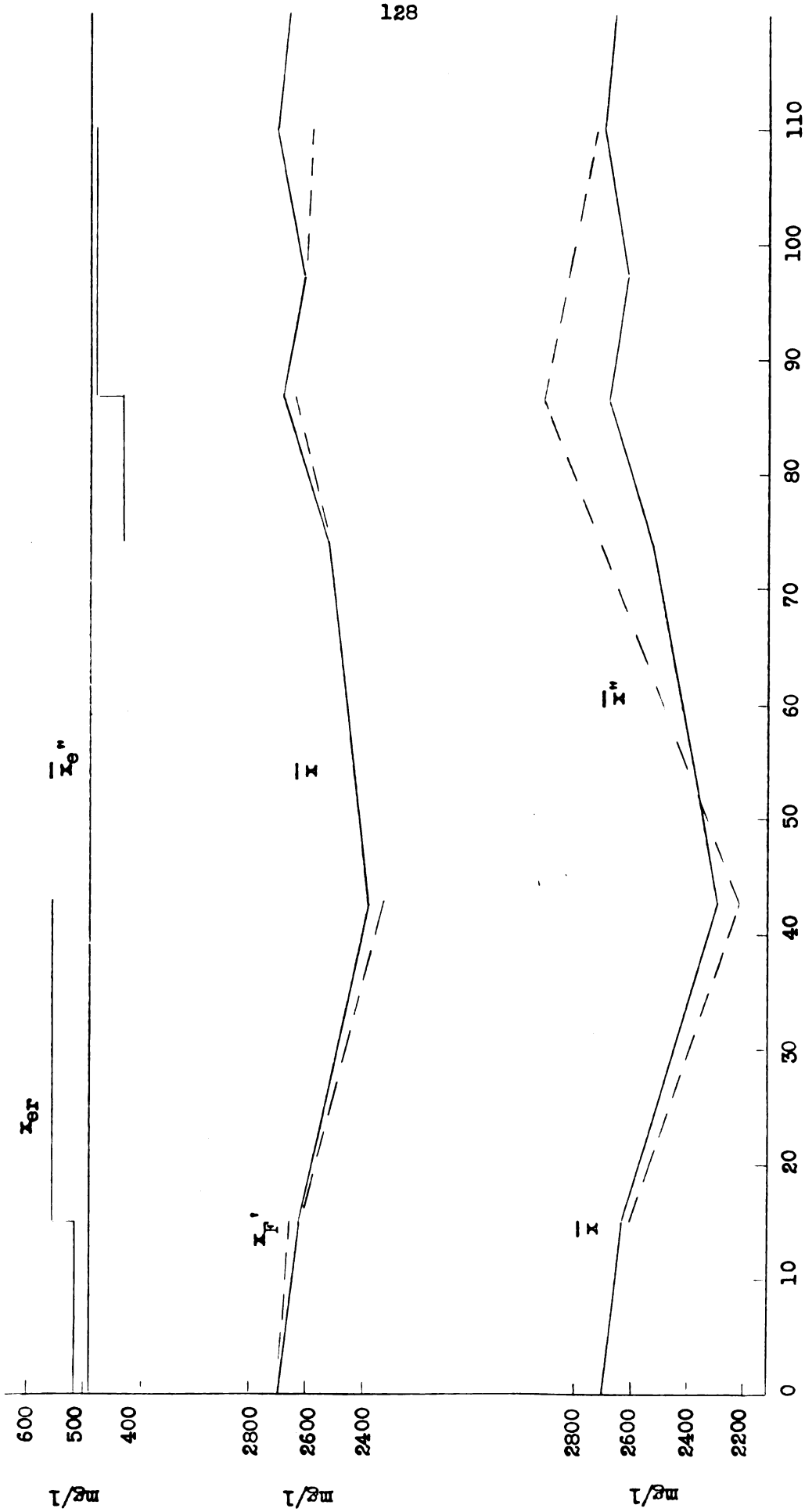


Figure 18. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 7.

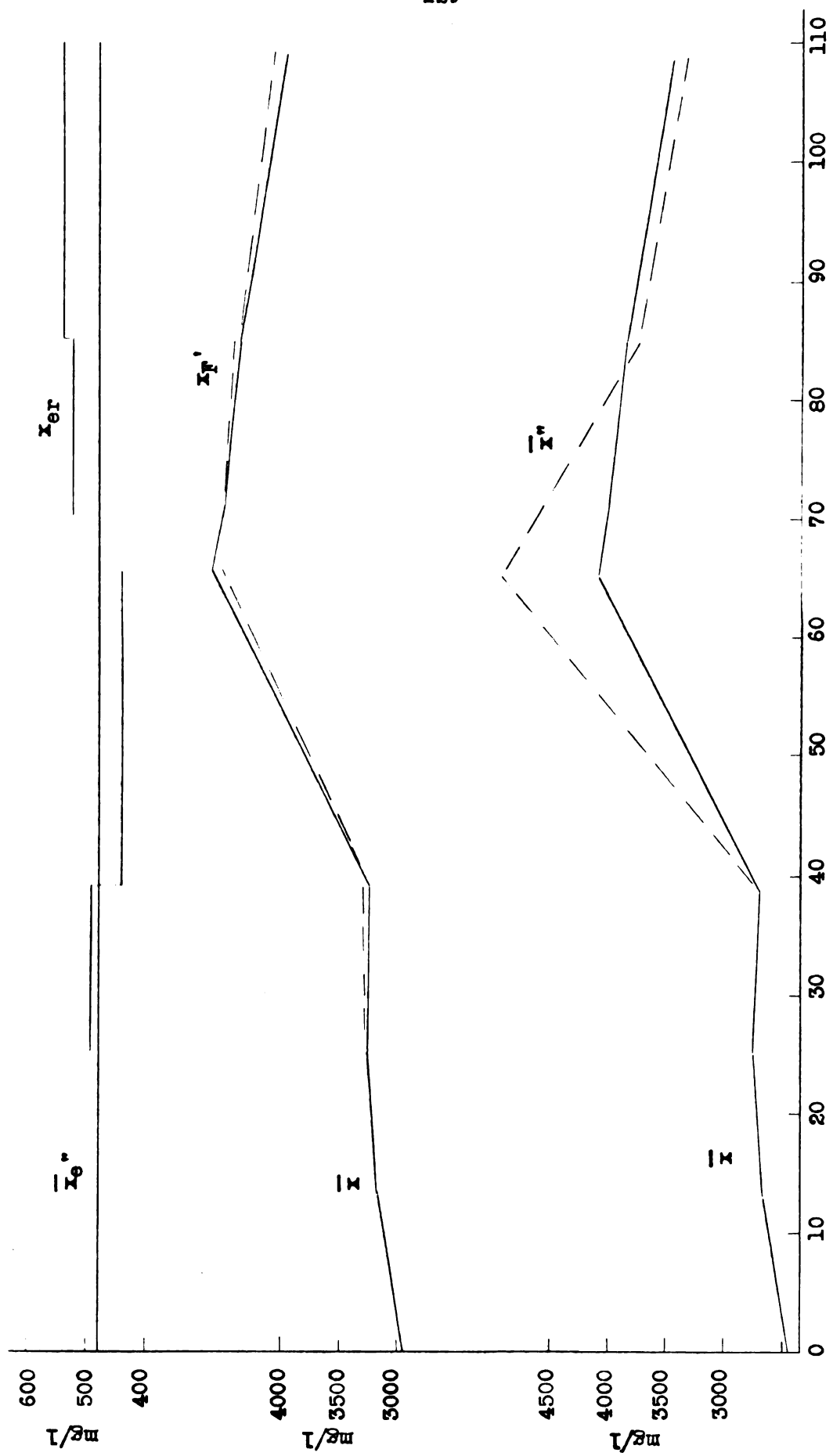


Figure 19. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 8.

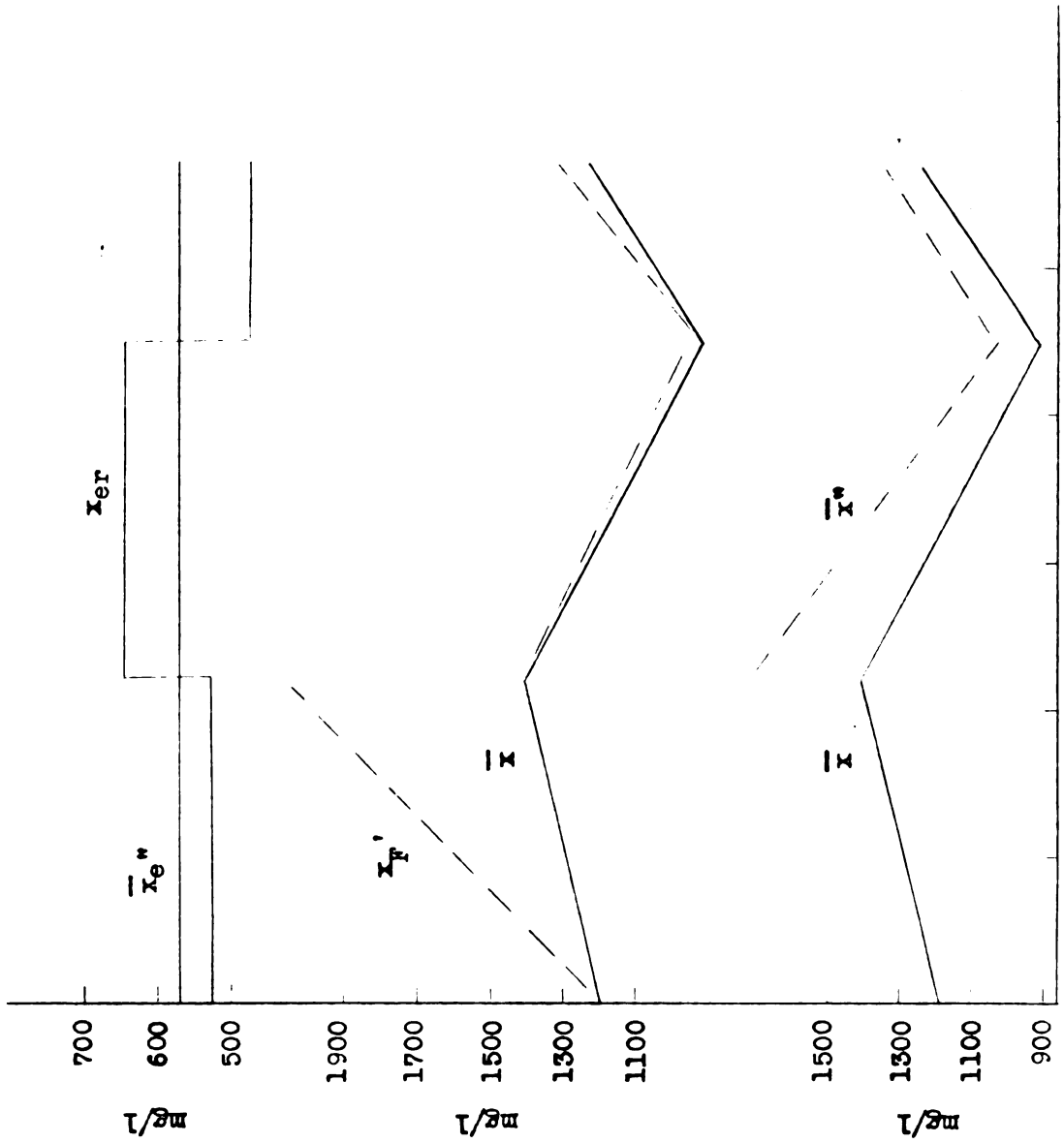


Figure 20. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}''$ and x_{er} - Run No. 8a.

