A STUDY OF THE GROWTH KINETICS OF ACTIVATED SLUDGE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

F - Flow rate, l/day

F_w - Sludge wastage flow rate, l/day

k - Maximum specific substrate utilization rate, day^{-1}

k_d - Cell maintenance coefficient in continuous flow system, day^{-1}

k_{dB} - cell maintenance coefficient derived from series of batch experiments, day^{-1}

k_e - Eckenfelder's specific substrate removal constant, l/mg·day

K_m - McKinney's substrate removal constant, day^{-1}

K_s - Substrate saturation constant, mg/l

m - specific substrate utilization rate for cell maintenance, day^{-1}

S - Substrate concentration, mg/l

S_e - Effluent substrate concentration from continuous flow system, mg/l

S_i - Initial substrate concentration in continuous flow system, mg/l

S_o - Initial substrate concentration in batch study, mg/l

S_t - Substrate concentration at time t, mg/l

t - time

U - Specific substrate utilization rate, day^{-1}

V - Volume, liters

X - Biomass concentration, mg/l

X_o - Initial biomass concentration in batch reactor or biomass concentration in continuous flow reactor after wasting sludge, mg/l

X_t - Biomass concentration at time t in batch reactor, mg/l

X_A - Average biomass concentration, mg/l

X_F - Biomass concentration in continuous flow reactor before sludge wastage, mg/l

X_e - Effluent biomass concentration, mg/l

Y - Cell yield coefficient

Y_B - Batch cell yield derived using seed drawn from a continuous flow system

Y_{B2} - Batch cell yield derived using seed drawn from batch which was seeded from continuous flow system

Y_o - Observed cell yield in continuous flow system

Y_t - True cell yield in continuous flow system

Y_{tB} - True cell yield derived from series of batch yields

μ - Specific growth rate, $hour^{-1}$ or day^{-1}

μ_m - Maximum specific growth rate, $hour^{-1}$

μ_n - Net specific growth rate, day^{-1}

θ_c - Mean cell residence time, day

bCOD - Readily biodegradable Chemical Oxygen Demand

COD_{min} - Residual Chemical Oxygen Demand

COD_e - Effluent Chemical Oxygen Demand

S/X - Ratio of initial substrate concentration to initial biomass concentration in batch reactor system

CHAPTER I

INTRODUCTION

The release of biodegradable organic compounds into rivers, lakes, and streams places a demand on the dissolved oxygen in the receiving waters, may make the receiving water aesthetically unsuitable as a source of potable water, may make the receiving water chemically unsuitable as a source of industrial process water, and may even make the water unsafe for or toxic to both man and other organisms living in or near the receiving body of water or dependent on it as a source of food or potable water. That fraction of organic, water-borne waste which is in particulate form is readily removable via physical-chemical methods. The physical-chemical processes available for removal of particulate wastes are efficient and economical - at least, when compared to the physical-chemical processes required for removal of soluble wastes. The cost of treating those soluble, organic wastes which are amenable to biological treatment by aerobic, biological processes is much less than the cost of achieving comparable treatment by physical-chemical means alone. When used as secondary treatment, the purpose of aerobic, biological waste treatment is removal of soluble organic compounds by conversion to water, carbon

dioxide, and new micro-organisms. The new micro-organisms can then be removed efficiently and economically by physical means. Biological waste treatment also removes various other chemical constituents - inorganic, organic, soluble, and particulate - by incorporation in new cell material, adsoption on cell surfaces, absorption into cells, and entrapment in intercellular matrices. Although these mechanisms occur in aerobic, biological waste treatment; the primary function of this process is removal of soluble, biodegradable, organic compounds. Organic carbon is generally the limiting nutrient for microbial growth in waste streams. Where this is not the case, other required nutrients are generally added to the waste in sufficient quantity to make organic carbon the limiting nutrient.

There are two major classes of aerobic, biological treatment processes. These are the fixed-bed reactor, in which the micro-organisms adhere to a solid surface and the waste stream passes over the stationary micro-organisms; and the fluidized-bed reactor, in which the micro-organisms are suspended in the waste stream and move with it. There are numerous variations and combinations of the two processes on the market. The most widely used process, and consequently the most important, is activated sludge - a fluidized-bed process.

The activated sludge process has been used for more than six decades. The design parameters for activated sludge were necessarily crude in the early years of appli-

cation of the activated sludge process. Standard ranges of such parameters as hydraulic detention time, BOD loading per unit volume, and BOD loading per unit mass of sludge per day were used. These parameters allowed little accuracy in control and prediction of effluent BOD. This was of no great consequence in the past, as standards for effluent BOD were neither demanding of the waste treatment process nor strictly enforced by the relevant governmental agencies. This is no longer true. High waste removal efficiencies are now required of treatment processes and effluent BOD standards are strictly enforced. The crude design and control methods of the past are no longer adequate.

Presently there exist a wide variety of sophisticated kinetic models purporting to describe the activated sludge process more precisely than the crude methods of the past. The most important of these models can be divided into two broad classes. The first class of kinetic models is based on first order, decreasing rate, substrate removal. The second class of kinetic models is based on the first order, increasing rate, microbial growth model of Monod (1).

All of the above-mentioned kinetic models are far more sophisticated than earlier empirical methods for design and control of the activated sludge process, but all of these newer models require laborious and time consuming pilot plant studies to determine system constants. The required pilot plant studies are in part so time consuming and laborious because continuous flow, pilot plant studies are

required to determine system constants. While batch studies are much simpler and less time consuming than are continuous flow studies, batch studies are generally not thought to produce results similar to those derived from continuous flow studies. Since the activated sludge process is generally operated as a continuous flow process, continuous flow pilot plant operation is the preferred method for gathering data for the design of a full size activated sludge plant.

The purpose of the present study is to explore, in a systematic way, the relationship(s) between batch systems and continuous flow systems. In order to design a full scale, activated sludge system; all of the kinetic models require that certain system constants describing microbial growth and waste removal be determined in smaller scale systems. These constants, once determined, can then be applied to design of the full scale activated sludge system. The system constants, as determined in a continuous flow bench scale system will be compared to the constants derived from batch experiments using sludge drawn from the continuous flow system at different sludge ages.

CHAPTER II

LITERATURE REVIEW

All of the activated sludge models contain system "constants" describing microbial growth and waste removal. The micro-organisms of greatest significance in the activated sludge process are aerobic, heterotrophic bacteria. A general discussion of the constants describing the growth of aerobic, heterotrophic bacteria will be presented first. This will be followed by a history of the development of quantitative descriptions of the activated sludge process. Finally, the major activated sludge models presently in use will be presented.

A. Bacterial Growth

The activated sludge process, like other aerobic, secondary waste treatment processes, is biological in nature. Any quantitative description of the activated sludge process must include not only a description of the hydraulics of the activated sludge system, but a description of microbial growth and substrate removal as well. A model purporting to describe the activated sludge process must allow, as a minimum, prediction and/or conrrol of the steady state values of sludge (microbial mass) production, sludge accumulation

in the system, and effluent waste concentration. The general discussions of the microbial growth and substrate removal constants is drawn from Kincannon and Gaudy (2) and Gaudy and Gaudy (3).

As mentioned above, the micro-organisms of primary significance in the removal of soluble, organic wastes in the activated sludge process are aerobic, heterotrophic bacteria. These bacteria feed on the waste, grow, and produce new micro-organisms, which can then be physically removed from the waste stream. The pseudo-equation often used to describe this process is given below. In the above

$$\text{Soluble organic matter} + O_2 \xrightarrow{\text{Micro-organisms}} CO_2 + H_2O + \text{Micro-organisms} \qquad (1)$$

"equation" the waste removed is partitioned by the feeding bacteria between the production of energy via respiration and the production of new bacterial mass. The oxygen on the left hand side of the equation is used by the bacteria to oxidize a portion of the organic waste and a portion of the energy released during the oxidation process is then stored and used in the production of new bacterial mass.

A quantitative description of the above partitioning process is the "cell yield" (Y). This relationship is presented below. The cell yield is important in the pre-

$$Y = \frac{dX}{dS} \qquad (2)$$

diction of sludge production and accumulation in the activated sludge process. Under continuous flow conditions, the observed cell yield (Y_o) has been found to decrease as specific growth rate is decreased. For this reason, a specific form of the cell yield must be specified as constant. This is the "true cell yield" (Y_t). A second constant is required to describe the variation of Y_o with specific growth rate. The "cell decay coefficient" or "cell maintenance coefficient" (k_d) is used to account for variation in observed yield. These two constants (Y_t and k_d) are shared by all of the activated sludge models to be discussed later. These constants are generally derived empirically from the operation of continuous flow, biological reactors at various specific growth rates (μ). Equations 3 and 4 describe this relationship. Various explanations

$$Y_o = \frac{Y_t \mu_n}{k_d + \mu_n} \tag{3}$$

$$\mu_n = \mu - k_d \tag{4}$$

have been proposed as "the explanation" for the relationship described by equation 3. These will be discussed later.

The metabolic pathways available for energy production in anaerobic micro-organisms are more limited than are the pathways available for energy production in aerobic micro-organisms. For this reason, no generalized statement can be made concerning cell yields in aerobic systems. Bauchop and Elsden (4) were able to find a general relationship

between yield and ATP production in several anaerobic bacterial cultures. The organic compound of interest was used almost exclusively for energy production in this study, while the carbon for cell synthesis was derived from other compounds in the medium. Prediction of yield in aerobic cultures is complicated by uncertainties as to the ATP yield from specific substrates in specific micro-organisms (the specific pathway used for energy production) and the degree of coupling between ATP procuction and synthesis of new cell material. Servizi and Bogen (5)(6) are among those who have attempted to relate yield and the free energy of oxidation of the substrate. Servizi and Bogen's equation for yield as a function of the COD of the substrate was 0.39 miligrams of dry cell mass generated per miligram of COD used (where COD is related to the free energy of oxidation). McCarty (7) recalculated the data of Siegel and Clifton (8) (9) and McKinney et al. (10) as molar growth yields and found these to correlate with the free energy of oxidation, although the different sets of data were in serious disagreement. Hetling et al. (11) criticized the conclusions of Servizi and Bogen on both theoretical grounds and because "endogenous metabolism" (variation in yield with specific growth rate) was not considered. Hetling et al. determined growth yields in continuous flow with several pure cultures and mixtures of pure cultures and several substrates. Hetling et al. concluded that the "heterogeneous metabolism rate" (same as endogenous metabolism) was not constant

for all organisms or all substrates, but even after correction for this factor, the yield (true yield) varied with substrate and with organism. Ramanathan and Gaudy (12), using a minimal medium with glucose as sole carbon source and heterogeneous microbial populations, found the average yield in batch systems to be 0.62 miligrams of dry cell mass per miligram of COD. The range of yields obtained was 0.36 to 0.88. Sawyer (13) found the yield on glucose to range from 0.44 to 0.64. All of this suggests that yield varies with the substrate, the micro-organisms, and the specific growth rate of the micro-organisms in a continuous flow system. The cell yield cannot be predicted on the basis of the free energy of oxidation of a substrate in aerobic systems.

The variation in cell yield with specific growth rate has been explained via cell death, predation, endogenous metabolism, cell maintenance, population changes in heterogeneous cultures or mutations in homogeneous cultures, and decreased efficiency in the capture or utilization of energy released during respiration. Many modifications to the major models discussed later have been published incorporating constants describing one or more of the above concepts. The cell maintenance coefficient (k_d), as used in the major models, is an empirical constant which accounts for the decrease in observed cell yield with decreasing specific growth rate in continuous flow systems. The reasons for this variation in cell yield are open to solution. Ramanathan and Gaudy (14) found yield to be constant during

the entire period of substrate removal and cell growth. This study was done with a heterogeneous microbial population using glycerol as the carbon/energy source. It should also be noted that the initial cell concentration was quite small, so that substrate removal was effected over a long period of time. Where a low S/X ratio is used, causing very rapid substrate removal, the yield is greater initially as substrate is removed from suspension and stored by the micro-organisms for later use as a carbon/energy source for synthesis (15). However, under the slower substrate removal conditions, Srinivasaraghavan (16) and Saleh (17) found that the batch values for cell yield obtained using seed from a continuous flow reactor were quite similar to the observed yield in the continuous flow reactor. Both of these authors used heterogeneous populations and a glucose minimal medium. The results of the above studies suggest that the traditional explanations of the variation of cell yield in continuous flow reactors questionable - at least those which imply a time dependent reaction such as predation, cell death, or even cell maintenance.

The range of values for the cell maintenance coefficient (k_d), as cited by Lawrence and McCarty (18), is 0.045 to 0.18 day^{-1}. The values experienced by various researchers in the Bioenvironmental Engineering laboratories at Oklahoma State University are also in this range.

The remaining major requirement of an activated sludge model is that it describe the rate of microbial growth and

waste removal. The relationship between microbial growth and waste removal is described by equation 5. If the rate

$$Y = \frac{dX/dt}{dS/dt} \tag{5}$$

of one is known, so is the rate of the other if the cell yield is known. The major models to be discussed vary significantly with respect to the reaction rates. The mathematical descriptions of the rates may be divided into two classes as noted earlier. The first class of activated sludge models assume first order, decreasing rate, substrate removal. This rate is described by equation six or, in its integrated form, equation seven. Equation six is a general

$$\frac{dS}{dt} = k_e XS = K_m S \tag{6}$$

$$K_m = k_e X = \frac{\ln S_o - \ln S_t}{\Delta t} \tag{7}$$

description of the rate. When applied to a complete-mix, continuous flow system; S becomes S_e. Equation seven applies to batch and plug-flow reactors.

Gaudy (19), Eckenfelder (20), Wilson (21), and Wuhrman (22) found linear substrate removal of specific substrates. One of these authors, Wuhrman, assumed that a pseudo-first-order removal rate would ensue with complex substrates (a mixture of organic compounds) when a non-specific measure of substrate concentration was used to measure substrate concentration remaining at various times

after the start of substrate removal. McCabe and Eckenfelder (23) assumed a declining growth phase in the activated sludge process. During this phase of growth, the kinetics described by equation six are assumed to apply. The instantaneous substrate concentration is assumed to control the rate of substrate removal and microbial growth. These authors also assumed and found first order kinetics to apply with both simple and complex substrates when a non-specific measure (COD) was used to measure substrate re-remaining. However, these authors also stated that simple substrates and a non-specific measure of substrate would theoretically yield first order kinetics, simple substrates and a specific measure of that substrate would theoretically yield zero order kinetics, and complex substrates with any measure of substrate concentration would yield some other order kinetics. Also, it was not made clear why the declining growth phase should begin at the start of a batch experiment at a high initial substrate concentration. Later, Tischler and Eckenfelder (24) found zero order kinetics to occur with simple substrates using both specific and non-specific (COD and TOC) measures of the substrate concentration. First order kinetics occured when the substrates were mixed. This conflicts with the findings and theorizations of McCabe and Eckenfelder. Chuboda (25), in a discussion of Tischler and Eckenfelder's paper, pointed out that apparent order of removal kinetics is related to S/X ratio and initial substrate concentration (at similar S/X ratios). The difference between first order and zero order kinetics

has nothing to do with declining growth, complexity of the substrate, or measure of substrate used.

It should be noted that first order kinetics are implied by the empirically useful, discontinuous linear function relating specific growth rate or specific utilization to effluent substrate concentration proposed by Garrett and Sawyer (26). This relationship implies also that the effluent substrate concentration is a function of detention time and bio-mass concentration. In fact, various authors (McCabe and Eckenfelder (23) and Rao and Gaudy (27), for example) have found removal rate in batch systems to be linearly related to bio-mass concentration. It has never been made entirely clear whether McKinney (28), who also uses first order, substrate removal kinetics, assumes the effluent substrate concentration to be a function of both bio-mass concentration and detention time or a function of detention time only. Goodman and Englande (29), in comparing k_e and K_m, apparently interpret McKinney's model as if K_m is a constant - i.e., removal rate is independent of bio-mass concentration. Unfortunately, the activated sludge operating data presented by these authors to show that K_m is a constant rather than k_e (the bio-mass concentration dependent, rate constant), demonstrates the reverse. However, these authors interpreted said data as if it did support their contention.

McKinney (28) and Goodman and Englande (29) suggest that a good value of K_m is 15 hour^{-1} (360 day^{-1}), while Eckenfelder suggests that the k_e for readily degradable wastes will vary

between 0.001 and 0.002 $hr^{-1}(mg/l)^{-1}$.

The quantitative description of cell growth or substrate removal used in the second class of activated sludge models is the first order, increasing rate, microbial growth model of Monod (1). The rate of increase of microbial mass is described by equation 8. The integrated form of this

$$\frac{dX}{dt} = \mu X \tag{8}$$

equation is equation 9. Equation 9 describes growth in

$$\mu = \frac{\ln X_t - \ln X_o}{\Delta t} \tag{9}$$

batch systems and plug-flow systems. The specific growth rate is related to substrate concentration by equation 10.

$$\mu = \frac{\mu_m S}{K_s + S} \tag{10}$$

An alternate form of equation 10, based on specific substrate utilization rate (U) instead of specific growth rate, is represented by equation 11. Equation 12 relates μ and U.

$$U = \frac{k S}{K_s + S} \tag{11}$$

$$Y = \frac{dX/dt/X}{dS/dt/X} = \frac{\mu}{U} = \frac{\mu_m}{k} \tag{12}$$

The appropriate value for substrate concentration (S) in equations 10 and 11 is the initial substrate concentration (S_o) when applied to batch systems and the effluent substrate concentration (S_e) when applied to continuous flow, complete

mix systems.

The relationship between μ and S was known to be curvilinear by Garrett and Sawyer (26) and McCabe and Eckenfelder (23), but activated sludge modellers like Eckenfelder found the discontinuous, linear relationship between μ and S to be of adequate precision for their purposes and more convenient for model development. Schulze (30) found that the curvilinear relationship of Teissier (32) fit the data obtained from a pilot plant, activated sludge system better than did Monod's equation. Gaudy et al. (33)(34) found Monod's equation a better fit. In addition to providing a superior fit of available data, Monod's equation is much more convenient and enables greater precision in determining the relevant constants than does Teissier's formulation.

In the early literature of both Microbiology and Sanitary Engineering it was assumed that exponential growth in batch reactors (described by μ) could neither be attained at values of μ less than μ_m nor could μ be sustained for any length of time. The values of K_s reported by Monod (1) were quite small. Although Monod (35) stated that μ could be measured in batch reactors using various values of initial substrate concentration (S_o) and that μ_m and K_s could be derived from the observed values of μ at the different values of S_o, he perhaps created some confusion when he suggested that the log growth equated to S_o occurred for a very brief period early in the substrate removal phase. Various researchers and engineers have since assumed that the observed

μ varies instantaneously with the remaining substrate concentration as it is removed. However, Gaudy et al. (36)(37) have shown that μ does occur at values less than μ_m when S_o is varied in batch reactors and that μ, once attained in a batch system, is sustained at a constant value for an extended period of time - even after the substrate concentration has decreased greatly from S_o. Further, if μ is calculated at various times during the removal of substrates, along with the substrate concentration remaining at that instant; the calculated value of K_s will be much smaller than if μ is determined versus S_o, due to the loose coupling between μ and S. The question arises as to which method of determining K_s is correct. Using heterogeneous cultures with glucose minimal medium, Gaudy et al. (33)(34) compared batch derived values of μ_m and K_s, using μ and S_o, with the observed values of μ and S_e (COD) from a chemostat. The batch values for μ_m and K_s were found to provide a reasonably good fit of the chemostat data.

The range of values for μ_m and K_s, using glucose minimal medium and a sewage-derived heterogeneous culture, reported by Gaudy and Gaudy (3) are 0.4 to 0.6 hour^{-1} and 50 to 125 mg/l respectively. Using seed drawn from a pilot plant, activated sludge reactor; Saleh (17), Esfandi (65), and Srinivasaraghavan (16) reported a wider range of values for these constants. The most notable variation was found by Esfandi. This author found K_s values above 2000 mg/l. Although not noted in the above references, the authors

found extended lag periods to occur prior to the log growth phase. Esfandi (65) found the lag period to range from 10 to 12 hours to as much as 30 to 36 hours. The lag period generally encountered prior to the log growth phase, using seed from a rapidly growing system, usually lasts less than an hour or two.

The values of μ_m and K_s reported by Peil and Gaudy (38) for a heterogeneous microbial culture grown on sewage were 0.46 hour^{-1} and 52 mg/l, respectively. These values are not dissimilar to those cited above for glucose.

B. History of Activated Sludge Design

The activated sludge process was developed early in the twentieth century. In 1912, the practice of aeration in the presence of micro-organisms was carried over to England by Dr. G. J. Fowler after his visit to Lawrence Experimental Station in Massachusetts. Ardern and Lockett (39) developed the activated sludge process in England in 1914. The process was called the activated sludge process as it involved the production of an activated mass of micro-organisms capable of aerobically stabilizing a waste. These early processes were fill-and-draw, but later activated sludge plants were continuous flow processes with recycling of bio-mass. Parameters for design and operation of the activated sludge process were understandably crude early in this century. For example, the "Ten State Standards" (40) set minimum requirements for sewage detention time in the

system and maximum BOD loading per aeration volume. Unfortunately, these parameters have little to do with BOD removal and allow no prediction of sludge production in the activated sludge process.

It was not untill the 1940's that Monod (1)(35) published his papers describing bacterial growth. Teissier (32) had earlier published a curvilinear relationship between cell growth rate and substrate concentration as represented in equations 13 and 14. Equation 14 is the integrated form of

$$\frac{d\mu}{dS} = c(\mu_m - \mu) \tag{13}$$

$$\mu = \mu_m(1 - e^{-cS}) \tag{14}$$

equation 13 (between the limits, 0 and S or μ).

Continuous flow models for microbial growth began with Monod (41) and Novick and Szilard (42), in 1950. Both of these models were based on Monod's kinetics (1)(35). Herbert, Ellsworth, and Telling (43) and Herbert (44) produced slightly more refined models similar to those of Monod and Novick and Szilard. Herbert (45) and later Pirt (46) added constants describing cell maintenance (k_d) and substrate needed for cell maintenance requirements (m), respectively. These two constants are related by equation 15.

$$k_d = m\ Y_t \tag{15}$$

The microbiological concepts underlying the activated sludge process began to be investigated in the early 1950's. In 1951, Helmers et al. (47) reported that cell growth was

proportional to BOD removal. In the same year Heukelekian et al. (48) proposed an empirical equation relating the sludge accumulation rate, sludge production due to BOD removal, and the oxidation of solids. In 1952, Hoover and Porges (49) presented an empirical formulation describing the elemental composition of activated sludge micro-organisms. This formula is often quoted and used today. The ratio of COD to dry cell mass for this formulation is approximately 1.42. In 1952, Garrett and Sawyer (26) and later McCabe and Eckenfelder (27) used a discontinuous linear function to describe the relationship between growth rate and substrate concentration. Garrett (50) applied this linear relationship to the operation of an activated sludge plant.

In 1955, Eckenfelder and O'Conner (51) proposed a mathematical model of the activated sludge process. Eckenfelder's early modelling was based on batch pilot plant studies, while his later efforts were based on continuous flow pilot plant studies (52)(53). Eckenfelder's model follows the discontinuous linear function of Garrett and Sawyer (26). That is, first order, decreasing rate, substrate removal is assumed.

In 1962, McKinney (28) proposed a mathematical model for a complete mixing activated sludge system. In this model, McKinney assumed a great many "constants" describing his conception of what occurs in the activated sludge process. McKinney's model apparently assumed effluent substrate concentration to be dependent on waste detention time and independent of the bio-mass concentration.

Busch (54)(55) developed what is essentially an empirical design procedure, requiring data from both an activated sludge pilot plant operating under non-steady state conditions and batch studies.

Weston et al. (56)(57)(58) have proposed a mathematical model and design procedure based on data obtained from bio-mass developed in fill and draw reactors. Batch experiments are performed on the acclimated bio-mass at various S/X ratios.

Schulze (30) proposed and tested and activated sludge model using the kinetic relationship of Teissier (32). Schulze used continuous flow reactors.

Jenkins and Garrison (59) proposed an activated sludge model based on Monod kinetics (1). Lawrence and McCarty (18) presented a more complete development of this activated sludge model. Sherrard and Schroeder (60)(61) presented a model which differed from that of Lawrence and McCarty in that the observed yield (Y_o) was used in place of the true yield (Y_t) and cell maintenance coefficient (k_d).

Gaudy et al. (34)(62)(63) have proposed both a mathematical model and a modified activated sludge system. The activated sludge model of Gaudy is based on Monod kinetics (1). The modification to the activated sludge process proposed by Gaudy and co-workers is an aerated sludge consistency tank between the secondary clarifier and the activated sludge tank.

C. The Major Activated Sludge Models

All of the activated sludge models to be discussed in this section of this chapter assume complete mixing in the bio-reactor and steady state conditions in the effluent substrate concentration, mixed liquor suspended solids concentration, and sludge wastage rate. The models to be discussed are those of Eckenfelder (52)(53), McKinney (28)(64), Lawrence and McCarty (18), and Gaudy (34)(62)(63). The notation and development of the models used here is from Kincannon and Gaudy (2). The models, as presented here, are faithful to the presentations by the original authors, except for McKinney's model. The many constants in McKinney's model have been dropped and kinetics similar to Eckenfelder's model have been assumed. In McKinney's model the effluent substrate concentration was apparently assumed to be dependent only on hydraulic detention time and independent of sludge concentration. In all of the other models the effluent substrate concentration is a function of both detention time and sludge concentration. A diagram of the activated sludge system is presented in Figure 1. The substrate and sludge mass balances are drawn around the entire system for all models; except those of Gaudy's model, which are drawn around the bio-reactor. Unlike the other models, Gaudy's model makes the assumption that the substrate concentration in the sludge recycle line is zero.

The materials balances for the various models are presented in Tables I and II. Table I presents the materials

Figure 1. Flow Diagram of Typical Activated Sludge Process

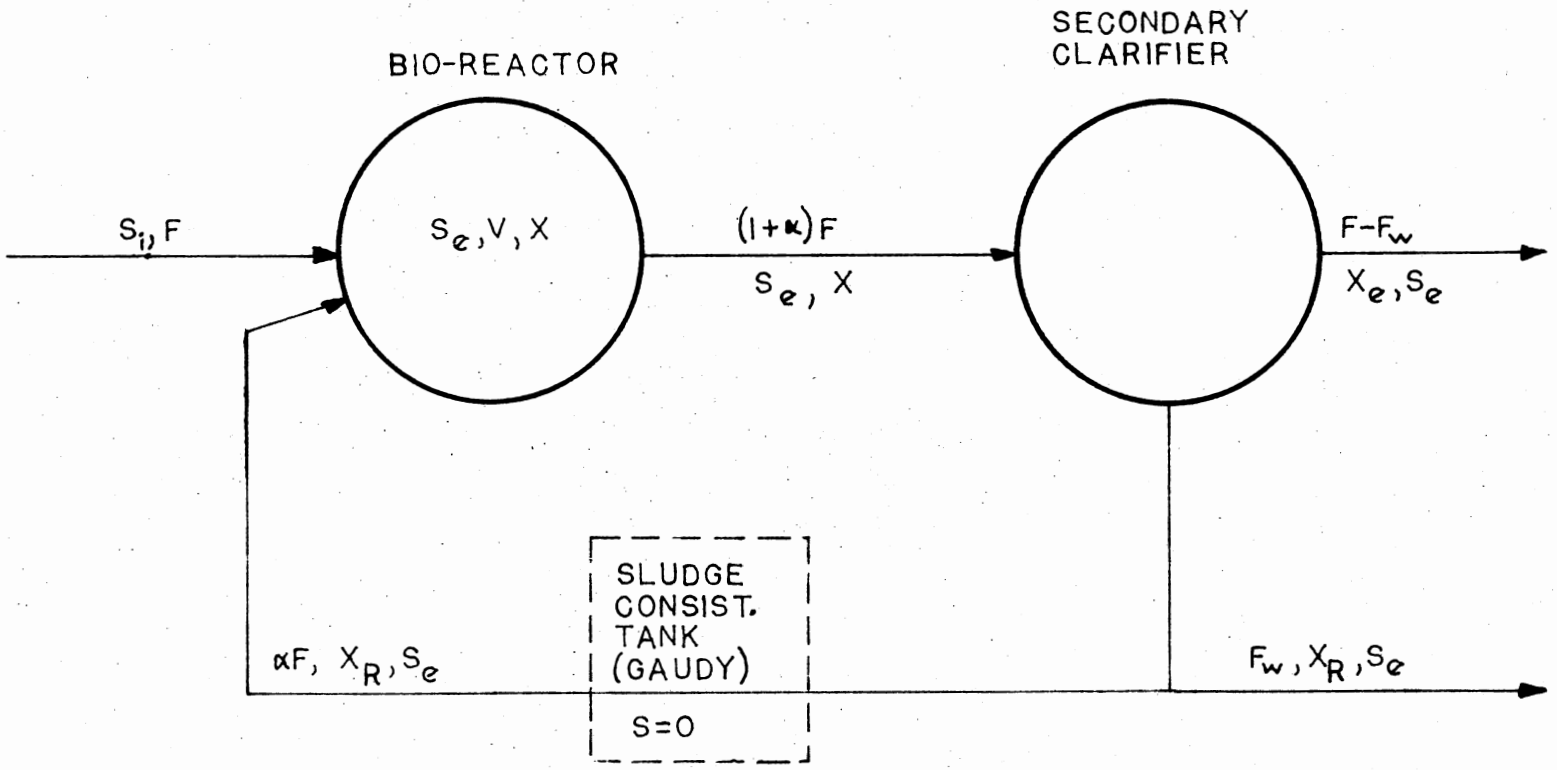

BIO-REACTOR
SECONDARY CLARIFIER
S_i, F
S_e, V, X
$(1+\alpha)F$
S_e, X
$F-F_w$
X_e, S_e
SLUDGE CONSIST. TANK (GAUDY)
$\alpha F, X_R, S_e$
S=0
F_w, X_R, S_e

TABLE I

STEADY STATE MATERIALS BALANCE FOR SUBSTRATE

Balance Model	Net Mass Rate of Change		Mass Rate due to Inflow		Mass Rate due to Outflow		Mass Rate due to Metabolism
Eckenfelder	$\frac{dS}{dt}V$	=	FS_i	-	FS_e	-	$k_e X S_e V$
McKinney	$\frac{dS}{dt}V$	=	FS_i	-	FS_e	-	$K_m S_e V$
Lawrence & McCarty	$\frac{dS}{dt}V$	=	FS_i	-	FS_e	-	$kX\frac{S_e}{K_s+S_e}V$
Gaudy	$\frac{dS}{dt}V$	=	$FS_i+\kappa FS_e$	-	$F(1+\alpha)S_e$	-	$\mu_m\frac{X}{Y_t}\frac{S_e}{K_s+S_e}V$

TABLE II

STEADY STATE MATERIALS BALANCE FOR BIO-MASS

Balance Model	Net Mass Rate of Change		Mass Rate due to Inflow		Mass Rate due to Growth		Mass Rate due to Autodigestion		Mass Rate due to Outflow
Eckenfelder	$\frac{dX}{dt}V$	=	FX_o	+	$Y_t(S_i-S_e)F$	-	k_dXV	-	$(F-F_w)X_e-F_wX_R$
McKinney	$\frac{dX}{dt}V$	=	FX_o	+	$Y_tK_mS_eV$	-	k_dXV	-	$(F-F_w)X_e-F_wX_R$
Lawrence & McCarty	$\frac{dX}{dt}V$	=	FX_o	+	$Y_t\frac{kS_eX}{K_s+S_e}V$	-	k_dXV	-	$(F-F_w)X_e-F_wX_R$
Gaudy	$\frac{dX}{dt}V$	=	FX_o+FX_R	+	$\frac{\mu_mXS_e}{K_s+S_e}V$	-	k_dXV	-	$F(1+\alpha)X$

balance for substrate, while Table II presents the materials balance for sludge or bio-mass. These balances are drawn around the system shown in Figure 1. The net mass rate of change is equal to zero at steady state. X_o is assumed to be equal to zero. The design and operational equations for each model may be derived for each model from the materials balance equations in Tables I and II. See Kincannon and Gaudy (2) for the relevant design equations.

