A BATCH BIOKINETIC STUDY OF THE PREFERENTIAL SEPARATION OF A MIXED CULTURE OF MICROORGANISMS USING SMALL-SIZE HYDROCYCLONES

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TITLE: A Batch Biokinetic Study of the Preferential Separation of a Mixed Culture of Microorganisms Using Small-Size Hydrocyclones

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SCOPE AND CONTENTS:

The thickening and classifying characteristics of small size hydrocyclones were investigated. Test slurries, consisting of mixed cultures of microorganisms in water, were partitioned into two fractions by hydrocyclones ranging in size from 2 mm to 10 mm body diameter which operated at inlet pressures ranging from 80 to 200 psi and volume splits ranging from 1.0 to 3.0.

The classifying response was defined in terms of the relative magnitudes of the specific growth rate of the two fractions as determined by coincident observation of duplicate batch biological reactors, each seeded with one portion of the partitioned slurry.

The thickening response was defined by the Rietema-Tenbergen separation efficiency.

The biokinetic aspects of this investigation are emphasized in this manuscript.

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TABLE OF CONTENTS

PAGE NUMBER

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

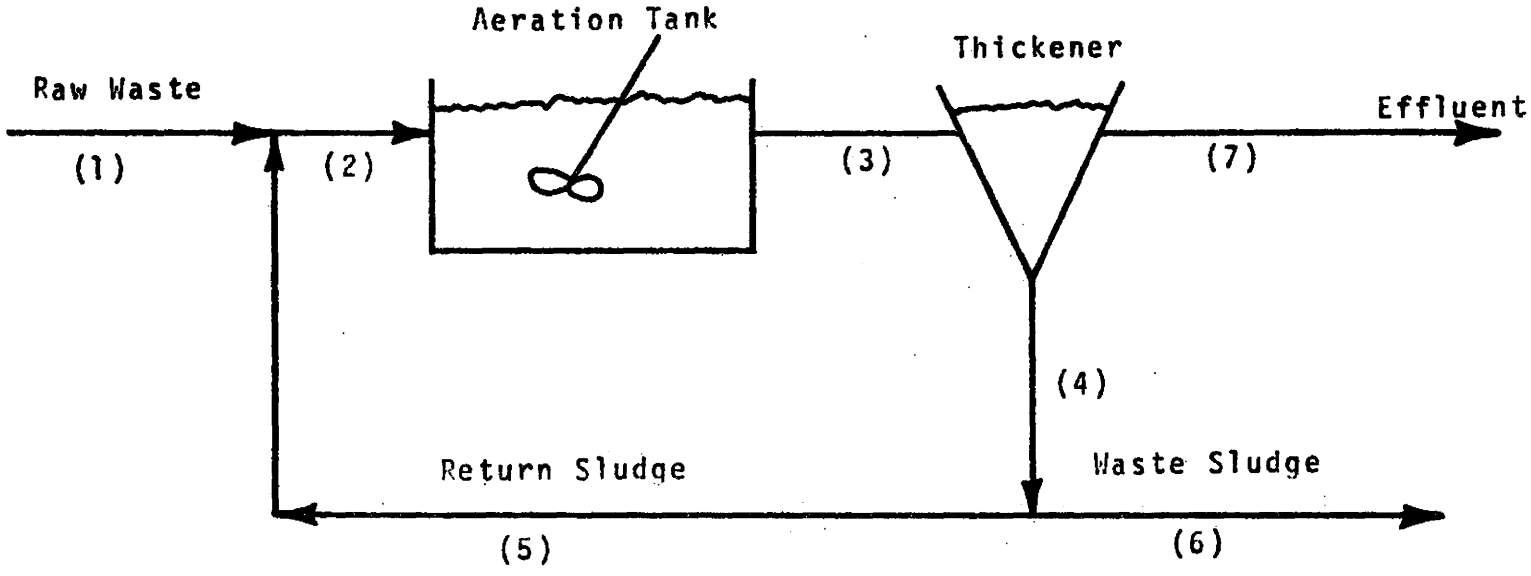
Since the work of Arden and Lockett (1914), the activated sludge biological treatment process has gained wide acceptance as a suitable vehicle for treating organic waste flows. This process, represented schematically in Figure No. 1 , is comprised of an aeration tank, in which organic material is removed from the waste flow by a resident population of micro-organisms, followed by a thickener, in which the microbial mass is separated from the aeration tank exit stream to provide a purified process effluent.

The aeration tank can be considered to be a well-mixed reaction vessel in which the reactants (organic material and micro-organisms) are contacted in an environment promoting a complex system of biochemical reactions which reduce the soluble organic content of the waste.

To operate this reactor most efficiently it is necessary to maximize the reduction of the organic content of the raw waste by the micro-organisms.

Previous studies have been directed to optimizing the rate at which micro-organisms can remove organic material with respect to such process parameters as residence time, rate of air addition, degree of mixing and reactant concentrations. Little consideration has been afforded the effect of optimizing the rate of substrate removal by improving the kinetic quality of the micro-organism population. This would afford a reduction in required reactor residence time and represent an increase in capacity for existing systems and a decrease in reactor volumes for new systems at a given effluent quality.

FIGURE 1



ACTIVATED SLUDGE PROCESS

It has been well established that the rate of removal of soluble organic material by micro-organisms is proportional to the microbial concentration. This suggests that increasing the solids concentration to a characteristic space limiting value would increase the velocity of organic disappearance to some maximum level. Thus, concentrated solids collected in the thickener are recycled to the entrance of the aeration tank to increase the velocity of substrate disappearance.

Helmers et al. (1951) and Heukelekian et al. (1951) have demonstrated that a section of the soluble organic fraction of the waste flow is converted to cellular protoplasm and is manifest as an increase in the mass and the number of micro-organisms. Since there exists an upper limit to the concentration of micro-organisms for which adequate gravity phase separation can be accomplished in the thickener, a portion of the microbial mass must be removed from the process as waste sludge to prevent microbial contamination of the process effluent. It is evident that process efficiency is optimized when the concentration of micro-organisms is maintained in a steady state condition at a level representing the capacity of the thickener.

The rate of increase of cell mass for the process is a function of type and relative numbers of micro-organism species present in the system, the characteristics of the raw waste stream, and the chemical and physical environment of the reaction vessel. Fluctuations in any of these entities can cause the process to drift from the optimum operating level by either overloading the thickener and contaminating the process effluent or by decreasing the concentration of micro-organisms in the reaction vessel and decreasing the overall rate of substrate removal.

By monitoring the effect of these fluctuations on microbial growth in the aeration tank and by providing a positive control on the amount of sludge wasted from the process it would be possible to maintain an activated sludge process at optimum operating conditions.

Activated sludge plants are designed so that it is virtually impossible to exercise positive control of the system relative to the concentration of the micro-organisms when fluctuations occur. In addition, the separation of micro-organisms from the carrier fluid of the aeration tank exit stream is usually accomplished by gravity thickening. Thickener performance is dependent on the type of micro-organisms present, micro-organism concentration and thickener residence time and, as a result, the phase separation efficiency is sensitive to variations in hydraulic and organic loading, (Busch, (1962)). Since these variations are common in field installations, the concentration of the thickener bottoms cannot be controlled and thus the quantity of sludge wasted from the system cannot be controlled. As a result, activated sludge plants are operated with no provision for maintaining optimum effluent purity in the event of variations in hydraulic and organic loading.

By replacing the gravity thickener with a phase separator capable of effecting positive control on the removal of suspended solids from the carrier medium, it would be possible to maintain effluent quality independent of hydraulic and organic variations in the raw waste flow. It would also be possible to increase the suspended solids concentration of the aerator and, thereby increase rate of disappearance of organics.

A study was therefore initiated to design and evaluate a phase

separator which would:

(1) provide a preferential separation in a biokinetic sense, of a mixed culture of micro-organisms to reduce the aeration tank capacity required for a given raw waste organic concentration.

(2) provide a positive control on the system concentration of micro-organisms whereby the rate of degradation of organics could be maintained at near maximum levels.

(3) provide a positive control on the separation of micro-organisms from the carrier medium independent of the hydraulic and organic loading to the process.

CHAPTER 2

PROBLEM DEFINITION

2.1 REACTOR GROWTH RATE CONTROL

Consider the schematic of the activated sludge process presented in Figure No. 1.

By assuming that solids neither enter the process in the raw waste flow (stream 1) nor leave the process in the effluent (stream 7) the volumetric flow rate and concentrations of micro-organisms and soluble organics in the various streams can be calculated:

Stream No.	Volumetric Flow Rate	Soluble Organic Concentration	Micro-organism Concentration
1	q	C_{C_o}	0
2	$(1 + \alpha\beta)q$	$\frac{C_{C_o} + C_C\alpha\beta}{1 + \alpha\beta}$	βC_B
3	$(1 + \alpha\beta)q$	C_C	C_B
4	αq	C_C	$(1 + \alpha\beta)\frac{C_B}{\alpha}$
5	$\alpha\beta q$	C_C	$(1 + \alpha\beta)\frac{C_B}{\alpha}$
6	$(1 - \beta)\alpha q$	C_C	$(1 + \alpha\beta)\frac{C_B}{\alpha}$
7	$(1 - \alpha + \alpha\beta)q$	C_C	0

where

α = the fraction of the total system flow which exits as thickener bottoms

β = the fraction of the thickener bottoms recycled

C_{C_o} = influent organic concentration of the waste flow

C_C = effluent organic carbon concentration

C_B = micro-organism concentration in the aeration tank

q = volumetric flow rate to the process

Performing a reactor mass balance on the micro-organisms:

$$V\frac{dC_B}{dt} = (1 + \alpha\beta)q\beta C_B - (1 + \alpha\beta)qC_B + r_{f_B}V \qquad (1)$$

where V = volume of the aeration tank

r_{f_B} = rate of production of the micro-organisms per unit reactor volume

Assuming steady state conditions: $\frac{dC_B}{dt} = 0$

$$\frac{r_{f_B}}{C_B} = \frac{q}{V}\left[(1 + \alpha\beta) - (1 + \alpha\beta)\beta\right] \qquad (2)$$

$$\frac{r_{f_B}}{C_B} = \frac{q}{V}\left[1 - \beta + \alpha\beta - \alpha\beta^2\right] \qquad (3)$$

where

$\frac{r_{f_B}}{C_B}$ = specific growth rate

A reactor mass balance on organic substrate gives

$$V\frac{dC_c}{dt} = V\, r_{f_c} + q[C_{c_0} + \alpha\beta C_c] - (1 + \alpha\beta)qC_c \qquad (4)$$

Assuming steady state conditions $\frac{dC_c}{dt} = 0$ and

$$r_{f_c} = -\frac{q}{V}(C_{c_0} - C_c) \qquad (5)$$

By assuming that some relationship exists between the rate of disappearance of organic substrate and the rate of increase of cell mass, the organic concentration of the reactor effluent can be expressed as a function of the specific growth rate.