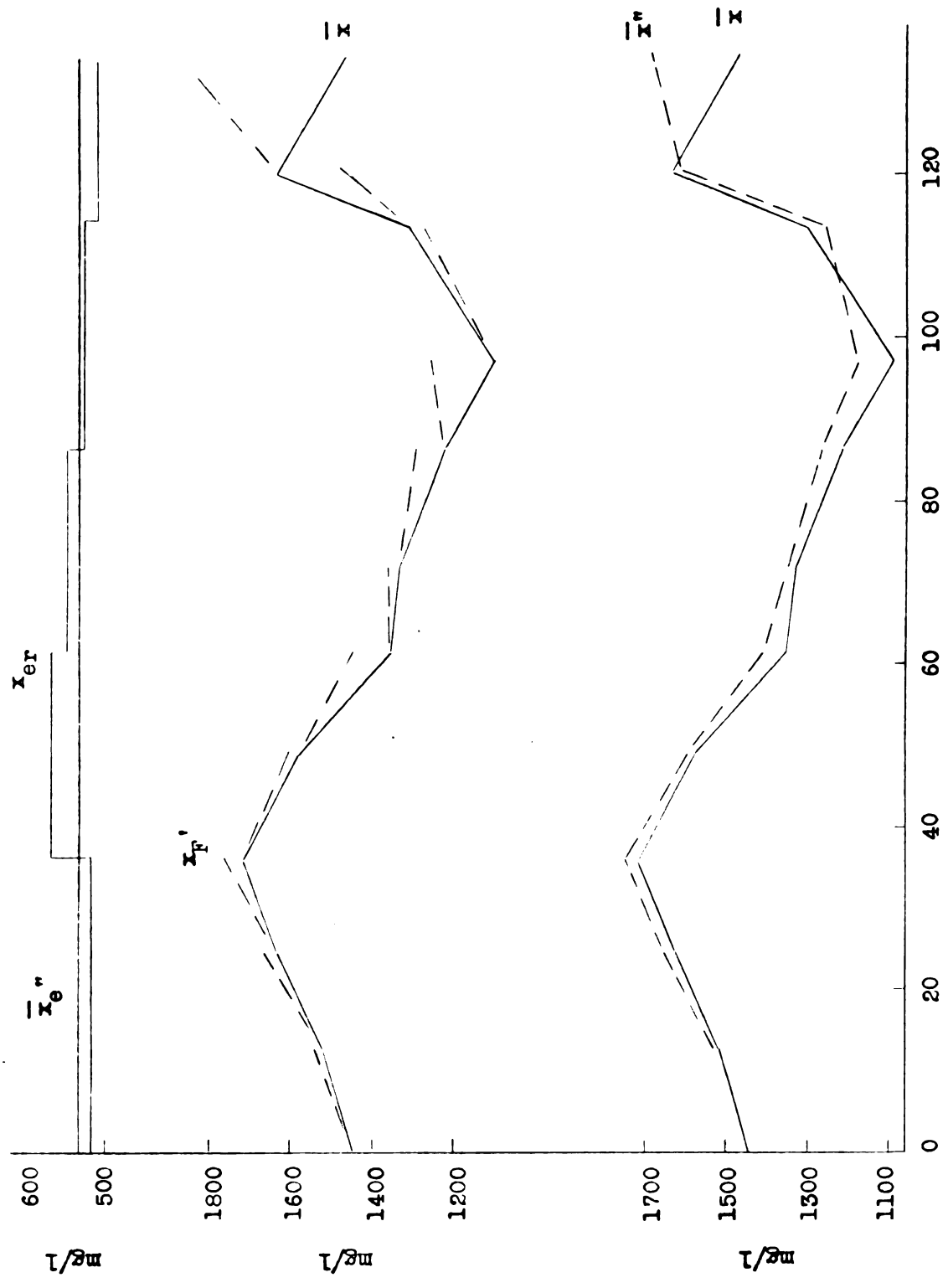


Figure 21. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 9.

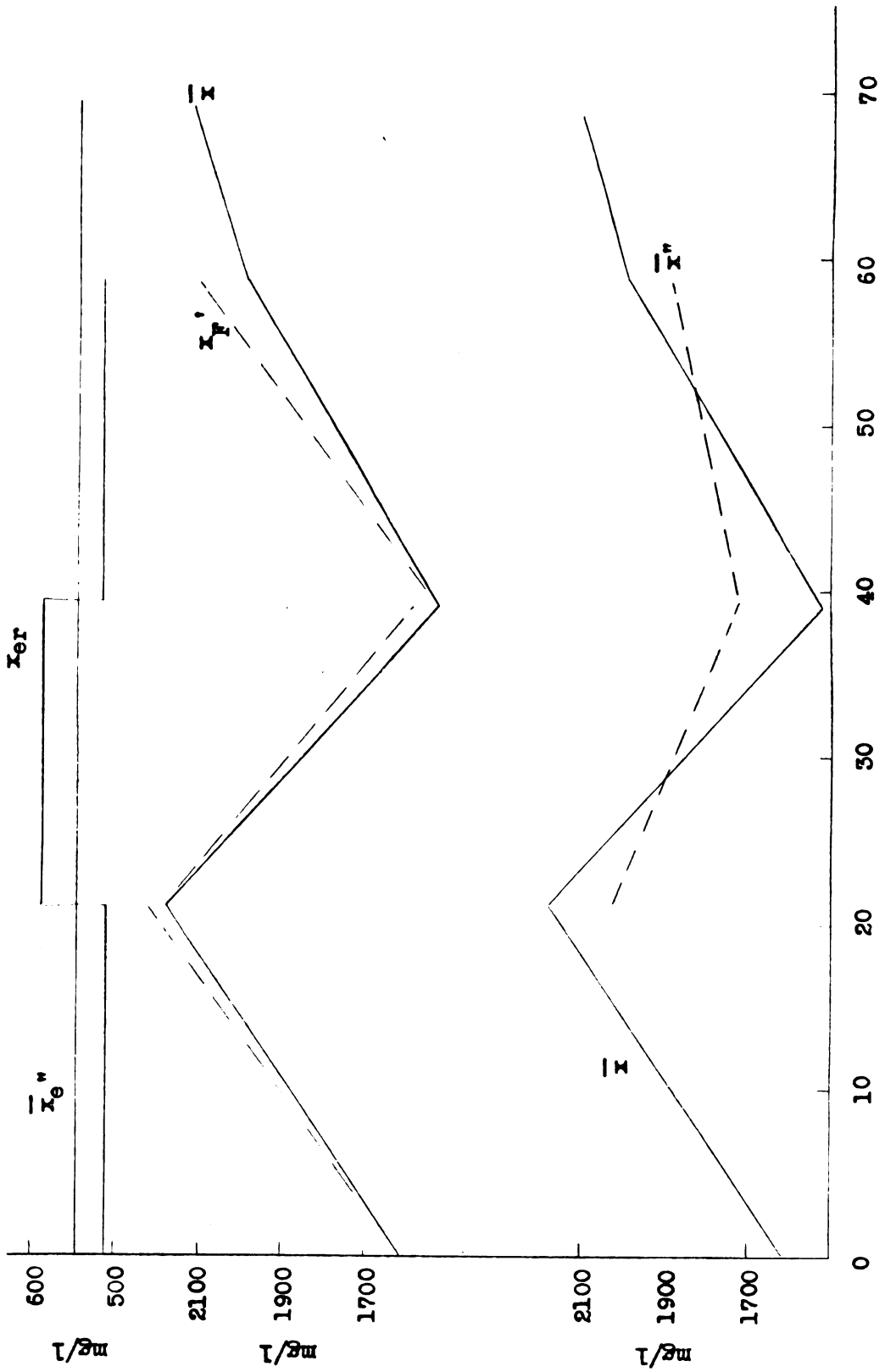


Figure 22. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} -Run No. 10.

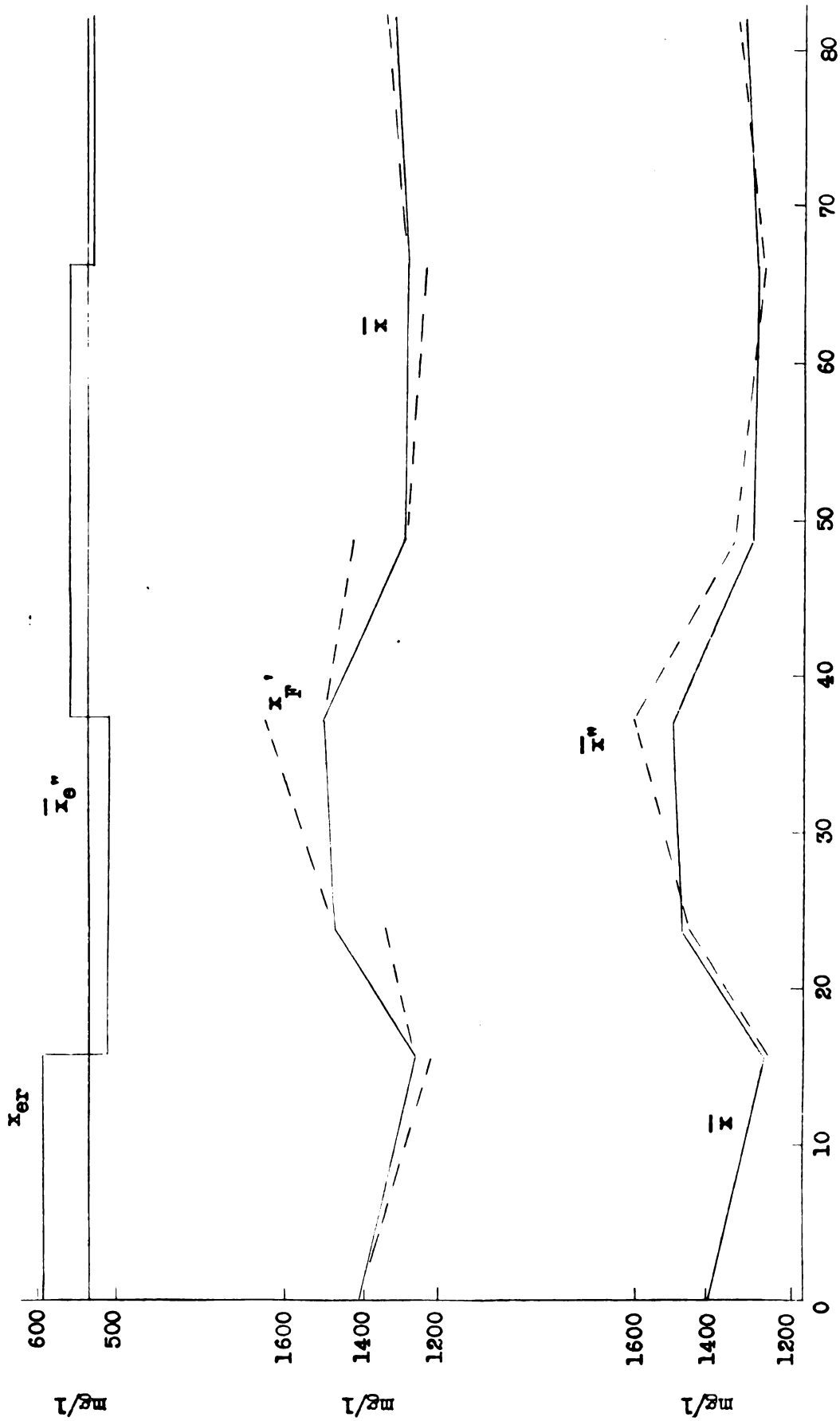


Figure 23. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 11.

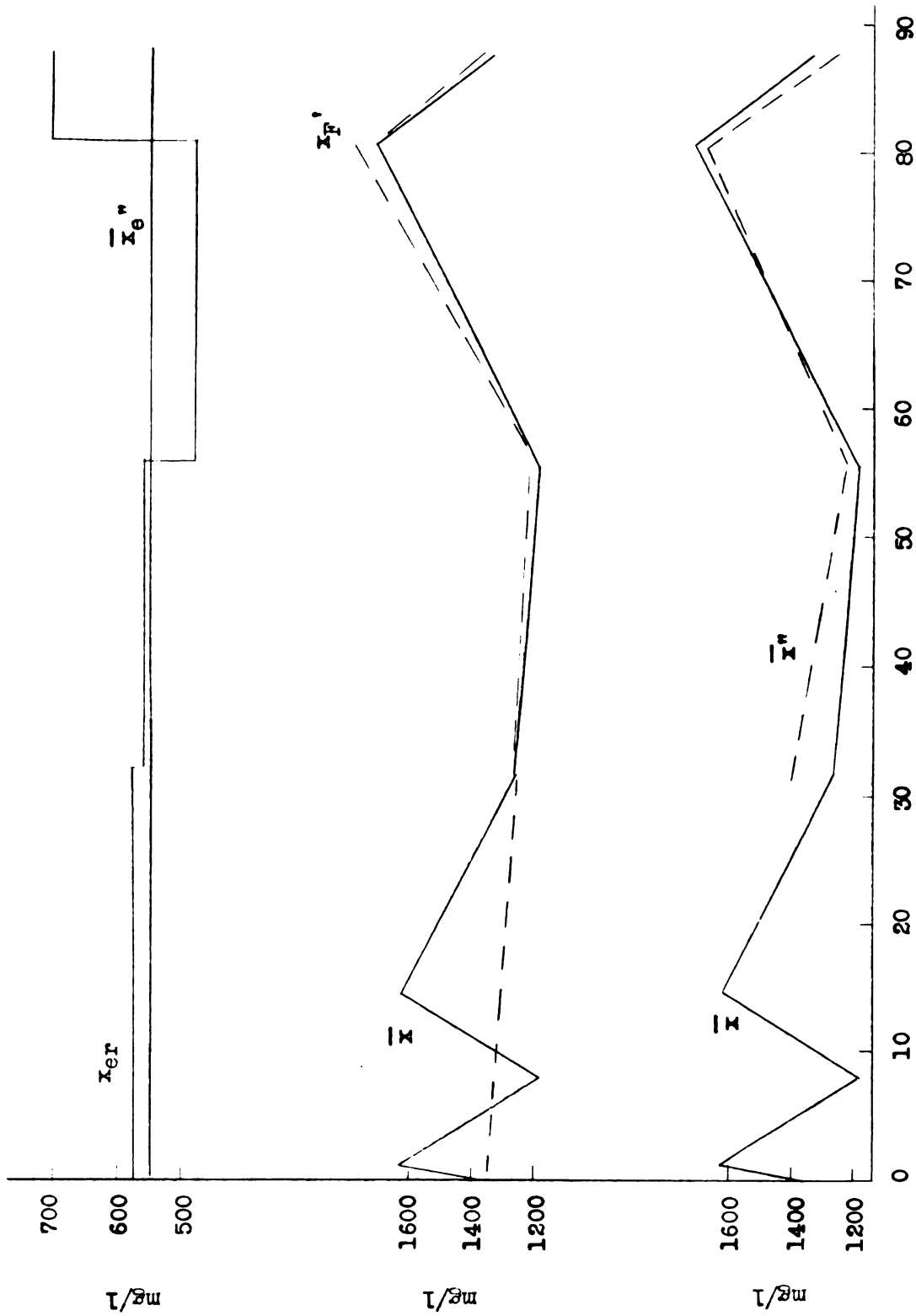


Figure 24. Comparison of $\bar{x}$ and $\bar{x}''$; $\bar{x}$ and x_F'; $\bar{x}_e''$ and x_{er} - Run No. 12.