CHAPTER III

MATERIALS AND METHODS

In order to observe the values of the various kinetic constants, bench scale activated sludge units were operated over a range of sludge ages (or net specific growth rates). At each sludge age, appropriate tests were conducted to allow evaluation of the various kinetic constants under continuous flow conditions. At each sludge age, some of the daily waste sludge was placed in batch reactors and various tests were performed with the sludge to allow evaluation of the kinetic constants in the batch reactors.

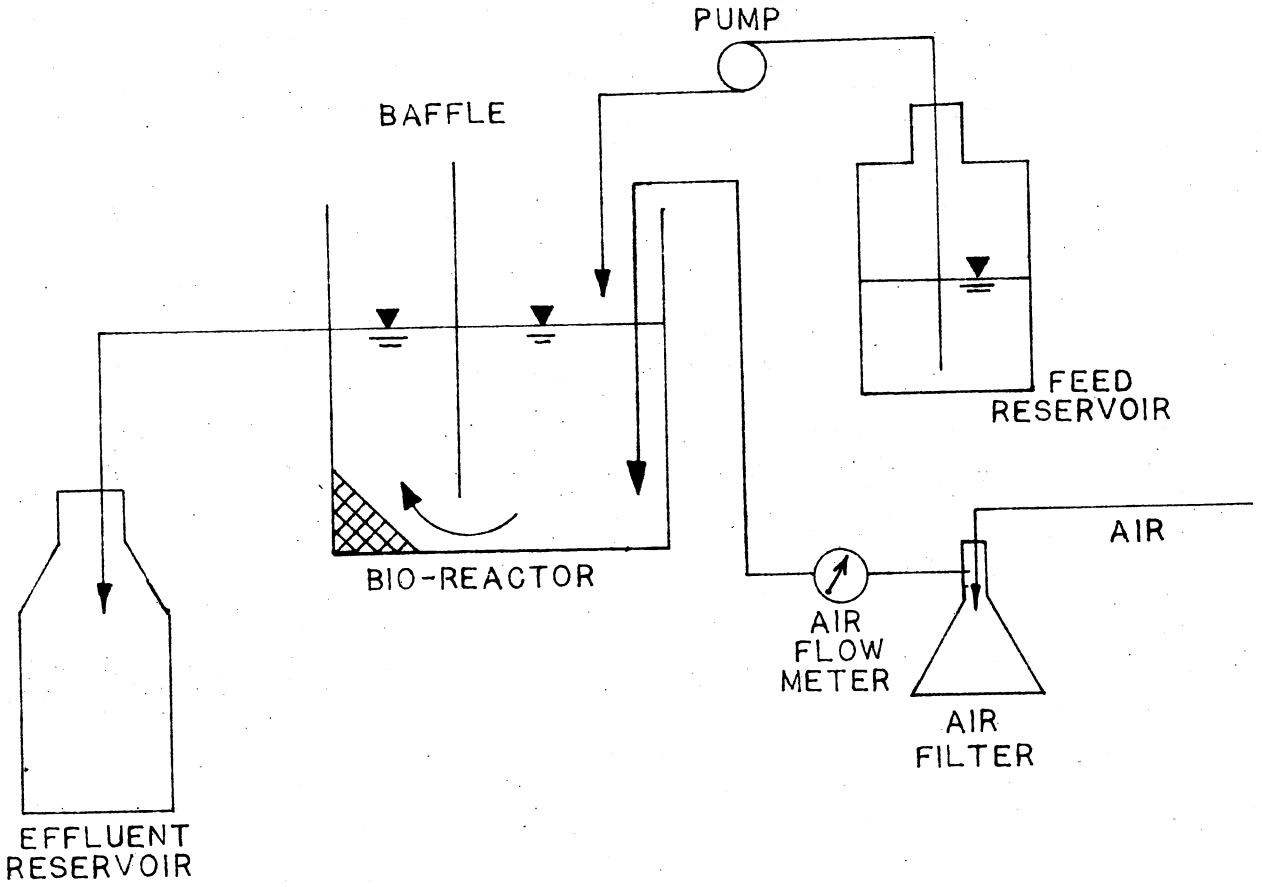
A. Laboratory Apparatus

1. Continuous Flow Apparatus

A diagram of the experimental apparatus is presented in Figure 2.

Two reactors were used in this study. The total volumes of the reactors were 8.1 and 8.4 liters. The volumes of the clarifier and aeration chamber of each of the reactors were 2.2, 2.2, 5.9, and 6.2 liters. The reactors were identical in design. Both reactors were rectangular in shape, had removable baffles separating aeration chamber and clarifier, and were constructed of clear plexiglass.

Figure 2. Laboratory Activated Sludge Unit


PUMP
BAFFLE
FEED
RESERVOIR
AIR
BIO-REACTOR
AIR
FLOW
METER
AIR
FILTER
EFFLUENT
RESERVOIR

A continuous feed rate of between 16 and 18 liters per day was supplied to the reactors via a pump. The feed rate was monitored and adjusted daily. The daily rate could be monitored, as 18 liter bottles - made of clear glass and marked in one liter graduations - were used as feed reservoirs. If the pumping rate was incorrect, a graduated cylinder and timer were used to adjust the pumping. Flow from the reactor to an effluent reservoir was accomplished via gravity flow.

Air was supplied to each reactor through two sintered glass diffusers. The air flow was monitored via air flow meters and maintained at 4 $\pm$ 0.5 liters per minute. A glass cotton filter was placed between the air diffusers and the air outlet to prevent any oil in the airlines from entering the experimental reactors.

The pH of the system was monitored daily with a pH meter. The pH of the system, both influent and effluent, was maintained at 7.2 $\pm$ 0.1 by means of a phosphate buffer system.

The temperature was monitored daily with a laboratory thermometer. The temperature in the reactor stayed at 22 $\pm$ 1 °C.

2. Batch Apparatus

Two different types of batch apparatus were used during the course of this study. The first type of batch reactor consisted of 500 and 1000 mililiter erhlenmeyer flasks. These

were kept aerobic and mixed with diffused air supplied through sintered glass diffusers. The second type of batch reactor consisted of 200 to 250 mililiter flasks. These were kept mixed and aerated via a shaker operating at a steady rate of 130 cycles per minute.

B. Feed Solution

Four stock solutions were made up in concentrated form. The composition of the stock solutions was as indicated in Table III. The chemical composition of the feed for the continuous flow reactors is also indicated in Table III. The feed for the various batch experiments was obtained by diluting the stock solutions as necessary. The same proportions of the stock solutions were used in the batch experiments as was used in the continuous flow reactors. Where yeast extract was used as a carbon/energy source, only phosphate buffer was added (in the same COD/buffer ratio used with glucose). Where yeast extract and glucose were used together, other stock solutions were added in the same ratio of glucose concentration to concentrations of other nutrients as was used in the continuous flow reactors.

C. Experimental and Analytical Procedures

The micro-organism seed was taken from the continuous flow reactors of Esfandi (65). Additional seed was added from the primary clarifier overflow of the Stillwater municipal treatment plant. The unit was operated at decreasing sludge ages from 11.5 days to 2.4 days. Seed for

TABLE III

COMPOSITION OF SYNTHETIC WASTEWATER

Solution	Component	Stock Solution Conc.	Volume per Liter of Feed	Feed Conc.
no.	-	(g/l)	(ml/l)	(mg/l)
1	glucose	143	2.2	318
2	$(NH_4)_2SO_4$	72	2.2	158
3	$MgSO_4 \cdot 7H_2O$	15	2.2	33
	$FeCl_3 \cdot 6H_2O$	0.15		0.33
	$CaCl_2$	1.5		3.3
	$MnSO_4 \cdot H_2O$	1.5		3.3
4	K_2HPO_4	215	2.2	473
	KH_2PO_4	28		62
5	Tapwater		991	

the 16.7 day sludge age was drawn from another continuous flow reactor operating at a sludge age of approximately seven days. This was done as it was felt that data for an additional sludge age, higher than 11.5 days, was needed. However, the original sludge had acquired characteristics at a low sludge age that made it unuseable (bulky and very sticky) and these characteristics persisted when an attempt was made to increase the sludge age to the 16 day sludge age.

Sludge age (or net specific growth rate) was selected as the independent variable for this study. The selected sludge age was maintained by wasting of microbial mass from the continuous flow reactor. This was accomplished daily at about the same time. The baffle was removed and the contents of the clarifier and the aeration chamber were mixed prior to wasting. At the 2.4 day sludge age, system effluent was returned to the reactor in a quantity equal to the volume of sludge removed from the reactor. The average total microbial mass in the reactor during each 24 hour period (median of the daily high and low values of microbial mass in both the clarifier and aeration basin of the reactor) was used to compute the sludge age and specific utilization rate.

Biological solids concentrations were monitored in the continuous flow reactors using Millipore filters having a 0.45 micrometer pore size, as described in Standard Methods (66). This method was also used, where required, for monitoring biological solids concentrations in the batch experiments. Where use of this method for determining suspended solids concentrations was impractical in the batch experiments, Absorbance readings at a wavelength of 600 nanometers were used. The approximate ratio of solids concentration to Absorbance was found to be 1050 miligrams/liter per Absorbance unit. While there seemed to be some variation in this ratio with differing microbial suspensions, the ratio seemed to be relatively constant up to an Absorbance of approximately 0.8.

Substrate concentration was measured by means of the Chemical Oxygen Demand Test (66). Where a specific test for soluble carbohydrate concentration was required, the Anthrone Test was used (67).

D. Data Analysis

Analysis of continuous flow data was accomplished using the complete mix equations presented below. The median sludge concentration (X_A) was determined using equation 16. The

$$X_A = \frac{X_F + X_o}{2} \tag{16}$$

specific utilization rate (U) was determined from equation 17.

$$U = \frac{F(S_i - S_e)}{VX_A} \tag{17}$$

Sludge age or net specific growth rate was determined via equation 18. The observed yield was determined via equation

$$\mu_n = \frac{1}{\Theta_c} = \frac{(F-F_w)X_e + F_w X_F}{VX_A} \tag{18}$$

19. Individual values of the substrate removal rate coeffi-

$$Y_o = \frac{(F-F_w)X_e + F_w X_F}{F(S_i - S_e)} \tag{19}$$

cient, K_m, were determined with equation 20. S_e in this case was the effluent COD. The specific substrate removal

$$K_m = \frac{((S_i/S_e) - 1)\ F}{V} \tag{20}$$

rate, k_e, were determined with equation 21. The value of k_e

$$k_e = \frac{K_m}{X_A} \tag{21}$$

(bCOD) was determined by plotting U versus S_e (where S_e is COD_e). The slope of the line of best fit through the points is k_e, while the intercept of the S_e axis is the non-biodegradeable COD (COD_{min}). The bCOD at each value of U can be determined from the equation below. This method is from

$$COD = COD_e - COD_{min} \tag{22}$$

Eckenfelder (52) and this value of k_e (using bCOD, rather than COD_e) is the one used in Eckenfelder's design method. The value of K_m (bCOD) at each value of U can be determined via equation 20, above. The true yield and cell maintenance coefficient were determined graphically via the relationship presented in the equation below. The values of k (and μ_m)

$$\mu_n = Y_t U - k_d \tag{23}$$

and K_s were determined from a Lineweaver-Burke plot of the Monod equation, using the continuous flow values of U and effluent bCOD.

The procedures used in the batch experiments varied considerably, as did the mathematical analyses used. In order to alleviate any confusion as to which mathematical analyses were used with which experimental conditions, the relevant mathematical analyses will be presented along with the relevant results in the "Results" chapter of this study.

CHAPTER IV

RESULTS

The results of this study are presented in this chapter. The observed operational parameters of the continuous flow reactor are presented first in order to provide a basis for evaluation of the batch reactor data. The operational parameters of interest are the constants: Y_t, k_d, μ_m (or k), K_s, k_e, and K_m. Since the continuous flow reactors are small-scale simulations of the activated sludge process (a continuous flow process); the values of the constants determined for the continuous flow reactors are the preferred or control values for the various constants, against which the batch data will be compared. The batch reactor data are presented in the following order: first, data from the substrate removal experiments; second, data from the various growth rate determinations made in shaker flasks; and third, batch yield data. The continuous flow data are represented, where appropriate, with the batch reactor data for ease of comparison.

A. Continuous Flow Data

The mean values of the observed and calculated operating parameters for the continuous flow reactor are presented in

Tables seven & eight. The remainder of this section of this chapter consists of graphic presentations of the data contained in the above tables.

Net specific growth rate is plotted versus specific utilization rate in Figure 3. The true cell yield and cell maintenance coefficient derived from Figure 3 via linear regression are 0.63 and 0.056 respectively. All of the major activated sludge design models - McKinney, Eckenfelder, Lawrence and McCarty, and Gaudy - employ these two constants and all employ a graphic method for determination of these constants similar to Figure 3. Equation 23 describes the relationship presented in Figure 3.

The specific utilization rate is presented versus effluent COD concentration in Figure 4. The graphic presentation in Figure 4 is from Eckenfelder's design procedure for determination of k_e. The relevant equation is presented below.

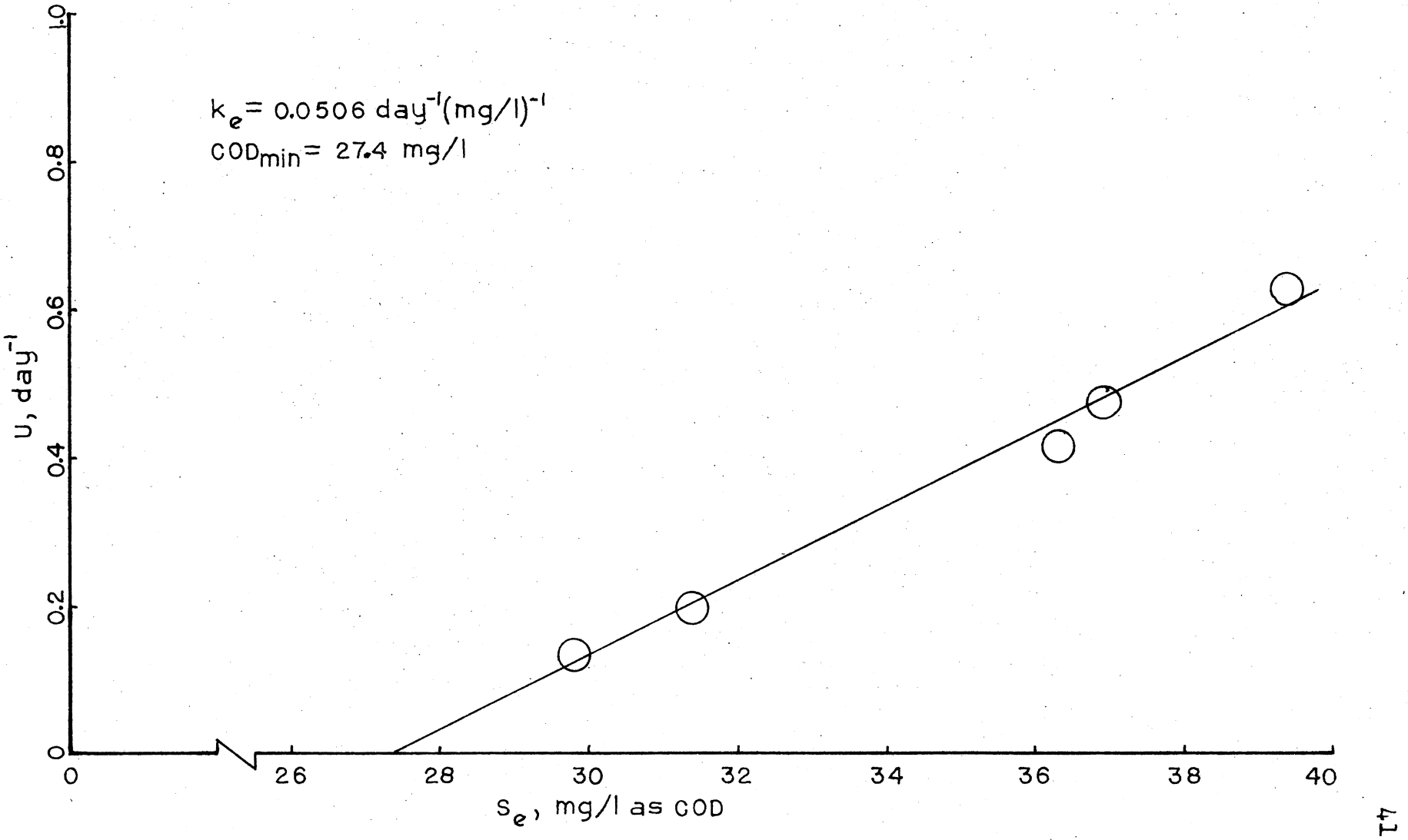
$$U = k_e(COD_e - COD_{min}) \qquad (24)$$

The slope of the line through the plotted values is Eckenfelder's substrate removal rate constant (k_e) and was found to be 0.0506 $day^{-1}(mg/l)^{-1}$ via linear regression. The point of intersection of the line with the x-axis is the theoretical minimum effluent COD. This value was found to be 27.4 mg/l. The difference between this value and observed effluent COD values is the effluent bCOD. The bCOD is the effluent substrate concentration used in Eckenfelder's design procedures. The effluent bCOD will be used later in this study.

Figure 3. Net Specific Growth Rate Versus Specific Substrate Utilization Rate

μ_n, day^{-1}
0.4
0.2
0
0
0.2
0.4
0.6
0.8
U, day^{-1}
$Y_+ = 0.634$
$k_d = 0.0562$ day^{-1}

Figure 4. Specific Substrate Utilization Rate Versus Soluble Effluent COD


k_e = 0.0506 day^{-1}(mg/l)$^{-1}$
COD_{min} = 27.4 mg/l
U, day^{-1}
0
0.2
0.4
0.6
0.8
1.0
0
26
28
30
32
34
36
38
40
S_e, mg/l as COD

Eckenfelder's design procedure employs only a single value for k_e, derived via the method described in the previous paragraph. However, multiple values of k_e can be calculated via equation 25, where S_e is either effluent bCOD or observed effluent COD. The calculated values of Eckenfelder's constant are presented versus sludge age in Figure 5. The values of Eckenfelder's constant in the upper portion of Figure 5 were computed using the bCOD values for S_e (COD_{min} minus the observed effluent COD). These values of k_e are similar to each other and are related to the value found in the previous figure. The mean for these values of k_e is 0.056 $day^{-1}(mg/l)^{-1}$. The values for the substrate removal constant plotted in the lower portion of Figure 5 were calculated using the observed effluent COD values for S_e and would not normally be considered (in Eckenfelder's design procedures). However, these values are presented here as they will be compared to similar values obtained in the batch experiments later in this study. These latter values of k_e show a tendency to decrease with increasing Θ_c.

$$k_e = \frac{S_i - S_e}{S_e X_A t} \qquad (25)$$

The calculated values of McKinney's substrate removal constant at each sludge age are presented in Figures 6 and 7. These constants were calculated via the following equation:

$$K_m = \frac{S_i - S_e}{S_e t} \qquad (26)$$

Figure 5. Specific Substrate Removal Constant (k_e) Versus Sludge Age

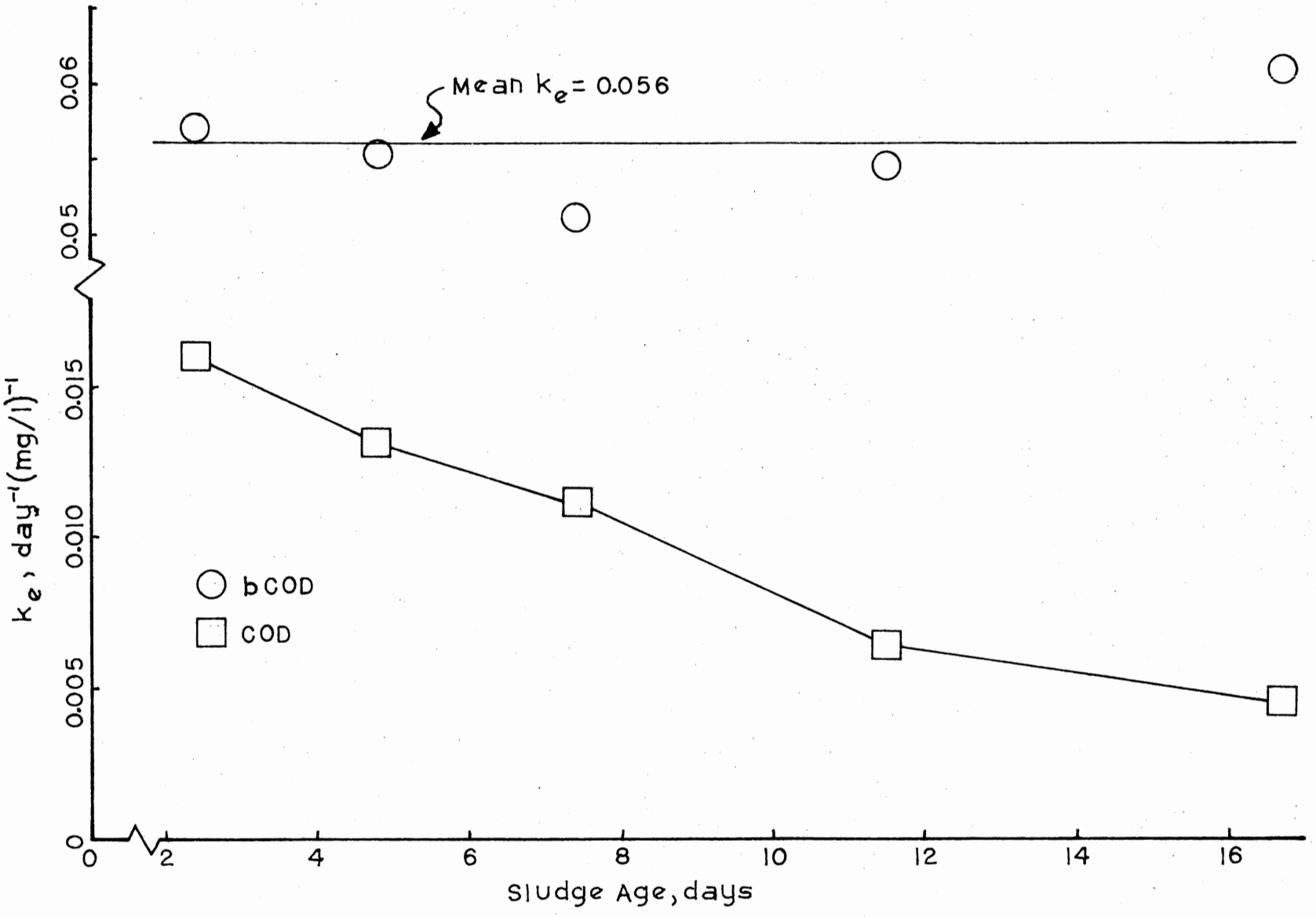
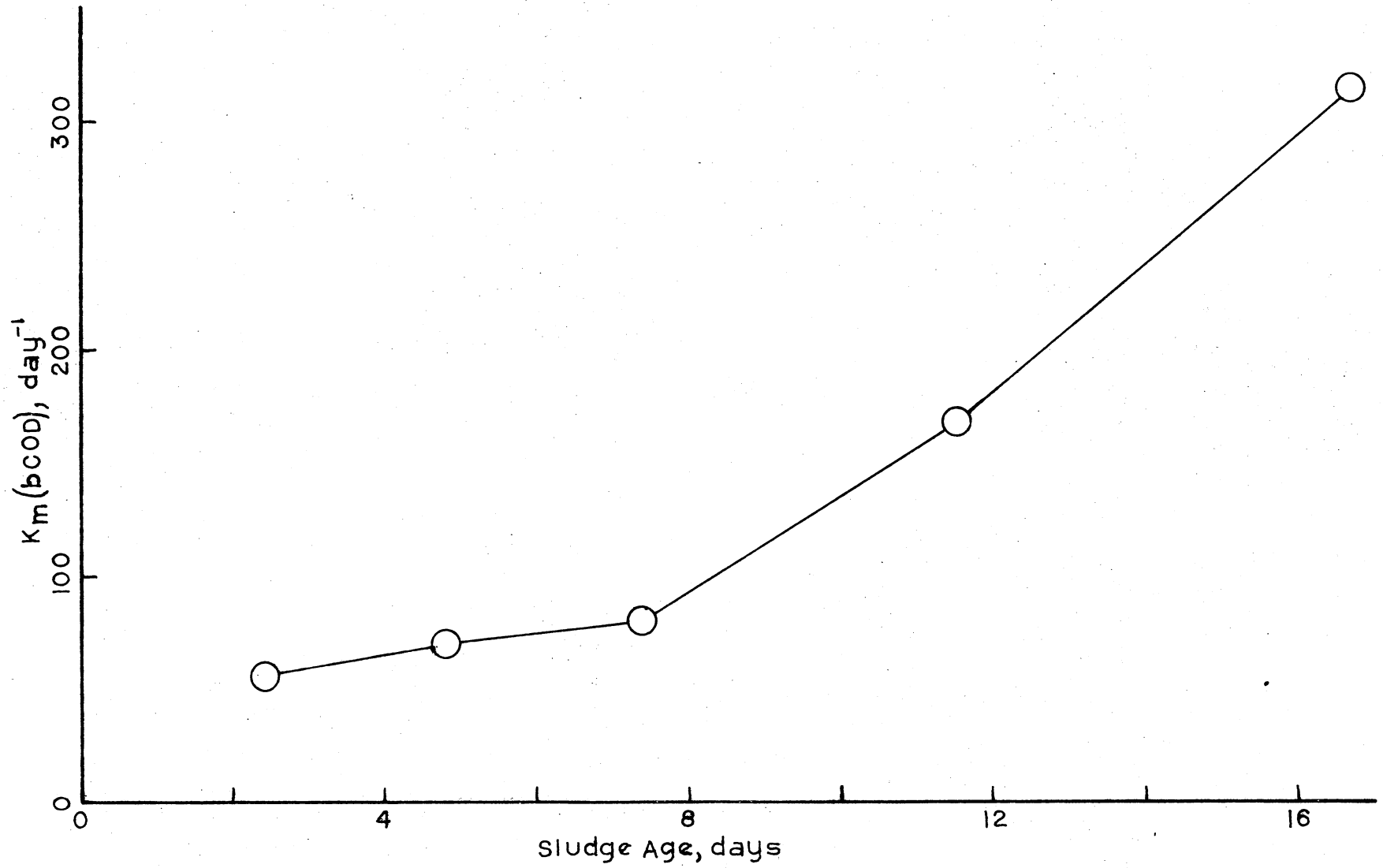

Mean k_e = 0.056
bCOD
COD
k_e, day^{-1}(mg/l)$^{-1}$
0.06
0.05
0.015
0.010
0.005
0
0
2
4
6
8
10
12
14
16
Sludge Age, days

Figure 6. Substrate Removal Constant (K_m, COD) Versus Sludge Age


K_m(COD), day^{-1}
0
5
10
15
20
25
0
4
8
12
16
Sludge Age, days

Figure 7. Substrate Removal Constant (K_m, bCOD) Versus Sludge Age


K_m(bCOD), day^{-1}
0
100
200
300
0
4
8
12
16
Sludge Age, days

The values of K_m in Figure 6 were calculated using the effluent COD concentration for S_e in equation 26, while the values in Figure 7 were calculated using the effluent bCOD for S_e in equation 26.