By defining

$$r_{f_B} = -Yr_{f_c} \qquad (6)$$

where Y is an unknown function called yield, equation No.(5) can be expressed:

$$C_B Y \left(\frac{r_{f_B}}{C_B}\right) = \frac{q}{V} \left(C_{C_o} - C_c\right) \qquad (7)$$

$$[1 - \beta + \alpha\beta - \alpha\beta^2] = \frac{C_{C_o} - C_c}{C_B Y} \qquad (8)$$

For a given value of C_{Co} characteristic of the raw waste flow and for variations of Y characteristic of the resident microbial population, the optimum level of micro-organism concentration required to optimize the organic concentration of the process effluent must be maintained by carefully manipulating the levels of the volumetric flow rates of thickener bottoms and waste sludge.

Garrett (1958) has commented that:

"The difficulty of this procedure is apparent from the reputation the activated sludge process has acquired of being easily upset and requiring highly skilled operators".

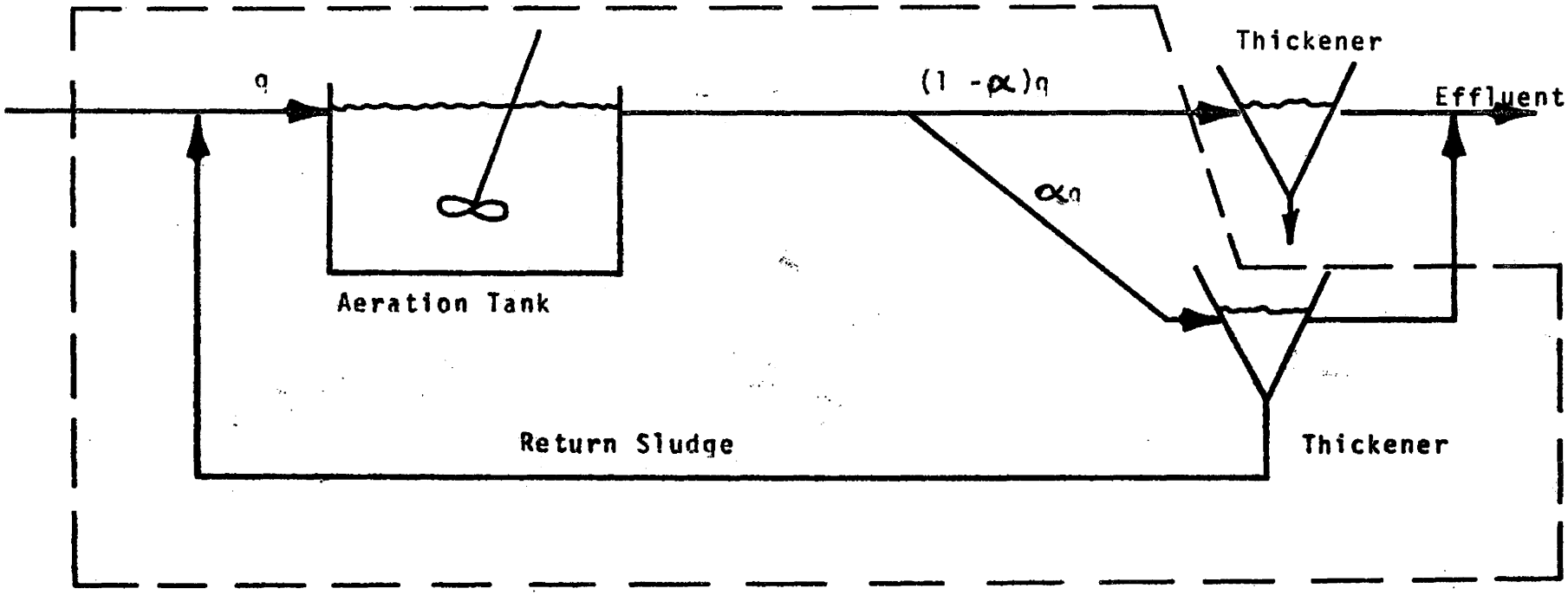
By using a scheme of solids control reported by Setter et al. (1945) and Wirts et al. (1951) in which solids are removed from the process by wasting all sludge settled in one or more settling tanks and returning all sludge from the rest of the settling tanks (Figure No. 2), Garrett presents a method of operation which provides a positive control on effluent purity.

Assuming steady state conditions, the mass balances around the designated facilities of Figure No. 2 can be expressed:

$$V \frac{dC_B}{dt} = -(1 - \alpha)qC_B + V r_{f_B} = 0 \qquad (9)$$

$$\frac{r_{f_B}}{C_B} = \frac{q}{V} (1 - \alpha) \qquad (10)$$

FIGURE 2



"GROWTH RATE CONTROL" MODIFICATION OF THE ACTIVATED SLUDGE PROCESS

(AFTER GARRETT)

assuming that no organisms enter the reactor in the raw waste flow, and

$$V\frac{dC_c}{dt} = q\,C_{c_o} - [(1-\alpha)q\,C_c + \alpha q C_c] + V\,r_{f_c} = 0 \quad (11)$$

$$r_{f_c} = -\frac{q}{V}(C_{c_o} - C_c) \quad (12)$$

Invoking the yield concept, equations No.10 and No.12 can be combined:

$$[1-\alpha] = \frac{C_{c_o} - C_c}{C_B Y} \quad (13)$$

The optimum level of micro-organism concentration in the reactor can be maintained for fluctuations in the soluble organic concentration of the influent and in the yield value of the resident microbial population by controlling the flow split from the reactor on a volumetric basis. Thus, the specific growth rate and hence the effluent purity can be controlled by the variation of a single operational parameter.

In order to maintain the purity of the process effluent independent of variations in the hydraulic and organic properties of the raw waste flow, it would be beneficial to be able to exercise the type of positive control inherent in the Garrett system. To optimize the rate of disappearance of the soluble organics, it would be beneficial to be able to increase the concentration of micro-organisms in the reactor to some level greater than that which represents thickener capacity.

Thus, this study will deal with an evaluation of a phase separator which would afford positive effluent quality control and which would be less sensitive to variations in micro-organism properties and concentration and hydraulic loading than gravity clarification.

2.2 PHYSICAL CHARACTERISTICS OF MICROBIAL GROWTH

When micro-organisms are inoculated into a suitable medium and incubated under appropriate conditions, an increase in numbers occurs to define a growth process. Nutrients from the medium are selectively taken into the cells and assimilated into protoplasmic material characteristic of the cell species. An increased amount of nuclear substance is produced and is manifest as an increase in cell volume. At a critical volume, defined as adult size, binary fission occurs resulting in daughter cells of approximately the same size and chemical consistency (Pelczar and Reid (1965)). Lamanna and Mallette (1965) indicate that the increase in size of micro-organisms is influenced by hereditary characteristics as well as environmental factors. This suggests that species subjected to the same environment may exhibit different growth characteristics. The differences in the growth of micro-organisms have been postulated to be indicative of a difference in the number of nuclei present per microbe: sigmoidal growth curves have been found to be characteristic of uninucleated organisms; exponential growth curves seem to be indicative of multinucleated organisms. (Lammana and Mallette (1965)).

The increase in bulk of an organism is accompanied by an increased uptake of water as well as synthesis of organic matter. Lammana and Mallette (1965) report that "in actively growing PROTEUS VULGARUS, a fivefold increase in volume is accompanied by only a twofold increase in dry weight". The rate of water uptake relative to dry weight increase is thought to be species dependent. Therefore, for a defined environment, the rate of increase in dry weight and the rate of increase in volume

(and therefore density) of a single micro-organism is a function of the species.

The importance of the physical dimensions of a cell relative to its rate of substrate uptake has been hypothesized in the Leuchart-Spencer principle. As the volume of a micro-organism increases, its surface area proportionately decreases such that the transport of toxic metabolic products out of the internal cell regions proceed at rates which are too slow to sustain maximum growth. This postulate indicates that for a given environment, the rate of utilization of substrate per organism would be proportional to the surface to volume ratio of the cell.

For the mixed cultures (i.e. several co-existing species) prevalent in waste treatment plants, it is evident that there exists a range of micro-organism sizes and densities due to interspecies and intraspecies growth. Thus, a range of surface to volume ratios exists and suggests that for a given time interval a spectrum of kinetic ability is present for the culture. Thus, if a mixed culture of micro-organisms could be preferentially partitioned on the basis of their relative sizes, it may be possible to obtain a micro-organism population possessing faster overall kinetic reaction rates than those of the original culture.

2.3 CRITERIA FOR SOLID-LIQUID PHASE SEPARATOR

From these considerations, the criteria established for the specification of a liquid-solid separator amenable to this investigation were that the device be capable of:

(1) performing the function of a selective classifier for a slurry containing a distribution of partial shapes, sizes and densities.

(2) affording positive control for the extent of slurry partitioning on a volumetric basis

(3) providing a high degree of solid-liquid phase separation.

Separation of the solid biological phase from the liquid carrier medium was expected to be dependent on the slurry characteristics and since no appreciable laboratory-prototype scaling was expected for slurry properties, the separator would have to be effective over a wide range of volumetric flowrates. These principles eliminated the amenability of the gravity or pressure filter separating methods prevalent in the waste-water industry as well as the techniques of centrifuging common to microbiology. The hydrocyclone appeared to be the only device to satisfy the established criteria.

2.4 DESCRIPTION OF THE HYDROCYCLONE

The hydrocyclone is a piece of equipment which utilizes fluid pressure energy to create rotational fluid motion. This rotational motion causes relative movement of materials suspended in the fluid, thus permitting separation of these material, one from another (classifying) or from the fluid (thickening). The rotation is produced by tangential injection of the fluid into a vessel. The outlet for the bulk of the fluid is usually located near to or on the axis of the vessel such that the rotating fluid is forced to spiral towards the center to escape. A rotational motion has thus built into it an inward radial motion. Particles of a suspended material consequently have two opposing forces acting on them, one in an outward radial direction due to the centrifugal acceleration, and one in an inward radial direction due to the drag force of the inward moving fluid.