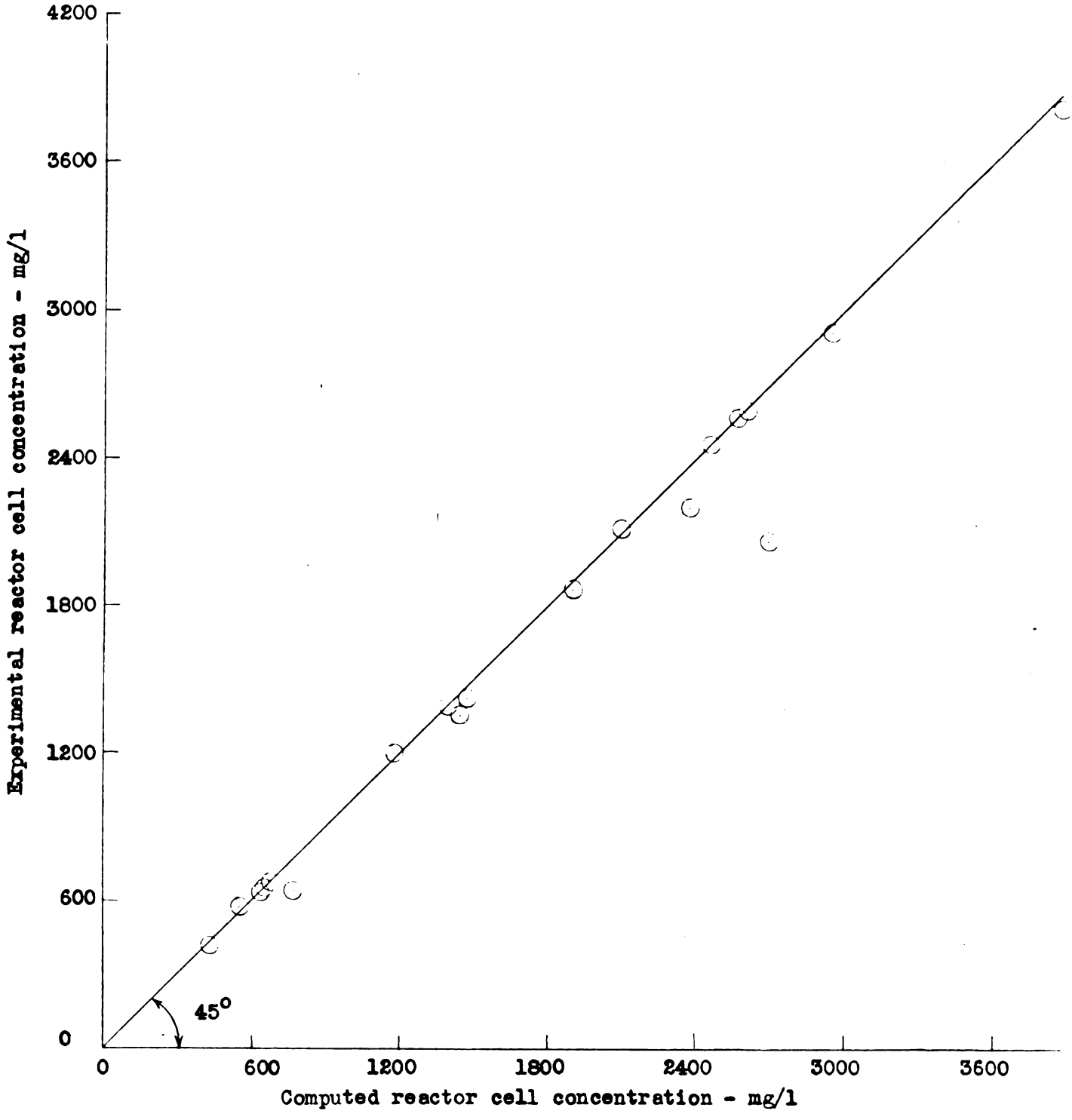


Figure 25. Comparison of average experimental and computed reactor cell conc.

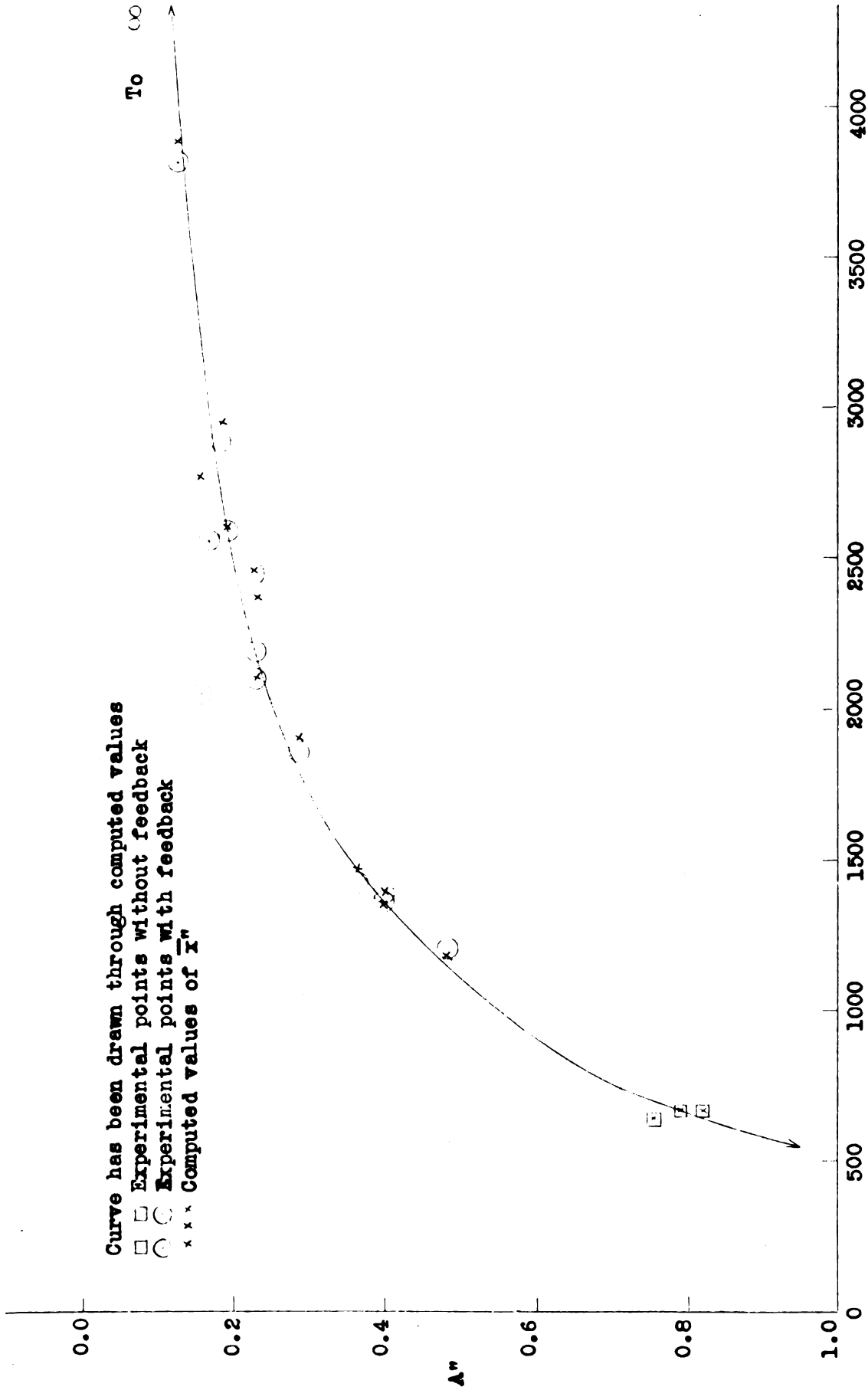


Figure 26. Relationship between $\bar{x}$ and A"

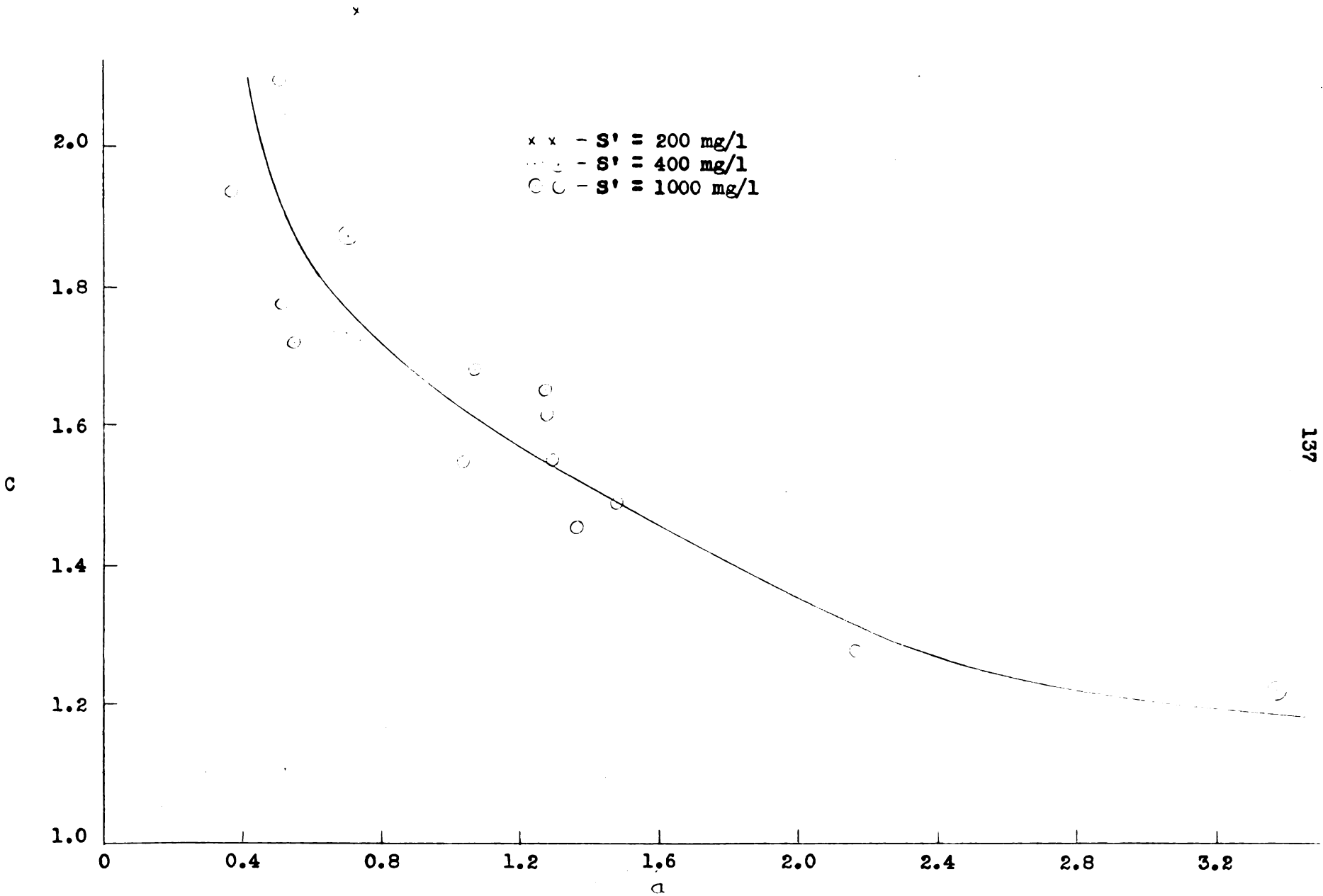


Figure 27. Experimental relationship between C and a.