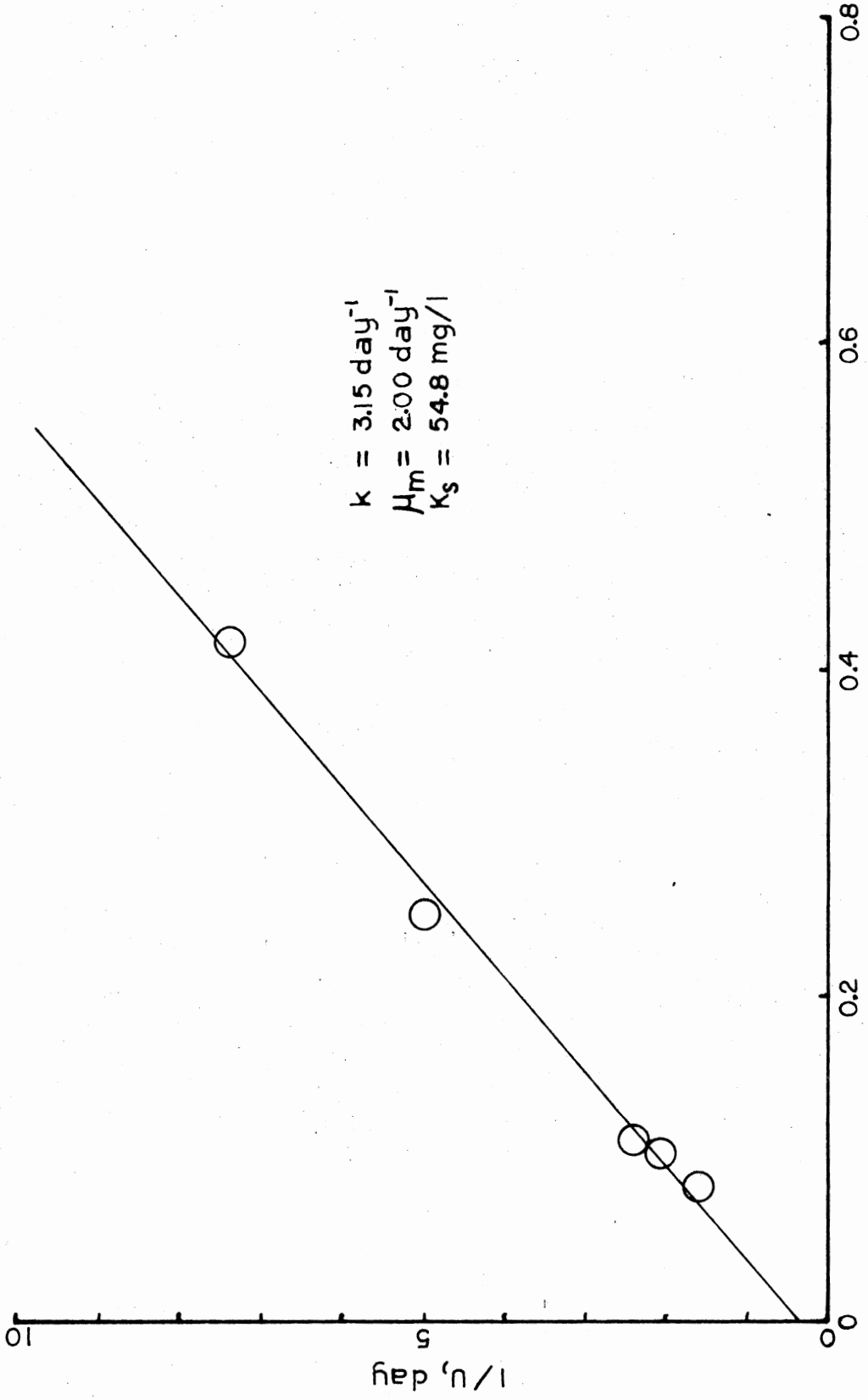
The reciprocal of the specific utilization rate is presented versus the reciprocal of the effluent bCOD in Figure 8. This figure presents a linear form of Lawrence and McCarty's kinetic equation relating U to S_e. This plot can be used to determine k, K_s, and μ_m (where μ_m is related to k via equation 12). The constants k and K_s are employed in Lawrence and McCarty's design model. The constants μ_m and K_s are employed in Gaudy's design model. The line of best fit in Figure 8 was derived via linear regression. The maximum specific substrate utilization rate (k) and the substrate saturation constant (K_s) were found to be 3.15 day^{-1} and 54.8 mg/l respectively. The maximum specific growth rate (μ_m) was found by multiplying k by the value of the true cell yield (Y_t) derived in Figure 3. The value of the maximum specific growth rate is 2.00 day^{-1} or 0.0833 $hour^{-1}$.

B. Batch Reactor Data

1. Substrate Removal Experiments

The experiments to be reported in this section consisted of measuring the soluble COD remaining at various times during the course of substrate removal in batch reactors. These experiments were performed with sludge drawn from the continuous flow reactors at each of the five sludge ages.

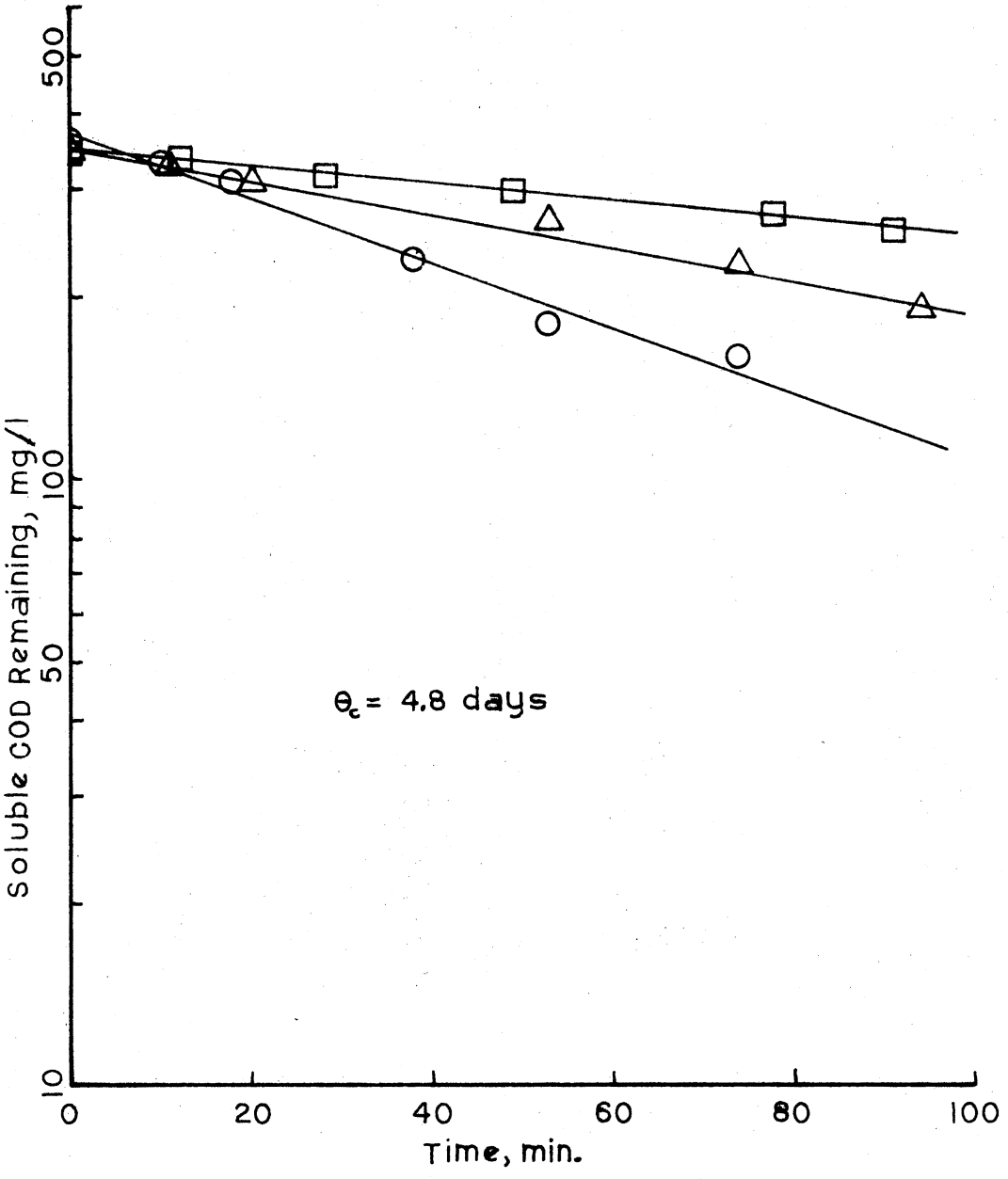
Figure 8. Inverse of Specific Substrate Utilization Rate Versus Inverse of Soluble Effluent Substrate Concentration


k = 3.15 day⁻¹
μm = 2.00 day⁻¹
Ks = 54.8 mg/l
1/U, day
0
5
10
0
0.2
0.4
0.6
0.8
1/Se, (mg/l as bCOD)⁻¹

The S/X ratios were sufficiently small so that the quantity of new cells produced from the COD removed was a fractional portion of the total micro-organism population in the batch reactors at any time. For both the above reason and because the cell yield under these conditions was unknown and unmeasurable, the specific substrate removal constant (k_e) was calculated using the initial sludge concentration. Later in this section of this chapter, batch yields found under higher S/X ratios were used to estimate specific growth rates.

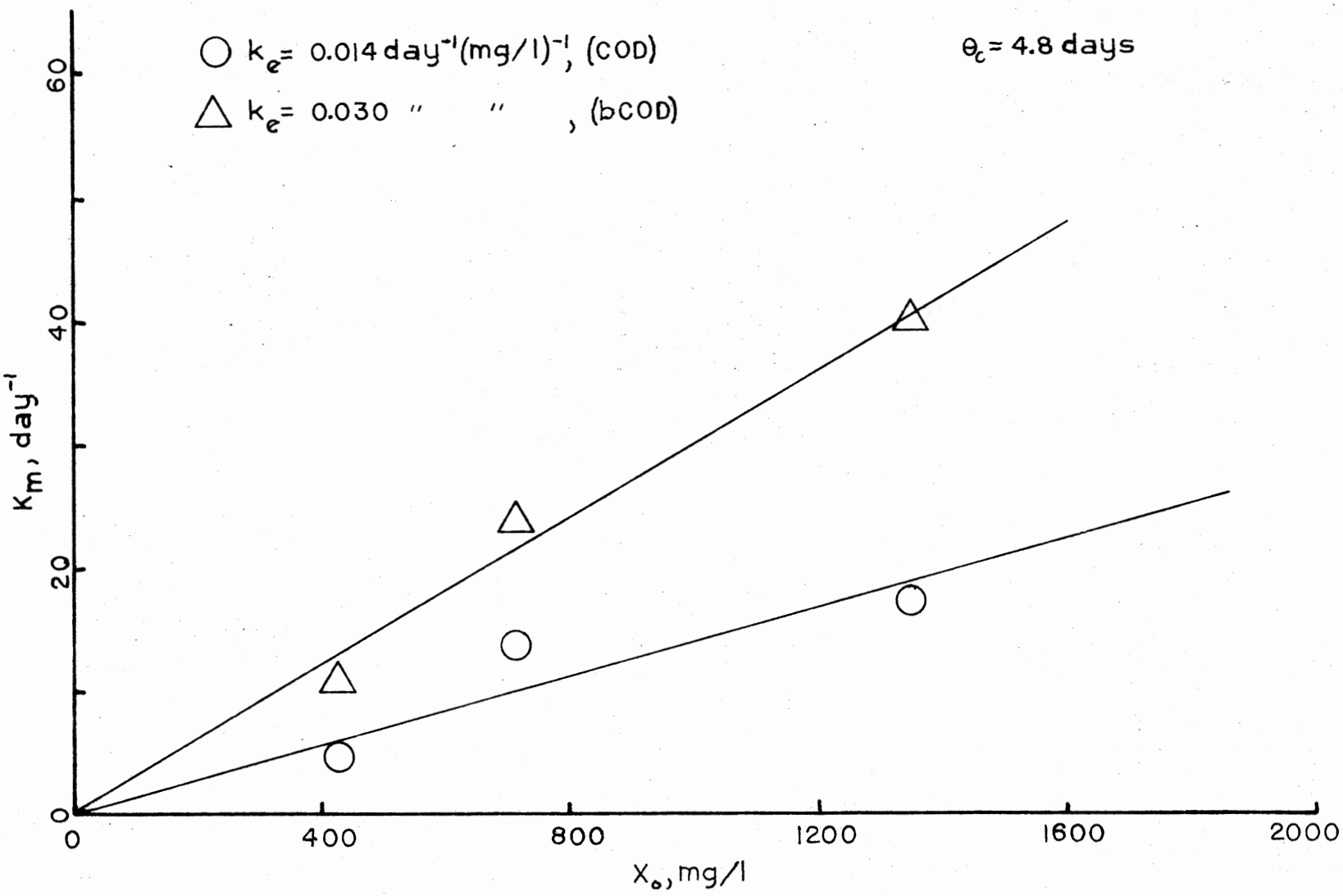
A typical set of substrate removal curves (θ_c=4.8 days) is shown in Figure 9. As the Eckenfelder and McKinney models assume first order, decreasing rate kinetics; a semi-log plot was used to find K_m and k_e. The relevant equation is equation 7. In addition to the removal rate constants calculated using the observed COD values; a second set of removal rate constants were derived, based on bCOD. This was done because the continuous flow values of K_m and k_e reported in the previous section of this chapter varied, depending on whether COD or bCOD values of effluent substrate concentration were used to calculate removal rates. The bCOD values for K_m and k_e were derived by finding the time to reach 28.4 mg/l COD remaining (where the minimum achievable effluent COD is 27.4), assuming that 1.0 mg/l bCOD remained at this point, and applying equation 7 to find the new value of K_m. The values for K_m are presented versus initial sludge concentration in Figure 10. These values were derived from the previous figure. The upper line is based on COD removal and the slope

Figure 9. Batch Substrate Removal Experiment



	X_o (mg/l)	K_m (day^{-1})	
		COD	bCOD
□	432	4.84	11.2
△	716	10.4	24.0
○	1348	17.3	40.0

Figure 10. Substrate Removal Constant (K_m) Versus Initial Sludge Concentration (From Figure 9)


○ k_e= 0.014 day^{-1}(mg/l)$^{-1}$, (COD)
△ k_e= 0.030 " " , (bCOD)
θ_c= 4.8 days
K_m, day^{-1}
0
20
40
60
0
400
800
1200
1600
2000
X_o, mg/l

of this line is k_e (batch, COD). The lower line is based on bCOD removal and the slope of this line is k_e (batch, bCOD). The remainder of the COD removal data (for other θ_c's) will be found in Table IX.

The values of k_e (batch) for all sludge ages are presented in Figure 11. The uppermost line represents the values of batch k_e calculated on the basis of bCOD as explained in the previous paragraph. While there is some fluctuation in these values of k_e, there seems to be no systematic variation of k_e with varying θ_c; i.e., k_e is a constant. The middle line represents the values of batch k_e found using COD. Again, there seems to be no systematic variation of these values of k_e with varying θ_c. The lowest line represents the batch k_e values calculated on the basis of bCOD plus COD_{min} (see Figure 12). These values of k_e represent the slope of a line from the origin to the relevant point on line B. These values do vary systematically with θ_c; i.e., k_e decreases with increasing θ_c. The value of S_e (bCOD plus COD_{min}) in this instance is an artificial value for effluent COD, which produces a relationship between U and S_e qualitatively similar to that in Figure 4. The predicted values for effluent COD, in this case, approach COD_{min} (as do the observed continuous flow, effluent COD's) rather than zero (as do the effluent COD's predicted without consideration of COD_{min}).

The values of S_e predicted by the k_e values presented in Figure 11 are presented versus specific substrate utilization

Figure 11. Batch Specific Substrate Removal Constant (k_e) Versus Sludge Age

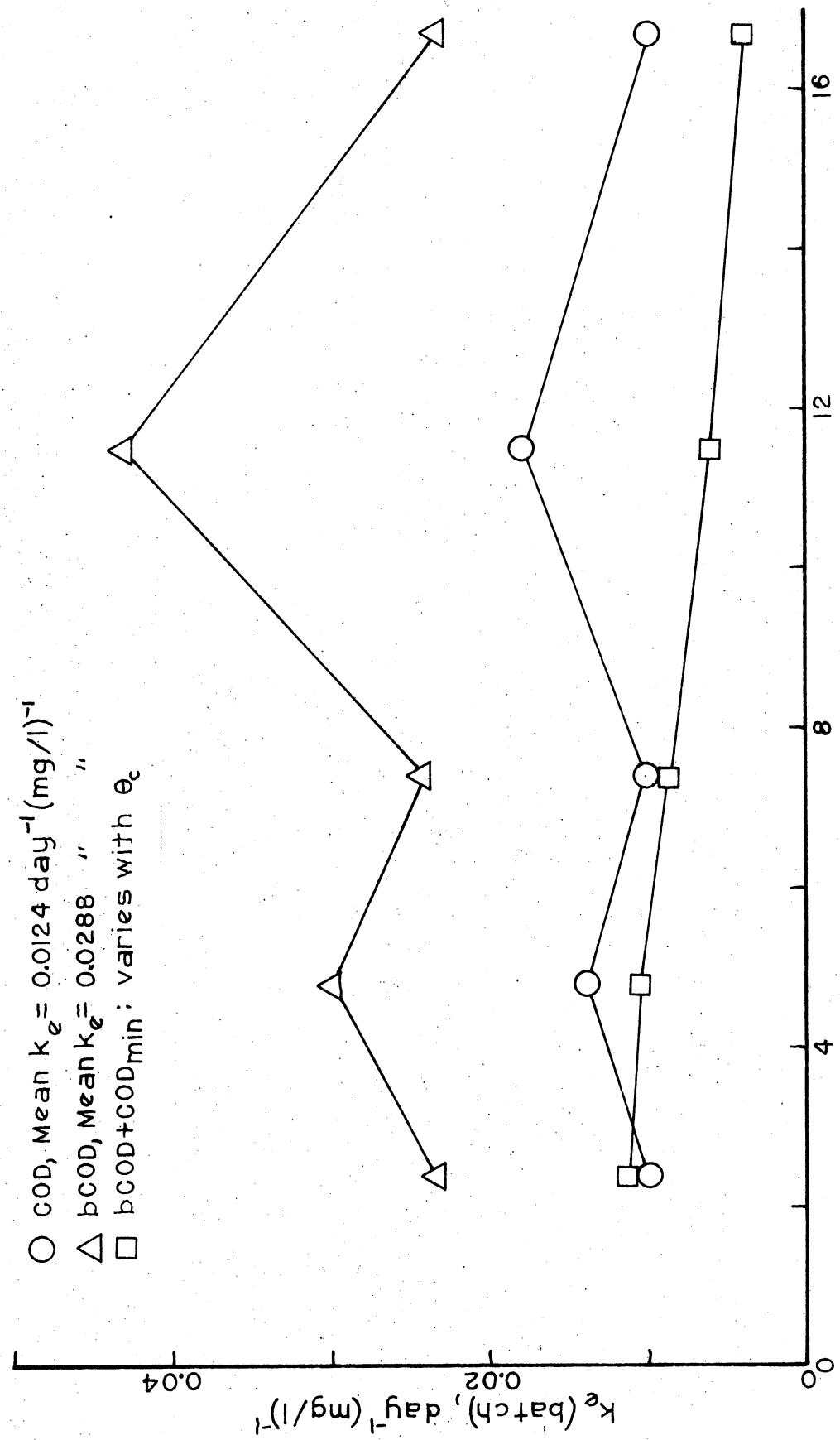
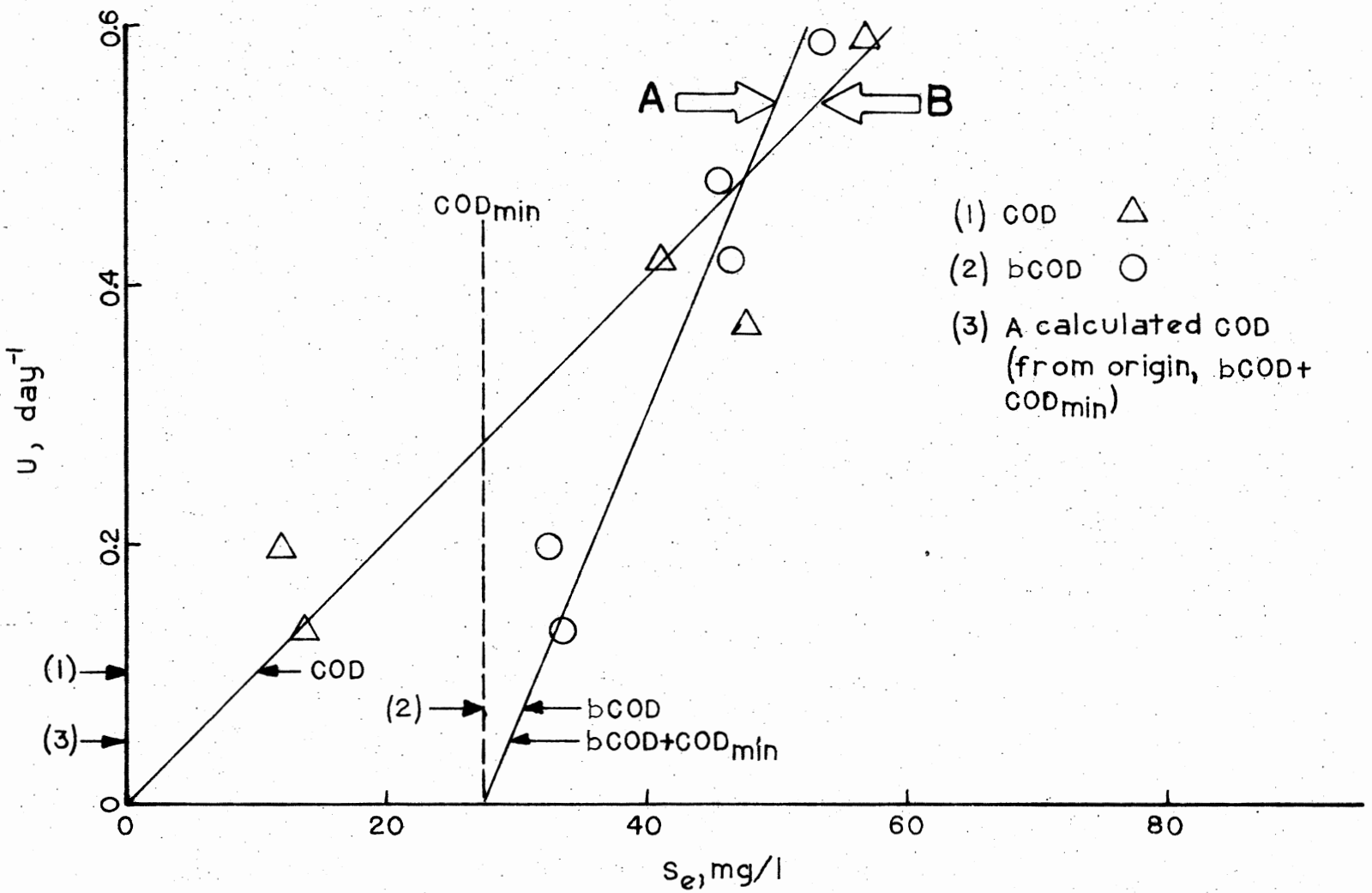

○ COD, Mean k_e = 0.0124 day^{-1} (mg/l)$^{-1}$
△ bCOD, Mean k_e = 0.0288 " "
□ bCOD+COD$_{min}$: varies with θ_c
k_e (batch), day^{-1} (mg/l)$^{-1}$
0
0.02
0.04
4
8
12
16
Sludge Age, days

Figure 12. Specific Substrate Utilization Rate Versus Soluble Effluent Substrate Concentration


0.6
0.4
0.2
0
U, day⁻¹
A
B
CODmin
(1) COD
(2) bCOD
(3) A calculated COD (from origin, bCOD+ CODmin)
(1)
(3)
COD
(2)
bCOD
bCOD+CODmin
0
20
40
60
80
Se, mg/l

rate (U) in Figure 12. The slope of the line through the origin (line A) is approximately equal to the mean batch k_e calculated using observed COD removal. The slope of the line which intersects the x-axis at 27.4 (COD_{min}) is equal to the mean value of the batch k_e calculated using bCOD removal. The values for the third type of batch k_e would be equal to the slopes of lines from the origin to the points along line B.

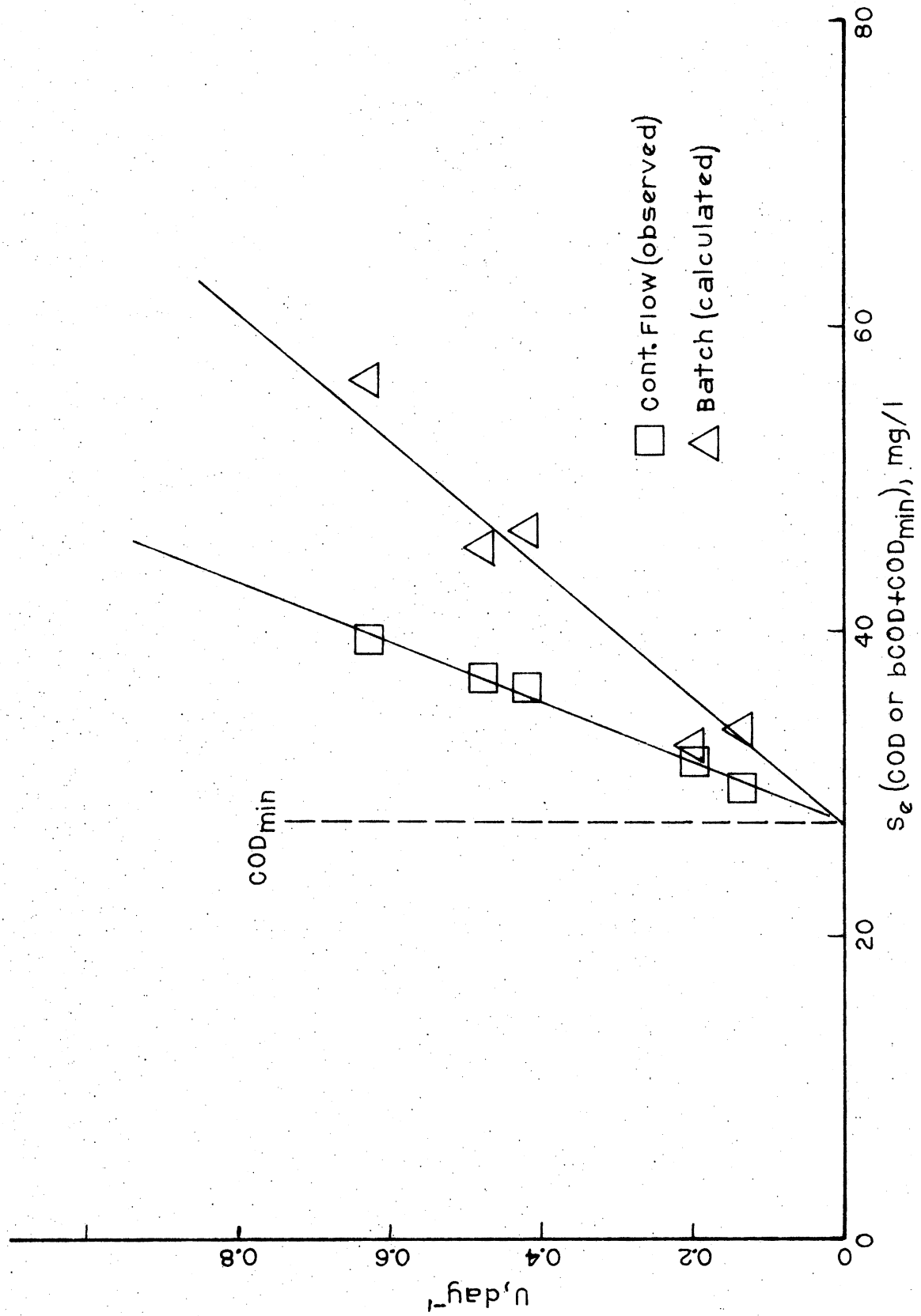
In Figure 13, the three types of batch k_e are presented versus the appropriate continuous flow k_e. The ratio of k_e (continuous flow, COD) to k_e (batch, bCOD+COD_{min}) is approximately 1.3. Since k_e (batch, COD) is essentially a constant, while k_e (continuous flow, COD) varies with U or θ_c; there is no relationship between k_e (batch, COD) and k_e (continuous flow, COD). The ratio of k_e (continuous flow, bCOD) to k_e (batch, bCOD) is approximately 1.9. This relationship is presented in the right side of Figure 13. Both of these values of k_e are essentially constant, resulting in a cluster of points.

In Figure 14, specific substrate utilization rate is presented versus the effluent substrate concentration for both the continuous flow reactor and the batch reactor (k_e, batch and continuous flow, are bCOD values). Since the two k_e values differ by a factor of approximately 1.9 (as mentioned in the above paragraph), the S_e values (bCOD) at each U vary by approximately the same factor. The batch values of k_e (bCOD) predict an effluent S_e at each U approximately

Figure 13. Continuous Flow Specific Substrate Removal Rate Constant Versus Batch Specific Substrate Removal Rate Constant

k_e(cont. flow)
0.005
0.010
0.015
0
0.01
0.02
k_e(batch)
k_e(cont. flow)
0.02
0.04
0.06
0
0.02
0.04
k_e(batch)
bCOD+COD$_{min}$
COD
bCOD

Figure 14. Specific Substrate Utilization Rate Versus Soluble Effluent Substrate Concentration


μ, day⁻¹
0
0.2
0.4
0.6
0.8
0
20
40
60
80
Se (COD or bCOD+CODmin), mg/l
CODmin
Cont. Flow (observed)
Batch (calculated)

double the bCOD values predicted by the continuous flow values of k_e (bCOD).

The values plotted in the above figure are replotted in Lineweaver-Burke form in Figure 15 (except that bCOD is plotted rather than bCOD+COD_{min}). The maximum specific growth rate (maximum specific utilization rate) and substrate saturation constant for these batch experiments are 0.885 day^{-1} (1.40 day^{-1}) and 41.6 mg/l respectively. The continuous flow values from Figure 8 are included in this figure for comparison.

The observed and predicted values of effluent substrate concentration (from the previous figure) at each sludge age are presented in Figure 16. The S_e (bCOD) predicted from the batch μ_m and K_s are again approximately double those predicted by the continuous flow μ_m and K_s.