The magnitude of these forces is dependent on the physical properties of both the fluid and the suspended material (particle size, particle shape, particle density, fluid density, fluid viscosity), and use of these properties can be made to effect separation of one material from another or of a single material from the fluid.

One product moves radially outwards while the other moves radially inwards. It is, therefore, necessary to provide two outlets. The usual design is shown in Figure No. 3 illustrating the tangential feed inlet, the main fluid outlet (overflow) and the peripheral fluid outlet (underflow). The overflow is taken out axially through a pipe (vortex-finder) which protrudes from the roof of the conical base. The underflow is taken out through an opening in the apex of the conical section.

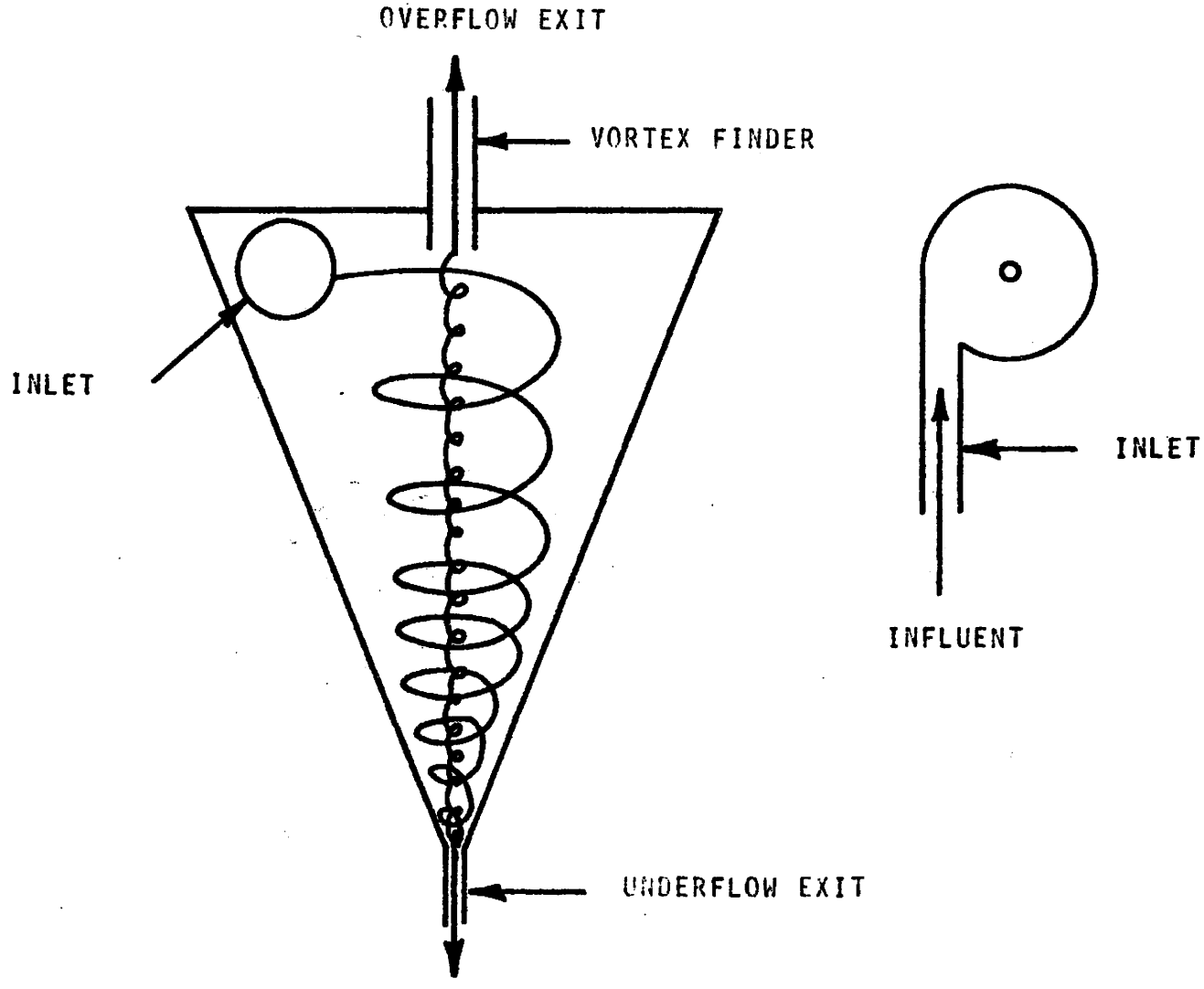
2.5 ACCEPTABLIITY OF HYDROCYCLONES FOR THIS STUDY

The use of the hydrocyclone as a tool for classifying and thickening solid-liquid streams has been well documented.

Fontein et al. (1962) reports that the size, shape and specific gravity of slurry particles influence the classification performance of hydrocyclones. A mixture of starch particles consisting of two equal density size fractions (50 - 75μ DIA and 15 - 35μ DIA) were partitioned into two exit streams representing a classification effectiveness of greater than 99%. Further testing with polystyrene spheres and discs having the same settling velocities indicated that similar classification efficiencies could be obtained based on particle shape differences.

Bradley and Pulling (1959) investigated flow patterns in hydraulic cyclones by dye injection to validate theoretical predictions for the

FIGURE 3



THE HYDROCYCLONE

mantle of zero vertical velocity and the classification surface. Using a particle size distribution of perspex spheres, they found that a well defined classification could be obtained based on particle diameter. These studies further indicated that almost 100% thickening efficiency could be obtained for particle diameters approximately four times larger than the cut size diameter.

Dahlstrom (1954) reports that particle size distribution, particle shape and feed concentration are the most important solid properties affecting the performance of the hydrocyclone. High feed-solid concentrations can exhibit significant particle-particle interaction and result in an increase in the particle cut size diameter.

Fitch and Johnson (1953) and Dahlstrom (1949) have indicated that "hindered settling" effects must be considered at solid feed concentration at approximately 11% by volume. Dahlstrom (1954) reports an increase in cut size diameter of 65% and a decrease in the sharpness of classification for 2.70 specific gravity silica sand operating at a volumetric solid feed concentration of 20%. The influence of particle size distribution on the sharpness of classification was investigated by Fitch and Johnson (1953). They observed a general increase in the cut size diameter as the coarseness of the feed solids was increased. In addition to particle size they found that particle density and particle shape had a significant effect on the size separation efficiencies. Particles with diameter less than cut size, but with high specific gravities were observed to migrate to the underflow exit rather than report to the overflow exit as expected. The shape of solids below the cut size diameter were observed to influence the amount of theoretical overflow solids which in fact become entrained in the voids of the underflow slurry and reported to that exit. These phenomena

decreased the sharpness of classification to the extent that the cut size particle had to be expressed as a range of diameters.

Moder and Dahlstrom (1952) studied the effect of particle size, particle specific gravity and volume split (the volumetric ratio of overflow discharge to underflow discharge) on the solid separation characteristics of a hydrocyclone. Using a test slurry consisting of spherical plastic beads with a size distribution in the range -25 to +100 ASTM mesh and with a specific gravity distribution bracketing that of the carrier medium, they performed a statistically designed experimental study. All main variables, plus the volume split-particle size and the volume split-specific gravity interaction were observed to be significant for both overflow (float solids) and underflow (sink solids) exit streams. The relative importance of the statistical variables were not the same for both exit slurries and were found to be a function of the weight ratio of sink and float solids present in the feed. Empirical performance characteristics were developed and tested against experimental data obtained on an industrial slurry. Good agreement was obtained between the calculated and experimental float solids separation, but appreciable deviations were exhibited for the sink solid separation. The latter was ascribed to hindered discharge at the underflow exit and to the extremely low sphericity of the sink material.

Bergman et al. (1956) discuss the use of the hydrocyclone in the separation of barite from clay in drilling fluid slurries. They report that 80 to 90 percent barite recovery with clay contamination ranging from 2 to 15 percent could be obtained under proper operating conditions. Although no data are presented, the influence of particle size, shape and density was felt to account for the range of classification efficiency.

Since the relative motion between particles and the carrier medium under the influence of the force fields generated by the hydrocyclone determine the separation or classification efficiency, the particle-medium density difference exerts an influence on hydrocyclone performance. Bradley (1965) indicates that for hydrocyclones of diameter greater than 10 mm, the cut size particle diameter (DP_{50}) is related to the solid-liquid density difference by the relationship:

$$D_{p_{50}} \alpha (\rho_{particle} - \rho_{liquid})^{-0.5}$$

For smaller hydrocyclones, the author found the relationship

$$D_{p_{50}} \alpha (\rho_{particle} - \rho_{liquid})^{-0.62}$$

to give a better correlation.

Therefore, for a given distribution of particle sizes, a hydrocyclone can be designed and operated to provide a sharp two-stream slurry partitioning based on particle diameter. The cut size particle diameter is expected to be dependent on particle density and the sharpness of the classification is expected to be a function of particle shapes. By defining the cut size diameter to be that of the smallest particle in the slurry, it would be possible to design and operate a hydrocyclone as a solid-liquid phase separator. The performance of the hydrocyclone can be made relatively independent of volumetric flowrate by using several units in parallel, each accepting a controlled fraction of the total flow.

Further, by valving the underflow exit, it would be possible to provide a positive control on the volumetric splitting of the influent. Although

much work has been done in evaluating the relationship between volume split and classification efficiency, this effect, being an operating variable, is germain to specific slurry properties and hydrocyclone geometries and must be evaluated for each application.

From these considerations, it is evident that the hydrocyclone satisfies the criteria established for a thickener-classifier amenable to this investigation.