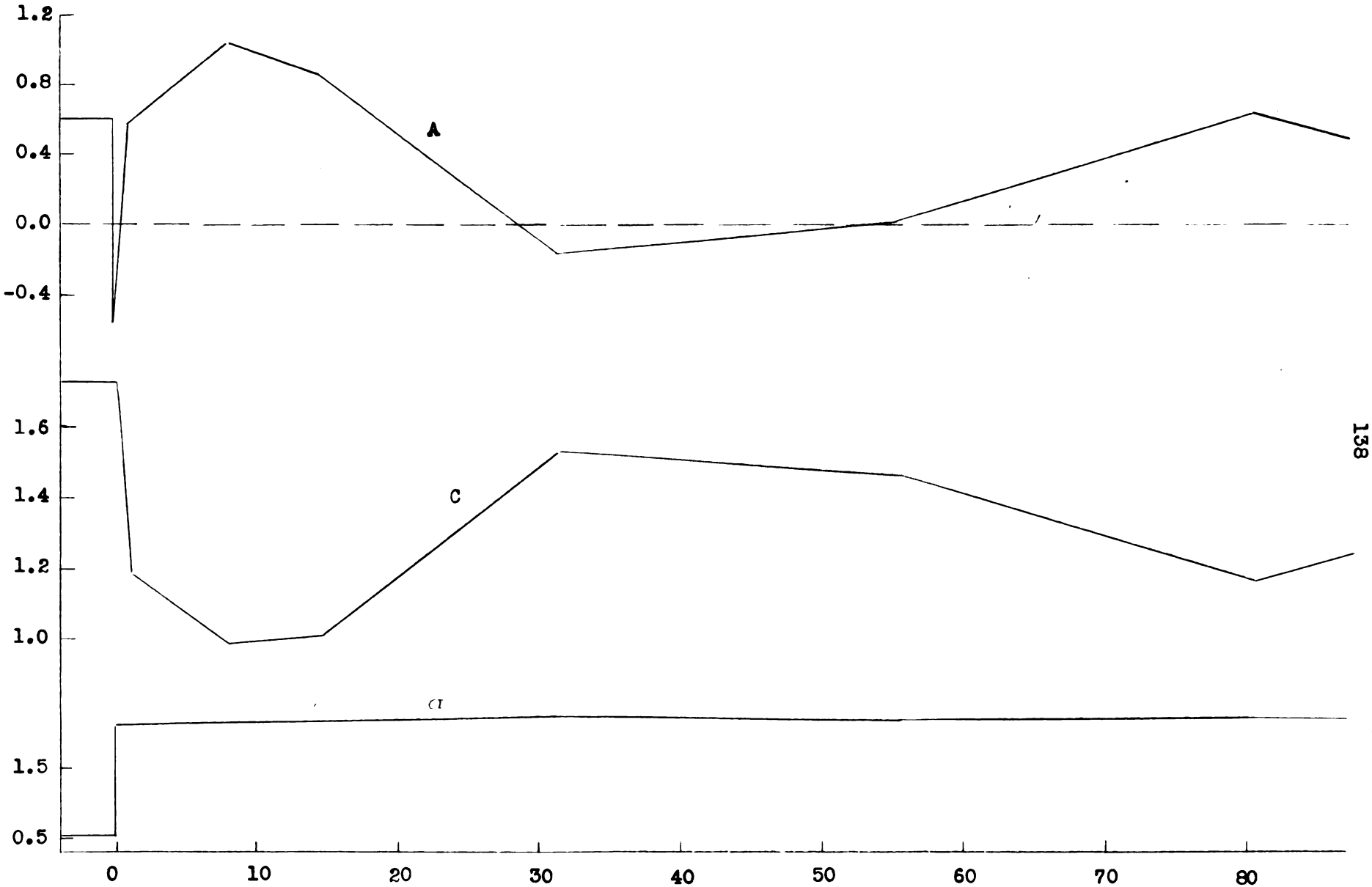


Figure 28. Variation in α, A, and C due to a negative A - Run No. 12.

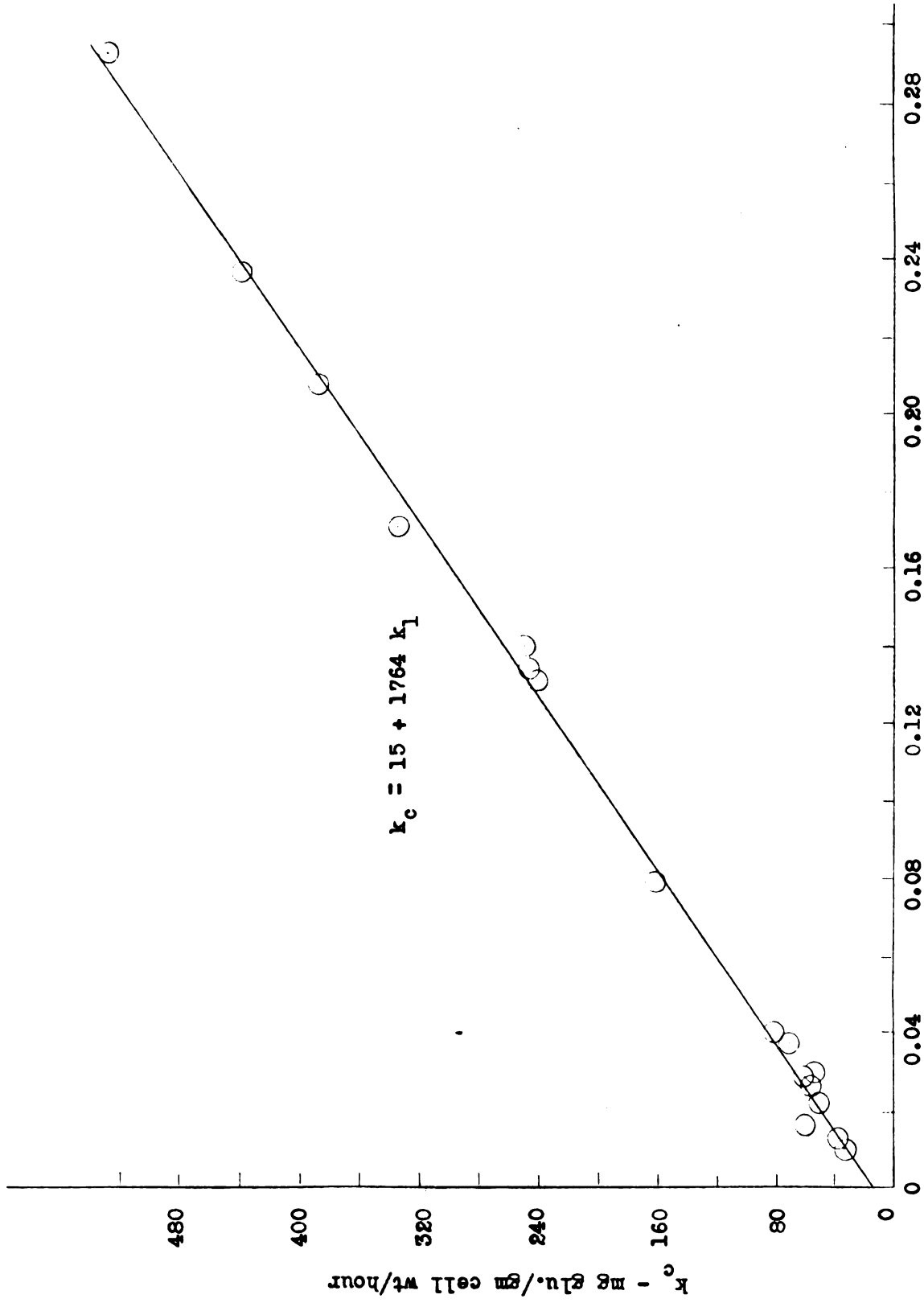


Figure 29. Comparison of k_c and k_1.

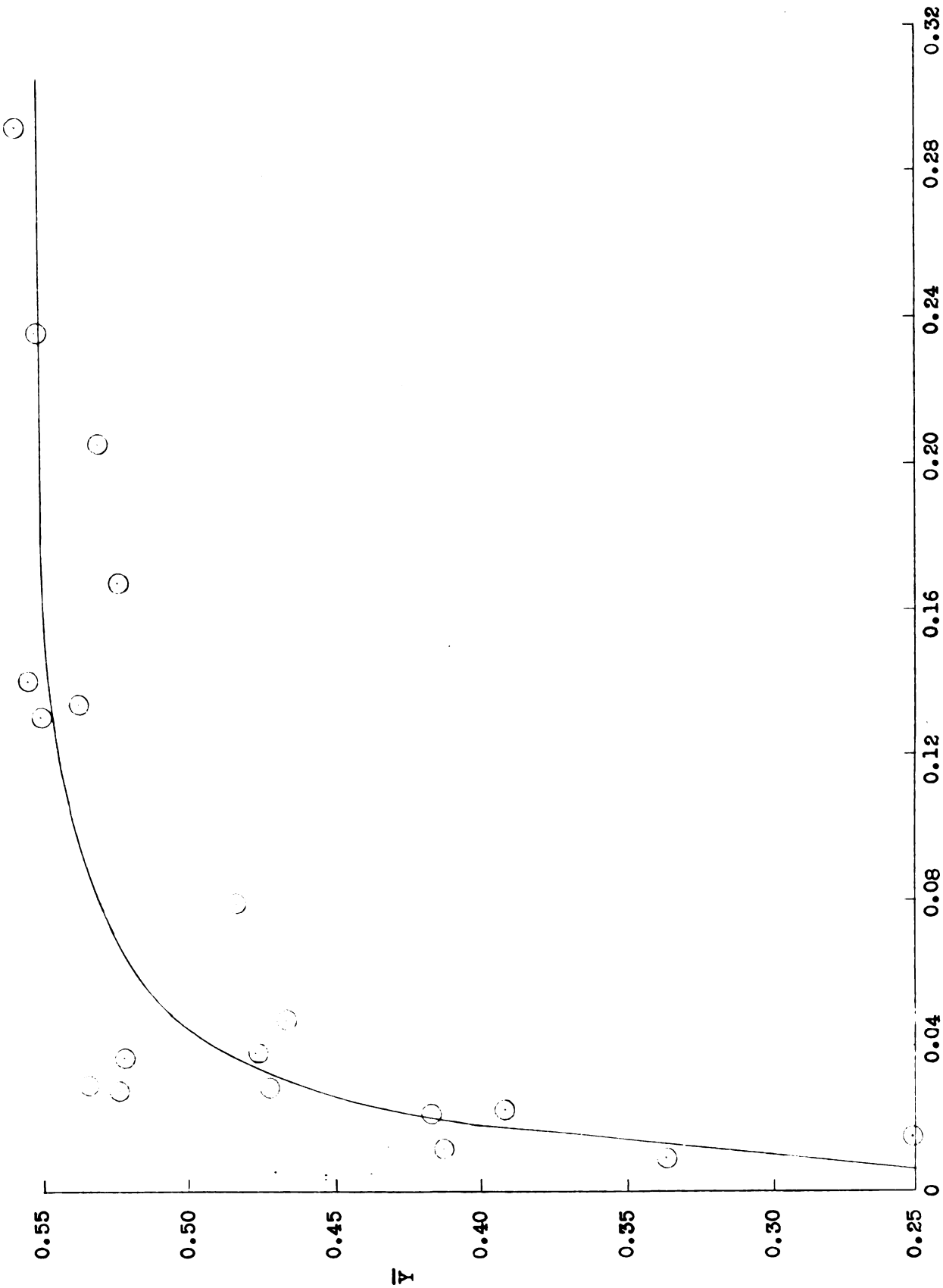
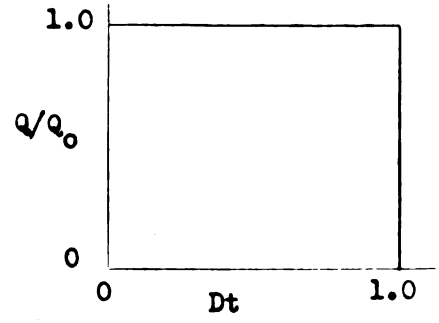
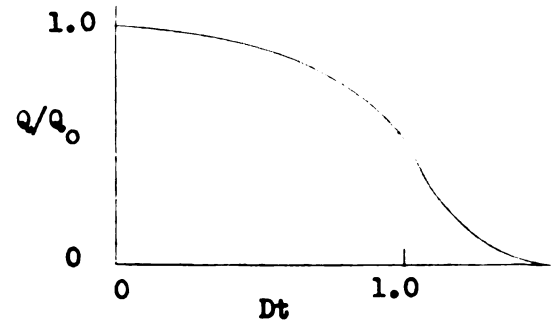


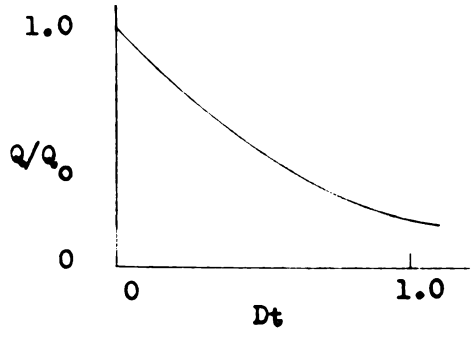
Figure 30. Relationship between $\bar{Y}$ and k_1.



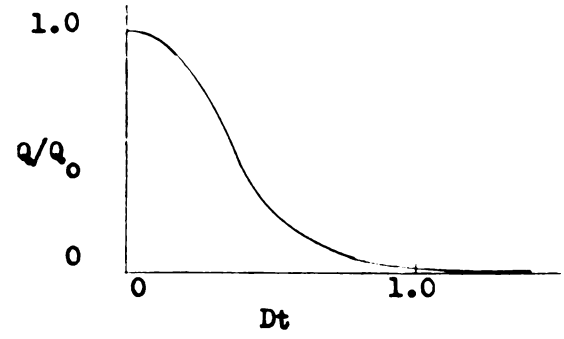
a) Piston flow



b) Piston flow with some longitudinal mixing



c) Complete mixing



d) Dead water

Figure 31-1. F diagrams of some continuous flow models.