The observed specific growth rates were calculated for the first thirty minutes of growth and substrate removal for all substrate removal curves. Equation 26 was used to calculate the specific growth rates. The means of the calculated

$$\mu = \frac{\ln\left[\frac{Y_B(S_o-S_t)}{X_o} + 1\right]}{t} \qquad (26)$$

where,

$$\mu = \frac{\ln X_t - \ln X_o}{t} \qquad (27)$$

and,

$$X_t = X_o + Y_B(S_o-S_t) \qquad (28)$$

Figure 15. Inverse of Specific Substrate Utilization Rate Versus Inverse of Soluble Effluent Substrate Concentration

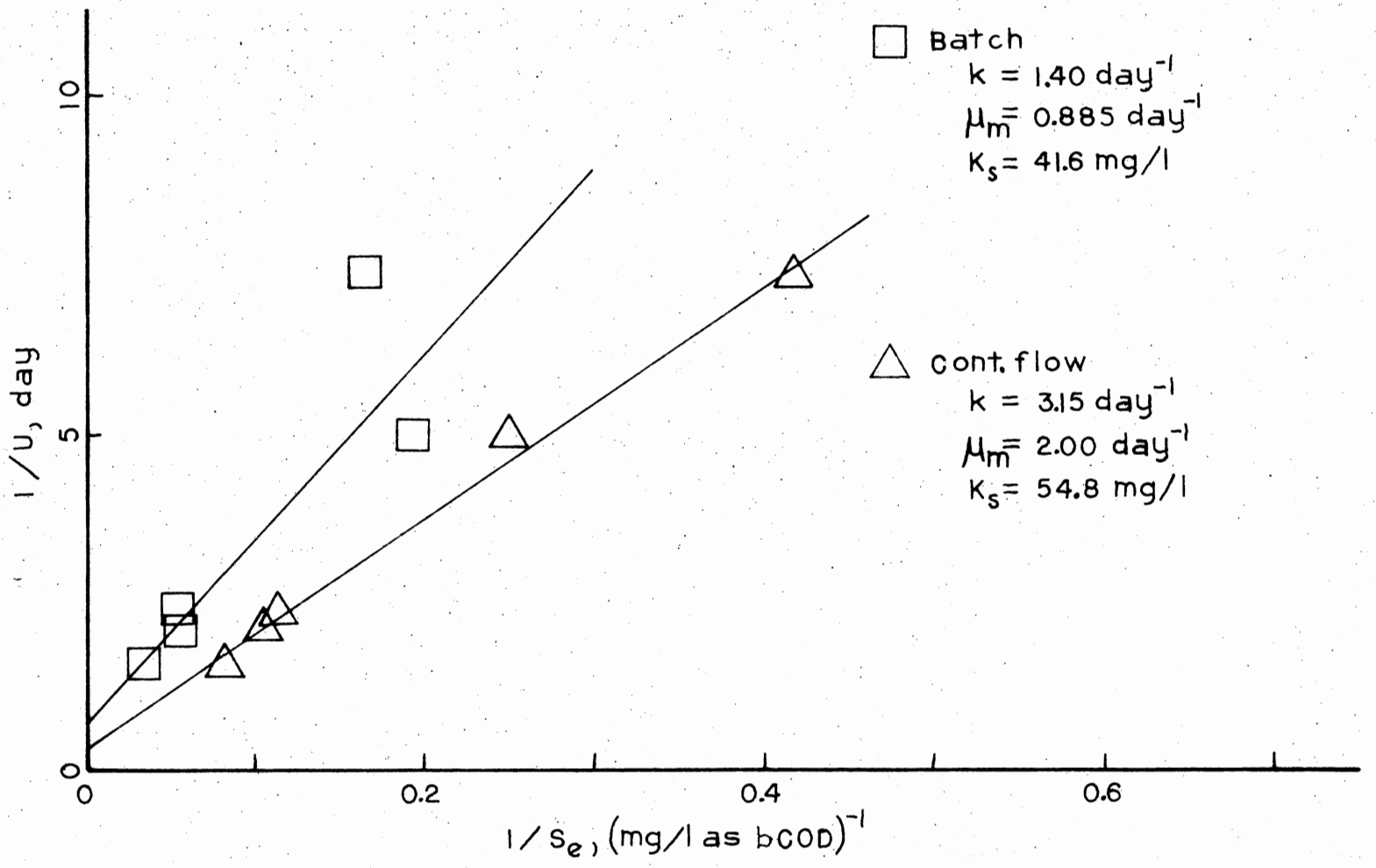
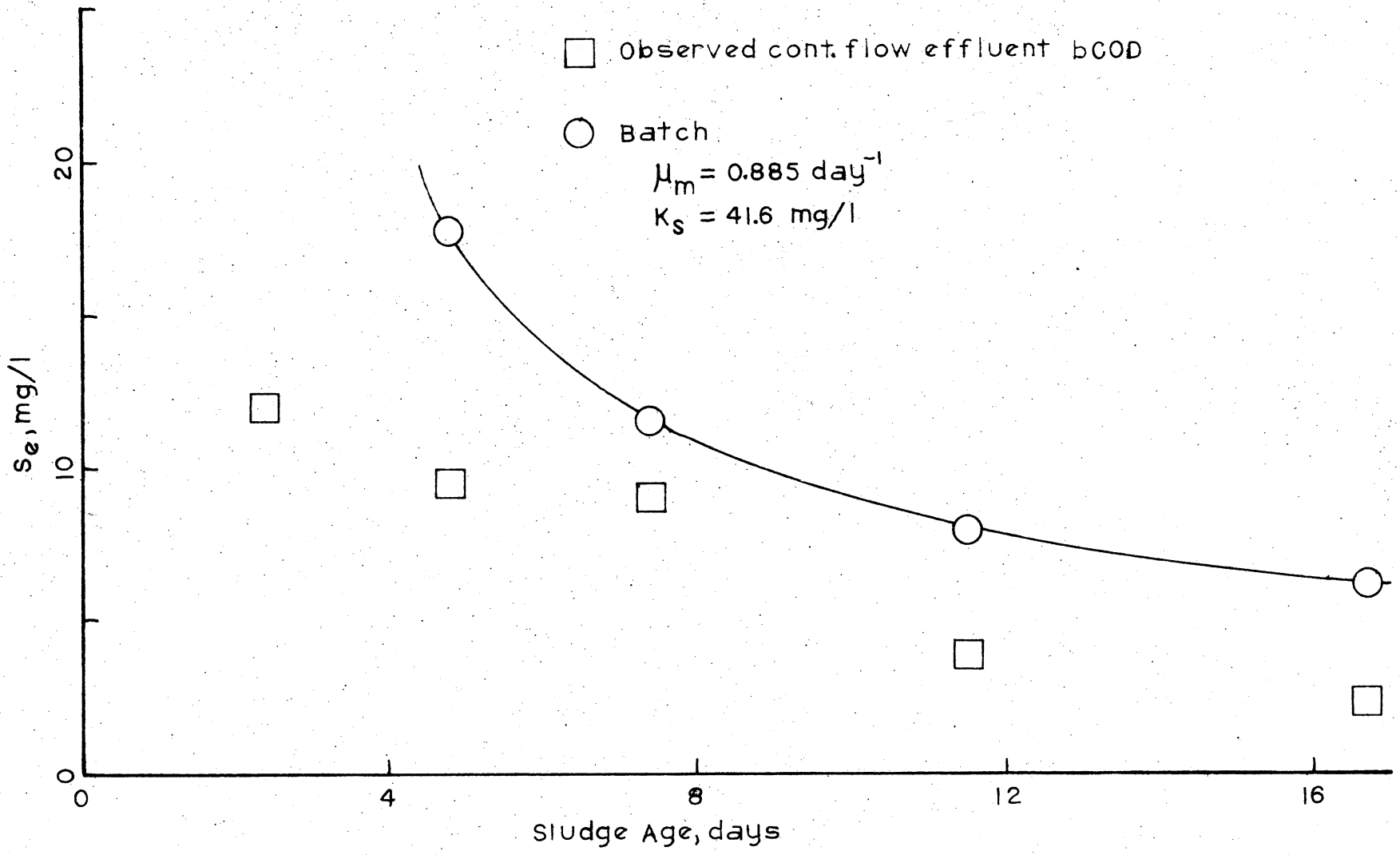

Batch
k = 1.40 day⁻¹
μm = 0.885 day⁻¹
Ks = 41.6 mg/l
cont. flow
k = 3.15 day⁻¹
μm = 2.00 day⁻¹
Ks = 54.8 mg/l
1/U, day
0
5
10
0
0.2
0.4
0.6
1/Se, (mg/l as bCOD)⁻¹

Figure 16. Effluent Substrate Concentration Versus Sludge Age


Observed cont. flow effluent bCOD
Batch
$\mu_m = 0.885\ day^{-1}$
$K_s = 41.6\ mg/l$
S_e, mg/l
0
10
20
0
4
8
12
16
Sludge Age, days

specific growth rates ($\Delta t < 30$ minutes) at each continuous flow, net specific growth rate are presented in Figure 17. The overall mean specific growth rate for all substrate removal experiments is 0.067 $hour^{-1}$ or 1.62 day^{-1}. Since S_o is approximately 350 mg/l COD, these values of μ may reasonably be interpreted as approximations of μ_m.

The substrate removal curves at a sludge age of 11.5 days extended to a sufficiently low level of remaining COD to allow recovery of the maximum specific growth rate and substrate saturation constant. The specific growth rate was calculated for successive COD sampling intervals via equation 26 and plotted versus the median soluble COD remaining during the relevant sampling interval (the mean of S_o and S_t) in Figure 18. The maximum specific growth rate and saturation constant, for all four substrate removal curves, are 0.0671 $hour^{-1}$ (1.61 day^{-1}) and 89 mg/l, respectively. This value of μ_m is, coincidentally, equal to the mean specific growth rate for all substrate removal curves at all θ_c's (see previous paragraph). The effluent substrate predicted by these values is compared with the observed continuous flow values of effluent bCOD in Figure 19.

2. Specific Growth Rate Experiments

Using seed drawn directly from the continuous flow reactors, small scale growth experiments were conducted. These experiments were conducted using 200 to 250 mililiter erhlenmeyer flasks, shaken at 130 cycles/minute. The biomass concentration was monitored via Absorbance at a wavelength of

Figure 17. Mean Initial Specific Growth Rate Calculated from Substrate Removal Experiments Versus Continuous Flow Net Specific Growth Rate

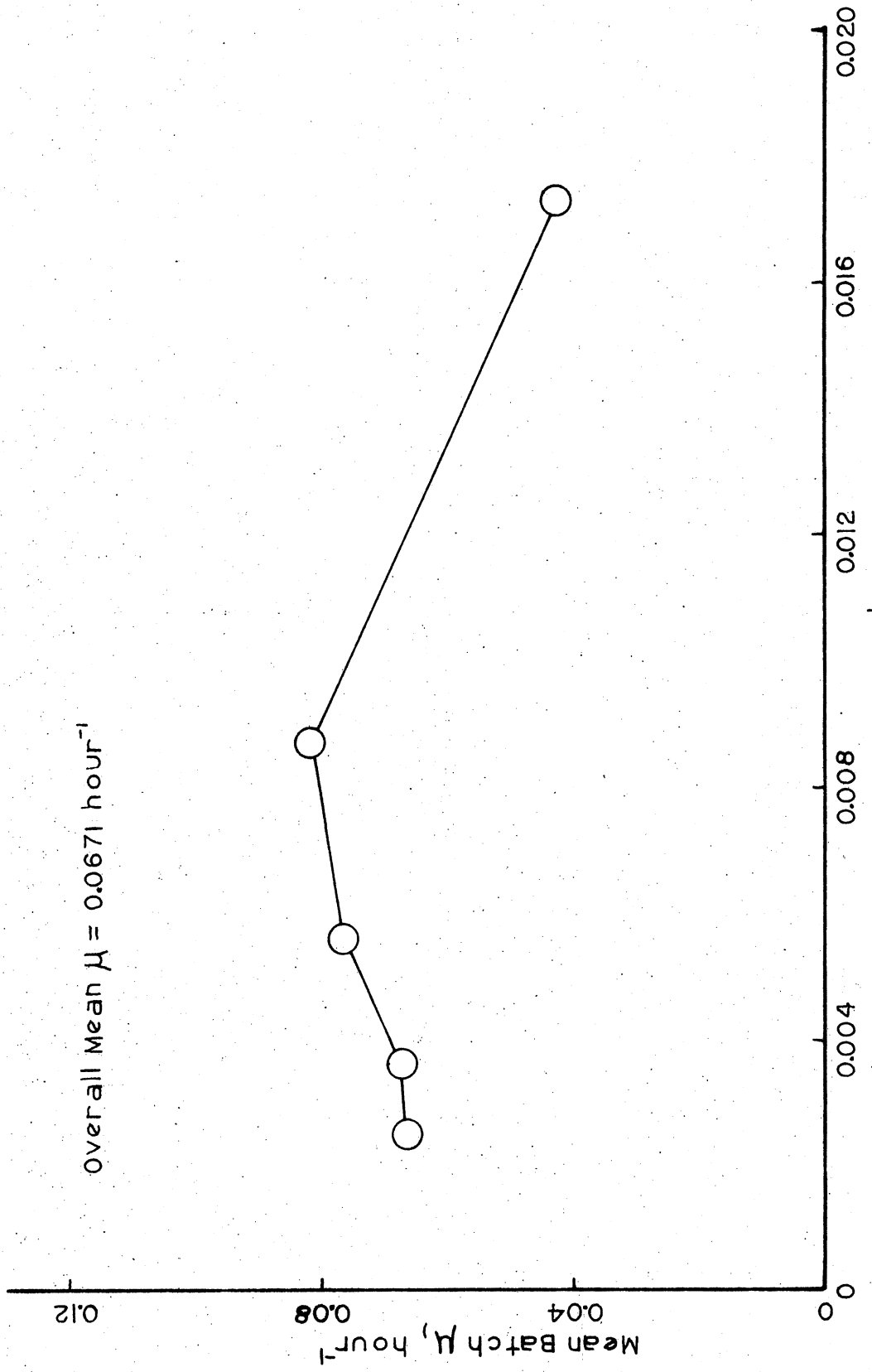

Mean Batch μ, hour⁻¹
0
0.04
0.08
0.12
Overall Mean μ = 0.0671 hour⁻¹
0
0.004
0.008
0.012
0.016
0.020
μn, hour⁻¹

Figure 18. Inverse of Specific Growth Rate During Sampling Interval Versus Inverse of Median Soluble COD Remaining in Sampling Interval

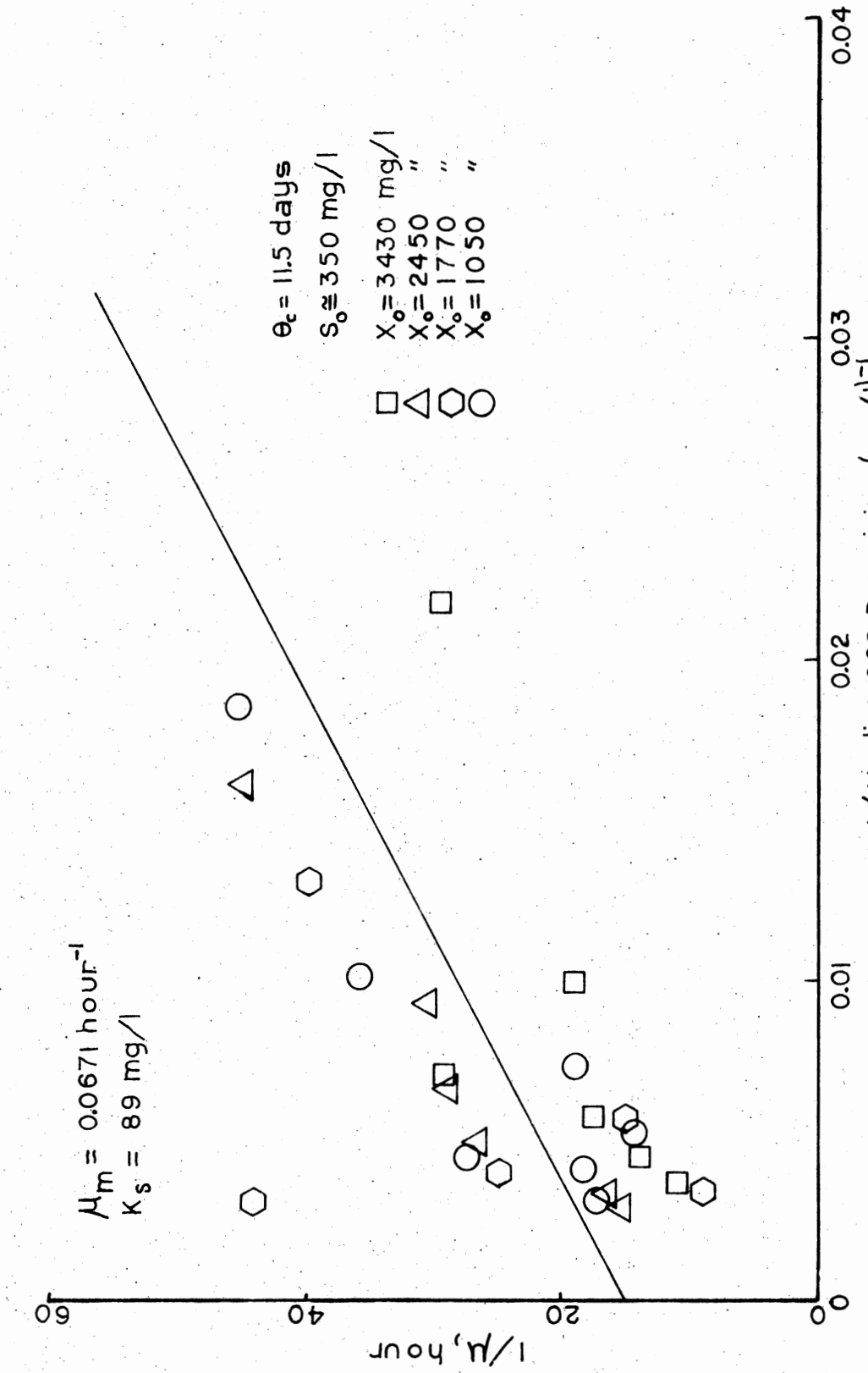
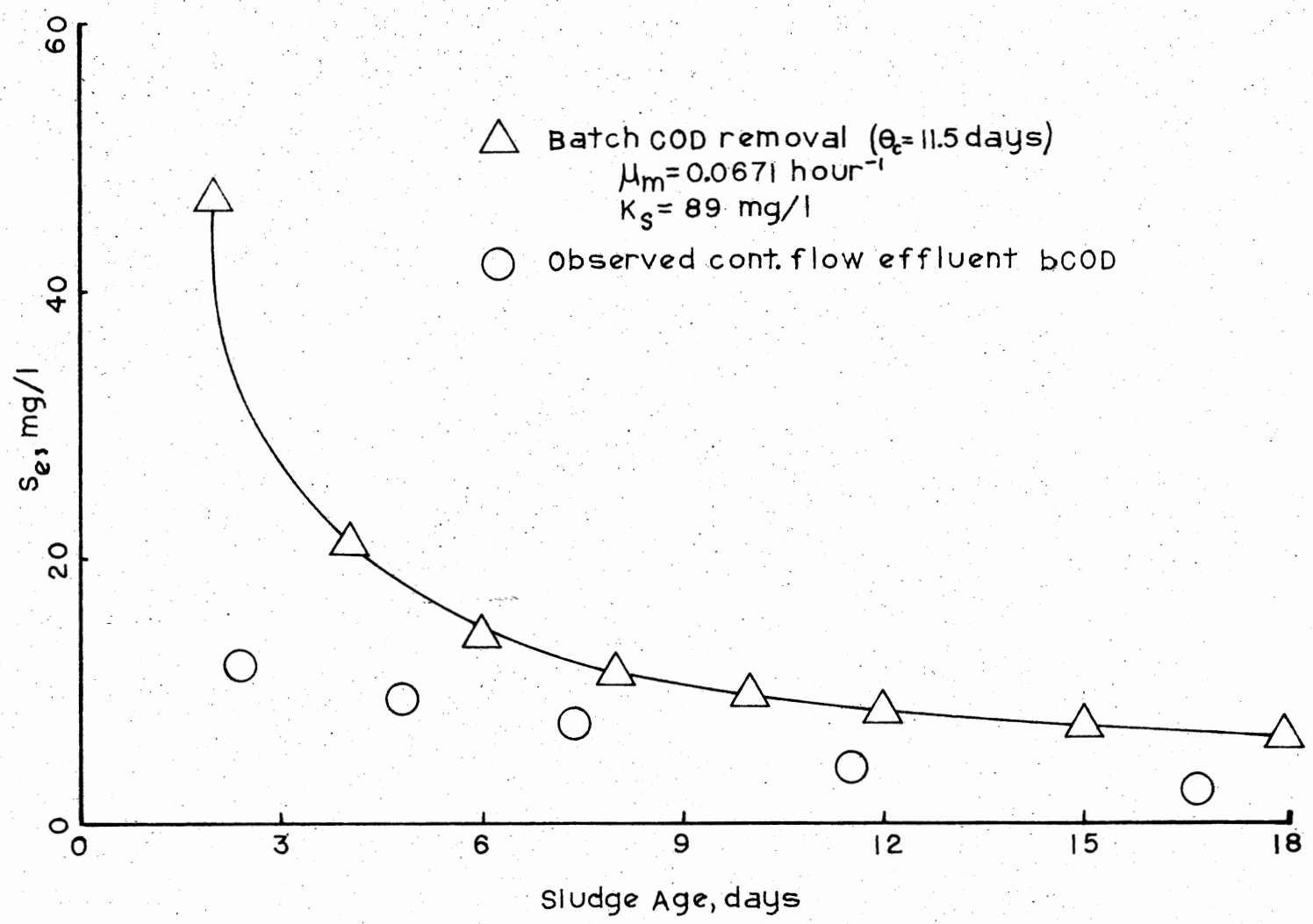

μ_m = 0.0671 hour^{-1}
K_s = 89 mg/l
θ_c = 11.5 days
$S_o \cong$ 350 mg/l
X_o = 3430 mg/l
X_o = 2450 "
X_o = 1770 "
X_o = 1050 "
1/μ, hour
1/ Median COD Remaining, (mg/l)$^{-1}$
0
20
40
60
0.01
0.02
0.03
0.04

Figure 19. Soluble Effluent Substrate Concentration Versus Sludge Age (From Figure 18)


Batch COD removal (θ_c = 11.5 days)
μ_m = 0.0671 hour^{-1}
K_s = 89 mg/l
Observed cont. flow effluent bCOD
S_e, mg/l
0
20
40
60
0
3
6
9
12
15
18
Sludge Age, days

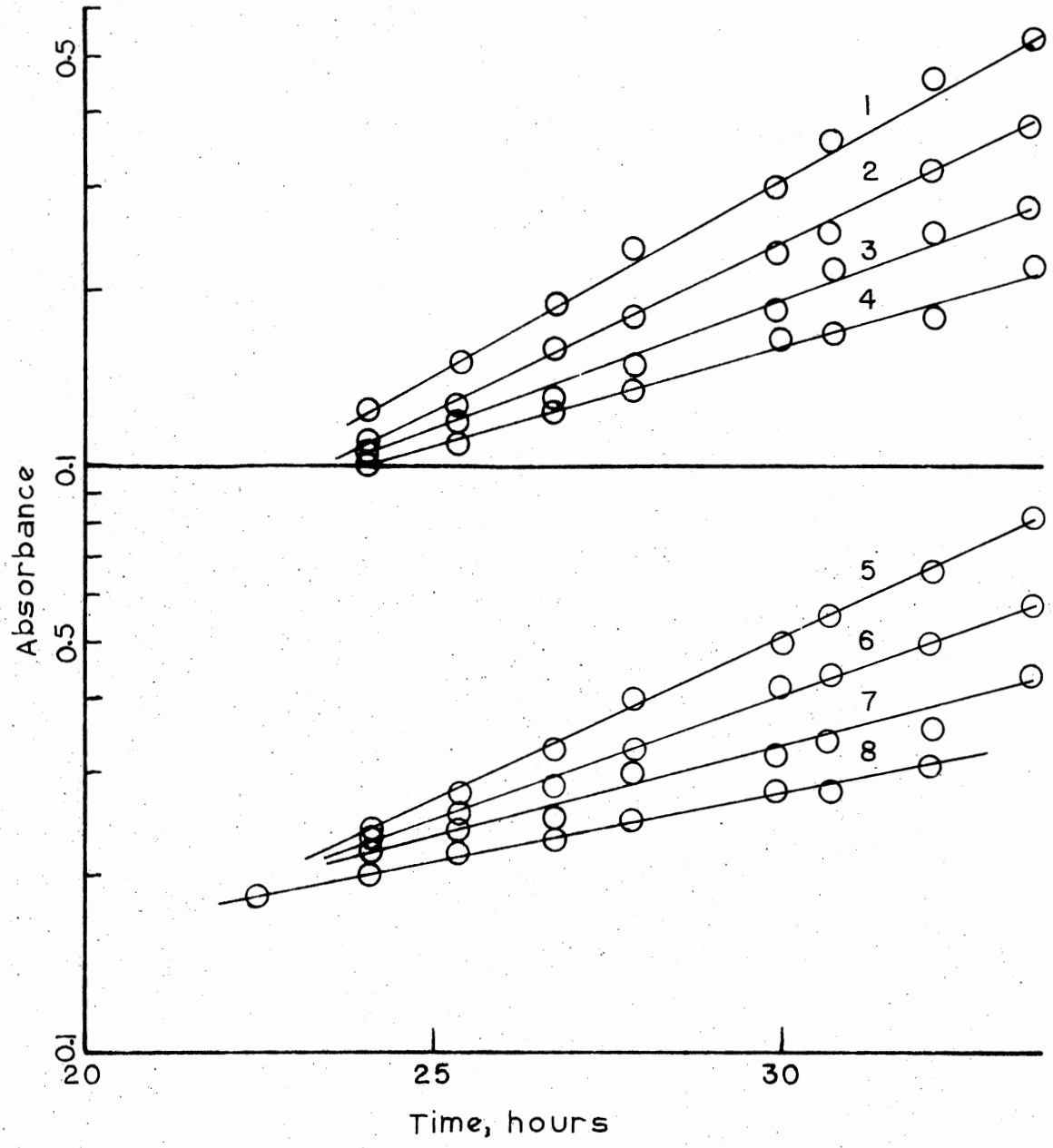
600 nanometers.

It was found that a rather long lag period, of variable length, occurred prior to logarithmic growth. The results of one of the many growth experiments is presented in Figure 20. The lag period, in this case, was approximately 24 hours. The seed for this experiment was drawn from a continuous flow reactor operating at a sludge age of 16.7 days. The results of the other experiments performed at other sludge ages are presented in tabular form in Table X in Appendix. Figure 21 is a Lineweaver-Burke plot of the data from Figure 20. It should be noted from this figure that the value of K_s seems to be dependent on the initial seed concentration (X_o). The values for observed K_s and X_o for all growth experiments (at the end of the lag period) are presented in Figure 22. Equation 29, derived via linear regression, is an empirical description of the observed relationship between K_s and X_o (both as mg/l). The observed values of μ_m

$$K_s = 6.79\ X_o + 365 \qquad (29)$$

for all experiments are presented versus X_o in Figure 23. The mean μ_m is 0.275 hour^{-1}. There was a systematic variation in μ_m with varying X_o. The values for predicted S_e (using μ_m=0.275 hour^{-1}, K_s=365 mg/l, k_d=0.056 day^{-1}) are presented in Figure 24, along with the observed effluent bCOD for the continuous flow reactors. Figure 25 presents both length of lag period (for those experiments where it could be determined) versus θ_c and X_o versus the Absorbance at the

Figure 20. Growth at End of "Lag Period" - Absorbance Versus Time



No.	X_o (mg/l)	S_o (mg/l)	μ (hour^{-1})
1	55	1980	0.153
2	"	990	0.128
3	"	594	0.102
4	"	396	0.078
5	110	1961	0.130
6	"	980	0.096
7	"	588	0.070
8	"	392	0.055

θ_c = 16.7 days

Figure 21. Inverse of Specific Growth Rate Versus Initial Substrate Concentration (COD) - From Figure 20

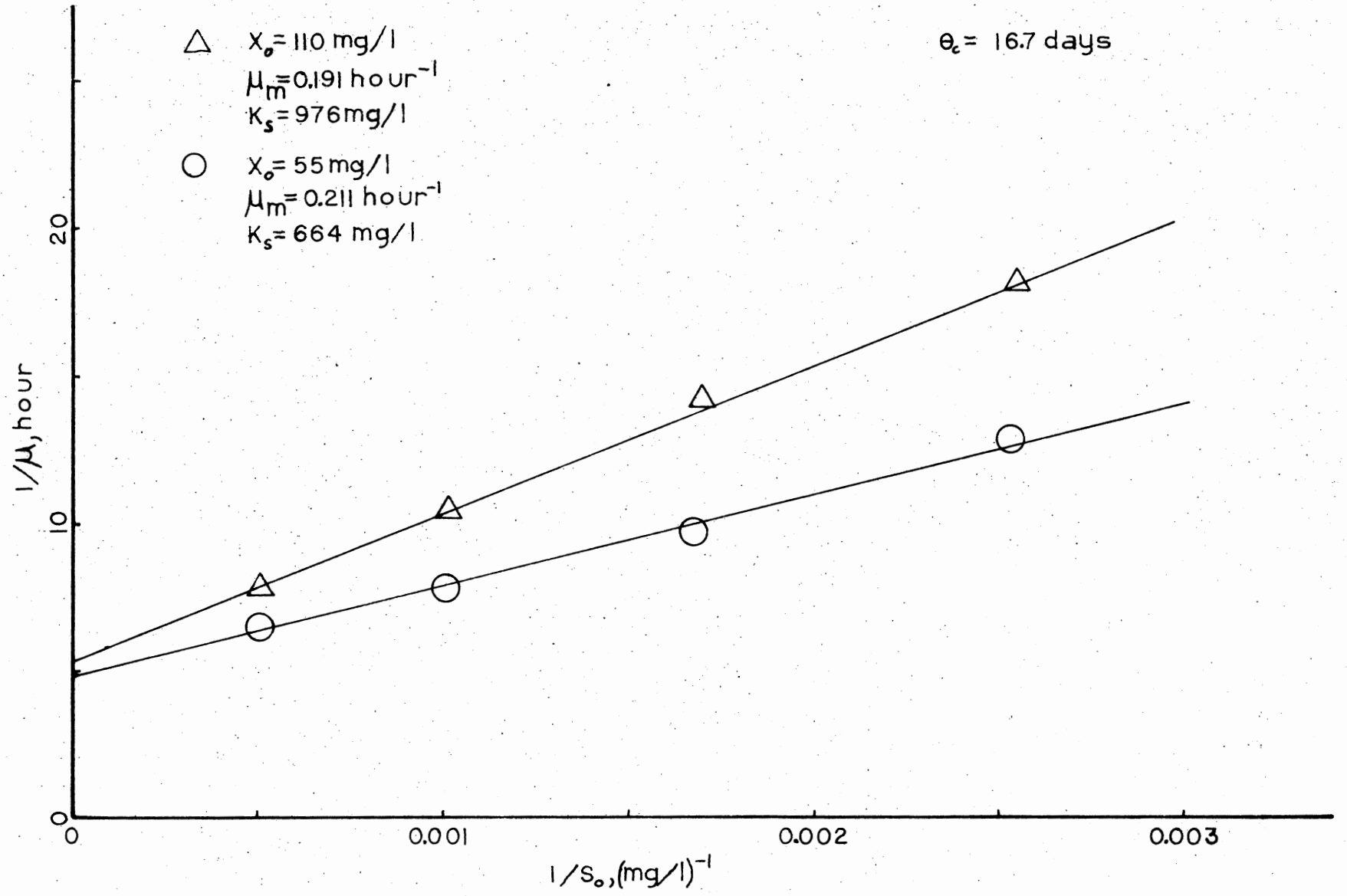

△ X_o= 110 mg/l
μ_m=0.191 hour^{-1}
K_s= 976mg/l
○ X_o= 55 mg/l
μ_m= 0.211 hour^{-1}
K_s= 664 mg/l
θ_c= 16.7 days
1/μ, hour
0
10
20
0
0.001
0.002
0.003
1/S_o, (mg/l)$^{-1}$