CHAPTER 3

BIOCHEMICAL KINETICS

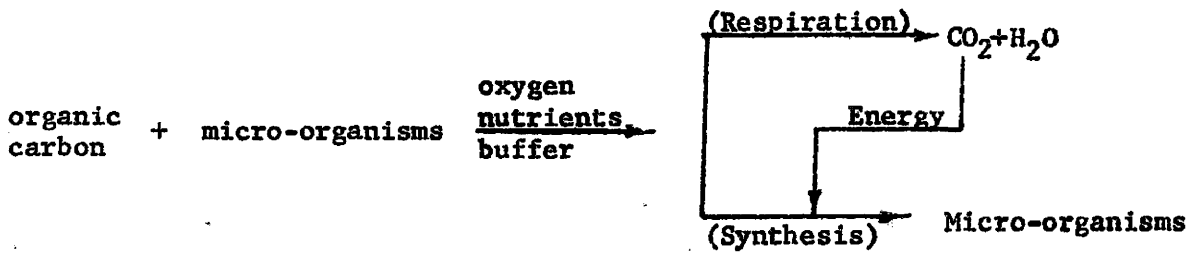
3.1 THE IMPORTANCE OF BIOCHEMICAL KINETICS TO THIS STUDY

In order to maintain a desired concentration of soluble organics in the aeration tank effluent for variations in the quantity of organic material entering the process, it is necessary to control the concentration of micro-organisms in the reactor. Thus, the velocity at which the resident microbial population utilizes the organic material and the rate at which the disappearance of organics is manifest as an increase in micro-organism concentration must be determined to afford a positive "growth rate control" on the quality of the exit stream.

To investigate the feasibility of optimizing reactor residence time by partitioning a mixed culture of micro-organisms, it is necessary to compare the rates of substrate utilization by the micro-organisms of the effected separation.

Therefore, this evaluation of the hydrocyclone will necessarily involve a study of the biochemical kinetics of the effected slurry separation.

Engineers have traditionally summarized the biochemical reactions occurring when soluble organic waste is contacted with acclimated micro-organisms as:



A portion of the organic waste reactant is converted into cellular protoplasm; the remainder is oxidized to provide energy (Heukelekian et al. (1951) and Helmers et al. (1951)).

Several "reaction kinetic mechanisms" have been proposed in an effort to model the activated sludge process.

3.2 FIRST ORDER KINETIC MODEL

Streeter and Phelps (1925) interpreted the Biochemical Oxygen Demand (BOD) test to be a batch bioassay technique measuring the progression of organic waste degradation. They found that the BOD data of Theriault could be represented by the first order reaction equation:

$$-\frac{dy}{dt} = ky$$

where y = substrate concentration as BOD
k = rate constant
t = time

Wilson (1967)

Subsequent investigation of many treatment plant data substantiated Streeter and Phelps findings and first order kinetics became widely accepted as a suitable model for the activated sludge process.

The first order kinetic model of Streeter and Phelps became suspect when it was discovered that specific organic wastes could not be characterized by unique levels of the rate constant "k". This led several workers to conclude that the rate of substrate removal was coupled to the rate of growth of the microbial population.

3.3 SECOND ORDER KINETIC MODELS

Keshavan et al. (1964) assumed the rate of change of volatile suspended solids to be proportional to the concentrations of suspended solids and organic substrate:

$$\frac{ds}{dt} = ksy$$

where "s" is the concentration of volatile suspended solids.

By further assuming that a constant fraction of the substrate removed was converted to volatile suspended solids (i.e. constant yield), these authors described the progression of substrate removal by:

$$-\frac{dy}{dt} = k'sy \quad \text{where} \quad k' = -\frac{dy}{ds}k$$

Using an acclimated mixed culture of activated sludge and a complex synthetic waste, the authors obtained a good empirical fit for the second order kinetic model for batch operation in which substrate concentrations were expressed as BOD_5.

Young and Clark (1965) proposed a second order kinetic equation to model the progression of BOD exertion:

$$-\frac{d(L-z)}{dt} = k\,(L-z)^2 \qquad \text{where } L = y_{max} \text{ and } z = \text{BOD exerted}$$

The authors advocated acceptance of the second order kinetic model based on the mathematical similarity to the Monod (1949) development, and the ease with which model constants could be evaluated. The first order model of Streeter and Phelps was not fundamentally rejected, but its use was discouraged due to the laborious calculations required for mathematical analysis.

Revelle et al (1965) used the second order autocatalytic kinetic expression:

$$\frac{dz}{dt} = k\,(z+b)\,(L-z)$$

where b = empirical constant related to the concentration of micro-organisms to fit data for the exertion of the BOD of a synthetic waste. They proposed that the empirical fit of the proposed model during the lag and exponential growth phases constituted proof of the kinetic mechanism. Wilson (1967) points out that the second order reaction equation has the well-known property that when the concentrations of the two reactions are very dissimilar, control is exercised by the one of lower concentration.

Thus, the autocatalytic model could be expected to fit data reasonably well only for low values of organism or substrate concentration.

3.4 TWO-PHASE MODEL

Eckenfelder (1959) proposed a three-phase mechanistic interpretation of batch bio-oxidation kinetics which coupled the various phases of sludge growth and BOD removal by a dynamic relationship between the mass transfer of nutrients into the cell and the use of these nutrients in the function of cell metabolism. Mathematically, Eckenfelder expressed the reaction model by a discontinuous function consisting of two intersecting linear curves. At high concentrations of organic substrate, the process was described as reaction limiting, for which the rate of disappearance of substrate was independent of substrate concentration but was a direct function of the generation time of sludge cells. By assuming this phenomenon to be indicative of logarithmic batch growth (constant generation time) and by invoking the concept of constant yield, the specific growth rate (rate of utilization of substrate per unit mass of micro-organisms) was a constant

$$-\frac{1}{s}\frac{dy}{dt} = k_1$$

At some level of substrate concentration, the rate of mass transfer of limiting nutrient into the cell and the rate of growth of the sludge cells were considered to be identical. At concentrations below this "equilibrium" value, the growth rate, and hence the BOD removal, was thought to be limited by the diffusive nutrient flux. By assuming a constant surface area per organism, a constant mass per organism,

and a constant yield coefficient, Eckenfelder suggested that the specific growth rate was a linear function of the limiting nutrient concentration:

$$-\frac{1}{s}\frac{dy}{dt} = k_2 y$$

Eckenfelder found the above equation adequately described the course of oxidation of most organic compounds in heterogeneous waste mixtures. This second order kinetic model was later modified (McCabe and Eckenfelder (1961)), to the first order kinetic expression:

$$-\frac{dy}{dt} = k_2 y$$

to describe the utilization of single organic compounds. The apparent first order kinetic response of the system was ascribed to the use of non-specific measurements of substrate concentrations as BOD and COD.

For heterogeneous waste mixtures, the authors observed a progressive decrease in the "reaction rate constant" k_2, indicating that the proposed kinetic models were not fundamentally correct in the regions of low substrate concentrations. The authors suggest that this phenomenon may be ascribed to auto-oxidation in which the auto-oxidation rate deviates from first order kinetics with time as the remaining cellular constitutents become more difficult to oxidize. The amount of oxidation is felt to be a function of the degree of initial BOD removal by adsorption, however, no attempt was made to correlate the dependence. In order to obtain a reasonable empirical correlation, the authors advocate the use of either a retardant-type reaction or a composite exponential for the substrate limiting region. Adequate mathematical fits were obtained using the retardant formula of

Fair et al (1941):

$$-\frac{dy}{dt} = \frac{ky}{(1+mt)}$$

and of Fair and Geyer (1954):

$$-\frac{dy}{dt} = ky\left(\frac{y}{y_{max}}\right)^n$$

where "m" is an empirical coefficient of retardation and "n" is an empirical non-uniformity coefficient. A more fundamental postulate for the observed phenomena involved the concept of sequential removal of several organic compounds, each with a characteristic kinetic mode. The latter approach admitted the impossibility of representing the progression of organic substrate disappearance, measured as BOD, by a single overall kinetic expression.

Deviations of the mathematical fits employed from empirical data are ascribed by McCabe and Eckenfelder (1961) to the release of cell components by lysing and the use of oxygen for "endogenous respiration", both of which may effect non-representative measurements of substrate concentration as BOD.

3.5 ENZYME KINETIC MODELS

Since the utilization of soluble organic substrate by micro-organisms is viewed as an enzyme catalyst process, many microbiologists have accepted the concept of the Michaelis-Menton (1913) kinetic model. These workers represented the reaction of enzyme with substrate as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.S$$

$$E.S \xrightarrow{k_3} E + P$$

where E = enzyme concentration

S = substrate concentration

E.S = enzyme-substrate complex

P = product

By defining a system in terms of a single enzyme, a single substrate, a single product and no inhibitors, and applying the Stationary State hypothesis to the enzyme substrate complex for a batch system with no substrate or nutrient limitation, they obtained the following expression for the proposed kinetic model:

$$v = \frac{VS}{K_m + S}$$

in which v = the rate of product formation

V = maximum model velocity

K_m = Michaelis-Menton constant = 0.5V

This expression indicates that the rate of product formation is gradually reduced from the maximum rate as substrate becomes limiting.

The Michaelis-Menton model was verified as a suitable mathematical representation of the batch growth rate of pure bacterial cultures by Monod (1949). In his studies of bacterial growth, Monod empirically obtained constant yield values and applied them to the observed exponential and declining growth characteristics to obtain a mathmetical model for the rate of batch substrate disappearance:

$$\mu = \mu_{max}\left[\frac{C_c}{K_m + C_c}\right]$$

where $\mu = \frac{1}{C_B}\frac{dC_c}{dt}$ = specific rowth rate

μ_{max} = maximum value of specific growth rate

K_m = Michaelis-Menton constant = $0.5\,\mu_{max}$

The Monod expression was verified for batch and continuous pure cultures systems by Novick (1955) and Herbert et al. (1956) and for mixed culture continuous systems by Grieves et al. (1963), with the result that it has been widely applied to the activated sludge process.

Rather than recognizing Monod's model as a curve fitting technique, several workers have interpreted it as a kinetic mechanism and have ascribed deviations of experimental data from the exponential equation to the biochemical characteristics of the biological population under test expressed by a kinetic rate expression.

Reynolds and Yang (1966), working with mixed microbial populations and complex synthetic wastes, obtained good empirical fits by augmenting the Monod expression with a kinetic expression, first order relative to micro-organism concentration, accounting for endogenous respiration under both batch and steady-state continuous operation. Middlebrooks and Garland (1968) developed a similar kinetic expression based on the assumption that continuous activated-sludge systems incorporating solids separation and recycle are susceptible to the effects of organism decay, thereby necessitating a decay rate in any kinetic model for the process. Similar kinetic models have been reported by Gram (1956), Stewart (1958), Andrews et al (1964) and Agardy et al (1963).