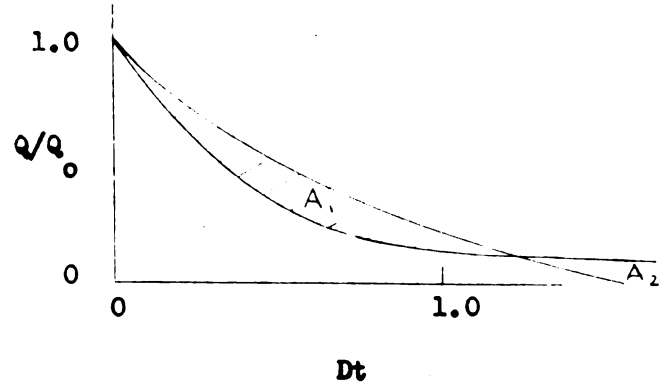


Figure 31-2. Comparison of completely and incompletely mixed models.

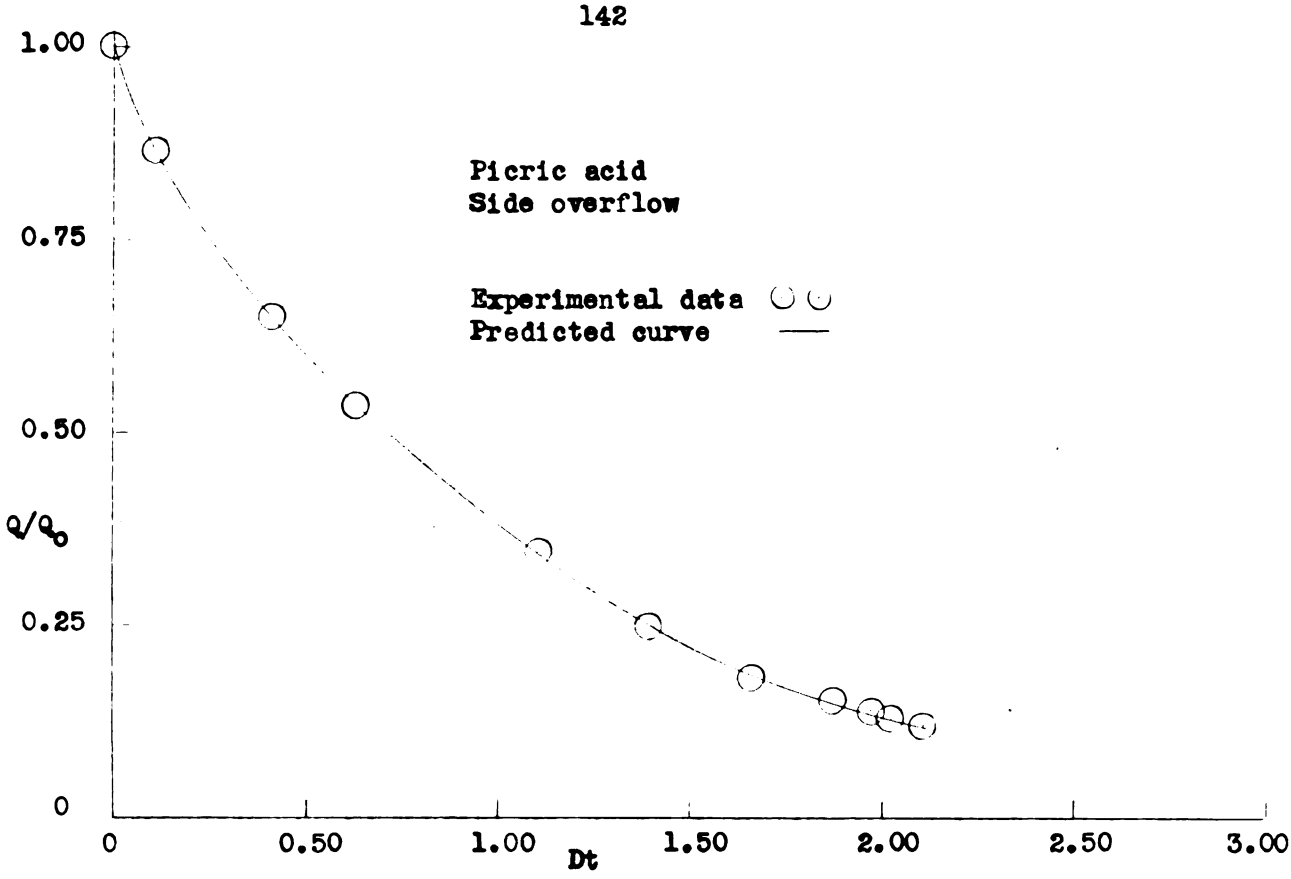


Figure 32. F diagram for Run W-1.

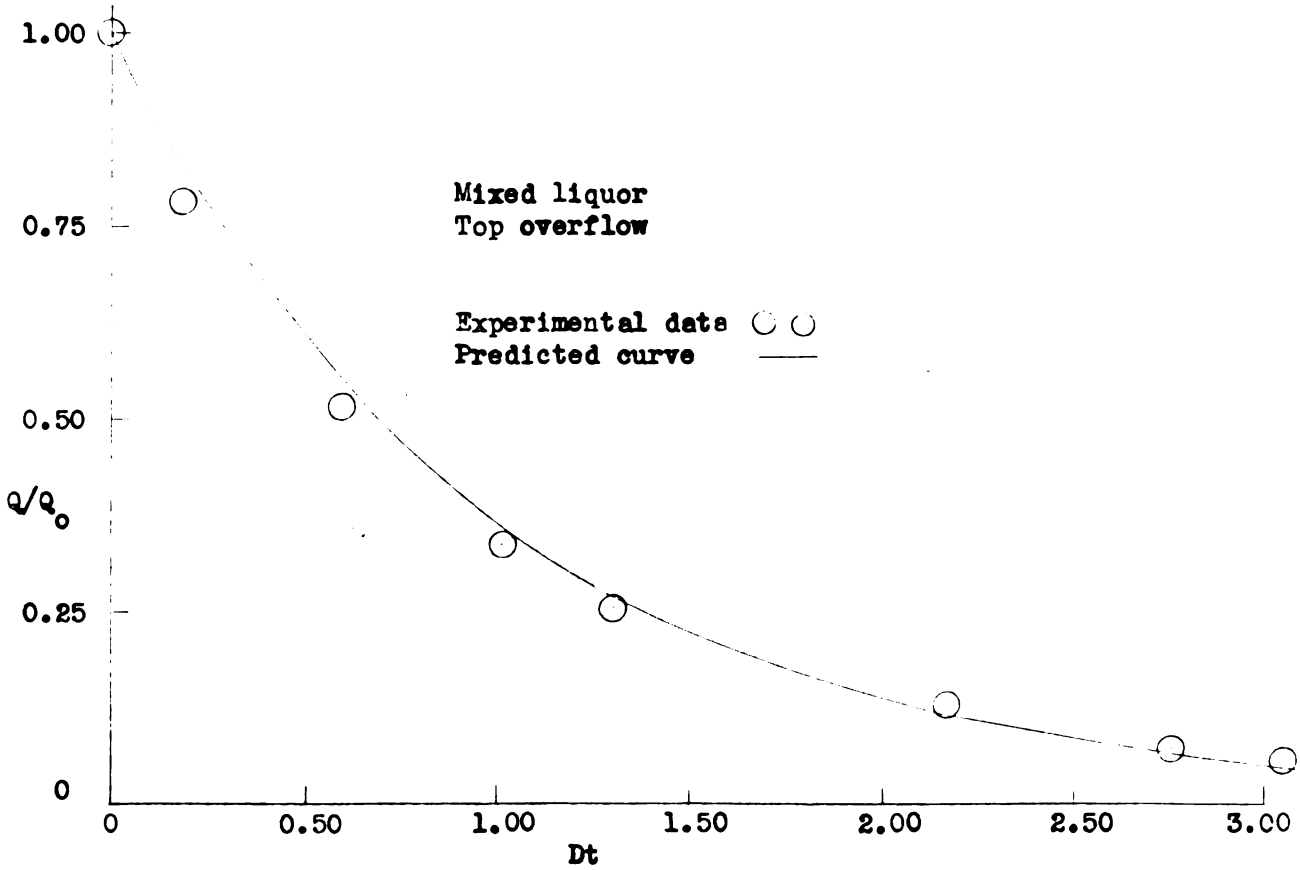


Figure 33. F diagram for Run W-2.

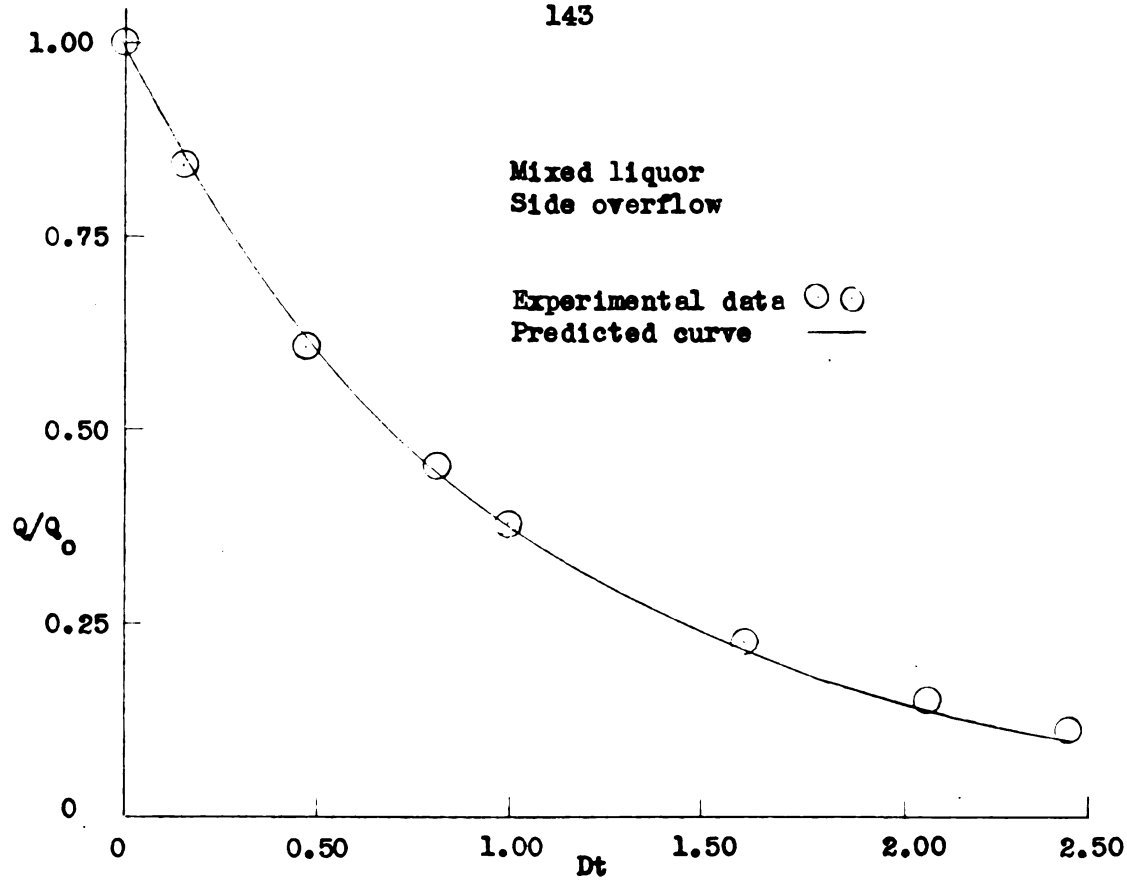


Figure 34. F diagram for Run W-3.

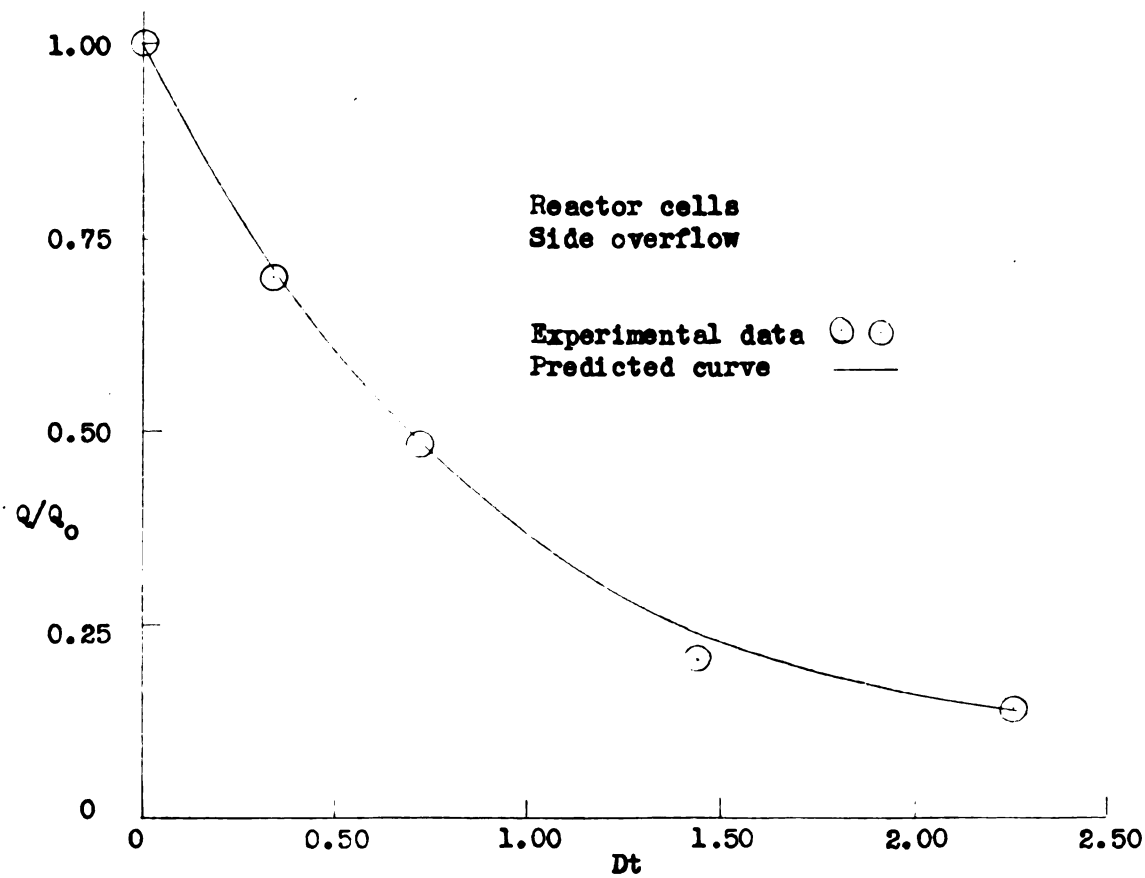


Figure 35. F diagram for Run W-4.