Figure 22. Substrate Saturation Constant Versus Initial Biomass Concentration

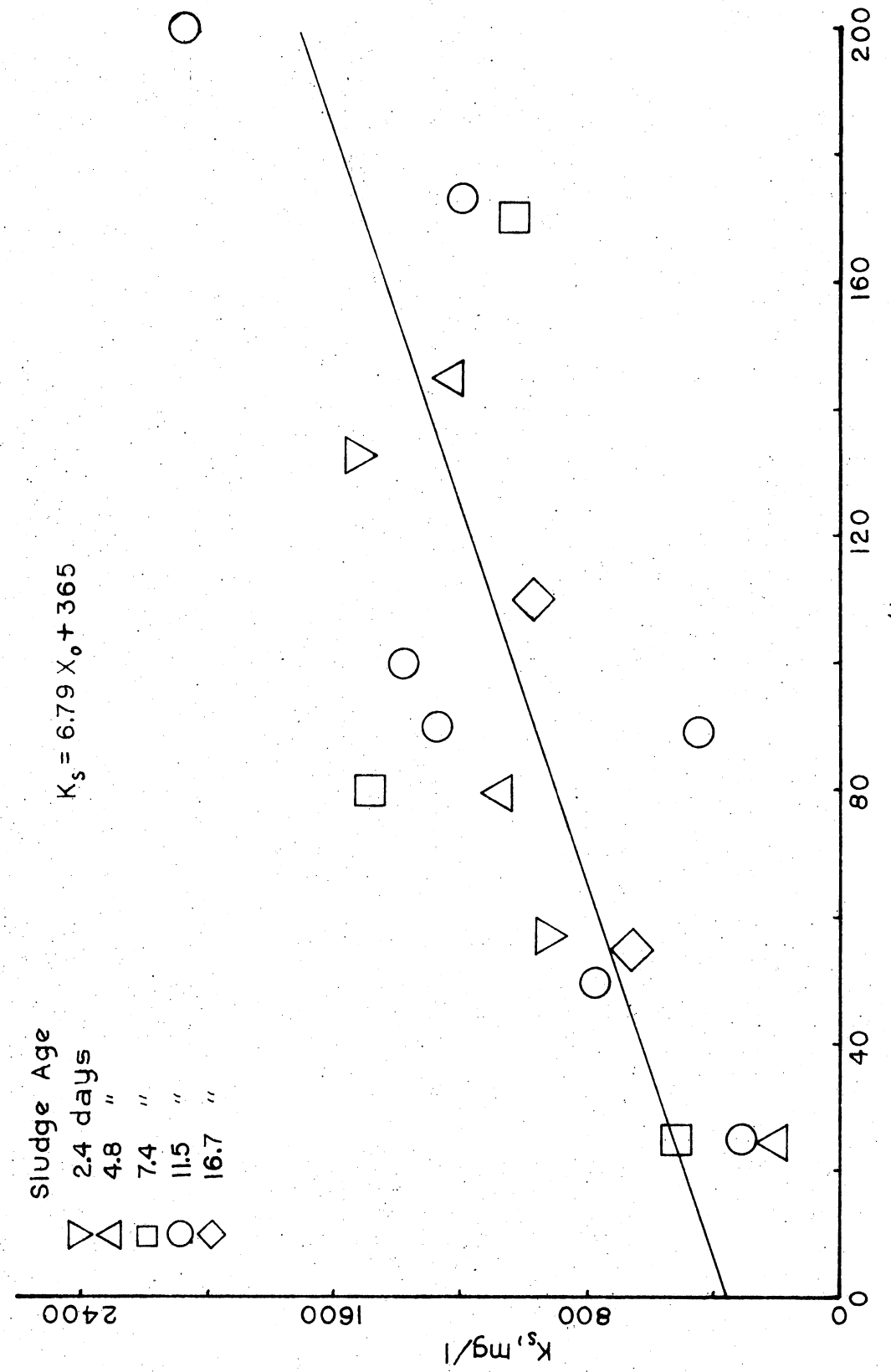

$K_s = 6.79\ X_o + 365$
Sludge Age
2.4 days
4.8 "
7.4 "
11.5 "
16.7 "
K_s, mg/l
0
800
1600
2400
X_o, mg/l
0
40
80
120
160
200

Figure 23. Maximum Specific Growth Rate Versus Initial Sludge Concentration

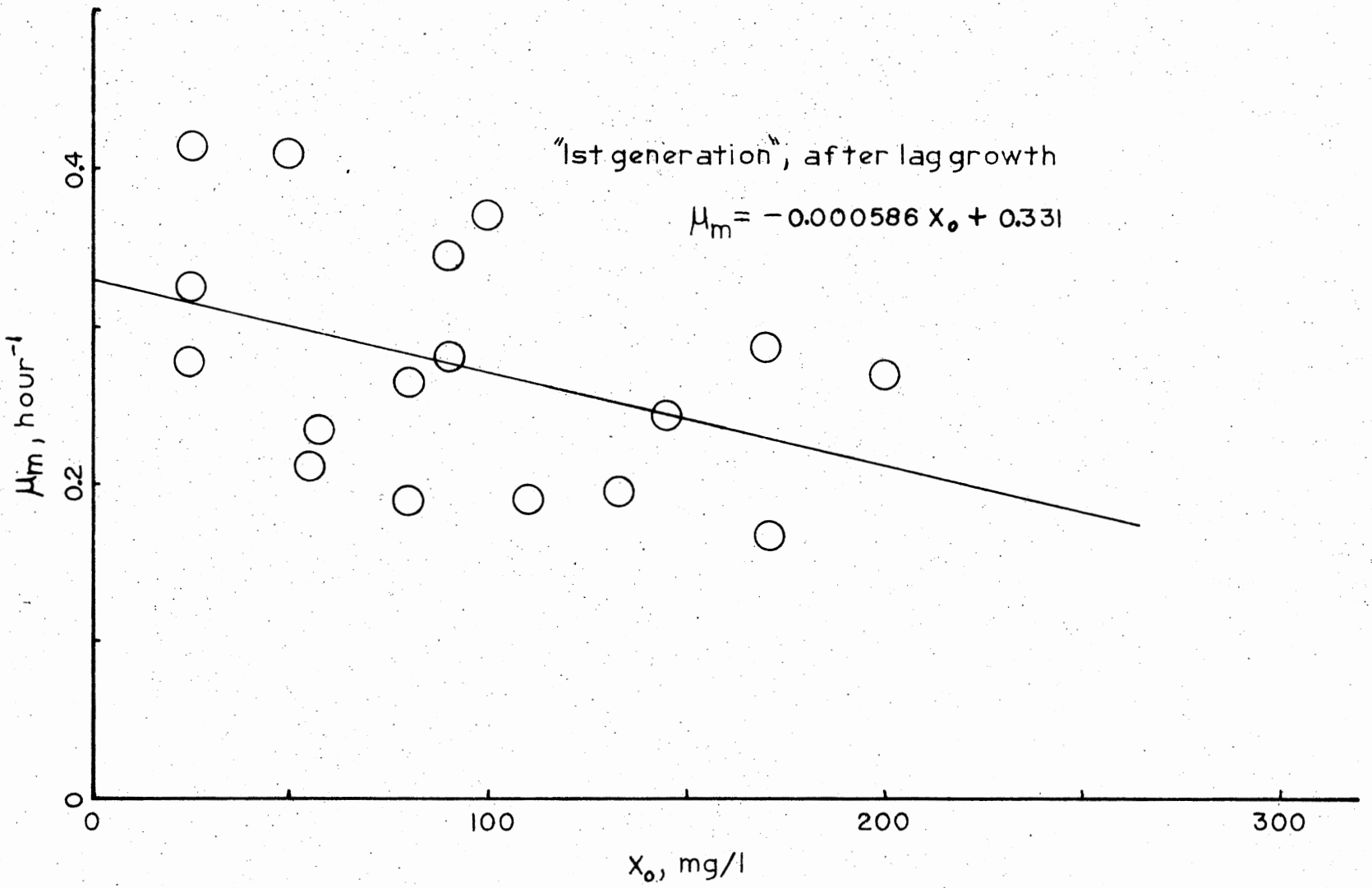

"1st generation", after lag growth
$\mu_m = -0.000586\, X_o + 0.331$
μ_m, hour^{-1}
0
0.2
0.4
0
100
200
300
X_o, mg/l

Figure 24. Effluent Soluble Substrate Concentration Versus Sludge Age (From Figures 22 and 23)

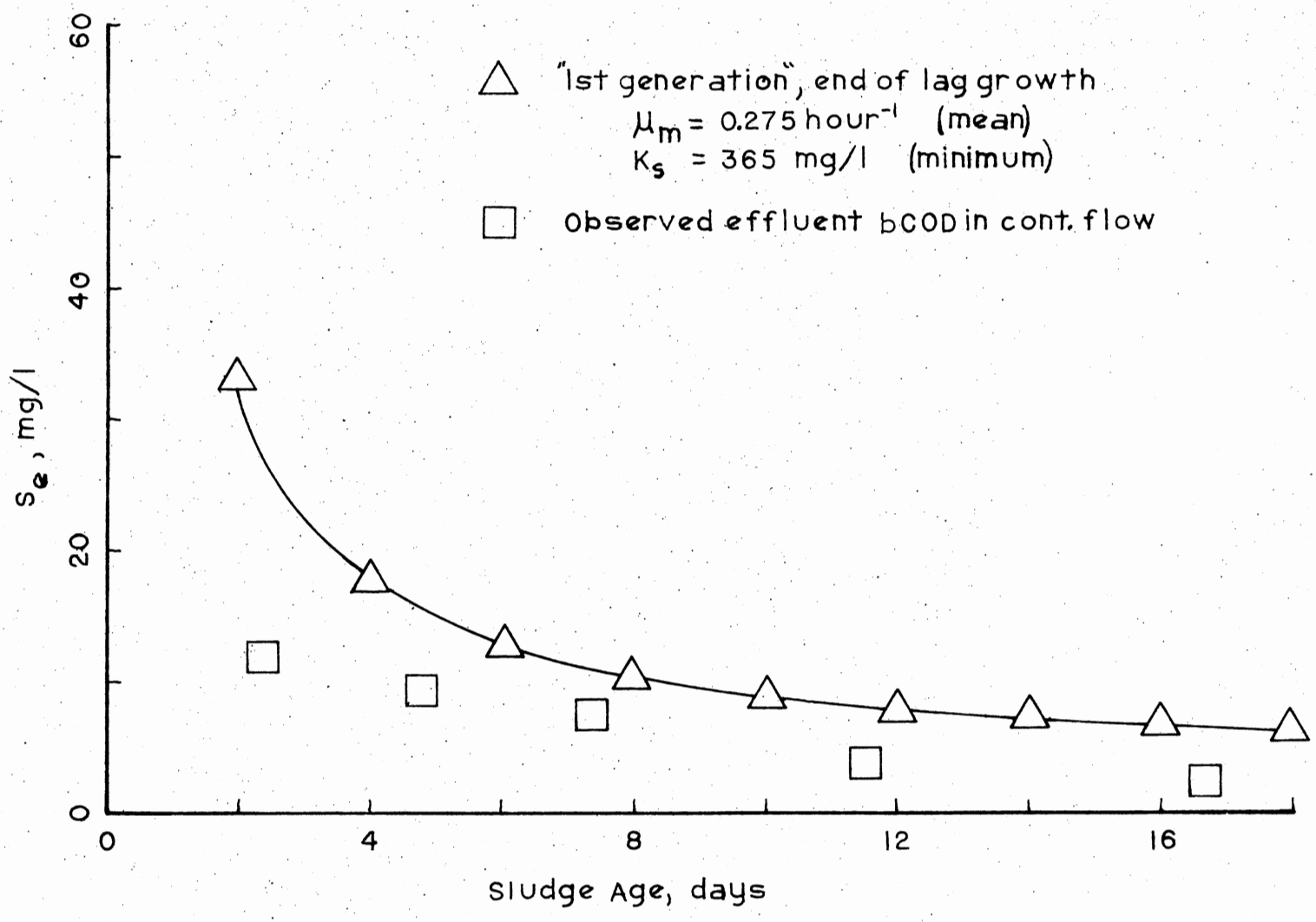
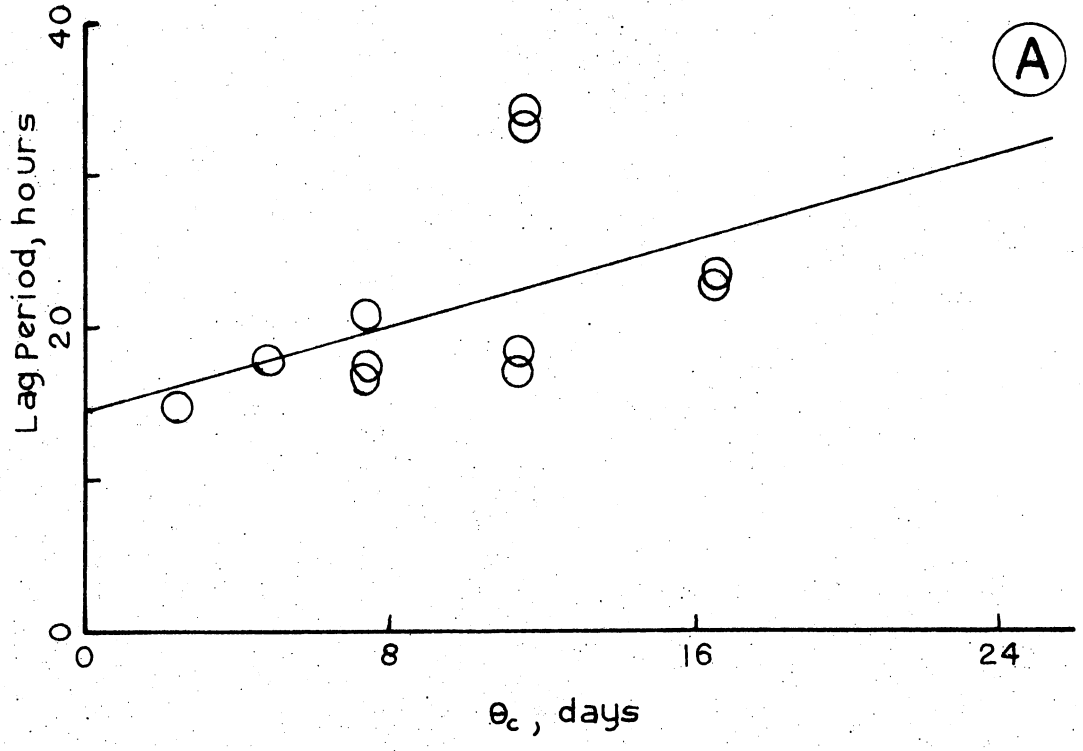
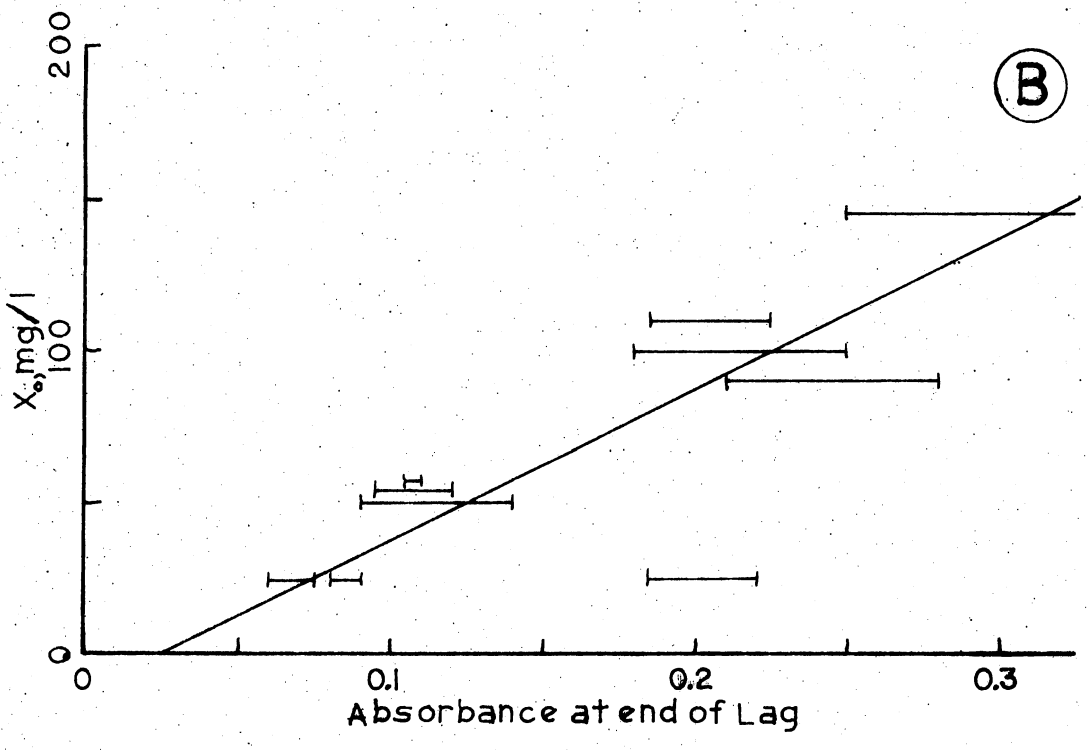

△ "1st generation", end of lag growth
μ_m = 0.275 hour^{-1} (mean)
K_s = 365 mg/l (minimum)
□ Observed effluent bCOD in cont. flow
S_e, mg/l
0
20
40
60
0
4
8
12
16
Sludge Age, days

Figure 25. Variation in Lag Periods:
A. Lag Period Versus Sludge Age
B. Initial Biomass Concentration Versus Absorbance at End of Lag Period


A
Lag Period, hours
0
20
40
0
8
16
24
θc , days


B
Xo, mg/l
0
100
200
0
0.1
0.2
0.3
Absorbance at end of Lag

end of the lag period (start of log growth period). The empirical equations, derived via linear regression, from this data are presented below. The significance of the first equation is evident. The units for t_L and θ_c are hours and days, respectively. The second equation suggests that at the start of log growth, the cell mass had slightly more than doubled ($\sim 2.4X_o$). The units for A_L and X_o are Absorbance units and mg/l, respectively. X and A are related by equation 32. The onset of the log growth phase (or the end of the lag

$$t_L = 0.669\ \theta_c + 14.8 \qquad (30)$$

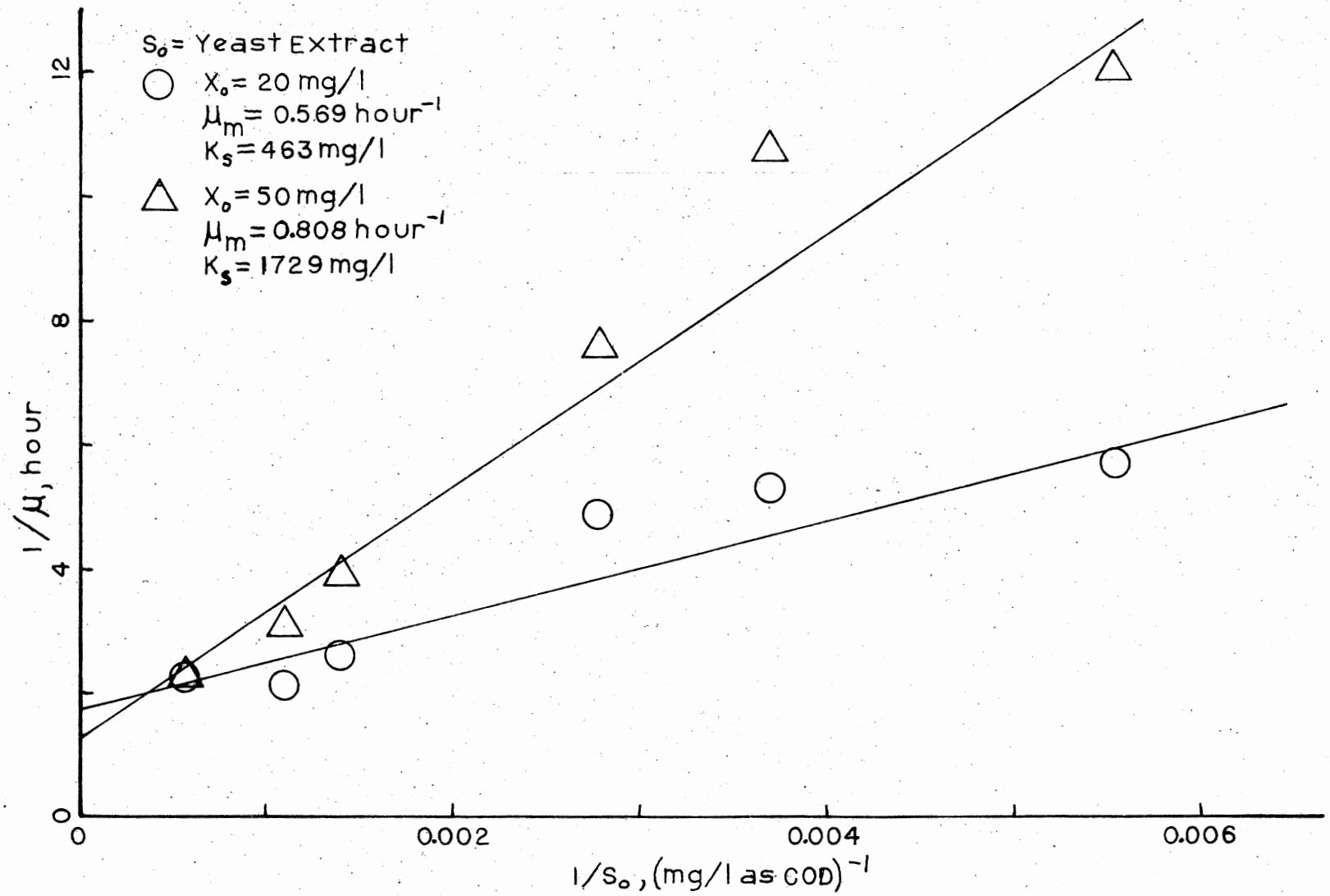
$$A_L = 0.00199\ X_o + 0.0245 \qquad (31)$$

$$X/A \cong 1050 \text{ mg/l / O.D. unit} \qquad (32)$$

period) was accompanied by an obvious change in physical character of the biomass. The cells suddenly became quite sticky. The cells formed clumps around the water line, which had to be physically scraped off the walls of the shaker flasks back into suspension at regular intervals.

Since the cells drawn from the continuous flow reactors existed in a medium which probably contained various growth factors not present in the glucose minimal medium and very little glucose, two other experiments were performed. Yeast extract was used as the sole carbon/energy source in one batch growth experiment and glucose/yeast extract (10/1) was used in the other experiment. Lineweaver-Burke plots of the results of these two experiments are presented in Figures 26

Figure 26. Lineweaver-Burke Plot of Growth at End of Lag Period with Yeast Extract as Carbon/ Energy Source


S_o = Yeast Extract
X_o = 20 mg/l
μ_m = 0.569 hour^{-1}
K_s = 463 mg/l
X_o = 50 mg/l
μ_m = 0.808 hour^{-1}
K_s = 1729 mg/l
1/μ, hour
0
4
8
12
0
0.002
0.004
0.006
$1/S_o$, (mg/l as COD)$^{-1}$

and 27. The seed for these experiments was drawn from the continuous flow reactor operating at a θ_c of 11.5 days. As with the growth experiments with glucose, a lengthy lag period was encountered in these two growth experiments (12 to 18 hours). It should also be noted that K_s varied with X_o in these experiments.

As noted in Figure 25, the cell mass had slightly more than doubled at the onset of log growth. In order to ascertain what was occurring during the lag period; Absorbance, soluble COD, and soluble carbohydrate were monitored for approximately one doubling time from time zero. Absorbance was monitored continuously in order to ascertain when one doubling time was reached. Soluble COD and carbohydrate were calculated for t_o and t_d. A summation of the observed growth rates are presented in Table IV. No μ_m or K_s could be obtained from the observed growth rates, although linear regression was used to calculate μ for all conditions. There seemed to be no systematic variation in μ with S_o, X_o, or sludge age of micro-organism seed. The mean μ is 0.103 hour^{-1}, with values ranging from 0.0675 to 0.1559. The values for μ are actually more similar than they appear to be in Table IV. The apparent variation is probably due to the limited number of points available for determination of each μ. Figure 28 demonstrates this graphically via example. The mean amounts of soluble COD removed during the doubling periods are presented in Figure 29. The mean changes in carbohydrate concentration are presented in Figure 30. Figures 29 and 30,

Figure 27. Lineweaver-Burke Plot of Growth at End of Lag Period Using Glucose/Yeast Extract in Ratio of 10/1 as Carbon/Energy Source

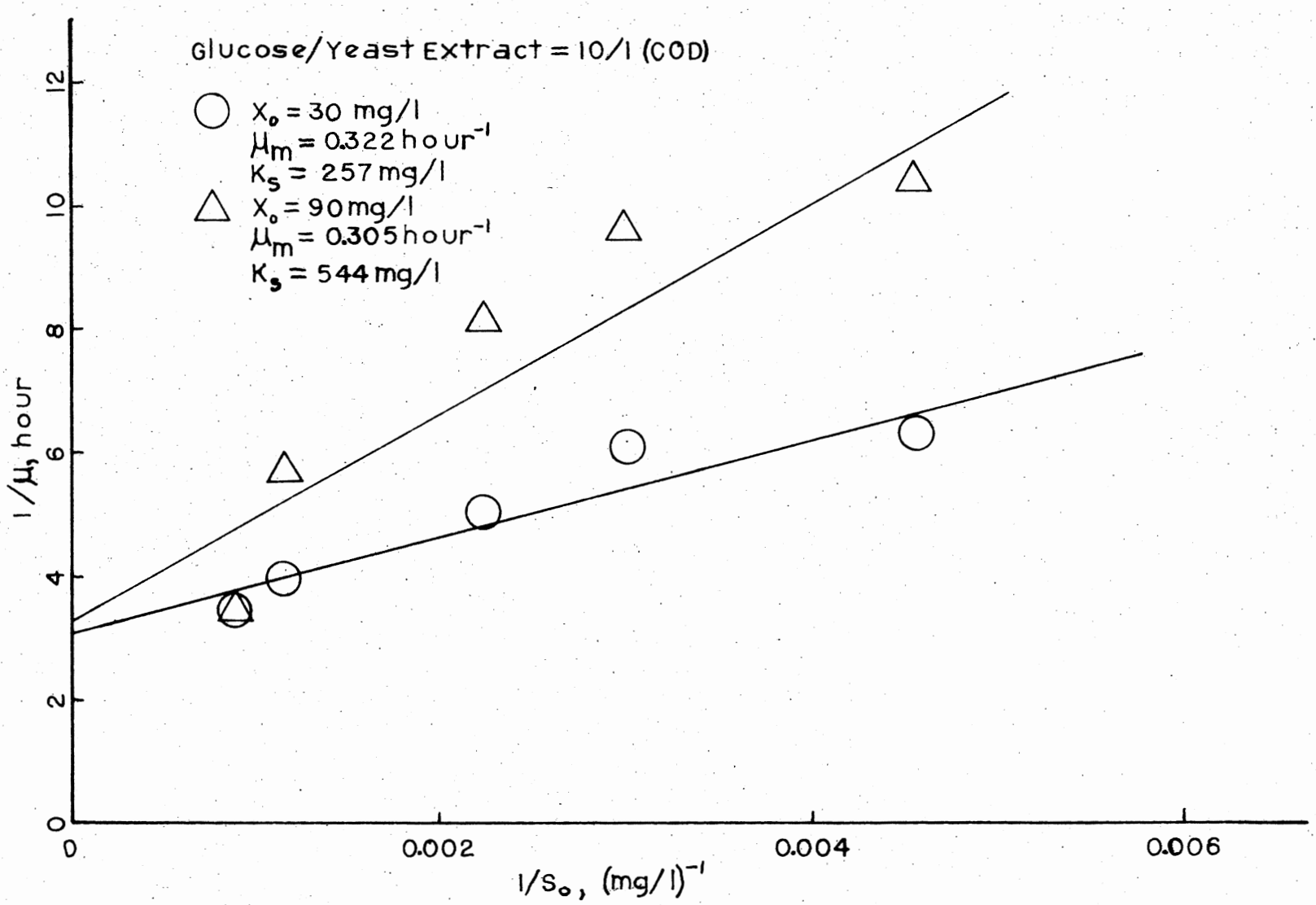

Glucose/Yeast Extract = 10/1 (COD)
X_o = 30 mg/l
μ_m = 0.322 hour^{-1}
K_s = 257 mg/l
X_o = 90 mg/l
μ_m = 0.305 hour^{-1}
K_s = 544 mg/l
$1/\mu$, hour
0
2
4
6
8
10
12
0
0.002
0.004
0.006
$1/S_o$, (mg/l)$^{-1}$