Other relationships between the rate of micro-organism growth and the substrate concentration have been proposed by microbiologists. The relationship

$$\mu = K_m\,(1 - e^{-zC_c})$$

where z and K_m are empirical constants, was proposed by Tessier (1936) and used by Spicer (1955) and Lipe (1961). Schulze (1964) studied the continuous growth of E. COLI, on an organic substrate of glucose and urea. His data on the removal of glucose was adequately fit by the Tessier expression and he suggested that this equation would also apply to activiated sludge cultures. Contois (1959) suggested that the parameter by which growth should be measured is not cell mass, but population density. He studied the specific growth rate of pure bacterial cultures in stead-state chemosats, and fit his data by the relationship

$$\mu = \mu_{max}\left(\frac{C_c}{FC_M + C_c}\right)$$

where $F = \frac{\mu}{C_c} = \frac{q}{VC_c}$

This equation expresses a dependence of specific growth rate on the ratio of cell mass to culture volume.

3.6 ADSORPTION MODELS

Dean and Hinshelwood (1966) consider the reactions of the microbial metabolic pathways to be mathematically analogous to the Langmuir Adsorption Isotherm. They mechanistically define biochemical reactions in terms of chemical reactions occurring on porous catalysts. By considering a matrix of high molecular weight enzymes to be representative of catalyst surfaces in which suitable reaction sites are determined by atomic spacing; they mathematically describe dynamic equilibrium between the rates of deposition and removal of substrate molecules in an adsorption context.

By assuming that the rate of deposition of free substrate molecules on to the enzyme "surface" is proportional to the amount of free surface $(1 - \sigma)$,

and to the concentration of substrate molecules in the environment (C_c) the rate of deposition is

$$K'C_c\ (1 - \sigma)$$

In further assuming that the inverse process is proportional to the density of adsorbed substrate moleules on the surface, the rate of removal is

$$K''\sigma$$

By defining the adsorption phenomenon to be in a state of dynamic equilibrium:

$$K'C_c(1 - \sigma) = K''\sigma$$

therefore

$$\sigma = \frac{bC_c}{1 + bC_c} \qquad \text{where } b = K'/K''$$

The authors stipulate that the adsorbed molecules enter biochemical reactions at a rate proportional to their surface density:

$$\text{rate} = \frac{KC_c}{1 + bC_c} \qquad \text{where K is a constant}$$

By assuming $\frac{KC_c}{1 + bC_c}$ to be specific growth rate, the authors justify the adsorption isotherm by noting that it is mathematically similar to the Monod expression. It should be noted that the shape of the "rate equation" can be altered to fit virtually any specific growth rate data by ascribing empirical deviations to adsorption inhibiting effects caused by (a) catalyst poisoning (b) electrostatic repulsion and attraction forces on moleules and (c) decreased substrate diffusion due to product formation at reaction sites.

Katz and Rohlich (1956) develop an adsorption mechanism to represent the removal of soluble organics by activated sludge. They consider a model

in which the diffusive flux of substrate through a water film surrounding a microbial floc is the rate controlling step for substrate removal.

Thus the diffusive flux is defined as the rate of adsorption per unit floc area and an empirical multiple of the coefficient of diffusivity is interpreted as the "kinetic reaction rate constant" characterizing the disappearance of substrate. Empirical justification for the adsorption mechanism is professed by the constancy of measured "rate constant" for a series of chemostats operating over a range of steady-state substrate concentrations. The data presented indicate that the value of the rate constant was not independent of steady-state substrate concentration refuting the use of the adsorption mechanism as a kinetic model as advocated by the authors.

Katz and Rohlich mathematically fit their data by the adsorption isotherm

$$\frac{x'}{M} = K' C_e^{\,n}$$

where x' = mass of adsorbate (as BOD) originally present in solution
M = mass of activated sludge
C_e = equilibrium concentration
$K', n,$ = empirical constants

3.7 ZERO ORDER KINETIC MODELS

Using the Jenkins modification of the Warburg apparatus, Wilson (1967) studied the oxygen uptake curves exerted by mixed cultures of micro-organisms on simple substrates. He found that uptake vs time curves for phthalic acid, glutamic acid, tyrosine and leucine were independent of organic substrate concentration and dependent only on micro-organism concentration to a sharp break-point. The break-point

in every case coincided with the exhaustion of extracellular substrate measured as BOD_5 and COD. Further studies with binary and ternary substrate mixtures indicated that the break-point coinciding with the elimination of each substrate constituent remained clearly defined.

Mixtures of amino acids comprised of more than three constituents resulted in compound uptake characteristics which approximated a first order kinetic response. The author concluded that the apparent first order rate plots widely reported for waste liquors are in fact summations of a number of curves of zero order with respect to substrate concentration.

The switch rate of micro-organisms from one food source to another was observed to have a significant effect on the shape of the oxygen uptake vs time curve for complex substrates. For a mixed microbial population in which each species was acclimated to a single constituent of a complex substrate, a net uptake curve resembling a second order autocatalytic kinetic mechanism was observed.

While the reported "zero order" phenomenon explains the variety of kinetic mechanisms prevalent in the literature, the use of oxygen uptake to predict kinetic response must be questioned.

Tishler and Eckenfelder (1968) observed zero order kinetic responses with respect to substrate as COD for mixed cultures utilizing synthetic feed mixtures of glucose, aniline and phenol. They found that the linear substrate removal rates characteristic of each compound remained unchanged when binary and ternary mixtures were tested, each being a function of solids concentration.

The overall organic removal rate for substrate mixtures was a summation of the linear removal rates of the constituent compounds producing a resultant removal characteristic similar to a first order kinetic response.

The authors postulate that the two-phase kinetic model reported by Garrett and Sawyer (1952) and McCabe and Eckenfelder (1960) "is probably the result of zero order removal of readily assimilable compounds, followed by a slower zero order removal of other compounds, the latter being mathematically approximated by a first order type equation".

Monod (1949) was the first researcher to observe linear substrate removal. Using pure cultures of E.COLI growing on a glucose substrate, he observed a value of the Michaelis-Menton constant of 4 mg/l. Thus, the Michaelis-Menton kinetic model

$$\mu = \frac{\mu_{max}\ C_c}{K_m + C_c}$$

reduced to $\mu = \mu_{max}$ for $C_c \gg K_m$. Wuhrmann (1956) was the first to suggest that the non-linear removal mechanism commonly associated with complex wastes are actually a summation of a number of zero order removals of the sample compounds which make up the complex substances.

3.8 MATHEMATICAL MODEL

Martin and Washington (1963) developed a mathematical model for the continuous-flow, steady-state bacterial culture. In applying a mass balance to the microbial culture, the rate of change of solids in the reactor was ascribed to two contributing phenomena. The mass of micro-organisms increased due to substrate utilization according to a "true" specific growth rate function and decreased due to microbial death. Both contributions were assumed to be linear functions of the steady state microbial mass and combined to define an "effective" specific growth rate, which could be measured experimentally. Pure cultures of PSEUDOMONAS FLORESCENS and ESCHERISHA COLI B. were studied on substrates of glucose, acetate and

glutamic acid in chemostat reactors. Steady-state concentrations of reactor substrates as COD were observed as a function of the reciprocal of reactor residence time (i.e. specific growth rate). Hetling and Washington (1964) found that these data were adequately fit by a rectangular hyperbola of the type:

$$\mu = \mu_m \frac{S_c - A}{S_c + B}$$

where S_c is a gross measure of organic concentration as COD

A and B are constants characteristic of the system under study. This formulation is equivalent to the Monod model if "A" is interpreted as the concentration of soluble organics due to metabolic end products and autolysis. The authors do not ascribe reaction kinetic validity to the empirical model but indicate that other parabolic or exponential functions could be derived with equal reliability.

3.9 EVALUATION OF REPORTED MODELS

Since most of the "kinetic mechanisms" advocated in the literature incorporate organic substrate concentration indexed as oxygen equivalents and since oxygen is postulated to participate in several of the unknown elementary reactions comprising metabolic pathways, the validity of an overall kinetic rate expression defined relative to oxygen has little meaning.

By defining a kinetic model in terms of the mass concentration of organic substrate, one makes the assumption that organic carbon participates in only one elementary reaction.

Since it is presently impossible to define the elementary reactions involving intracellular and extracellular organic carbon, it is not possible to define a kinetic mechanism representing substrate degradation. Therefore, the "kinetic" models reported in the literature have little reaction kinetic significance and represent, at best, curve fitting techniques.

The practice of ascribing empirical deviations from a proposed model to the "kinetic" characteristics of the system quantitized by the addition of terms in a kinetic mechanism has no fundamental justification. These techniques merely add flexibility to an empirical correlation such that a broad spectrum of experimental data tends to "validate" postulated models in a mathematical sense.

3.10 GLUCOSE METABOLISM BY MIXED MICROBIAL CULTURES

The biological process in which glucose is metabolized by microorganisms in an aerobic environment may be considered as a two-stage series process (Murphy(1966)). The first phase, glycolysis, is a fermentative process in which glucose is degraded to several products, notably pyruvate, with the liberation of energy usually stored in high-energy phosphate bonds. Traditionally, the carbohydrate anerobic metabolism of all organisms has been represented by the Embden-Meyerhof mechanism, with species differences in the ability to utilize substrate appearing in steps beyond the formation of pyruvate. Lamanna and Mallette (1965) report that although many bacteria ferment carbohydrates to pyruvates by the Embden-Meyerhof scheme, others possess alternative pathways and may utilize one or several. Pelczar and

Reid (1965) support this contention and ascribe interspecies variations in the glycolysis metabolic pathway to differences in the enzymatic composition of different bacterial species.

Differences in the metabolic abilities of micro-organisms are also manifested by the manner in which they further utilize the pyruvate in the second phase of glucose degradation. Several possible pathways exist leading to a number of end products. (Pelczar and Reid (1965)). In the presence of oxygen, the most likely scheme is the cyclic terminal oxidation of pyruvic acid to CO_2 by the Krebs tricarboxylic acid cycle.

From these considerations it is evident that not all micro-organisms metabolize the same substrate in exactly the same manner. The component species of a mixed microbial population may degrade glucose by one or several pathways effecting an unknown network of possible series and parallel reactions. The impossibility of predicting or measuring the rate controlling step precludes the formulation of a realistic kinetic model for substrate degradation.