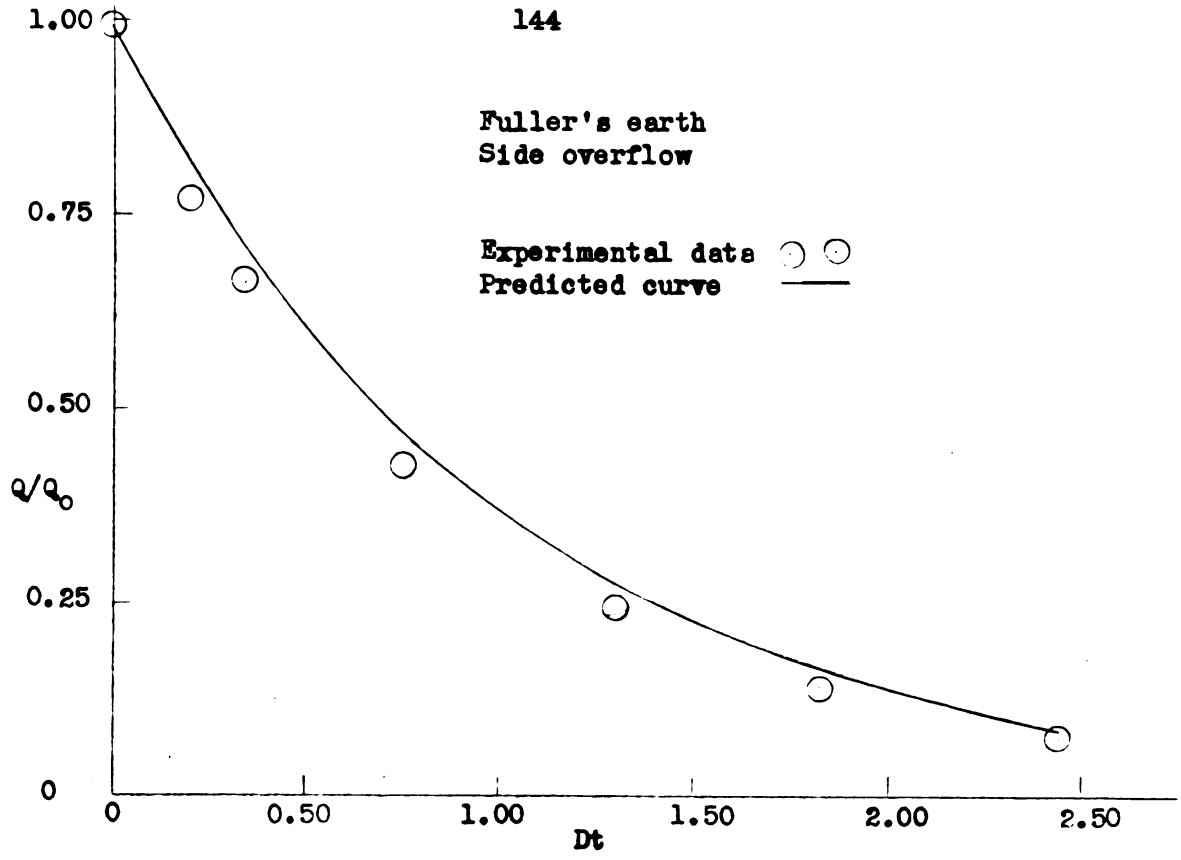


Figure 36. F diagram for Run W-5.

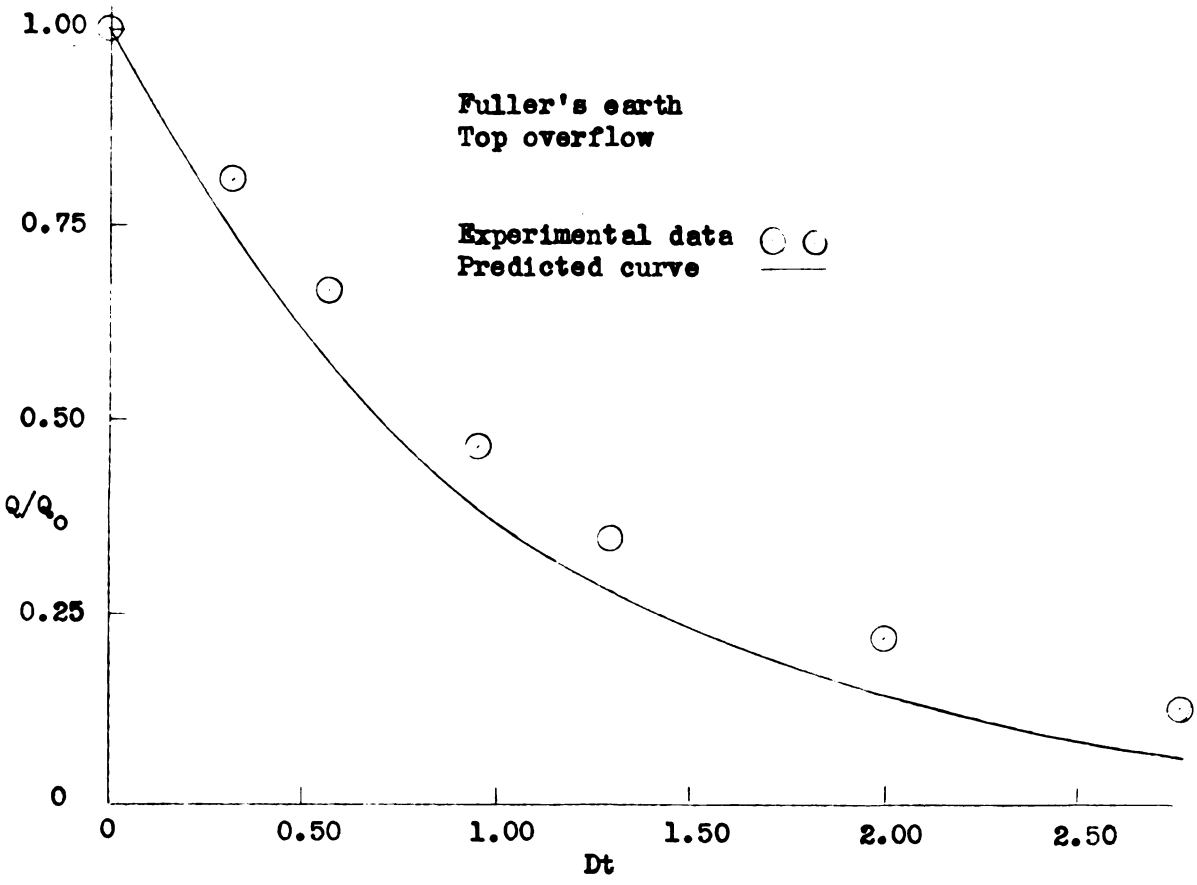


Figure 37. F diagram for Run W-6.

VI. DISCUSSION

The purpose of this dissertation was to determine if the mathematical model presented in Section III could be used to describe the operation of the continuous flow feedback system. It was found that the predicted values of $\bar{x}$ were less than the measured values in each case due to evaporation and reactor effects. Other workers have noted the same type of discrepancy. Herbert et al. (1956) and Herbert (1959) working with a continuous flow system without feedback found that at high dilution rates $\bar{x}$ was greater than that predicted by theory. The same type of relation was obtained by Schulze and Lipe (1963). It is quite possible that the difference between theoretical and actual cell concentration found by these workers was due to causes similar to those found in this investigation. The original mathematical model was modified as shown in Section V and it was shown that the behavior of the system could be successfully analyzed.

It was found that the concentration factor decreased with increasing recycle ratio. To see why this occurred, and to see what are some of the practical limitations on the system, the inter-relations of C and α are discussed below using a hypothetical situation.

Assume a continuous flow feedback system is initially operating under equilibrium conditions (condition A) as given in Table 35.

Table 35. Inter-relation of $\bar{x}$, C and α.

Given: $\bar{Y} = 0.5$, $S' = 1005$ mg/l, $\bar{S} = 5$, $L = 1.0$ $(A = A'')$

Cell concentration produced = 0.5(1000) = 500 mg/l

Condition	F_i ml/min	F_r ml/min	C	α	A	x mg/ml	x_r mg/ml
A	10	5.0	2.00	0.50	0.500	1.000	2.000
B	10	7.5	2.00	0.75	0.250	1.000	2.000
C	10	7.5	1.75	0.75	0.438	1.142	2.000
D	10	7.5	2.00	0.75	0.250	2.000	4.000

The recycle ratio is then increased to 0.75 by increasing F_r from 5.0 to 7.5 ml/min producing condition B. However, since cells are now being returned to the reactor at a higher rate, state B is only a temporary condition. To see what immediate effects the increase in F_r has on the system, cell balances will be taken around the reactor and the settling tank under condition B.

Reactor cell balance

Rate of return cells + rate of cells formed = rate of effluent cells

$$2.0\,(7.5) + 0.5\,(10.0) = 1.0\,(17.5)$$

$$20.0 \text{ mg/min} = 17.5 \text{ mg/min}$$

Hence the reactor is receiving cells at a faster rate than it is losing them and the reactor solids will increase at a rate of 2.5 mg/min.

Settling tank cell balance

Cells entering settling tank = cells returned to reactor + cells lost in effluent

$$1.0\,(17.5) = 2.0\,(7.5) + 0.5(10.0)$$

$$17.5 \text{ mg/min} = 20.0 \text{ mg/min}$$

The settling tank is therefore losing cells at a rate of 2.5 mg/min. The above balances are made assuming that the cells produced in the reactor will be lost in the effluent (no storage in the settling tank).

If the system is allowed to run as in condition B and there are enough cells in the settling tank to maintain x_r at 2000 mg/l, the reactor will eventually come to a new equilibrium which may be found by solving the above cell balance equation for a new $\bar{x}$ as

$$\bar{x} = F_r x_r + F_i \frac{[\bar{Y}(S' - \bar{S})]}{F_i + F_r}$$

The new equilibrium condition is shown as condition C in Table No. 35. It may be noted that the result of increasing a was to increase $\bar{x}$ to 1142 mg/l and to decrease C. The value of A also decreased slightly. The same pattern of results was obtained experimentally as reported in Section V. When a was increased from 0.549 in run 11 to 2.163 in run 12, the value of C dropped from 1.72 to 1.28 while A'' decreased slightly from 0.397 to 0.393. A'' is used instead of A since L is not equal to 1.0 in run 12. The average reactor cell concentration increased slightly from 1361 in run 11 to 1866 mg/l in run 12. However, one important factor observed in the experimental run was not considered in the above example.

The average return cell concentation decreased from 2337 mg/l in run 11 to 1780 mg/l in run 12. This reduction in x_r is responsible for the large reduction in C and the small reduction in A''.

In the above hypothetical example, if it had been possible to maintain C at 2.0, the new equilibrium would be as in condition D in Table No. 35. However, x_r would have had to be doubled to 4000 mg/l. One possible method of increasing x_r would be to purposely waste less solids than are being produced allowing the sludge blanket to increase in the settling tank. Thismethod, of course, has limitations since the properties of normal sludge allow only a limited range of compaction.

The above paragraphs have shown how C, A'' and $\bar{x}$ will respond to an increase in α. An equivalent decrease in α in the above example situation would bring about the reverse sequence. That is, the values of A'' and C would increase, while $\bar{x}$ would decrease.

In operation of the experimental system, the value of $\bar{x}$ was found to vary from day to day in spite of a constant feed and return rate. Equation 54 was derived for a non-steady state or transient type of system operation. The formulation predicts variation in x due to a difference between the concentration of cells in the effluent x''A'' and the concentration of cells produced $\bar{x}_e$''. As reported in Section V, equation 54 was used to compute the daily variation in $\bar{x}$. The computed daily cell concentration (termed x'_F) was plotted against $\bar{x}$ in Figures 9 to 24 showing good agreement in most cases. It was also noted that the measured system effluent concentration x_e varied significantly from day to day.