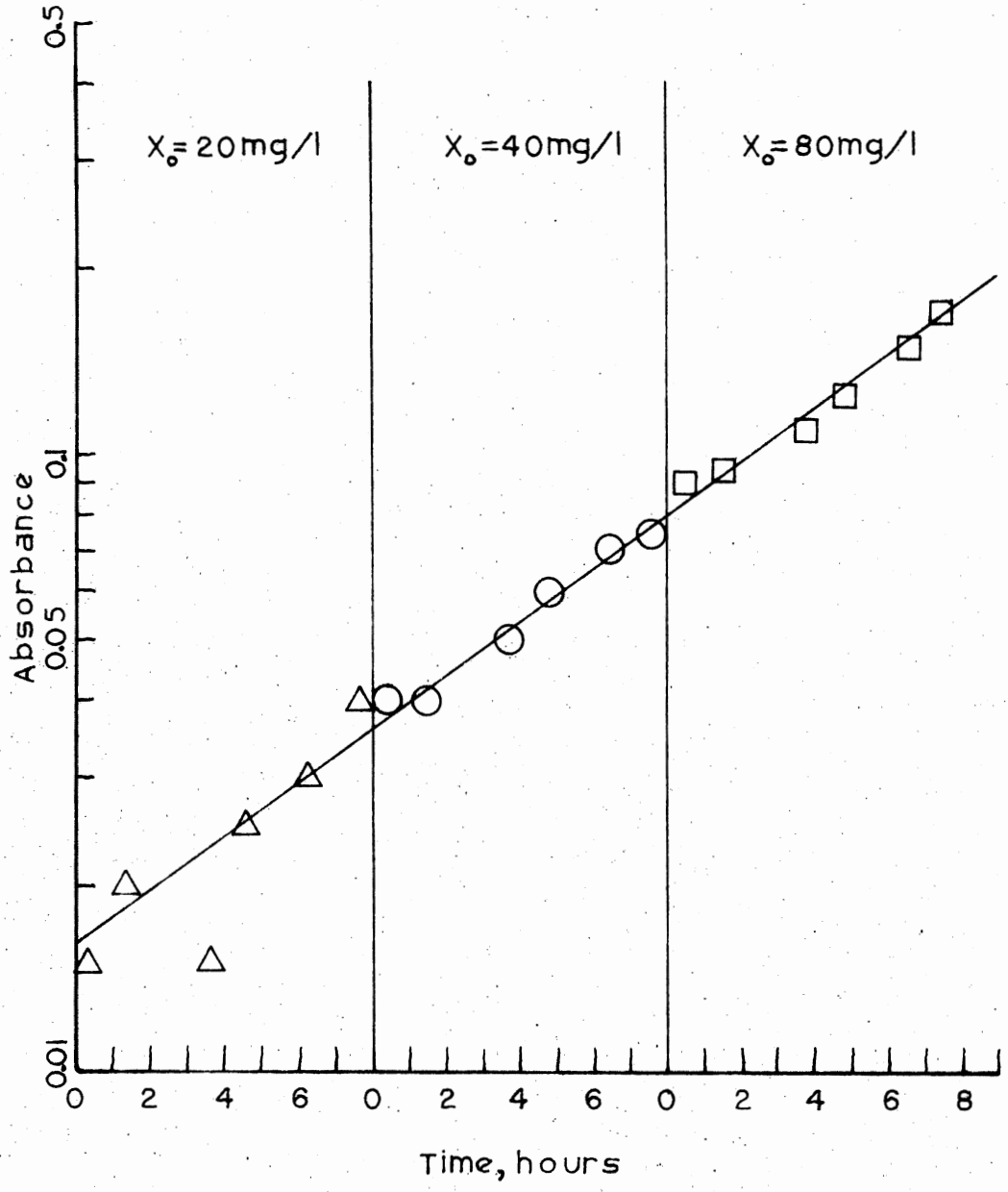
TABLE IV

SPECIFIC GROWTH RATES DURING FIRST DOUBLING TIME

Initial Bio-mass Conc. (mg/l)	Initial Substrate Conc. (mg/l glucose as COD) 1000	600	400	200
25	0.0675	0.0908	0.1204	0.1317
50	0.0897	0.1003	0.1029	0.1470
100	0.1038	0.1006	0.1185	0.1094
	θ_c = 2.4 days^{-1}		$\bar{\mu}$ = 0.1069 hours^{-1}	
25	0.1176	0.1192	0.1559	0.1475
50	0.0944	0.1119	0.1222	0.1027
100	0.0903	0.0985	0.0840	0.0951
	θ_c = 4.8 days^{-1}		$\bar{\mu}$ = 0.1116 hours^{-1}	
25	0.0995	0.0941	0.0835	0.0967
50	0.0885	0.0921	0.0865	0.0871
100	0.0928	0.0918	0.0924	0.0878
	θ_c = 7.4 days^{-1}		$\bar{\mu}$ = 0.0911 hours^{-1}	

Overall Mean μ = 0.1032 hours^{-1}

Figure 28. Absorbance Versus Time for First Doubling Period


X_o= 20mg/l
X_o=40mg/l
X_o=80mg/l
Absorbance
0.5
0.1
0.05
0.01
0 2 4 6 0 2 4 6 0 2 4 6 8
Time, hours

$S_o = 1000$ mg/l

Figure 29. Soluble COD Removed During First Doubling Period Versus Initial Soluble COD Concentration

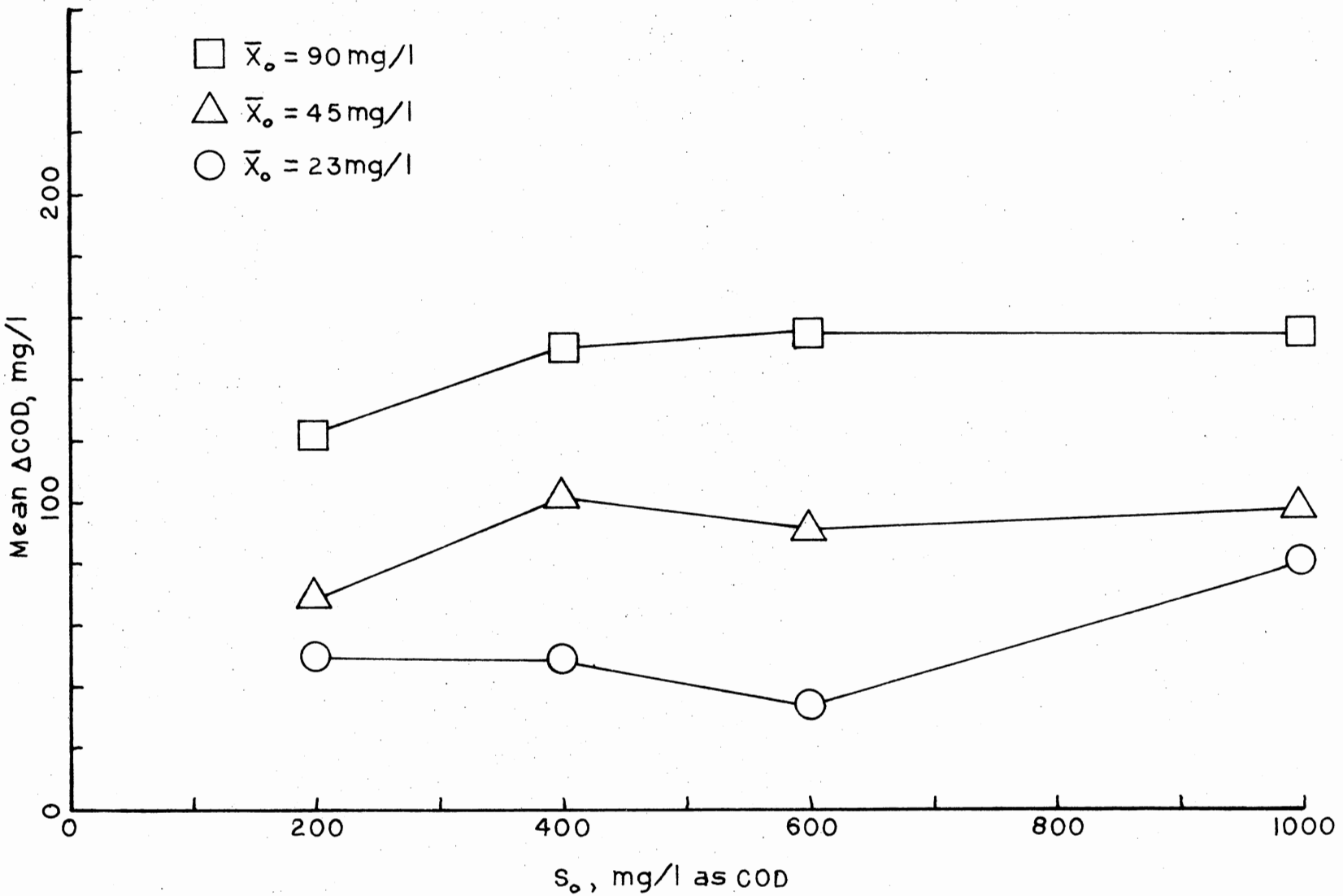
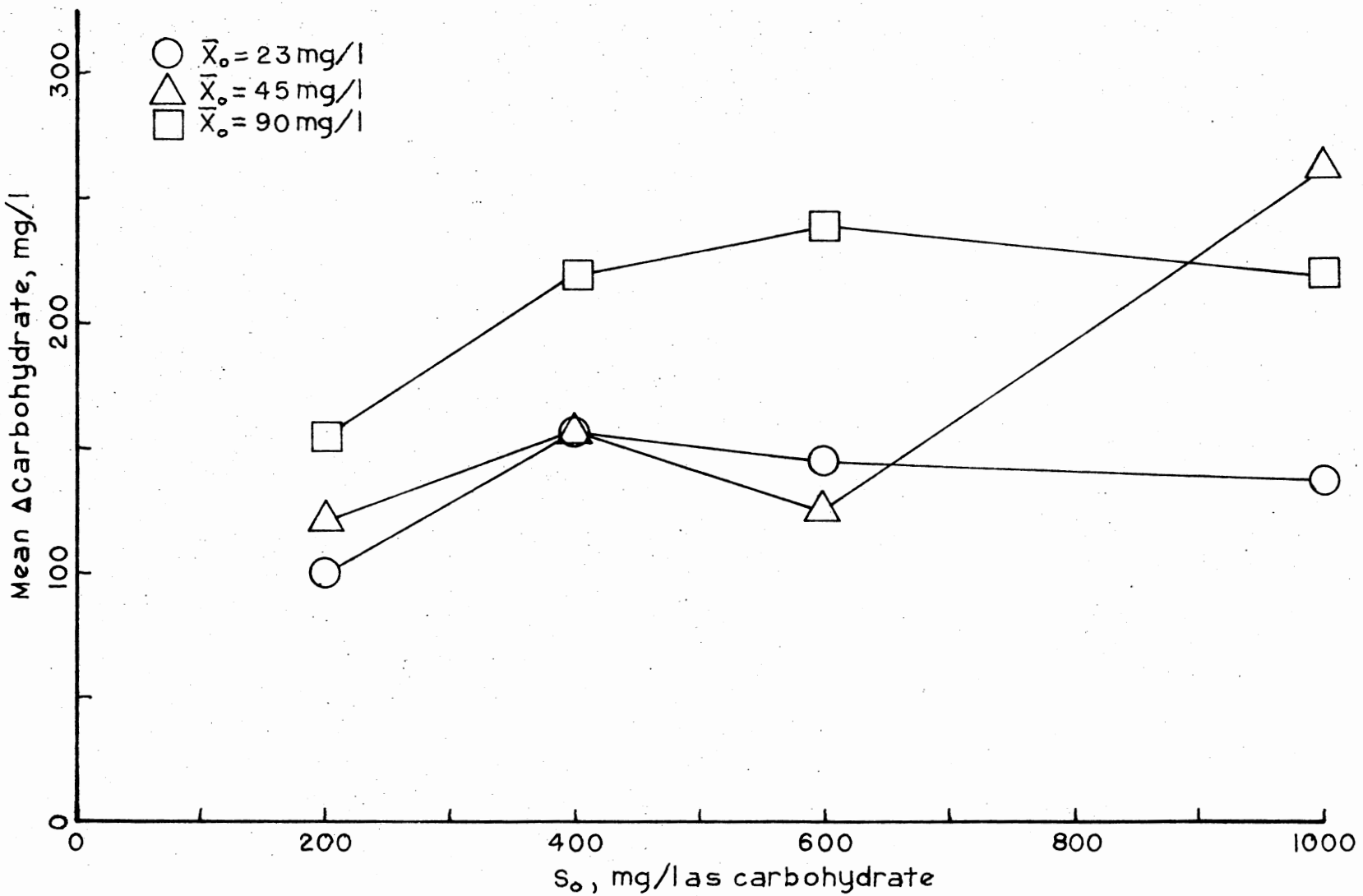

$\bar{X}_o$ = 90 mg/l
$\bar{X}_o$ = 45 mg/l
$\bar{X}_o$ = 23 mg/l
Mean ΔCOD, mg/l
0
100
200
0
200
400
600
800
1000
S_o, mg/l as COD

Figure 30. Soluble Carbohydrate Removed During First Doubling Period Versus Initial Soluble Carbohydrate Concentration


$\bar{X}_o$ = 23 mg/l
$\bar{X}_o$ = 45 mg/l
$\bar{X}_o$ = 90 mg/l
Mean ΔCarbohydrate, mg/l
0
100
200
300
0
200
400
600
800
1000
S_o, mg/l as carbohydrate

unlike the values of μ, seem to indicate a Monod-like relationship between growth (substrate removal) and initial substrate concentration. The amount of substrate removed is also dependent on X_o. The approximate value for the mean cell yield, calculated on the basis of ΔA and ΔCOD, is 0.55.

In order to establish whether a μ_m and K_s could be determined for the lag period, two additional experiments were performed using seed again drawn from the continuous flow reactors (at a θ_c of 2.4 and 16.7 days). The Absorbance was monitored from time zero to the apparent end of substrate removal. Linear regression was used to obtain values for μ. The calculated values for μ_m and K_s, at θ_c's of 16.7 and 2.4 days, are 0.0543 hour^{-1}, 7.6 mg/l, 0.0660 hour^{-1}, and 7.3 mg/l respectively. The predicted values of S_e, using μ_m and K_s equal to 0.0543 hour^{-1} and 7.6 mg/l respectively, are compared with the observed values of effluent bCOD for the continuous flow reactors in Figure 32.

There seemed to be two distinct growth phases. The first phase (the "lag period") seemed to have a μ_m less than 0.1 hour^{-1}, while the second phase (after the "lag period") seemed to have a μ_m equal to or greater than 0.2 hour^{-1}. However, no apparent increase in μ was observed during the course of the experiments reported in the previous paragraph. The initial substrate concentrations were all below 600 mg/l COD. For this reason, an experiment was performed in which Absorbance was monitored over an extended period of time. The initial substrate concentration was 2000 mg/l

Figure 31. Lineweaver-Burke Plot of Growth During "Lag Period"

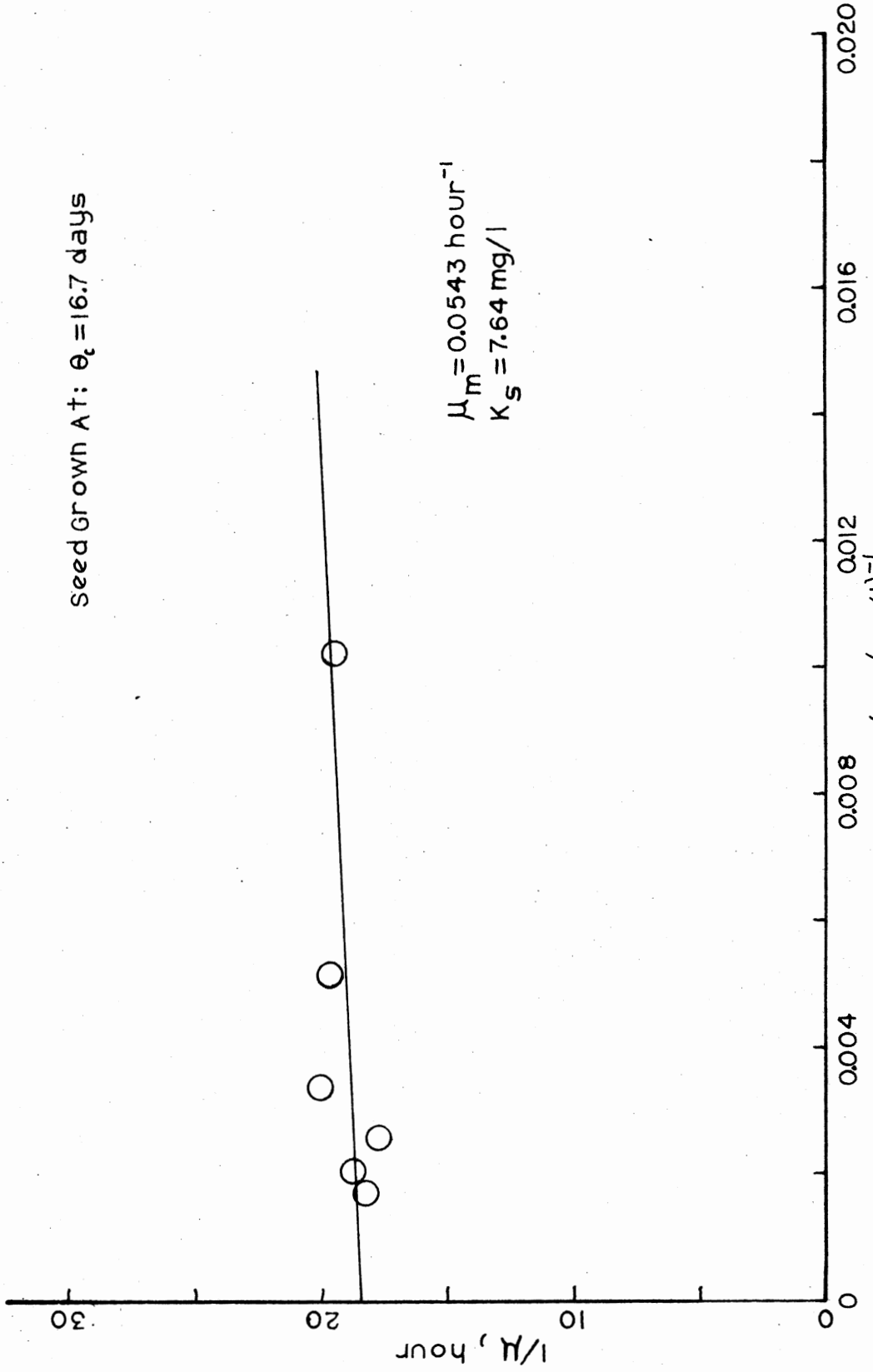
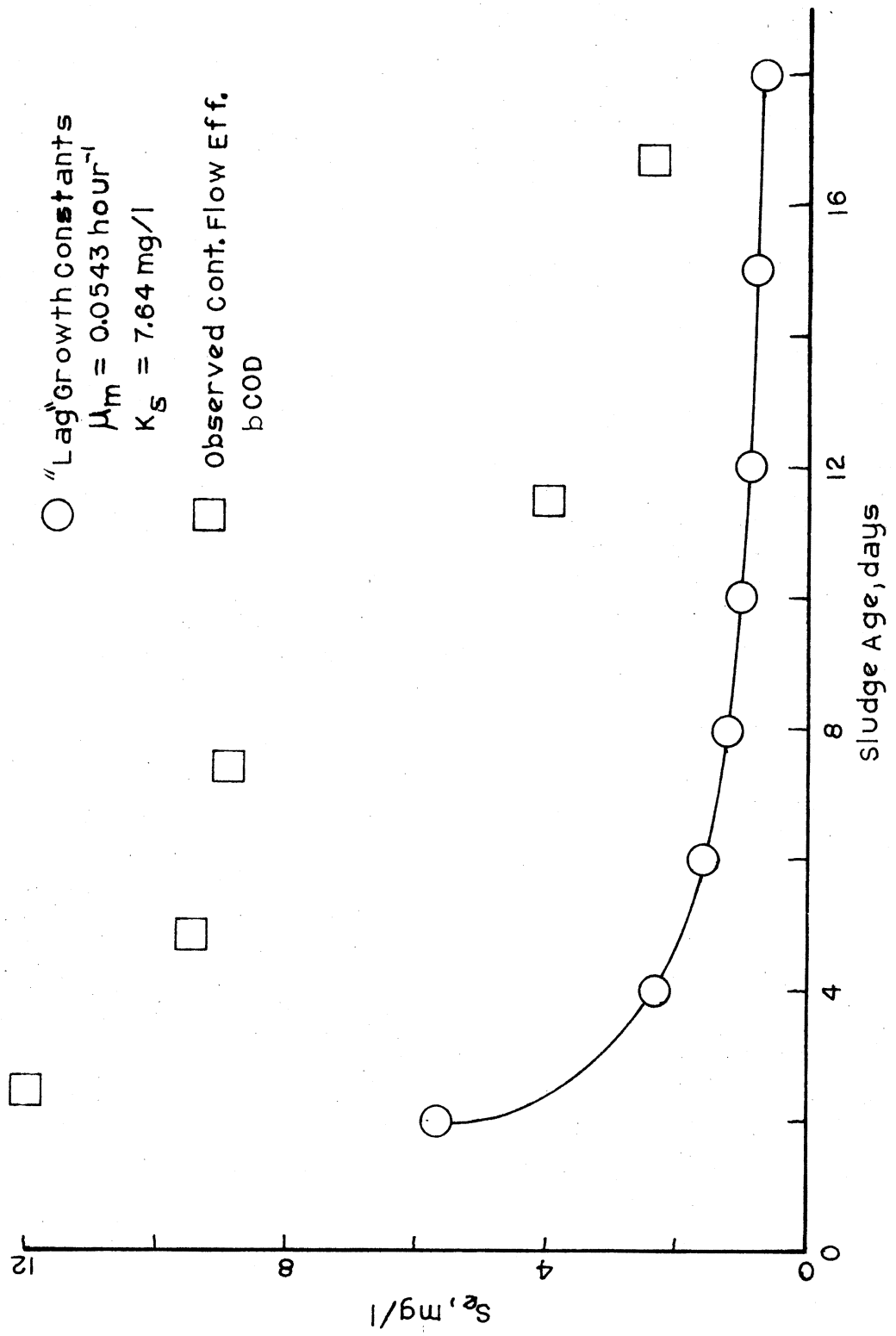

Seed grown At: θ_c=16.7 days
μ_m=0.0543 hour^{-1}
K_s=7.64 mg/l
1/μ, hour
$1/S_o$, (mg/l)$^{-1}$
0
10
20
30
0.004
0.008
0.012
0.016
0.020

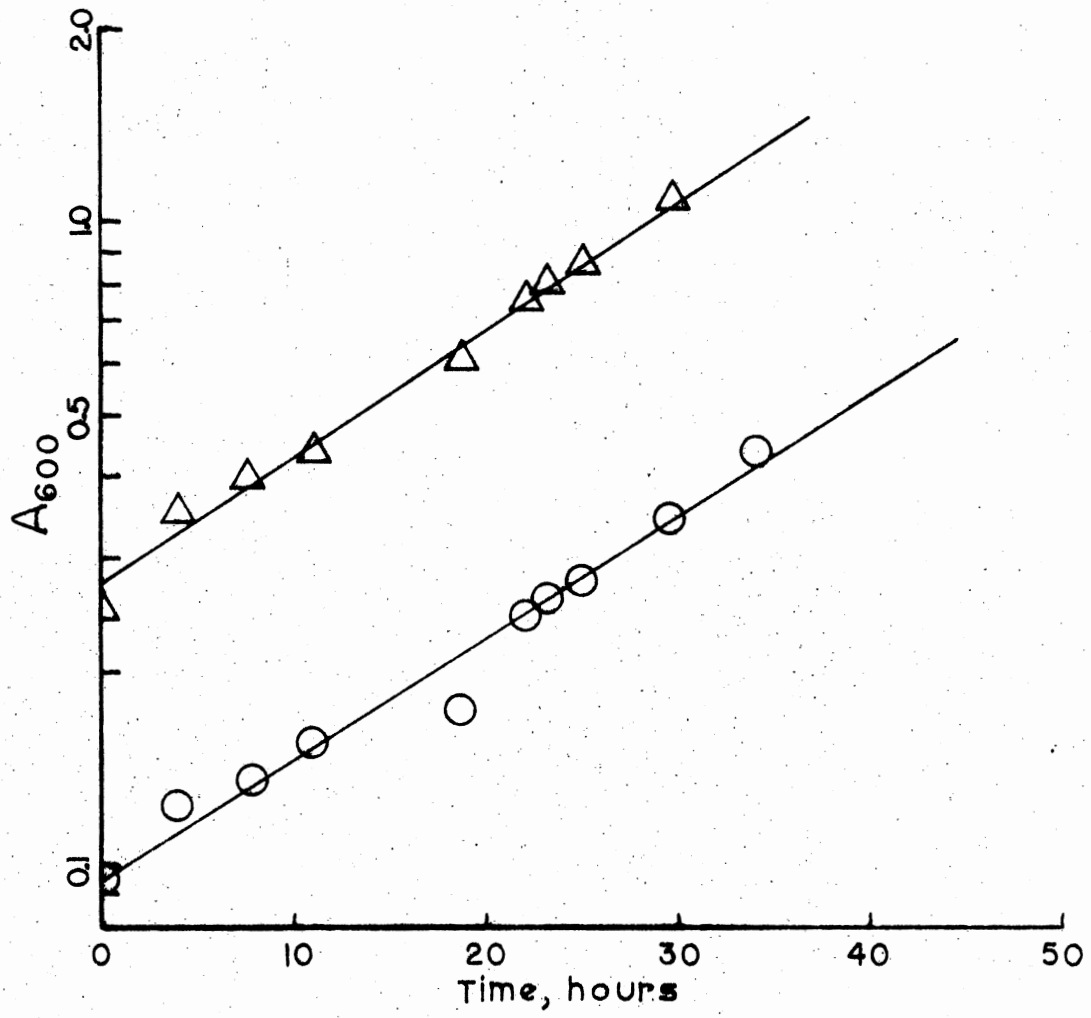
Figure 32. Effluent Soluble Substrate Concentration Versus Sludge Age (From Figure 31)


"Lag" Growth Constants
μ_m = 0.0543 hour^{-1}
K_s = 7.64 mg/l
Observed Cont. Flow Eff.
bCOD
S_e, mg/l
0
4
8
12
Sludge Age, days
4
8
12
16

glucose (as COD). The results are presented in Figure 33. The soluble COD and suspended solids concentration were determined at the times indicated by the arrows. The ratio of X to A at A=0.85 was 1000 - similar to the ratio observed at lower levels of X and A. No increase in μ after an extended period was observed. The observed μ remained essentially constant at approximately 0.045 $hour^{-1}$ for 34 hours; except that at the beginning of growth and substrate removal, the apparent μ was somewhat greater than it was later. The yield will be presented later.

An additional set of growth experiments were carried out at θ_c's of 2.4 and 16.7 days. Extra flasks containing high substrate concentrations (1000 and 2000 mg/l glucose as COD) were incubated along with the growth experiments done to determine the growth constants for growth during the log growth phase (after the lag period). At the peak of growth in these flasks, these flasks were used as a source of seed for an additional set of growth experiments. A Lineweaver-Burke plot for the results of this "second generation" growth experiment is presented in Figure 34. The original seed for this experiment was grown at a θ_c of 16.7 days. μ_m and K_s are, respectively, 0.200 $hour^{-1}$ and 85 mg/l. The "second generation" values of μ_m and K_s for seed drawn originally from the continuous flow reactor at a θ_c of 2.4 days are 0.210 $hour^{-1}$ and 37 mg/l, respectively. The S_e predicted from the constants for a θ_c of 16.7 days are compared with the observed values of S_e (bCOD) for the continuous flow

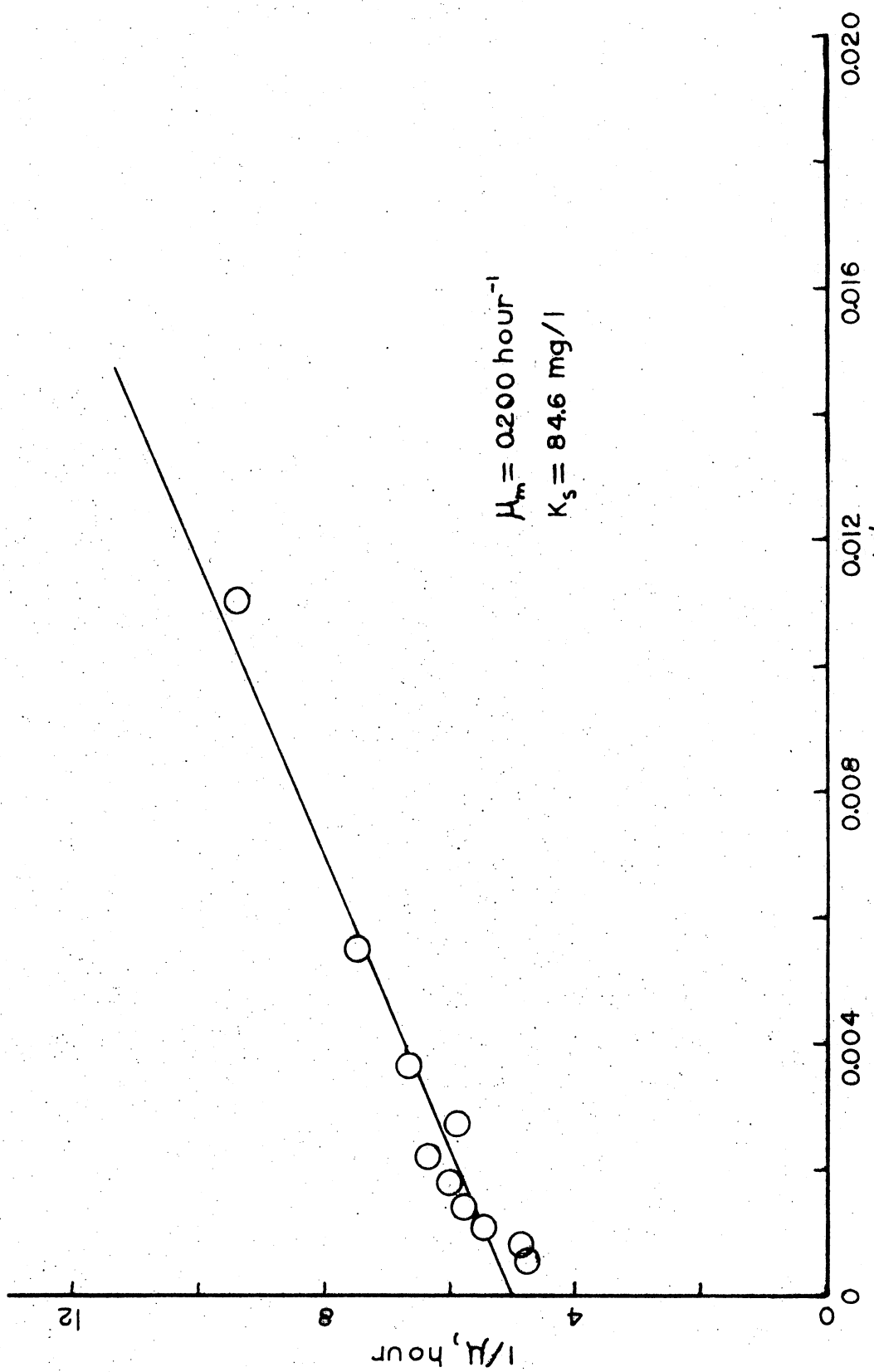
Figure 33. Growth During "Lag Period"



○ $X_o = 103$ mg/l, $S_o = 2000$ mg/l, $\mu = 0.044$ hour^{-1}

△ $X_o = 272$ mg/l, $S_o = 2000$ mg/l, $\mu = 0.046$ hour^{-1}
$\theta_c = 16.7$ days

Figure 34. Lineweaver-Burke Plot of "Second Generation" Growth


$\mu_m = 0.200$ hour^{-1}
$K_s = 84.6$ mg/l
1/μ, hour
$1/S_o$, (mg/l)$^{-1}$
0
4
8
12
0.004
0.008
0.012
0.016
0.020

reactors in Figure 35.

The cell yield was calculated for the continuous flow reactors (Y_o), in batch reactors during the "first generation" growth (Y_B), and in batch reactors during "second generation" growth (Y_{B2}). The means for all experiments are presented versus θ_c in Figure 36. Since the continuous flow reactor operated at a θ_c of 16.7 days was started from a different seed than the continuous flow reactors operated at other θ_c's, Y_{tB} and k_{dB} for the batch yields was calculated both with and without the values for batch yields at a θ_c of 16.7 days. A linear form of equation 3 and linear regression were used to calculate batch yield constants. The constants, Y_{tB} and k_{dB}, with and without the batch yields for the 16.7 day sludge age, were 0.58, 0.0029 day^{-1}, 0.67, and 0.034 day^{-1}. A summary of the batch yield data may be found in Table X in the Appendix.