Because of the relationship between utilization of soluble organics and cell growth established by Garrett and Sawyer (1952), attempts have been made to model the growth phenomenon for biological waste treatment systems. Weston and Eckenfelder (1955) and Eckenfelder and O'Connor (1961) have proposed the use of the sigmoidal batch grow characteristic reported for pure cultures, (Pelczar and Reid (1965)). This approach takes no account of the competitive interaction effects prevalent in mixed cultures and further presupposes the absence of non-active biomass formed by imperfect cell reproduction. The data of Lamanna and Mallette (1965), reported for

studies on the exponential growth of SALMONELLA PULLORUM in a nonsynthetic nutrient broth, indicate that non-viable cells may comprise from approximately 8 to 25 per cent of the total microbial population. Kountz et al. (1959) and McKinney (1963) concluded that up to 25% of the volatile solids formed in the extended aeration process may be inert non-biologically oxidizable solids. These considerations, coupled with the presence of a dynamic environment, precluded the selection of a microbiological response model for the mixed culture used in this investigation. With no fundamental basis for response modelling, interpretation of the biological raw data was, therefore, based on a mathematical response giving equal weight to all data.

CHAPTER 4

EXPERIMENTATION

4.1 SYSTEM OPERATION

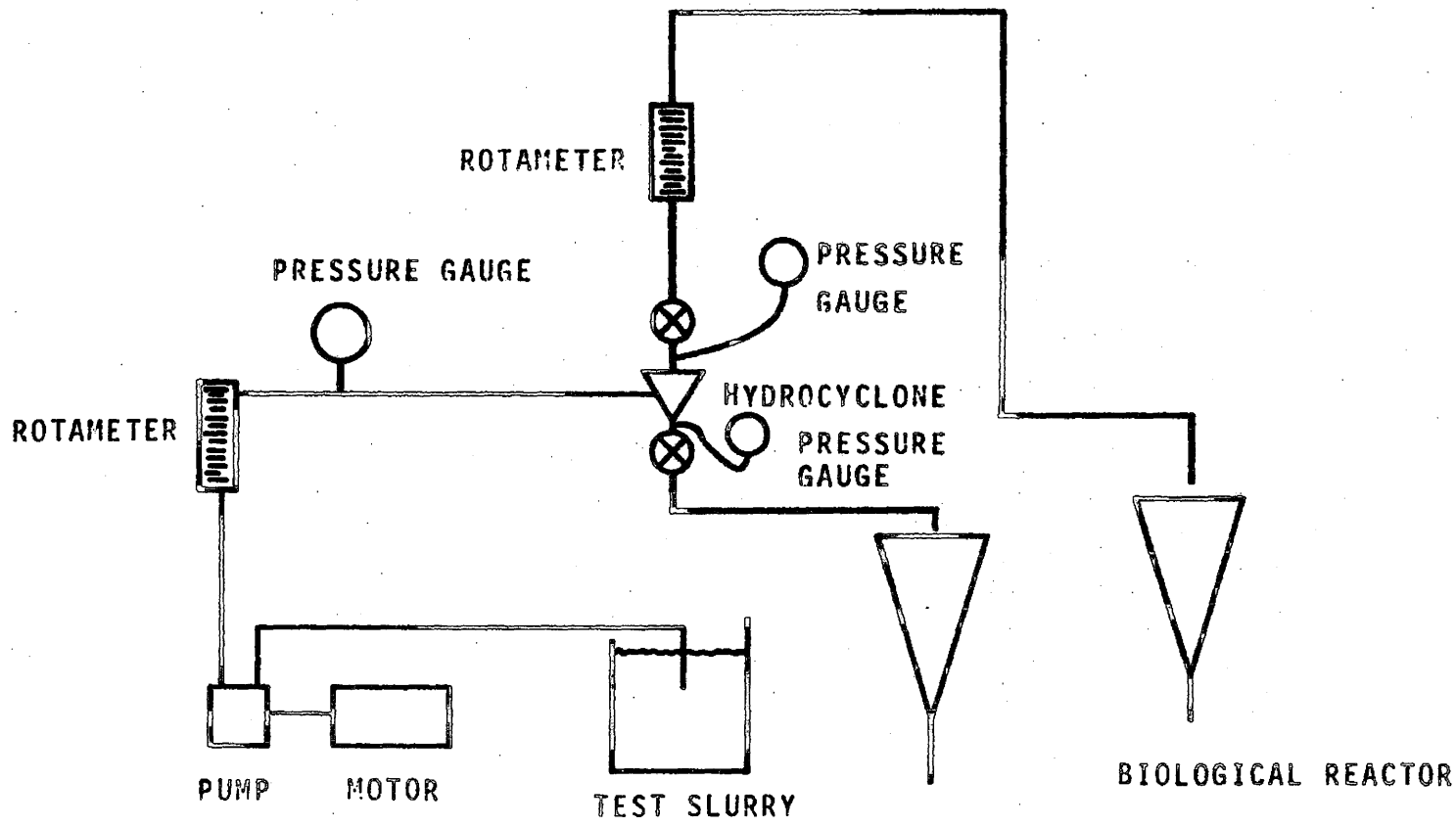
The micro-organic slurry under test, cultivated in a biological reaction vessel, was pumped through the hydrocyclone in which the generated force fields separated the influent into overflow and underflow exit streams. Representative grab samples were collected from both discharge streams and used to inoculate "identical" batch reactors for an evaluation of the biological characteristics of the separation. The operational characteristics of the system were observed by means of pressure gauges mounted in the entrance and exit streams of the hydrocyclone to determine the energy dissipation across the unit, and rotameters inserted in the influent and the overflow lines to quantitize the operating and separation level of the slurry flow.

Swagelock valves on the hydrocyclone exit ports provided positive control over both discharge pressures and volume split. The infinite speed control for the DC pump drive afforded positive control for variation of flow rate to the hydrocyclone or inlet pressure. The experimental apparatus is illustrated schematically in Figure No. 4.

4.2 BIOLOGICAL REACTOR OPERATION

In order to evaluate differences in metabolic activity of the separated exit fractions, random variation of environmental influences, both physical and chemical, were minimized by coincident evaluation of "identical" biological reactions with "identically" controlled chemical environments. To ensure that the biological responses would be representative of the characteristics of the micro-organism populations under

FIGURE 4



APPARATUS

study, the level of environmental contamination was reduced by employing batch rather than continuous operation. This provided the added benefit of a rapid system response, not possible with a continuous biological process with its rather slow approach to a "unique" steady-state level of operation.

4.3 SYSTEM DEFINITION

4.3.1 Hydrocyclone Design

The lack of theoretically sound criteria for the design of hydrocyclones has been ascribed by Bradley (1965) to the unresolved performance contributions effected by: liquid velocity profiles, non-ideal particle behaviour (short-circuiting, recirculation), non-ideal fluid behaviour (eddy propagation due to wall friction), air core formation, and location of the mantle of zero vertical velocity. Present design concepts have their genesis in empirical considerations which correlate hydrocyclone behaviour in terms of an efficiency of separation and in terms of a particle size (diameter) which has equal probability of migrating to either exit.

The problems of hydrocyclone design have been further compounded by the wide variety of definitions and interpretations applied to both separation efficiency and critical particle diameter. The efficiencies reported in the literature have, for most cases, been selected to most accurately describe the specific purpose of the unit under investigation. The cumulative efficiency suggested by Kelsall (1953), the clarification number used by Fontein et al. (1962) and the weight or gross efficiency used by several workers, all defy fundamental considerations by yielding 100% efficiency when the total feed flow exits through the underflow with no separation having

taken place at all. Tengbergen and Rietema (1961) discuss the impossibility of defining a single number to correctly relate how two exit streams can each take some fraction of two components. They attempt to define an efficiency that considers both streams, dependent only on the physical shape of the cyclone and independent of the fluid, the concentration, and the temperature:

$$\text{EFFICIENCY} = \left[\frac{C_3 Q_3}{C_1 Q_1} - \frac{\langle\rho\rangle_3 Q_3 - C_3 Q_3}{\langle\rho\rangle_1 Q_1 - C_1 Q_1}\right]$$

where

$\langle\rho\rangle$ = mixture density 1 = inlet

C = mass concentration 2 = overflow

Q = volumetric flowrate 3 = underflow

All of the correlations presently available for predicting the separation particle size require prior knowledge of either specific hydrocyclone performance or the range of operating levels available for a unit of unspecified design. The equilibrium approach suggested by several workers, (Lilgé, (1962); Bradley, (1965); de Gelder, (1957)) considers a particle whose locus of zero radial velocity coincides with the mantel of zero vertical velocity for the fluid. The wide disagreement as to the form of the correlations for the equilibrium consideration arise from the uncertainty as to the shape of the locus of zero axial velocity. Acceptance or rejection of these theories must be made empirically and the use of these formulations for hydrocyclones beyond the spectrum of unit size and slurry characteristics reported by the investigators is questionable. Empirical correlations reported by such workers as Haas et al (1957), Yoshioka and Hotta (1955) and Matschke and Dahlstrom (1959)

have exhibited wide discrepencies one to another, and application beyond the region of slurry characteristics reported is considered dangerous.

The residence time approach advocated by Rietema (1961) assumes prior knowledge of the available energy, the total flow rate, the desired 50 per cent separation diameter and the physical properties of the slurry to be separated. Because this method formulates hydrocyclone design on slurry characteristics and operational flexibility with no restriction on unit size, it was felt to be the most fundamentally acceptable and was used in this investigation. A detailed design analysis is presented in Appendix No. I.

Preliminary hydrocyclone design resulted in unit dimensions too small to be practicable for fabrication or operation. The specific gravity differential between the biological solid phase and the liquid carrier medium, and the average microbial particle size were selected from the data of Lamanna and Mallette (1965); these were fixed physical properties of the test slurry. Further, the input energy level was restricted by the equipment available. Since these considerations had a significant influence on hydrocyclone size requirements, and could not be altered, it was decided to select the smallest hydrocyclone which could be made and operated for this investigation. Since no precedent was available to uniquely establish this lower size limit, a group of hydrocyclones were fabricated ranging in size from 10 mm to 2 mm body diameter, the latter representing the size minimum for construction.

The hydrocyclones were fabricated from materials capable of withstanding the high pressures expected and amenable to machine finishes with low surface roughness (to impede eddy formation and particle recirculation). Cyclones larger than 5 mm D_c were made of plexiglass, those less than

5 mm D_c of brass.