According to equation 54, when the concentration of cells produced is greater than the concentration of cells in the effluent ($\bar{x}_e''$ greater than x_e), the reactor cell concentration will rise. When the reverse is true the value of x will fall. It was shown in Section V that the weight of cells in the settling tank varied from day to day due to changes in the settling properties of the floc. The weight of cells stored or the excess loss of cells from the settling tank in a time Δt is termed W_c. The weight of cells, W_c, stored in a given period has, in effect, been temporarily lost from the system because it has not appeared in the system effluent as measured by x_e. The actual concentration of cells lost from use in the system including storage or excess loss from the settling tank will be termed x_{er}. The value of x_{er} can be determined by

$$x_{er} = x_e \pm W_c / V_s$$

where V_s is the volume of feed solution that passed through the system during Δt. If cells were stored, the term W_c / V_s is added to x_e and if an excess of cells were lost, the term is subtracted. The computed daily values of x_{er} have been recorded in column 15 of Tables 1 to 16. x_{er} is also plotted vs $\bar{x}_e''$ in Figures 9 to 24. It may be seen that when x_{er} is greater than $\bar{x}_e''$, $\bar{x}$ falls proportionately. The reverse is also true. Hence the plots of x_{er} vs $\bar{x}_e''$ give complete support for equation 54.

The above considerations show that the daily variations in reactor solids concentration were due to wasting too much or too little cell mass over a given period of time. The variation in the effluent

solids concentration is thought to be a result of the constantly varying settling properties of the culture. An index of the settling properties is given by the return cell concentration, x_r. As may be seen in Tables 1 through 16 (column 5), x_r varied widely throughout a given run.

The following sequence of events is suggested to describe the effect of a change in the settling properties of the culture on system operation.

1. The system is initially in equilibrium.
2. The culture begins to lose some of its settling properties.
3. A concentration of cells begins to flow out in the system effluent which is greater than that being formed in the reactor.
4. The mass of cells in the final tank decreases.
5. The value of x_r begins to decrease and as a result, $\bar{x}$ begins to fall.
6. The system may then level off at a new equilibrium with a lower C and a slightly lower $\bar{x}$.
7. At this new equilibrium, the system is again losing a concentration of cells equal to that produced in the reactor.

Organisms other than bacteria were present during all of the runs. These included species of rotifers, vorticella and other protozoa. As observed microscopically, the population of these organisms was usually quite small when compared to that of the bacteria, as is normally true in an activated sludge plant. However, at times, quite unpredictably, one or more species of the protozoa would increase to the point of destroying the floc either directly by preying upon it or indirectly by competing with the bacteria for the substrate.

Macroscopically, the cultural variation could be noted from a change in some physical floc characteristic such as its color, settling property, degree of foaming, or tendency to adhere to the walls of the apparatus. Two factors are thought to be responsible for the varying organism population. First, both the reactor and settling tank were subject to airborne contamination, as well as contamination from equipment routinely used for sampling and flow measurement. Secondly, as experimental conditions were changed from run to run, it would be expected that certain organisms would find the environment more favorable to their growth than others. These variations in culture are thought to be largely responsible for changes in the settling property of the floc, which in turn affects the equilibrium of the system. Gaudy et al. (1960) also reported that the settling characteristics of their mixed culture were extremely variable during operation of a continuous flow feedback unit. They used a glucose based medium.

Obviously, by returning to the reactor only those organisms which would readily settle, flocculent species would be favored over those which are dispersed. However, both flocculent and dispersed organisms were present at all times during the runs with feedback. During some periods, the supernatant above the settled system effluent was quite cloudy, showing a high concentration of dispersed organisms, while at other times the supernatent was relatively clear. It should be also noted that the presence of various types of higher organisms probably influenced the relative population of dispersed and flocculent organisms present.

The three runs without feedback were carried out with culture formed during the feedback runs. During the three runs, the percentage of flocculent growth in the system rapidly decreased. This is shown in column 8 of Table 19 under the heading of "Settled floc volume". The floc volume was obtained by settling a one liter sample of the system effluent for 30 minutes in a one liter graduate and reading the volume of floc thus formed. It may be seen that the settled floc decreased from 800 ml to almost zero during the tests. It is felt that when the feedback was discontinued, the growth of the flocculent bacteria was no longer favored and they were displaced by dispersed types better adapted to the existing conditions. In run 21, the protozoa population increased greatly, destroying some of the flocculent growth.

A contributing factor to the loss of flocculent growth in run 22 may have been the inability of the organisms to produce flocs at a higher growth rate of 0.292. However, this could not explain the loss of floc in runs 20 and 21. In both of these runs the growth rate was below that used in run 10, in which no loss of floc was observed.

The temperature of the reactor was controlled by immersing the vessel in a water bath. During a given run the temperature was maintained at the desired level $\pm$ 0.5°C by means of a Fenwal thermo-regulator and heater. Temperatures of 24, 25, and 27°C were used during the course of the experiments. The temperature used for each run as well as the range of growth rates during the respective runs are given in the following table.

Run Nos.	Tem perature ^{o}C	Range of growth rate - hr^{-1}
1 - 4a	27	0.009 - 0.028
5 - 8	24	0.029 - 0.048
8a - 22	25	0.079 - 0.292

The first temperature change (from $27^{o}C$ to $24^{o}C$) was made in an attempt to reduce evaporation from the system. The second change ($24^{o}C$ to $25^{o}C$) was necessitated when it was found that the room temperature sometimes approached $25^{o}C$ ($77^{o}F$) during the night due to a faulty heating system. It is well known that temperature influences biological activity. A 10 degree rise in temperature is often found to approximately double the rate of growth if substrate is present in excess. However, as may be seen in the above table, the maximum value of k_1 at the 27^{o} C temperature was only 0.028. During the rest of the runs (runs 5 - 22), k_1 varied from 0.029 to 0.292 and the temperature from 24 to 25^{o} C. Since the bath temperature has a $\pm$ 0.5^{o} C variation, runs 5 to 22 may be considered to have been performed at essentially the same temperature. Because of this and because k_1 reached a value of only 0.028 in runs 1 to 4, it is not felt that an attempt to adjust the data for temperature variation is justified.

The theoretical relation between the dilution rate and the reactor and effluent cell concentrations is plotted in Figure 38 for the continuous flow systems with and without feedback. The curves have been plotted using equations 35, 42, 48 and 49. Curves showing the

rate of cell loss have also been included for both systems. The curves for the system without feedback are shown using dashed lines. It may be seen that the cell concentration for the feedback system is twice that for the system without feedback due to the use of an A'' value of 0.5. Since A'' is 0.5, the growth rate for the feedback system is 0.5 D while $k_1 = D$ for the system without return. Hence the former system may be operated at twice the dilution rate and still maintain a much lower effluent substrate concentration. This characteristic is of paramount importance to the operation of the activated sludge process. Since k_1 and $\overline{S}$ are directly related (see Figure 7), an effluent low in BOD may be produced by keeping A'' small which in turn lowers the value of the growth rate. It should also be noted that the initial BOD or substrate concentration will not affect the effluent concentration as long as A'' is not changed. The effect of a higher or lower S' is merely to vary the concentration of solids carried in the aerator as may be seen from equation 34

$$\overline{x} = \frac{\overline{Y}(S' - \overline{S})}{A''}$$

Thus doubling of S' will approximately double $\overline{x}$. In the experimental runs, values of S' of 200, 400 and 1000 mg/l were used. However all but three of the runs were made at the highest influent substrate concentration. Because of variation in both A'' and $\overline{Y}$ from run to run, no quantitative conclusion can be reached concerning the relation between S' and $\overline{x}$. However, as may be seen from Tables 17 and 18, as S' was increased from 200 to 1000 mg/l, $\overline{x}$ increased from 426 to a maximum of 3822 mg/l. Hence the general trend was as expected.

It is possible to have complete washout of the culture in the reactor at a given A'' as shown in Figure 38. Theoretically, it is also possible to run the unit at any dilution rate without washout by decreasing the value of A''. The theoretical relation between $\bar{x}$ and the feedback factor has been plotted in Figure 39 using equation 48. The curve has been plotted for a dilution rate of 0.5. It may be seen that the cell concentration approaches infinity as A'' decreases toward zero and that $\bar{x}$ approaches zero as A'' increases past 1.0. However, high values of $\bar{x}$ are limited in actual operation by the transfer rate of dissolved oxygen. It should also be noted that the only way that A'' may become greater than unity is for the cell concentration factor to become less than 1.0. Hence A'' values above 1.0 seem of little practical significance.

In addition to these practical restrictions, there are a number of other basic factors to be considered before applying the analysis used in this dissertation to the full-scale plant. One of the most obvious is that in formulation of the models, it has been assumed that the influent contained only dissolved substances. However, in almost all domestic wastes, there is a significant concentration of suspended organic and inorganic solids. Fair and Geyer (1958) state that the average domestic sewage contains 235 mg/l of suspended solids. Even assuming 50 per cent of the above solids are removed during primary sedimentation the remaining approximately 100 mg/l of solids will certainly have a large effect on the concentration of solids in the system.

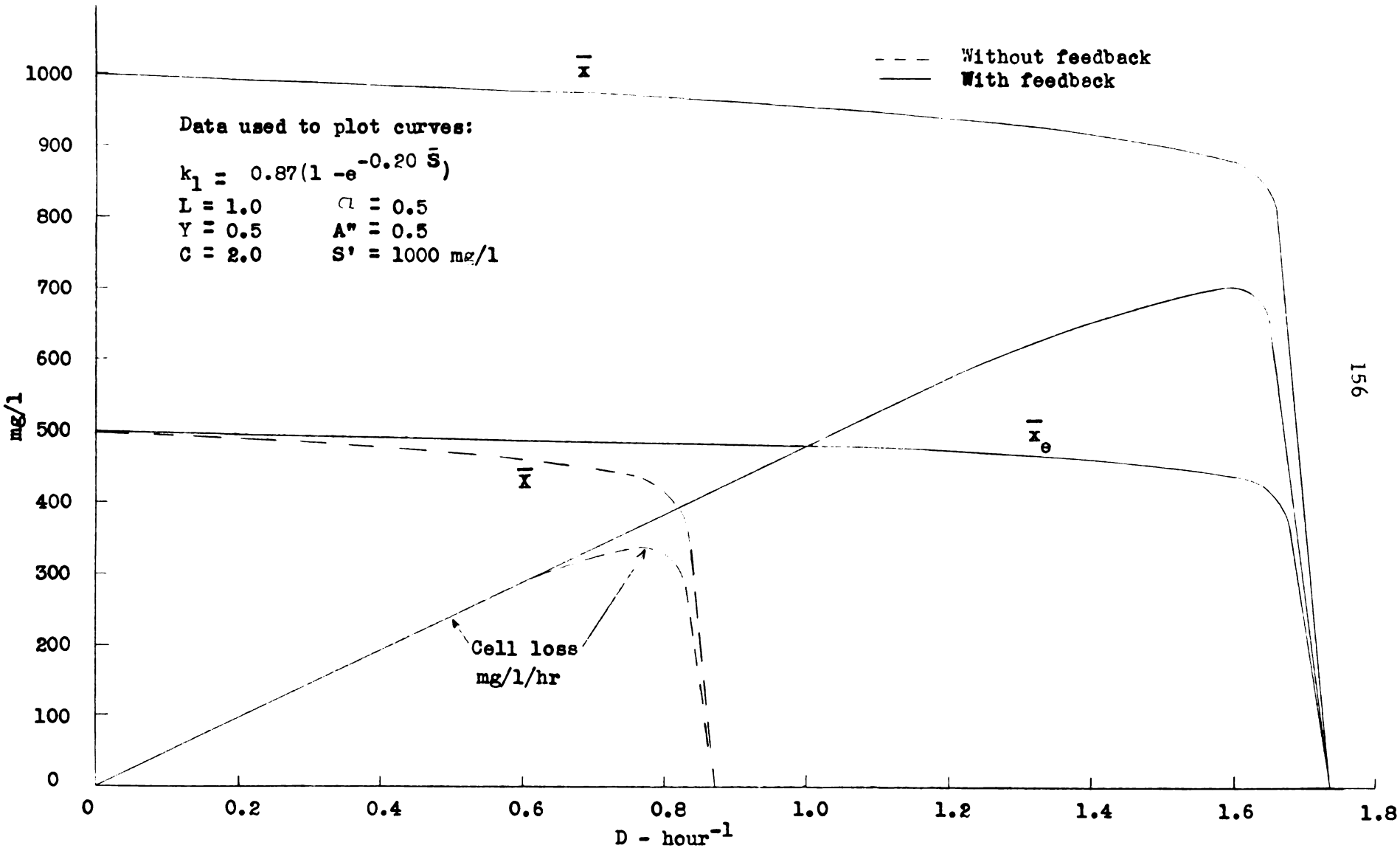


Figure 38. Theoretical relation between D and reactor and final effluent cell concentration for systems with and without feedback.