Figure 35. Soluble Effluent Substrate Concentration Versus Sludge Age (From Figure 34)

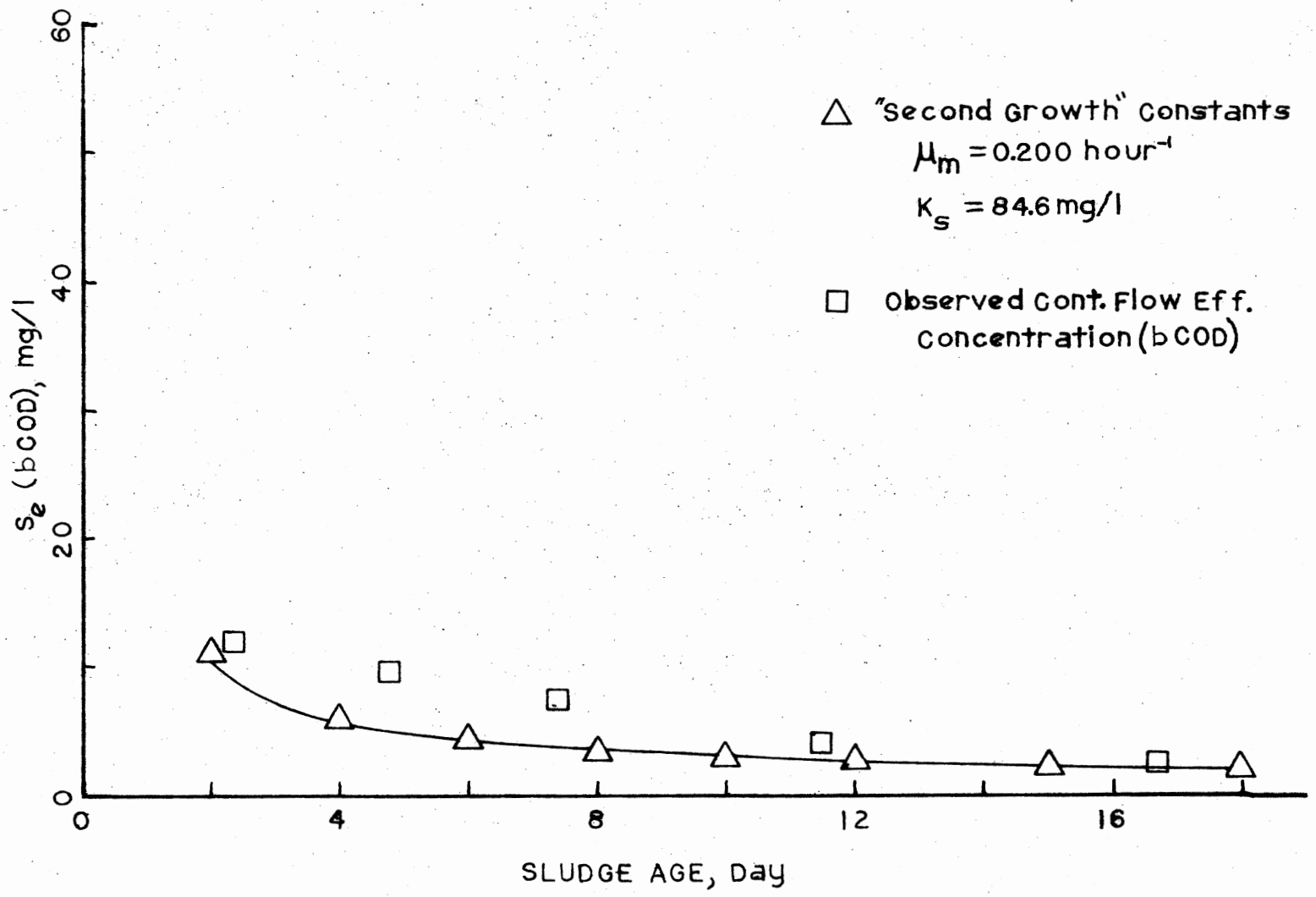
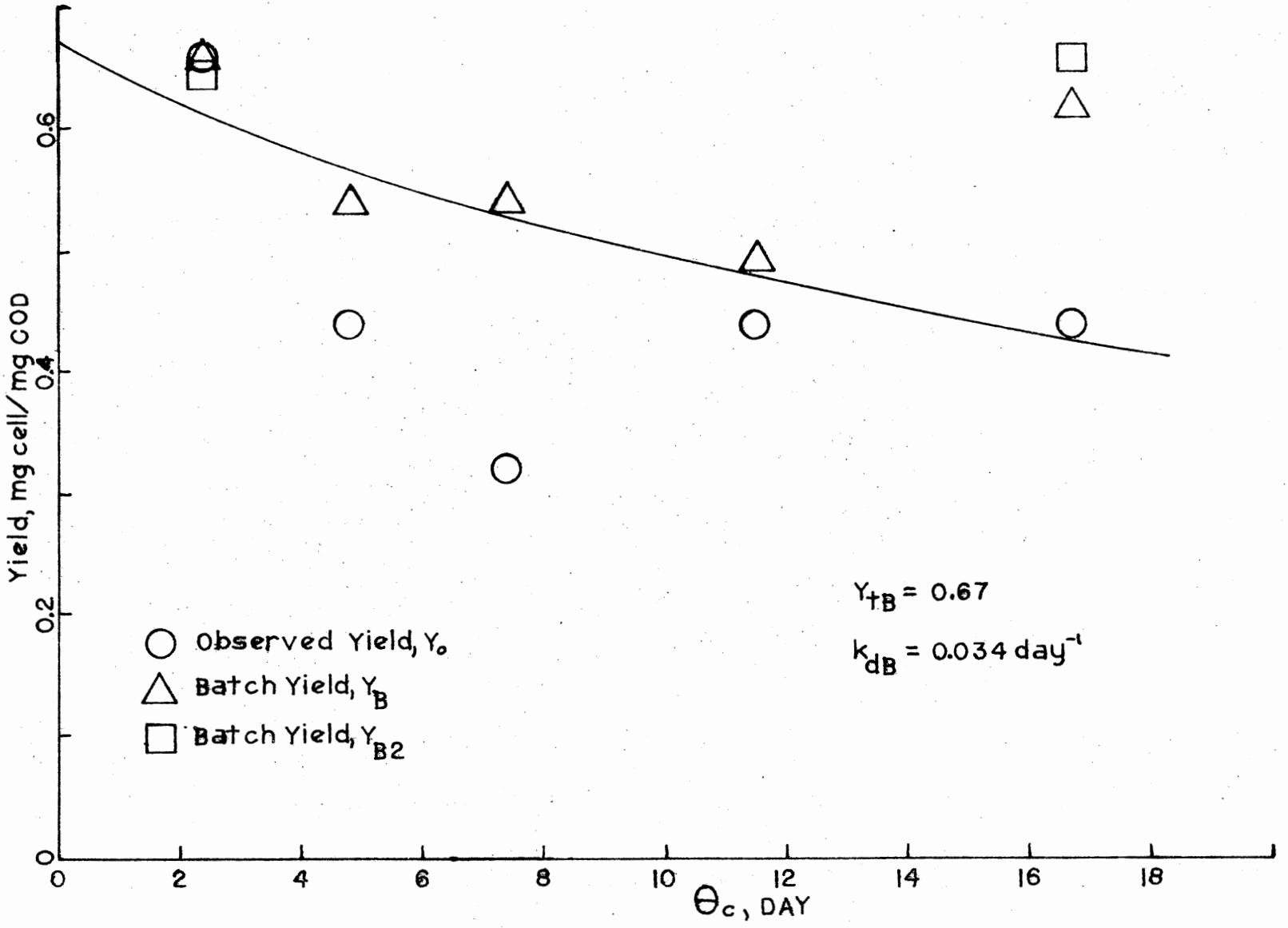

△ "Second Growth" Constants
$\mu_m = 0.200$ hour^{-1}
$K_s = 84.6$ mg/l
□ Observed Cont. Flow Eff. Concentration (bCOD)
S_e (bCOD), mg/l
0
20
40
60
0
4
8
12
16
SLUDGE AGE, Day

Figure 36. Batch and Continuous Flow Cell Yields Versus Sludge Age


Yield, mg cell/mg COD
0
0.2
0.4
0.6
0
2
4
6
8
10
12
14
16
18
θ_c, DAY
Observed Yield, Y_o
Batch Yield, Y_B
Batch Yield, Y_{B2}
$Y_{tB} = 0.67$
$k_{dB} = 0.034\ day^{-1}$

CHAPTER V

DISCUSSION

A. Continuous Flow Growth Constants

All of the continuous flow growth constants were within the relevant ranges discussed in Chapter II, except for the maximum specific growth rate. The expected range for μ_m, from Gaudy and Gaudy (3), is 0.4 to 0.6 hour^{-1} (9.6 to 14.4 day^{-1}); while the value found in this study was 2.00 day^{-1}. It was necessary to use bCOD to determine μ_m and K_s. The values of μ_m and K_s are sensitive to the point selected for COD_{min} - which determines the effluent bCOD's. Also, since the observed values of S_e and U cover a small range within the range of possible values; μ_m and K_s are determined by a very small apparent curvature of the relationship between U and S_e. Because there is some scatter of paired S_e and U or μ values around a line describing either a Monod or Lawrence and McCarty relationship between S_e and U or μ, recovery of the "true" values of k, μ_m, and K_s is unlikely. However, the observed μ_m for the continuous flow reactors lies within the range of μ values observed in the batch experiments for the lag period of growth (approximately 1.2 to 2.4 day^{-1}). Whether this is coincidence or an indication of some relationship between the low specific growth rates which occur during

the lag period and low μ_m calculated for the continuous flow reactors is unclear.

B. Comparison of Growth Constants

One of the major purposes of this study was to explore the possibility of predicting continuous flow operation of an activated sludge reactor from determinations of the growth constants made in batch reactors.

Before making any comparisons or discussing these comparisons, a couple of preliminary points should be made clear. bCOD was employed as the measure of the effluent substrate concentration from the continuous flow reactor. However, there are other measurements which might have been justifiably employed. Examples of alternative measurements of S_e are observed COD or glucose concentration. While calculation of bCOD is based on a questionable assumption - i.e., that there is an invariant and non-biodegradable residual produced at all sludge ages - there is some justification for its use as a measure of S_e. The bCOD, as used in this study, is probably a better indicator of the concentration of readily biodegradable organic compounds present in an effluent; than is COD or any measurement of a specific organic compound. The readily degradable organics present in the effluent stream of a secondary waste treatment plant are generally of greater interest than is the total COD concentration or concentration of a specific organic compound. Another equally significant factor is that it is necessary to subtract any apparent

residual before k_e, k, μ_m, or K_s can be calculated. A plot of S_e versus μ or U must pass through the origin, in order that a linear form of the Monod equation may be used to calculate the constants μ_m or k and K_s. Whether the continuous flow effluent bCOD can be related to the growth constants, as determined in batch experiments, was an empirical question to be determined in this study. The other preliminary point that needs to be made is that the various operating parameters - U, μ_n, etc. - and the growth constants can be calculated in either of two ways. The two methods involve inclusion of differing sludge masses in calculating the operating parameters or growth constants. In one method, the total sludge mass in the system is employed in the calculations. In the other method, only the sludge mass in the aeration chamber at any moment is employed in the calculations. There has been some speculation as to which is the "correct" method (68). Regardless of one's criteria for "correctness", there is no empirical evidence supporting one method over the other. The former method (using total sludge mass in the system) was employed in the previous chapter for calculation of the various constants. The sludge mass in the aeration chamber was not monitored, so it was not possible to accurately calculate the operating parameters and growth constants of the continuous flow system using only the sludge mass in the aeration chamber. However, if one assumes that the sludge in the system was evenly distributed between the clarifier and aeration chamber proportional to their volumes; the

operating parameters and the resultant growth constants can be calculated. The ratio of aeration chamber volume to clarifier volume was approximately 2.7 to one. The continuous flow growth constants, calculated via both methods, are presented in Table V; in order that both sets of continuous flow growth constants may be compared with batch growth constants.

TABLE V

CONTINUOUS FLOW GROWTH CONSTANTS

Constant (units)	Calculated with Total Solids	Calculated with only Aeration Solids
Y_t (mg cell/mg COD)	0.63	0.63
k_d (day^{-1})	0.056	0.076
k_e (l/mg·day)	0.051	0.07
k (day^{-1})	3.2	4.3
u_m (day^{-1})	2.0	2.7
K_s (mg/l)	55	55

Can Y_t and/or k_d be determined via batch experiments? Batch yield is often interpreted as a constant equivalent to the true cell yield (Y_t). The cell maintenance coefficient

is often treated as a reaction rate constant which can be determined in batch reactors via some measurement, such as the respiration rate or the rate of disappearance of sludge mass in a batch reactor in the absence of an exogenous carbon/energy source. The studies of Saleh (17) and Srinivasaraghavan (16) suggest that the batch yield varies systematically with the continuous flow growth rate at which the microbial seed for the batch determination of yield is grown. Their data further suggest that the variation in observed yield and batch yield with specific growth rate is not a time-dependent reaction, but rather a fixed characteristic of the cells such that the efficiency of conversion of substrate to cell material is affected. The batch yield determined using seed grown at a given continuous flow growth rate was found to be equivalent to the observed yield in the continuous flow system. Esfandi (65), under similar conditions, found the batch yield to be much larger than either the observed yields or the true cell yield.

The "first generation" batch yield was found to vary slightly from the observed yield, but both yields were found to be dependent on the continuous flow growth rate. The yield constants - Y_t, k_d, Y_{tB}, and k_{dB} - were calculated via equation 3, using μ_n calculated on the basis of total sludge in the system. The cell yield constants, Y_t and k_d, found in the continuous flow reactors were dissimilar to the constants found in the batch reactors. The true cell yield found in the continuous flow reactors was 0.63, while the

true cell yields found in the batch reactors (using sludge seed drawn from the continuous flow reactors at each sludge age) were 0.58 (with the yields found in the batch system at a sludge age of 16.7 days) and 0.67 (without the 16.7 day sludge age data). The cell maintenance coefficients, in the same order, are 0.056, 0.0029, and 0.034 day^{-1}. The two sets of batch reactor data were calculated, because the continuous flow reactor operated at a sludge age of 16.7 days was started with a different micro-organism seed than were the continuous flow reactors operated at other sludge ages. The batch true yield and batch cell maintenance coefficient, calculated without the batch yield for the sludge age of 16.7 days, seem to fit the observed batch yields plotted in Figure 36 better than do the constants calculated with the batch yield found at a sludge age of 16.7 days. That is, the batch yield at a sludge age of 16.7 days does not follow the trend observed in the other batch yields. A comparison of the batch yields and the trend of the observed yields in Figure 36 suggests that the cell yields in batch and continuous flow reactors approach the same maximum cell yield as net specific growth rate increases, but tend to vary increasingly more widely as net specific growth rate decreases. As was noted earlier, Saleh and Srinivasaraghavan found Y_o to be equivalent to Y_B - i.e., Y_t is equivalent to Y_{tB} and k_d is equivalent to k_{dB}. Such was not found to be the case in this study. However, the findings of this study are in agreement with the findings of Saleh and Srinivasaraghavan in that Y_B does seem to be dependent on the growth rate in

the continuous flow reactor from which the seed for the batch growth study is drawn. The findings of Esfandi - that Y_B is much larger than Y_o and independent of the continuous flow growth rate - are not supported. However, unlike Saleh, Srinivasaraghavan, and the author of the present study; Esfandi was working with a microbial population which included nitrifying bacteria with the carbonaceous bacteria. The nitrifying bacteria may have affected the batch yields observed by Esfandi. The "second generation" batch studies performed by the present author produced batch yields, which did seem to be independent of the continuous flow growth rate and also approximated the true yield.

The yield data obtained in this study suggest that the variation in yield (both Y_o and Y_B) might be better explained via some internal mechanism of the individual cell linking yield with the environment or some property of the cell population dependent on the environment, rather than via some simple time-dependent reaction. It seems not unreasonable to suppose that utilization of substrate by the cell might be different when substrate is scarce (as at a low continuous flow growth rate), than when substrate is plentiful (high continuous flow growth rate). The influence of continuous flow growth rate on "first generation" batch yields may reflect some slippage in the linkage between environment and cell yield. If this supposition is correct; the batch cell yield should vary more widely from the observed yield in the direction of the true yield with increasing distance in time and cell generations from the environment producing the

observed yield (continuous flow reactor at low μ), along with the presence of a plentiful supply of carbon/energy souce. That is, Y_{B2} would be expected to approach Y_t. Since a heterogeneous population was used, a change in predominance could also account for the observed variation in yields. Whatever the correct explanation is for the observed variation in yields (Y_o and Y_B versus μ_n), it does not appear to be a simple time-dependent reaction (such as "cell decay" or "endogenous respiration") which can be determined in a single batch experiment - i.e., k_d should probably be determined in a continuous flow system. The true cell yield is best determined in a continuous flow system, but might be approximated via determination of "second generation" batch yield. As the values of Y_{B2} and Y_t found in this study are similar to the mean batch yield cited by Ramathan and Gaudy (12) for an acclimated seed of sewage origin grown on glucose, the value of Y_{B2} might be interpreted as the batch yield one would expect to find using a "young", acclimated microbial seed.

Can the effluent substrate concentration from a continuous flow reactor be predicted by batch determinations of the growth constants - k_e, μ_m, k, and K_s? The batch determinations of the cell growth and substrate removal constants are presented in Table VI, along with the continuous flow values of these constants. The values of k for the batch experiments were determined using the true cell yield. While the batch yield may have been more correct in some cases, use of Y_t provides for some measure of uniformity and does not

alter the calculated value of k very much. The presented values of k are close enough for comparison purposes. The values of k_e in Table VI were computed by dividing k by K_s. When S_e is very low, as it generally is in the activated sludge process, this gives a good approximation of k_e. Closeness of predicted S_e at any U or μ is more readily seen via k_e, than via μ_m (or k) and K_s. At any U (when S_e is low), k_e is approximately inversely proportional to S_e.

TABLE VI

COMPARISON OF CELL GROWTH AND SUBSTRATE REMOVAL CONSTANTS

Source - Figure	μ_m (day^{-1})	k (day^{-1})	K_s (mg/l)	k_e (l/mg·day)
Cont. Flow (X_{TOT}) - 8	2.0	3.2	55	0.058
Cont. Flow (X_{AER})	2.7	4.3	55	0.078
Batch - 15	0.89	1.4	42	0.033
Batch - 18	1.61	2.6	89	0.029
Batch - 24	6.6	10.5	365	0.029
Batch - 31	1.3	2.1	7.6	0.28
Batch - 34	4.8	7.6	85	0.089

It should be apparent from Table VI that the constants derived from "second generation" growth provide the best fit of observed continuous flow data (using either X_{AER} or X_{TOT}). The Monod constants for this "second generation" growth are more closely comparable to those cited by Gaudy and Gaudy (3), than are the other sets of Monod constants. The reason for this is that the ranges of the Monod constants cited by Gaudy and Gaudy were derived using young, active cell populations; as was the case with the "second generation" growth experiments in this study. Before the reader gets too excited about this result, it should be recalled that a second "second generation" growth experiment was performed. The values of μ_m (k) and K_s found in that experiment were 0.21 $hour^{-1}$ (8.0 day^{-1}) and 37 mg/l, which give an approximation of k_e of 0.22 l/mg·day. This value of k_e is grossly different from that observed in the continuous flow system (0.058 or 0.078 l/mg·day). Repeated determinations of μ_m and K_s in "second generation" growth experiments may possibly have favored the greater K_s, giving a good approximation of the k_e for the continuous flow system. The values of μ_m probably would have been similar to those found in "first generation" log growth (after the lag period) - i.e., 0.2 to 0.4 $hour^{-1}$. For the two determinations of "second generation" Monod constants, μ_m was found to be approximately equal to μ_m for the "first generation" growth (after the lag period). The values of K_s varied greatly, but that will be discussed later.

It should be noted that direct determination of k_e was attempted in batch reactors. An initial substrate concentration equal to that in the continuous flow systems was used, along with high initial sludge concentrations. The batch determinations of k_e at all sludge ages were found to be consistently lower than the k_e values obtained in the continuous flow system (Figures 9 thru 14). The reasons for this are unclear.

The Monod constants, μ_m and K_s, derived from the substrate removal experiments at a sludge age of 11.5 days were found to be 0.067 $hour^{-1}$ and 89 mg/l (Figure 18). The k_e derived from these constants is about 0.03 l/mg·day, which is about the same as the values of k_e derived directly from substrate removal versus time. This is as it should be. What makes the values of μ_m and K_s of interest is the difference between the continuous flow k_e and the batch k_e. The K_s found above is questionable. According to Gaudy (36) (37), there should be "slippage" during growth. The K_s derived via comparison of the instantaneous values of μ and S should be smaller than the K_s derived via comparison of μ and S_o. The value of K_s found above is comparable to the "second generation" value of K_s and much larger than the K_s derived for the lag period (the lag period is applicable to the low S/X substrate removal experiments). The reason that this probably does not happen (small K_s) is that in order to calculate μ from substrate removal, a Y_B must be assumed. The value of Y_B assumed was the value of Y_B observed in high

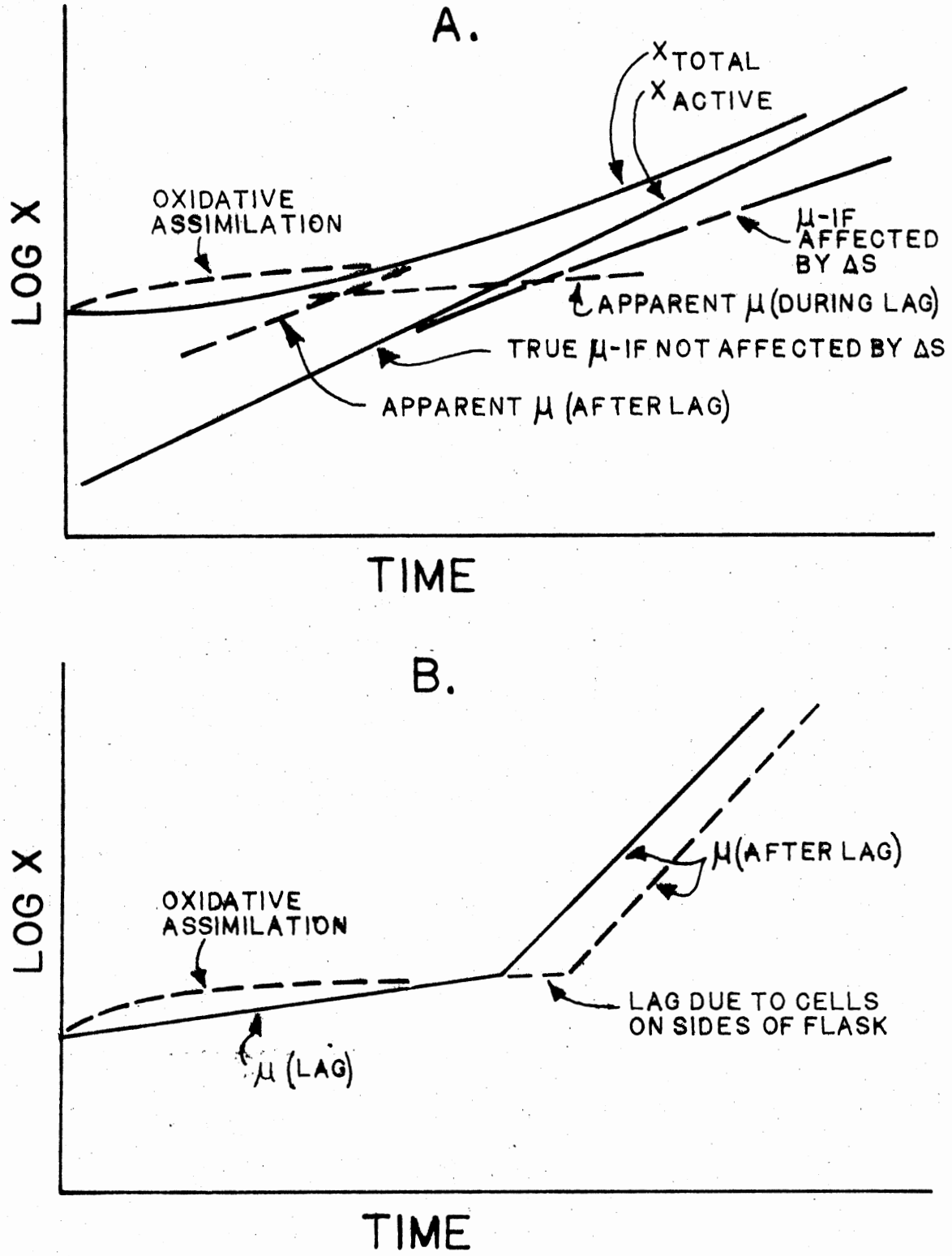
S/X growth experiments at the sludge age of 11.5 days. Yield under these low S/X conditions was probably close to one initially (due to oxidative assimilation), while synthesis lagged behind substrate removal (substrate removing mass). Assumption of a constant (and probably incorrect) yield results in a variable μ (more variable than it really is), making K_s larger than it should normally appear to be. Figure 17 presents the calculated mean initial μ for the various substrate removal experiments. The Y_B used to calculate these values of μ was the value of Y_B observed in high S/X growth experiments - such that synthesis had begun long before Y_B was determined. The mean of the values of μ in Figure 17 is 0.067 hour^{-1}. The growth rates presented in Table IV were observed growth rates where time of observation was approximately one doubling time. These values of μ represent observed changes in cell mass (via Absorbance). The mean μ here was 0.103 hour^{-1}. Both values of μ reflect growth during the lag period. Figure 33 also suggests an initially high μ, which decreases with time. This "hump" suggests a varying yield (oxidative assimilation). Perhaps, as an alternative explanation, μ does in fact start very high and decrease rapidly - as opposed to a variable yield. This variation of μ or Y_B does not appear to be related to S or ΔS. Whatever the reason for the observed effects, the value of μ_m and/or K_s is probably not the "true" value of that constant. Consequently, the value of k_e observed in batch substrate removal experiments is not the "true" value of

k_e - i.e., the value of k_e observed in continuous flow systems. The discussion below, of the high S/X growth experiments, may help to clarify the above discussion.

The Monod constants, μ_m and K_s, derived in the high S/X batch systems will be discussed at this point. Explanation and understanding of the varied findings will hopefully be made easier by the graphic presentation in Figure 37. Figure 37 will be referred to during the course of this discussion; along with other figures as they become relevant.

There are at least two good alternatives for explaining what occurred during "first generation" growth - both during and after the apparent lag period. These are presented in parts A and B of Figure 37. Represented in part A of Figure 37 is the concept of "viability", "active fraction", or any other hypothesis which purports to explain the observed decrease in specific activity of the sludge mass at high sludge ages whereby a portion of the sludge is assumed to consist of dead or inactive suspended solids. Represented in part B of Figure 37 is an alternative concept whereby the sludge is assumed to consist of bacterial cells whose metabolic processes are operating slowly both in the activated sludge system at high sludge age and initially in the batch reactor. After a period of time, the presence of a high substrate concentration produces a change within the cells - the apparent μ_m is greatly increased. These concepts are not mutually exclusive and either or both may in fact occur. It is also recognized by the author that the former concept is probably the preferred one. There have been innumerable

Figure 37. Growth of Seed from Activated Sludge System in High S/X Batch Reactor Systems ("First Generation" Growth)


A.
X TOTAL
X ACTIVE
OXIDATIVE ASSIMILATION
μ-IF AFFECTED BY ΔS
APPARENT μ (DURING LAG)
TRUE μ-IF NOT AFFECTED BY ΔS
APPARENT μ (AFTER LAG)
LOG X
TIME
B.
μ (AFTER LAG)
OXIDATIVE ASSIMILATION
LAG DUE TO CELLS ON SIDES OF FLASK
μ (LAG)
LOG X
TIME

papers published concerning "viability" and many activated sludge modellers, including McKinney, who have included the concept of active/inactive sludge fractions. While the concept of "viability" is questionable, there are readily perceivable quantities of intercellular slime/matrix material in activated sludge, which must be considered "inactive".

The apparent lag period can be interpreted in accordance with either of the above hypotheses. If the observed specific growth rates during the lag are interpreted as being a consequence of the existence of a sizeable inactive fraction in the biomass, the small K_s found for this period is understandable. Both the range and values of the specific growth rates observed during the lag period would be very small - giving both a small apparent μ_m and small apparent K_s (Figure 31). The occurrence of oxidative assimilation during the lag period, as discussed earlier, would tend to further overshadow the variation in μ with S_o. Both μ_m and K_s would be quite meaningless under these circumstances. The alternative hypothesis is that the metabolism of the individual cells within the biomass is much decreased. In this instance, the values of μ_m and K_s (Figure 31) may have some significance with respect to cell metabolism - at least, the same significance that is generally attributed to μ_m and K_s.

It should also be noted that the apparent rate of growth during the lag period seemed to be retarded in growth flasks which were left unattended until the end of the lag period. This was probably due to accumulation of cells on the walls

of the flask, where they were washed up by the shaking action. Access to substrate was probably limited by this occurrence.