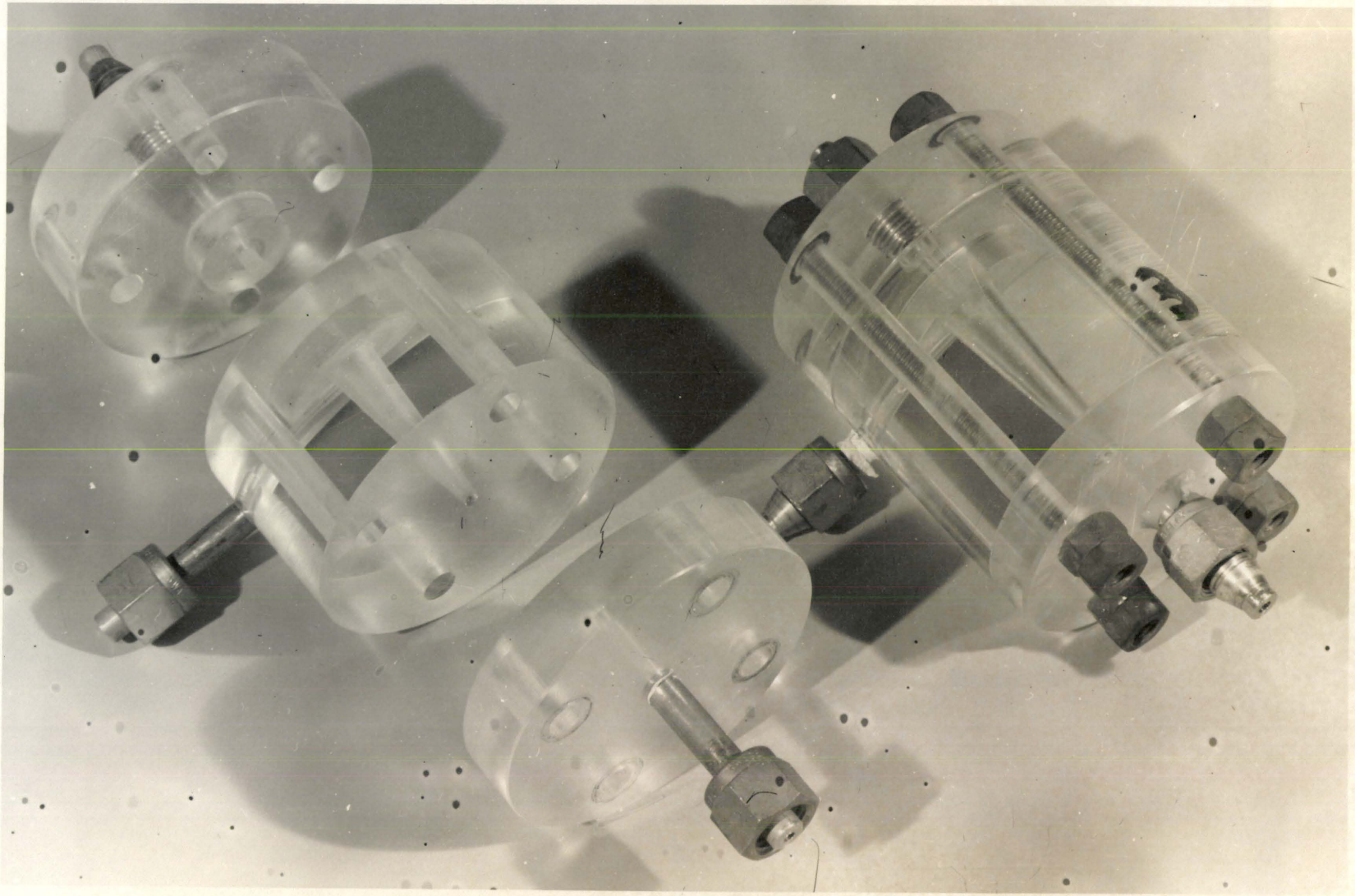
To facilitate construction, the hydrocyclones were constructed in three sections as illustrated in Figure No. 5. The upper and lower sections, containing the exit ports,were machined and bolted to the central body section section to provide a sealed unit. The stainless-steel inlet and exit ports were threaded into the unit to provide convenient connection to system piping.

4.3.2 Biological Reactor Design

The biological reactors were fabricated by glass-welding 350 ml Buchner funnels with fritted glass discs to the apex of 4-litre pyrex percolators. Compressed air, supplied at 20 psi to the stem of a Buchner funnel escaped from the fritted discs as finely dispersed bubbles satisfying the design criteria of providing a completely mixed, hydraulic regime for the reactor contents and of providing sufficient gas-liquid surface area per unit reactor volume to maintain near saturation levels of dissolved oxygen for the spectrum of biological solids levels carried during the investigation. Preliminary testing indicated that the level of mixing provided by air addition sufficiently approached complete mixing to eliminate the need for additional mechanical agitation.

To minimize the loss of reactor contents by evaporation, a water-cooled condensor was mounted above each reactor and secured by means of an inverted funnel adaptor sealed with air-tight, plastic membrane connections to both the reactor and the condensor. This effected the condensation and return of most of the water vapour being stripped from the reactor via exit gases.

FIGURE 5



PHOTOGRAPH OF TYPICAL HYDROCYCLONE

A one inch diameter hole was cut in each funnel adaptor to accomodate a large volume pipette during sample collections and an airtight, rubber stopper between sample collections to facilitate testing and to minimize the possibility of external contamination.

4.3.3 Organic Carbon Source

The reported work on biological growth kinetics have assumed growth limitation by a single organic or inorganic nutrient so that the rate of degradation of the subject nutrient can be related to the rate of growth of biological solids. The growth process can be defined as a biochemical mechanism in which micro-organisms change soluble organic substrate into final products along a network of possible reaction paths all of which may consist of several combinations of consecutive and parallel reactions. The unique path is probably most dependent on process reactants (the species of bacteria, the composition of organic substrate) and the system environment (pH, concentration of trace elements, temperature, pressure). Since the component reactions of any conversion pathway may be highly sensitive to environmental factors beyond experimental control, and since the reaction characteristics of the micro-organisms was of prime importance in this investigation, it was decided to employ an organic substrate with a relatively short reaction path. Thus, the use of complex wastes prevalent in field installations was considered undesirable and a simple dextrose solution was employed as the organic reactant.

Traditionally, the strength of organic wastes has been measured in terms of the five-day Biochemical Oxygen Demand. Schroepfer et al (1960) has indicated that this is not a conserved parameter, but one which will differ for similar abolute concentrations of substrate. Murphy (1966)

recommends the use of conserved parameter such as organic carbon or chemical oxygen demand to measure biological substrate utilization. For this investigation, organic carbon was used as the limiting nutrient to observe the growth phenomenon.

4.3.4 Mixed Microbial Cultures

Helmers et al. (1951) and Heukelekian et al. (1951) have reported that the products of the biochemical reactions (when acclimated micro-organisms and a soluble organic waste are contacted in the presence of oxygen and nutrients) are cellular protoplasm and energy. These observations led Garrettand Sawyer (1952) to conclude that organism growth, indicated by an increase in microbe weight, is evidence of the utilization of food and thus, micro-organism growth must be considered in the investigation of the kinetics of the substrate degradation.

Several workers have investigated the growth phenomenon using pure cultures where only one micro-organic species is permitted. This technique has the advantage of simplifying the kinetic interpretation of data but is based on the erroneous assumption that the culture under study typifies the mixed cultures prevalent in prototype waste treatment facilities. The competitive and mutuallistic interactions among the various species in a mixed culture which preclude the formulation of a kinetic model were tolerated in order that this laboratory investigation would have some relevance for field application. The use of mixed cultures provided the added benefit that the effects of interspecies as well as intraspecies size and shape distributions on slurry classification could be studied.

4.4 EXPERIMENTAL TECHNIQUES

4.4.1 Initial Microbial Inoculum

Two continuous reactors were seeded with the filtered effluent (Whatman No.1) of a bench scale continuous reactor which had operated for several months in a chemical environment similar to that selected for this investigation.

One reactor was operated in a substrate adequately rich in nutrients to encourage growth of predominately rod-shaped bacteria. The other reactor was operated in an identical nutrient environment save for a lack of buffering capacity which promoted low pH levels favouring the growth of yeast. After a period of 10 days, the contents of the two reactors were mixed to provide approximately equal numbers of bacteria and yeast and experimentation commenced. The two distinct micro-organism shapes were maintained throughout the experimental period in approximately the same proportion by numbers in an effort to qualitatively evaluate the contribution of particle shape to the classification performance of the hydrocyclones.

4.4.2 System Preparation

Prior to each run, the biological reactors were washed with soap and tap water, purged with fifty ml of concentrated sulphuric acid and rinsed with distilled water to reduce the level of micro-organic contamination both in the porous diffuser and on the walls of the reactor. To remove any biological solids build-up in the hydrocyclone piping network, ten litres of tap water were pumped through the system at maximum flow rate before and after every run. The test slurry was allowed to recycle through the apparatus for approximately ten minutes prior to sample collection to promote steady-state system operation.

A one litre aliquot of prepared nutrient solution was added to each reactor from a common batch supply prepared prior to each run and seeded with a two litre grab sample from the appropriate hydrocyclone exit stream at which time the experimental run commenced.

4.4.3 Sampling

Three replicate grab samples from the centroid of the reactor contents were pipetted through the sampling port of the funnel adaptor at regular time intervals. Prior to each run the filtering properties of the test biological floc were observed to determine optimum sample volumes which were selected on the basis of a maximum allowable filtration time of fifteen minutes. This precaution was necessary to reduce the possibility of non-representative measurements of biological solids and substrate levels as a function of time. Preliminary experimentation indicated that refrigerated sample storage at $4^{o}C$ did not preserve the biological and chemical characteristics of the slurry. These observations are in accord with those of Ellison (1932) who found evidence of psychrophilic bacteria metabolism at temperatures as low as $5^{o}C$. Agardy and Kiado (1966) report similar findings and conclude that biological activity can change the biological, physical and chemical characteristics of the waste when stored at low temperatures (0 - $10^{o}C$). It has been fairly well established that freezing will curtail biological activity (Agardy and Kiado, (1966)). However, experiments performed by Morgan and Clarke (1964) and Fogarty and Reeder (1964) indicate that significant errors will occur in certain analyses performed on the thawed samples. The colloidal character of a sample will be altered by the freezing and thawing process promoting coagulation, which phenomenon will affect the dissolved and suspended solids content of the waste. (Agardy and Kiado (1966)). Having eliminated

the possibility of sample storage, it became essential that filtration time not exceed sampling intervals. Triplicate ten or twenty ml samples were used throughout this investigation. In all cases, the filtrate of the first replicate collected was used for an organic carbon analysis.