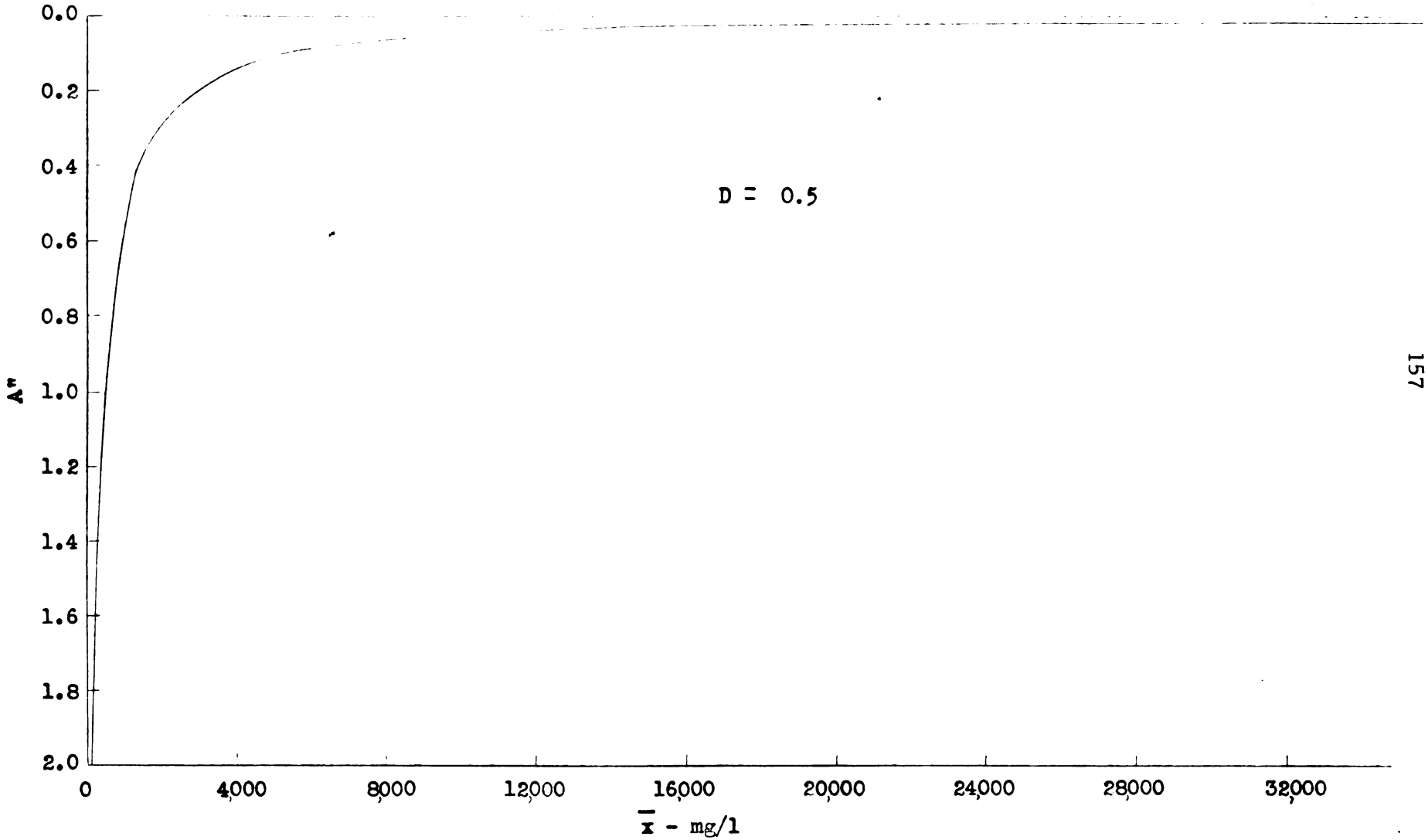


Figure 39. Theoretical relationship between $\bar{x}$ and A".

In the following analysis, S_i is the incoming solids concentration and S_s is solids concentration in the reactor exclusive of the cell contribution, x. The total reactor solids concentration is thus $T_s = S_s + x$. The following balance is taken around the aerator.

Solids balance

Increase = feedback - outflow + growth + incoming

$$\frac{dT_s}{dt} = DC(x + S_s) - (1 + \alpha) DL(x + S_s) + k_1 x + DS_i \qquad 58$$

The corresponding substrate balance (equation 19) would be unchanged.

At equilibrium, equation 58 may be solved for k_1 as :

$$k_1 = \frac{D}{x}(T_s A'' - S_i) \qquad 59$$

The steady state value of T_s may be found by combining equations 59 and 19 as:

$$T_s = \frac{S_i + Y(S' - \bar{S})}{A''} \qquad 60$$

The effluent solids concentration at equilibrium $\bar{T}_e$, may be found from a solids balance around the settling tank as

Reactor solids effluent - system effluent = cells in feedback

$$LDT(1 + \alpha) - DT_e = \alpha DCT$$

or

$$\bar{T}_e = A'' \bar{T} \qquad 61$$

Expressions for the equilibrium substrate concentration in the aerator may be obtained by substitution of equation 59 into equation 6 to give:

$$\overline{S} = \frac{\ln\left(\frac{k_m}{k_m - \frac{D}{x}(T_s A - X_i)}\right)}{c} \qquad 62$$

From equation 60, it may be seen that a suspended solids concentration of 100 mg/l entering the aerator will have a large effect on the concentration of suspended solids in the aerator. The magnitude of the increase will depend on the value of the feedback factor.

Another important consideration in using the preceeding analysis is the magnitude or even the existence of the retention factor, L for full-scale-plants. The L factor was introduced in Section V to include the effects of evaporation and the reactor effect. The reactor effect is the term applied to the retention of solids in the aerator due to a film of cells clinging to the walls and to the partial plugging of the reactor effluent tube. The relatively large surface area and high aeration rates characteristic of activated sludge plants would suggest that evaporation may be a significant factor. The film of sludge on the tank walls would probably be negligible because of the relatively large volume per unit of wall surface. On the other hand, the higher overall specific gravity (due to influent suspended solids) might lead to a solids concentration gradient in some areas of the aeration tanks particularly near the tank bottom. This lack of complete mixing would result in retention of solids in the tank and could be analyzed using a retention factor in the same manner as was done for the laboratory-scale unit.

In the theoretical analysis, it has been assumed that a concentration of cells equal to that formed will be continuously wasted from the system. The effect of wasting too low or too high a concentration of cells from the laboratory-scale unit was to produce variation in the reactor cell concentration. In most full-scale plants, sludge is wasted only intermittantly from the settling tanks. "Bulking" of sludge is also a common problem which would tend to cause fluctuation in the settling properties and hence the concentration of return sludge. However, over a period of time, the sludge carried in the system will be approximately constant. It is also very probable that because of the large quantity of solids carried in a full-scale plant, minor short duration variations in return cell concentration would have little effect on the aerator solids concentration. Hence, it is felt that in most cases, intermittant sludge wasting will not seriously limit the applicability of the proposed equations.

The relation between growth and reactor substrate concentration was found to follow the Teissier equation for the experimental unit. The use of this equation for computing substrate concentration depends on the determination of the constants k_m and c. The Michaelis-Menten equation has been used to relate k_1 and $\bar{S}$ by most authors. In the application of this equation the value of k_m and S_n have to be known. The problems associated with the determination of k_m for a mixed culture from batch tests have already been discussed in Section V. It was found that the batch value of k_m determined using one of the pre-dominant organisms

of the culture did not agree with the k_m obtained from continuous flow data. The maximum growth rates of pure cultures have been widely studied. However, it would seem that there is need for more work in the area of growth of heterogeneous cultures at high rates.

In many cases, the most expedient approach to obtain the relation between k_1 and $\bar{S}$ might be to operate a continuous flow bench-scale unit without feedback. The unit would be loaded with the waste to be treated in the full-scale plant and an acclimated mixed culture. The experimental systems with and without feedback were found to have approximately the same $\bar{S}$ at a given growth rate. In addition, at the low growth rates used in sewage treatment, the relation between k_1 and $\bar{S}$ is approximately linear. Hence the experimental data obtained from such a unit would supply the needed relation and the constants k_m and c would not be required. It is obvious that some of the above considerations relating to the application of the theory discussed in Section V to full-scale plants will require further study.

VII. CONCLUSIONS

A continuous flow system with feedback was constructed and operated under steady state conditions at growth rates ranging from 0.009 to 0.236. The system was also operated without feedback under steady state conditions at three growth rates ranging from 0.079 to 0.292.

The steady state cell concentration $\bar{x}$ in each case was found to be greater than that predicted by the mathematical model based on the equations of Herbert (1961). From a material balance around the system and from direct measurements, it was found that cells were being retained in the reactor due to their sticking to the walls and due to a partial blocking of the effluent port. Evaporation tended to have the same effect in that it increased reactor cell concentration. When the model was modified to include the retention of solids, good agreement was obtained between measured and computed steady state reactor cell concentrations. The retention of solids in the reactor was expressed as a retention factor L which varied from 0.727 to 0.999 during the runs.

It was found that $\bar{x}$ varied inversely with the feedback factor A'' as was expected. A'' in turn was a function of the recycle ratio α, the cell concentration factor C and L as expressed in equation 33. Values of A'' ranging from 0.120 to 0.480 were produced during the runs by adjusting α. The value of C was found to decrease from 2.20 to 1.28 when α was increased from 0.36 to 3.37.

It was found that daily fluctuations in $\bar{x}$ during a given run were due to differences between the weight of cells produced and that lost from the system. The daily fluctuations were predicted reasonably well using a transient condition analysis instead of a steady state type of analysis. The yield coefficient $\overline{Y}$ was found to increase as the rate of growth increased. For runs made at S' = 1000 mg/l, $\overline{Y}$ increased from 0.413 to 0.556 as k_1 increased from 0.01 to 0.29. A sharp decrease in $\overline{Y}$ occurred at low growth rates reaching a value of 0.25. With increasing k_1 values $\overline{Y}$ probably tended toward a maximum value of 0.56.

The steady state reactor substrate concentration $\overline{S}$ was found to increase as k_1 increased. The relationship between the two parameters was correctly expressed by a first order equation by Teissier (1936) as

$$k_1 = k_m(1 - e^{-c\overline{S}})$$

where $k_m = 0.87$ and $c = 0.02$. When the substrate consumption rate k_c was plotted against k_1, a linear relation was obtained. The curve showed that 15 mg glucose per gram cell weight per hour were being consumed at a k_1 of zero. Hence a certain rate of substrate uptake was necessary to sustain cell metabolism without producing growth.

The settling properties of the mixed culture were quite variable. However flocculent growth was present during all the runs with feedback.

Mixing studies conducted using the reaction chamber showed that a dye solution exhibited perfect mixing while various suspensions showed slight departures from theoretical complete mixing. The departure from mixing was greatly influenced by the specific gravity of the particles in suspension.

VIII. APPENDIX

1. Computation of evaporation

When dry air is bubbled through liquid at constant temperature, the gas becomes saturated with the liquid and the partial pressure of the vapor in the resulting mixture is equal to the vapor pressure of the liquid. If Dalton's law of partial pressure holds, then

$$p = \frac{n_1 P}{n_1 + n_2} \qquad 63$$

where p = partial vapor pressure

P = total pressure of air and vapor

n_1 = moles of vapor

n_2 = moles of air in gas leaving saturator

The value of n_2 may be calculated assuming the ideal gas law holds as:

$$n_2 = \frac{PV}{Rt} \qquad 64$$

where R = the ideal gas constant = 0.082 $\frac{\text{liter-atm}}{\text{mole-degree Kelvin}}$

Using t = 297°K, V = 1.0 liters, and P = 1.0 atm, n_2 equals 0.0411 moles.

At 24° C (297°K) the vapor pressure of water is 23.24 mm or 0.0306 atm. Substituting into equation 63 then yields a value for n_1 equal to 0.0013 moles or 0.0234 gms of vapor. Since the computation is based on 1.0 liters of air passing through the reactor per minute, the evaporation rate is 0.0234 ml/minute per liter of air.

2. Estimate of weight of cells adhering to reactor walls

In the following computations, a typical bacterium is taken to have a wet weight of 1.6×10^{-2} gms and have a volume of 1.5×10^{-9} mm^3 (Lamanna and Mallette, 1959). The layer of cells adhering to the reactor wall could readily be observed with the naked eye. Hence it is conservatively assumed to be 0.05 mm thick. Assuming the reactor to contain 2 liters of culture, a volume of cell material equal to 3504 mm^3 would be contained in a layer 0.05 mm thick adhering to the reactor walls. There would then be 2338×10^9 bacteria contained in this layer since

$$\frac{3504 \text{ mm}^3}{1.5 \times 10^{-9} \text{ mm}^3} = 2338 \times 10^9$$

Taking the cells to have a water content of 75 per cent, there would be

$$2338 \times 10^9 \times 0.25 \times 1.6 \times 10^{-12} = 9352 \times 10^{-4} \text{ gm}$$

or 935 mg of bacteria on a dry weight basis. If all these cells were scraped from the walls, it would add 935 mg/2 liters = 467 mg/l to the reactor concentration.

The calculation was checked by assuming that the density of cell mass is 1.1 gm/cc (Lamanna and Mallette, 1959). Then

$$3.504 \text{ cc} \times 1.1 \text{ gm/cc} \times .25 \text{ (per cent dry weight)} = 962 \text{ mg}$$

In two liters there would be 481 mg/l added to the reactor cell concentration which checks with the above figure of 467 mg/l.

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