The Monod constants, μ_m and K_s, observed during growth after the lag period were affected as shown in Figures 22 and 23. The observed value of K_s was found to be dependent on the initial biomass concentration, while μ_m was found to vary to a much lesser extent with variation of the initial biomass concentration. Again, this finding can be explained via either of the hypotheses pictured in Figure 37. If the proper hypothesis is the active/inactive fraction hypothesis; the initial sludge concentration would be expected to affect the observed value of K_s, but not the observed value of μ_m to any great extent. In accordance with this hypothesis, the range of initial substrate concentrations (via S/X ratio) and distribution of the biomass between the active and inactive fractions would both also affect the observed K_s. The range of initial substrate concentrations used in these growth determinations remained between 200 mg/l and 2000 mg/l glucose as COD. Where X_o was very high; the minimum S_o used was generally 300 or 400 mg/l, as the substrate was often exhausted before the end of the lag period when S_o was less than double X_o. It should also be noted that reaching the end of the lag period required from 15 to 34 hours and the remaining growth required from 8 to 12 hours more. The question arises as to whether a truly constant μ can be maintained over this period, with the substrate concentration changing constantly. This question is relevant

to both hypotheses, but in a different sense in each case. The change in metabolic activity hypothesis assumes that at the end of the lag period a certain amount of substrate has been removed - the amount of substrate removed being dependent in large part on X_o and to a lesser extent on S_o (Figures 29 and 30). The substrate remaining at the end of the lag period (S_t) replaces S_o in the Monod expression. The apparent K_s (K_{sA}) is displaced from the "true" K_s (K_{sT}) because S in the Monod equation is decreased before the "log" growth period begins; so that cell metabolism is "set" at S_t, rather than S_o. These are related quantitatively via the equation below. It can be seen that this hypothesis also

$$\mu = \frac{\mu_m S_t}{K_{sT}+S_t} = \frac{\mu_m S_o}{K_{sA}+S_o} \qquad (33)$$

which reduces to:

$$\frac{K_{sT}}{K_{sA}} = \frac{S_t}{S_o} \qquad (34)$$

where,

$$S_t = f(X_o, S_o) \qquad (35)$$

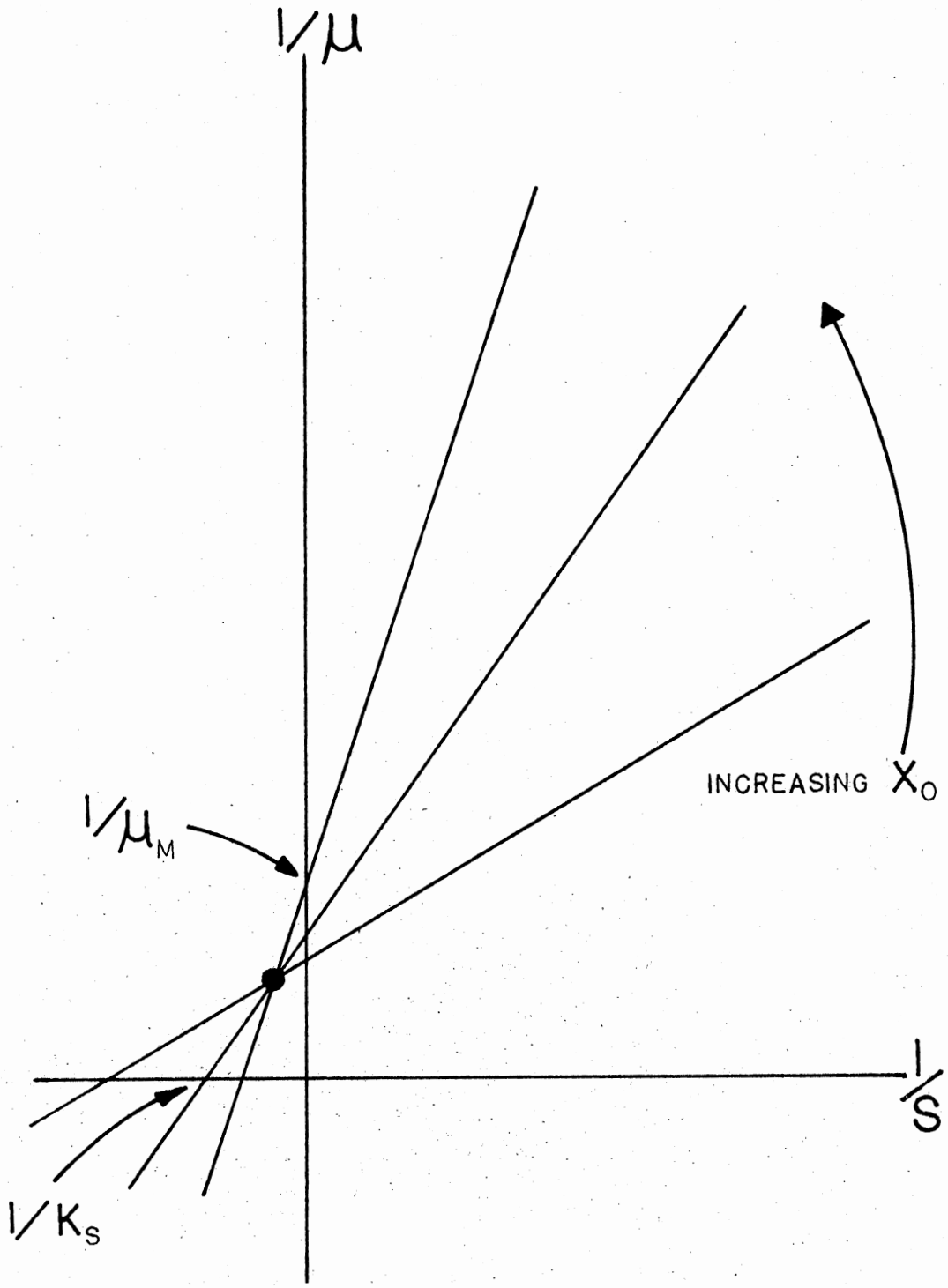
suggests that K_s is sensitive to X_o and S_o, but not to percentage inactive fraction (since it is not considered). Both hypotheses predict variation in K_s. The values of μ_m also varied slightly with X_o (Figure 23), although to a lesser extent than did the values of K_s. A graphic representation of the variation of both K_s and μ_m with varied X_o is

presented in Figure 38. The μ_m values observed at a given sludge age approximated the "second generation" values of μ_m at the relevant sludge age.

The values of K_s found in this study during "first generation" growth are higher than those cited by Gaudy and Gaudy (3) for a heterogeneous population grown on glucose. The values of K_s cited by Gaudy and Gaudy - 50 to 125 mg/l - were derived using "young", active microbial seed. A much "older" microbial seed drawn from an activated sludge reactor was used in the determinations of K_s presented in Figure 22. Saleh (17), Srinivasaraghavan (16), and Esfandi (65), using "older" seed also drawn from activated sludge reactors, found values of K_s ranging from the values cited by Gaudy and Gaudy to much higher values - Esfandi found a high K_s of 2000+ mg/l. These authors cited no values of initial sludge concentrations. The effect of X_o on K_s found here almost certainly explains the wide range of K_s values found by these authors. The values of K_s observed in "second generation" growth might reasonably be assumed to be the "true" value of K_s; which the values of K_s derived for "first generation" growth after the lag period approach. The "second generation" value of K_s is probably comparable to the values of K_s cited by Gaudy and Gaudy for "young", active seed.

The growth at the end of the lag period, using yeast extract and yeast extract/glucose as the carbon/energy source exhibited the same dependence of K_s on X_o as discussed above. The discussion above, concerning "first

Figure 38. Effect of X_o on K_s and μ_m During "First Generation" Growth After the Lag Period as Demonstrated by Lineweaver-Burke Plots of Hypothetical Growth Curves at Various Values of X_o


1/μ
1/μM
INCREASING X0
1/S
1/KS

generation" growth, applies here as well. However, μ_m did not vary with X_o as it did with growth on glucose alone. The Lineweaver-Burke plots of this data (Figures 26 and 27) suggest some sort of complex inhibition - perhaps substrate inhibition.

Although the results of this study suggest that the true cell yield and growth or substrate removal constants for a continuous flow system may be approximated by "second generation" batch experiments ("young", acclimated seed), batch determinations of the constants are subject to considerable variation. While batch determinations of the yield and growth/substrate removal constants were made at a number of sludge ages in this study, such would not be the case if batch determinations of the constants were made for design purposes. There would be no reason for batch experiments; if continuous flow data were available, since the continuous flow data would be the preferred source of the kinetic constants. The source of seed for batch determinations of the kinetic constants would be "young", acclimated biomass. These batch determinations are subject to considerable variation. The range of yields on glucose, with young heterogeneous populations, cited by Ramanathan and Gaudy (12) is 0.36 to 0.88 mg cell/mg COD. The ranges of values for K_s and μ_m, under similar conditions, cited by Gaudy and Gaudy (3), are 0.4 to 0.6 hour^{-1} and 50 to 125 mg/l, respectively. Predictions of sludge mass produced and effluent substrate concentrations vary over a wide range. The variations of yield are evident

above. The possible variation in predicted S_e, using the values of μ_m and K_s cited above, is evident if one uses the ratio of μ_m to K_s. The high value of μ_m/K_s is 0.29 l/mg·day, while the low value is 0.08 l/mg·day. The S_e predicted at a given μ is approximately inversely proportional to μ_m/K_s (at low S_e). The variation in S_e predicted at a given μ is nearly four-fold. At a given design value of S_e, the required sludge mass (volume times concentration) is also approximately inversely proportional to μ_m/K_s (at low values of S_e). If concentration of sludge in the aeration basin is assumed constant, the range of design values for required aeration basin volume is again nearly four-fold. The significance of this is that the design values of the kinetic constants should be carefully determined, preferably in a continuous flow system. The variation one is liable to encounter by using batch determinations of the kinetic constants or simply choosing "good engineering approximations" of the growth constants is, or should be, intolerable.

CHAPTER VI

CONCLUSIONS

1. The value of Eckenfelder's specific substrate removal constant (k_e) observed in batch experiments, using seed drawn from a continuous flow system operated at various sludge ages, remains essentially constant and consistently lower than the value of k_e observed in the continuous flow system.

2. Batch growth experiments, using seed drawn from a continuous flow system operating at a high sludge age, exhibit a very long lag period.

3. Substrate removal occurs during the lag period, but the apparent μ_m and K_s are very small.

4. The apparent value of K_s - and to a lesser extent μ_m - derived from growth rates observed after the long lag period are dependent on the initial microbial seed concentration.

5. The "second generation" batch values of μ_m and K_s are the "true" values of these constants and the best batch-derived predictors of the observed continuous flow values of effluent substrate concentration.

6. The value of the batch yields (Y_B) derived using seed drawn directly from a continuous flow reactor are

dependent on the net specific growth rate of the continuous flow system.

7. The value of the batch yield (Y_{B2}) derived using seed drawn from a batch experiment seeded from the continuous flow system is independent of the net specific growth rate of the continuous flow system and approximates the true cell yield (Y_t).

CHAPTER VII

SUGGESTIONS FOR FUTURE STUDY

Based on the results of this study, the following studies are suggested for future investigation.

1. Study the effect of S/X ratio in batch experiments, using seed drawn from continuous flow reactors operated at various sludge ages, on the batch yield.

2. Study the effect of varied μ_n on "first generation" values of μ_m and K_s, after the lag period, at a set value of X_o and a set range of values of S_o.

3. Study the effect of variation of S_o on "first generation" values of μ_m and K_s, after the lag period, at a set value of μ_n and a set value of X_o.

4. Compare values of "second generation" batch μ_m and K_s derived at various continuous flow sludge ages with each other and with the continuous flow μ_m and K_s.

5. Run high S/X, "first generation" batch experiments using activated sludge seed grown at high sludge age and monitor increase in biomass protein and carbohydrate content, along with biomass concentration, in order to determine if oxidative assimilation does in fact account for the "hump" in growth rate observed during the early hours of the lag period.

6. Run the above experiment using seed grown at various sludge ages in order to determine the quantitative effect of μ_n on the "hump" in growth rate which occurs during the lag period.

SELECTED BIBLIOGRAPHY

1. Monod, J., Recherches sur la Croissance des Cultures Bacteriennes. Hermann & Cie, Editeurs, Paris (1942).

2. Kincannon, D. F., and Gaudy, A. F. Jr., Functional Design of Aerobic Biological Waste Water Treatment Processes. Manual M-3, Center for Water Research in Engineering, Oklahoma State University, Stillwater, Oklahoma (1977).

3. Gaudy, A. F. Jr., and Gaudy, E. T., Biological Concepts for Design and Operation of The Activated Sludge Process. Project Report # 17090 FQJ 09/71, Environmental Protection Agency (1971).

4. Bauchop, T., and Elsden, S. R., "The Growth of Microorganisms in Relation to Their Energy Supply." J. Gen. Microbiol., 23, 457 (1960).

5. Servizi, J. A., and Bogen, R. H., "Free Energy as a Parameter in Biological Treatment." J. Sanitary Engineering Division, ASCE, 89, SA3, 17 (1963).

6. Servizi, J. A., and Bogen, R. H., "Thermodynamic Aspects of Biological Oxidation and Synthesis." J. Water Pollution Control Federation, 36, 607 (1964).

7. McCarty, P. L., "Discussion: Free Energy as a Parameter in Biological Treatment." J. Sanitary Engineering Division, ASCE, 89, SA6, 65 (1963).

8. Siegel, B. V., and Clifton, C. E., "Energy Relationships in Carbohydrate Assimilation by E. Coli." J. Bacteriol., 60, 573 (1950).

9. Siegel, B. V., and Clifton, C. E., "Energetics and Assimilation in the Combustion of Carbon Compounds by E. Coli." J. Bacteriol., 60, 585 (1950).

10. McKinney, R. E., Tomlinson, H. D., and Wilcox, R. L., "Metabolism of Aromatic Compounds by Activated Sludge." Sewage and Industrial Wastes, 28, 547 (1956).

11. Hetling, L. J., Washington, D. R., and Rao S. S., "Kinetics of the Steady State Bacterial Culture - Variation in the Rate of Synthesis." Proceedings, Industrial Waste Conference, Purdue University, Lafayette, Indiana, 687 (1965).

12. Gaudy, A. F. Jr., and Ramanathan, M., "Variability in Cell Yield for Heterogeneous Microbial Populations of Sewage Origin Grown on Glucose." Biotechnology and Bioengineering, XIII, 113 (1971).

13. Sawyer, C. N., "Bacterial Nutrition and Synthesis." In Biological Treatment of Sewage and Industrial Wastes - Vol. I, Reinhold Publishing Corp., New York (1956).

14. Ramanathan, M., and Gaudy, A. F. Jr., "Studies on Sludge Yield in Aerobic Systems." J. Water Pollution Control Federation, 44, 441 (1972).

15. Gaudy, A. F. Jr., and Engelbrecht, R. S., "Basic Biochemical Considerations During Metabolism in Growing versus Respiring Systems." Advances in Biological Waste Treatment, Vol. II, Pergamon Press, New York, 11 (1963).

16. Srinivasaraghavan, R., "Studies on a Kinetic Model for Design and Operation of an Activated Sludge Process Employing Constant Cell Feedback." Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma (1974).

17. Saleh, M. M. A. M., "Studies on the Behavior of a New Activated Sludge Process under Shock Loading Conditions." Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma (1976).

18. Lawrence, A. W., and McCarty, P. L., "Unified Basis for Biological Treatment Design and Operation." J. Sanitary Engineering Division, ASCE, 96, SA3, 757 (1970).

19. Gaudy, A. F. Jr., "Shock Loading Activated Sludge with Spent Sulfite Pulp Mill Wastes." J. Water Pollution Control Federation, 34, 124 (1962).

20. Eckenfelder, W. W. Jr., "Designing Biological Oxidation Systems for Industrial Wastes - Part 1." Wastes Engineering, 32, 216 (1961).

21. Wilson, I. S., "Concentration Effects in the Biological Oxidation of Trade Wastes." In Advances in Water Pollution Research, Vol. 2, Pergamon Press, New York, 27 (1964).

22. Wuhrman, K., "Factors Affecting Efficiency and Solids Production in the Activated Sludge Process." In Biological Treatment of Sewage and Industrial Wastes, Vol. I, Reinhold Publishing Corp., New York, 49 (1956).

23. McCabe, J., and Eckenfelder, W. W. Jr., "BOD Removal and Sludge Growth in the Activated Sludge Process." J. Water Pollution Control Federation, 33, 3, 258 (1961).

24. Tischler, L. F., and Eckenfelder, W. W. Jr., "Linear Substrate Removal in the Activated Sludge Process." Proceedings, 4th International Conference on Water Pollution Research, Prague, 361 (1968).

25. Chuboda, J., "Discussion: Linear Substrate Removal in the Activated Sludge Process." Proceedings, 4th International Conference on Water Pollution Research, Prague, 375 (1968).

26. Garrett, M. T., and Sawyer, C. N., "Kinetics of Removal of Soluble BOD by Activated Sludge." Proceedings, 7th Industrial Waste Conference, Purdue University, Lafayette, Indiana, 51 (1952).

27. Rao, B. S., and Gaudy, A. F. Jr., "Effect of Sludge Concentration on Various Aspects of Biological Activity in Activated Sludge." J. Water Pollution Control Federation, 38, 5, 794 (1966).

28. McKinney, R. E., "Mathematics of Complete-Mixing Activated Sludge." J. Sanitary Engineering Division, ASCE, 88, SA3, 87 (1962).

29. Goodman, B. L., and Englande, A. J. Jr., "A Unified Model of the Activated Sludge Process." J. Water Pollution Control Federation, 46, 2, 312 (1974).

30. Schulze, K. L., "The Activated Sludge Process as a Continuous Flow Culture - I." Water and Sewage Works, 11, 12, 526 (1964).

31. Schulze, K. L., "The Activated Sludge Process as a Continuous Flow Culture - II." Water and Sewage Works, 12, 1, 11 (1965).

32. Teissier, G., "Les Lois Quantitative de la Croissance." Ann. Physiol. Physicochim. Biol., 12, 527 (1936).

33. Gaudy, A. F. Jr., Ramathan, M., and Rao, B. S., "Kinetic Behavior of Heterogeneous Populations in Completely Mixed Reactors." Biotechnology and Bioengineering, IX, 387 (1967).

34. Ramanathan, M., and Gaudy, A. F. Jr., "Effect of High Substrate Concentration and Cell Feedback on Kinetic Behavior of Heterogeneous Populations in Completely Mixed Systems." Biotechnology and Bioengineering, XI, 207 (1969).

35. Monod, J., "The Growth of Bacterial Cultures." Ann. Rev. Microbiol., 3, 371 (1949).

36. Gaudy, A. F. Jr., Yang, P. Y., Bustamante, R., and Gaudy, E. T., "Exponential Growth in Systems Limited by Substrate Concentration." Biotechnology and Bioengineering, XV, 589 (1973).

37. Gaudy, A. F. Jr., Obayashi, A., and Gaudy, E. T., "Control of Growth Rate by Initial Substrate Concentration at Values Below Maximum Rate." Applied Microbiology, 22, 1041 (1971).

38. Peil, K. M., and Gaudy, A. F. Jr., "Kinetic Constants for Aerobic Growth of Microbial Populations Selected with Various Single Compounds and with Municipal Wastes as Substrates." Applied Microbiology, 21, 253 (1971).

39. Ardern, E., and Lockett, W. T., "Experiments on the Oxidation of Sewage without the Aid of Filters." J. Soc. of Chem. Ind., 33, 523 (1914).

40. Great Lakes - Upper Mississippi River Board of State Sanitary Engineers, Recommended Standards for Sewage Works (Ten State Standards) (1960).

41. Monod, J., "La Technique de Culture Continue. Theorie et Applications." Ann. Inst. Pasteur, 79, 390 (1950).

42. Novick, A., and Szilard, L., "Experiments with the Chemostat on Spontaneous Mutation of Bacteria." Proceedings, Nat. Acad. Sci., U.S., Washington, 36, 708 (1950).

43. Herbert, D., Ellsworth, R., and Telling, R., "The Continuous Culture of Bacteria. A Theoretical and Experimental Study." J. Gen. Microbiol., 15, 601 (1956).

44. Herbert, D., "A Theoretical Analysis of Continuous Culture Systems." Continuous Culture of Micro-organisms, Soc. Chem. Ind., Monograph No. 12, 21 (1961).

45. Herbert, D., "Some Principles of Continuous Culture." In Recent Progress in Microbiology, G. Tunewell (Ed.) Blackwell Scientific Pub., Oxford, England (1958).

46. Pirt, S. J., "The Maintenance Energy of Bacteria in Growing Cultures." Proc. Roy. Soc., Ser. B, 163, 224 (1965).

47. Helmers, E. N., et al., "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, II. Treatment with Domestic Sewage." Sewage and Industrial Wastes, 23, 884 (1951).

48. Heukelekian, H., et al., "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process." Sewage and Industrial Wastes, 23, 945 (1951).

49. Hoover, S. R., and Porges, N., "Assimilation of Dairy Wastes by Activated Sludge, II. The Equation of Synthesis and Rate of Oxygen Utilization." Sewage and Industrial Wastes, 24, 306 (1952).

50. Garrett, M. T. Jr., "Hydraulic Control of Activated Sludge Growth Rate." Sewage and Industrial Wastes, 30, 3, 253 (1958).

51. Eckenfelder, W. W. Jr., and O'Connor, D. J., "The Aerobic Treatment of Organic Wastes." Proceedings, 9th Industrial Waste Conference, Purdue University, W. Lafayette, Indiana, Ext. Ser. 89, 39, 2, 512 (1955).

52. Eckenfelder, W. W. Jr., and Ford, D. L., Water Pollution Control, Pemberton Press, Austin, Texas (1970).

53. Eckenfelder, W. W. Jr., Industrial Water Pollution Control, McGraw-Hill Book Co., New York (1966).

54. Busch, A. W., "Laboratory Units for Bench Scale Studies." Water and Sewage Works, 106, 6, 254 (1959).

55. Busch, A. W., "Treatability versus Oxidizability of Industrial Wastes and Formulation of Process Design Criteria." Proceedings, 15th Industrial Waste Conference, Purdue University, W. Lafayette, Indiana, 109 (1961).

56. Bhatla, M. N., Stack, V. T. Jr., and Weston, R. F., "Design of Waste Water Treatment Plants from Laboratory Data." J. Water Pollution Control Federation, 38, 4, 601 (1966).

57. Weston, R. F., and Stack, V. T. Jr., "Prediction of the Performance of Completely Mixed Continuous Biological Systems from Batch Data." Advances in Biological Waste Treatment, W. W. Eckenfelder Jr. and Brother J. McCabe (Ed.), Pergamon Press, Oxford, England (1963).

58. Weston, R. F., "Design of Sludge Reaeration Activated Sludge Systems." J. Water Pollution Control Federation, 33, 7, 748 (1961).

59. Jenkins, D., and Garrison, W. E., "Control of Activated Sludge by Mean Cell Residence Time." J. Water Pollution Control Federation, 40, 1905 (1968).

60. Sherrard, J. H., and Schroeder, E. D., "Relationship Between the Observed Yield Coefficient and the Mean Cell Residence Time in the Completely Mixed Activated Sludge Process." Water Research, 6, 1039 (1972).

61. Sherrard, J. H., and Schroeder, E. D., "Cell Yield and Growth Rate in Activated Sludge." J. Water Pollution Control Federation, 45, 1889 (1973).

62. Ramanathan, M., and Gaudy, A. F. Jr., "Steady State Model for Activated Sludge with Constant Recycle Sludge Concentration." Biotechnology and Bioengineering, XIII, 125 (1971).

63. Srinivasaraghavan, R., and Gaudy, A. F. Jr., "Operational Performance of an Activated Sludge Process with Constant Sludge Feedback." J. Water Pollution Control Federation, 47, 7, 1946 (1975).

64. Burkhead, C. E., and McKinney, R. E., "Application of Complete-Mixing Activated Sludge Design Equations to Industrial Wastes." J. Water Pollution Control Federation, 40, 557 (1968).

65. Esfandi, A., "Inhibition of Nitrification in Biological Systems." Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma (1977).

66. Standard Methods for the Examination of Water and Wastewater, 14th Edition, American Public Health Association, New York (1975).

67. Ramanathan, M., Gaudy, A. F. Jr., and Cook, E. E., Selected Analytical Methods for Research in Water Pollution Control. Manual M-2, Center for Water Research in Engineering - Bioenvironmental Engineering, Oklahoma State University, Stillwater, Oklahoma (1968).

68. Deaner, D. G., and Martinson, S., "Communication: Definition and Calculation of Mean Cell Residence Time." J. Water Pollution Control Federation, 46, 10, 2422 (1974).

APPENDIX

TABLE VII

RAW DATA FOR THE CONTINUOUS FLOW REACTORS

θ_c (days)	Date	V (liters)	F (l/day)	F_w (l/day)	X_F (mg/l)	X_e (mg/l)	S_i (mg/l)	S_e (mg/l)
2.4	10-11	8.1	17.5	2.7	1344	2	337	40
	10-13		15.0		1160	4	365	36
	10-14		16.5		1096	4	341	48
	10-26		17.0		1232	14	349	32
	10-29		16.5		1200	16	353	28
	11-1		16.0		1200	8	339	40
	11-3		17.0		1192	8	347	36
	11-5		16.0		1184	10	347	40
	11-6		17.0		1144	12	339	52
	11-8		16.5		1188	10	355	44
	mean		16.5		1194	9	347	39.6
	s.d.		0.7		64	4.5	8.8	7.2
	s.d.x100/mean		4.3		5.4	52	2.5	18.1
4.8	8-1	8.4	18.0	1.4	1460	22	345	28
	8-2		17.0		1340	26	333	36
	8-5		18.0		1348	22	351	44
	8-8		16.5		1432	12	291	56
	8-16		18.0		1440	22	350	28
	8-17		17.0		1325	14	346	31
	8-18		18.0		1436	10	346	35
	mean		17.3		1397	18	337	36.9
	s.d.		0.7		57	6.2	21.3	10.1
	s.d.x100/mean		4.0		4.1	34	6.3	27.4

TABLE VII (Continued)

θ_c (days)	Date	V (liters)	F (l/day)	F_w (l/day)	X_F (mg/l)	X_e (mg/l)	S_i (mg/l)	S_e (mg/l)
7.4	8-1	8.1	18.0	0.9	1584	20	353	44
	8-2		18.0		1616	22	361	28
	8-5		16.5		1648	6	347	44
	8-8		16.5		1780	20	355	48
	8-16		18.0		1672	10	327	24
	8-17		17.0		1654	12	339	31
	8-18		16.5		1692	6	350	35
	mean		17.2		1664	14	347	36.3
	s.d.		0.8		62	6.9	11.3	9.2
	s.d.x100/mean		4.4		3.8	49	3.3	25.3
11.5	5-26	8.1	18.0	0.6	3152	36	348	37
	5-28		17.5		3432	20	336	12
	5-30		17.0		3548	24	344	21
	6-2		16.5		3648	20	342	60
	6-3		17.0		3600	18	342	40
	6-4		18.0		3472	8	346	44
	6-5		16.5		3324	16	340	30
	6-6		17.0		3276	6	334	30
	6-9		17.0		3208	2	341	20
	6-11		17.5		3304	14	325	20
	mean		17.2		3396	16	340	31.4
	s.d.		0.5		169	9.8	6.7	14.3
	s.d.x100/mean		2.9		5.0	61	2.0	45.5

TABLE VII (Continued)

θ_c (days)	Date	V (liters)	F (l/day)	F_w (l/day)	X_F (mg/l)	X_e (mg/l)	S_i (mg/l)	S_e (mg/l)
16.7	11-29	8.4	18.0	0.45	5456	12	360	32
	12-1		17.0		5576	6	360	28
	12-8		18.0		5356	8	364	24
	12-10		18.0		5260	22	358	28
	12-15		18.0		5108	10	352	36
	12-18		17.0		5052	14	369	31
	mean		17.7		5301	12	360	29.8
	s.d.		0.5		202	5.7	5.7	4.1
	s.d.x100/mean		2.9		3.8	48	1.6	13.8

X_F and X_e were monitored for from 2 to 3 θ_c's prior to collection of the data in this table in order to ascertain that steady state conditions had prevailed in the reactor for said period prior to collection of the data in this table.

TABLE VIII

OPERATING DATA FOR THE CONTINUOUS FLOW REACTORS

Operating Parameter (Means)	Operating Condition No. 1	2	3	4	5
V (liters)	8.1	8.4	8.1	8.1	8.4
F_w (l/day)	2.7	1.4	0.9	0.6	0.45
F (l/day)	16.5	17.3	17.2	17.2	17.7
S_i (mg/l COD)	347	337	347	340	360
S_e (mg/l COD)	39.4	36.9	36.3	31.4	29.8
X (mg/l)	1194	1397	1664	3396	5301
X_e (mg/l)	9	18	14	16	12
X_A (mg/l)	995	1281	1572	3270	5159
t (days)	0.491	0.486	0.471	0.471	0.475
U (day^{-1})	0.630	0.483	0.420	0.200	0.135
Y_o (mg/mg)	0.660	0.432	0.323	0.435	0.444
μ_n (day^{-1})	0.415	0.208	0.136	0.087	0.059
θ_c (days)	2.41	4.80	7.38	11.5	16.7
K_m (COD) (mg/l)	15.9	16.7	18.2	20.9	23.3
k_e (COD) (day^{-1}(mg/l)$^{-1}$)	0.016	0.0131	0.0116	0.0064	0.0045

TABLE IX

BATCH SUBSTRATE REMOVAL EXPERIMENTS

θ_c (days)	X_o (mg/l)	K_m(COD) (day^{-1})	k_e(COD) (day^{-1} $(mg/l)^{-1}$)	K_m(bCOD) (day^{-1})	k_e(bCOD) (day^{-1} $(mg/l)^{-1}$)
2.4	739	7.56		17.6	
	1160	12.2		28.5	
	1232	11.6	0.0101	26.8	0.0235
4.8	432	4.84		11.3	
	716	10.4		24.3	
	1348	17.3	0.0136	40.3	0.0320
7.4	890	8.62		20.0	
	1648	19.1		44.2	
	502	3.80	0.0102	8.92	0.0242
11.5	1050	18.1		42.3	
	1750	32.6		75.9	
	3450	62.2	0.0180	150	0.0430
16.7	2104	20.2		47.4	
	5356	56.5	0.0101	128	0.0232

TABLE X

MONOD CONSTANTS FOUND DURING "FIRST GENERATION" GROWTH AFTER LAG PERIOD WITH HETEROGENEOUS POPULATION GROWN ON GLUCOSE

θ_c (days)	X_o (mg/l)	K_s (mg/l COD)	μ_m ($hour^{-1}$)
2.4	57	933	0.236
	133	1539	0.196
4.8	25	208	0.326
	80	1084	0.191
	145	1227	0.245
7.4	25	522	0.278
	80	1488	0.264
	170	1039	0.167
11.5	25	316	0.414
	50	783	0.411
	90	1283	0.344
	90	449	0.283
	100	1391	0.372
	170	1196	0.288
	200	2076	0.271
16.7	55	664	0.211
	110	976	0.191

TABLE XI

BATCH YIELD

θ_c (days)	S_o (mg/l COD)	S_f (mg/l COD)	X_o (mg/l)	X_f (mg/l)	Y_B (mg/mg)
2.4	1909	84	57	1188	0.62
	1909	68	57	1292	0.67
	2000	72	50	1324	0.66
	1000	48	50	696	0.68
4.8	1268	189	300	884	0.54
	2000	39	10	948	0.48
	2000	441	10	924	0.59
7.4	1204	299	310	780	0.52
	2000	244	10	1004	0.57
	2000	142	10	1012	0.54
11.5	988	157	96	504	0.49
	1137	755	100	296	0.51
	1000	266	25	372	0.47
16.7	1846	962	272	844	0.65
	1846	1523	103	324	0.68
	980	32	110	676	0.60
	980	52	55	652	0.64
	1960	79	110	1276	0.62
	1960	75	55	1176	0.59

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