4.4.4 Air Supply

A constant and equal supply of air was supplied to both reactors to provide an adequate dissolved oxygen level (approximately six mg/l) and a high degree of mixing. A cylinder of glass wool inserted in the air line prevented air contamination of the porous diffusers which could result in non-uniform air flow rates and reactor contamination. Throughout the investigation, individually calibrated rotameters were valved to maintain a constant air flow rate of 6 l/min at one atm pressure and 75 ^{o}F.

4.5 ANALYTIC TECHNIQUES

During the course of an experimental run, the concentration of soluble organic carbon and non-filterable suspended solids were monitored as a function of time for both biological reactors. Microscopic examinations of the test slurry, and overflow and underflow exit streams were performed to provide an indication of the relative distribution of microorganism shape, size and type effected by the hydrocyclone.

4.5.1 Determination of Suspended Solids Concentration

The suspended soldis concentration was determined gravimetrically using Sartorius weightconstant membrane filters (47 mm DIA; o.45 μ pore DIA) in conjunciton with a six stall millipore vacuum filtration apparatus. Preparatory to each run, the membrane filters were individually washed with one hundred ml of distilled water to ensure the absence of weight contributing

and carbon contributing soluble contaminants. Each filter paper was then placed in a tagged aluminum foil dish, placed in a mechanical convection oven at 103°C for 0.5 hours (Standard Methods (1965)) and dessicated to room temperature. As they were required during a run, the filter papers were individually removed from the desiccator, weighed to the nearest 10^{-4} gms on a Mettler type No. 15 balance and mounted on the filtration apparatus to accept a known volume of reactor contents. After filtration, each membrane filter was returned to its aluminum foil dish, redried and reweighed as per above. The sample volume and the weight of non-filterable suspended solids were combined to define the suspended solids concentration and expressed as mass per unit volume. Three replicate measurements were made for every suspended solids determination.

4.5.2 Determination of Soluble Organic Carbon Concentration

The filtrate from one of the replicate determinations of suspended solids concentration was immediately collected, treated and analyzed for soluble organic carbon content using a Beckman model IR 315 infrared carbonaceous analyzer.

Inorganic carbon present in the samples was converted to CO_2 by titration with two drops of concentrated hydrochloric acid and liberated by means of a five minute helium purge, (Schaffer et al. (1965)). Twenty microlitre aliquots were then syringe-collected and injected into the analyzer until three successive determinations produced output signals which differed by less than 1% of full scale output. The total organic carbon concentration as mg/l was determined by linear interpolation from five point analyzer calibration curves made for each run using solutions

of a stable organic compound (sodium oxalate in distilled water) of known carbon concentration. These standard solutions were capped and stored at 4°C between runs. All glassware used for the collection and storage of the test samples was washed and dried in an automatic laboratory washer employing a distilled water rinse cycle.

4.5.3 Microscopic Examination

An approximate quantitative measure of the nature of population separation relative to size, shape and type of organisms present in the test slurry and the overflow and underflow exit streams was made using an Olympus microscope with a phase contrast attachment.

4.5.4 Additional Testing

Periodic pH determinations were made during the course of each run using a Beckman expanded scale model 76, pH meter.

One hundred ml samples collected from overflow and underflow streams were allowed to quies for approximately one hour to determine the settling characteristics of each exit.

4.5.5 Run Duration

Testing continued until the substrate carbon concentration reached a level which remained constant for at least 0.5 hours (i.e. steady-state).

4.6 VARIABLES UNDER STUDY

Based on an evaluation of the literature (Hsiang (1967)), the operational variates exerting the most significant influence on hydrocyclone classification and thickening performance were selected to be:

(1) volumetric flow rate

(2) volume split

(3) slurry characteristics (particle size distribution and concentration)

(4) inlet pressure

(5) pressure drop

Preliminary testing revealed several predictor variates to be mutually dependent for the system operational levels of greatest interest, negating the use of all operational variables as system parameters:

Variate	Independent of				
	Volumetric Flowrate	Volume Split	Slurry Characteristics	Inlet Pressure	Pressure Drop
Volumetric Flowrate	-	No	Yes	No	No
Volume Split	No	-	Yes	No	No
Slurry Characteristics	Yes	Yes	-	Yes	Yes
Inlet Pressure	No	No	Yes	-	No
Pressure Drop	No	No	Yes	No	-

Since the test slurry consisted of a dynamic, non-equilibrating, living biomass, no positive control could be exercised on its properties, thereby precluding its status of a predictor variate. Preliminary evaluation of the test system, therefore, indicated no experimental variables of interest which could be selected as mutually independent test parameters. The operational characteristics of the apparatus were such, however, that inlet pressure and volumetric split were relatively mutually independent and controllable over the range of interest for this investigation; the former being a function of the level of influent volumetric flow rate

and the latter being a function of the level of underflow-exit valve throttle.

Inlet pressure and volume split, being mutually independent in an operational sense and controllable over a discrete range of interest for hydrocyclone geometry were selected as predictor variates and were investigated with hydrocyclone size in a three variable experimental analysis.

4.7 EXPERIMENTAL DESIGN

Investigation of the thickening and classification responses for a meaningful number of predictor variate levels suggested the use of statistically designed experimentation in the interest of research efficiency. Hunter and Wu (1967) have noted that this experimental philosphy provides a measure of the interactions between variables, the simultaneous action of variables on the response variates and the most promising direction of change for predictor variates to maximize the response.

The implementation of this approach presupposes the existence of a smooth, functional relation correlating measured response to various levels of the predictor variates which can be represented to any required degree of approximation by employing a sufficient number of terms in its polynomial representation (Box, (1967)). Since the upper and lower bounds of predictor variate levels were fixed by the operational characteristics of the test system, the steepest ascent approach to response maximization could be employed; but, system responses could only be measured over a well-defined spectrum of variable levels. Since the experimental region under investigation may have represented a near stationary section of the

response surface, in which case a first order polynomial would not adequately define the surface, provision was made for inclusion of second order effects by employing a second order central composite design. The five level, three variable, statistical design illustrated geometrically in Figure No. 6 was employed for the predictor variates of hydrocyclone diameter (x_1), inlet pressure (x_2) and volume split (x_3) at the following factor levels (Hunter, (1960)).

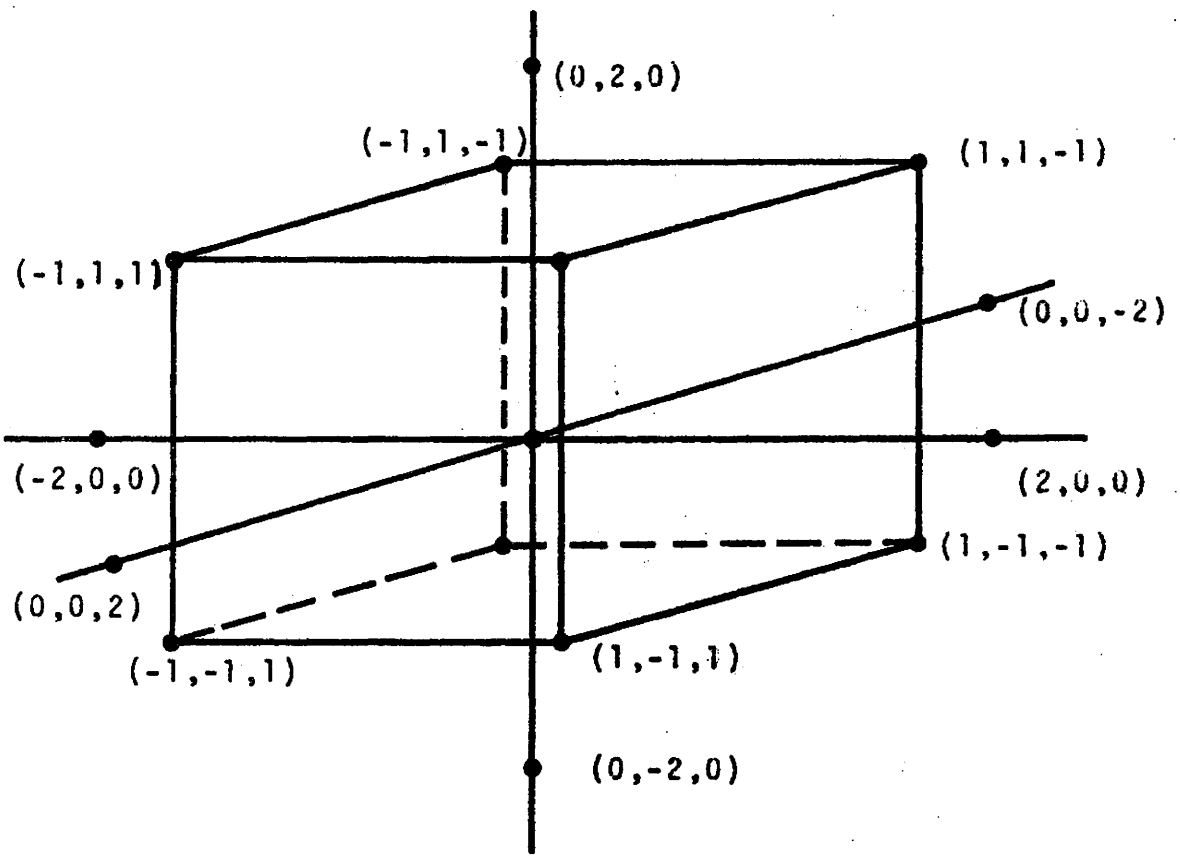
Coded Levels

Run No.	x_1	x_2	x_3
1	1	-1	1
2	1	-1	-1
3	1	1	-1
4	1	1	1
5	-1	1	-1
6	-1	1	1
7	-1	-1	1
8	-1	-1	-1
9	-2	0	0
10	2	0	0
11	0	2	0
12	0	-2	0
13	0	0	2
14	0	0	-2
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0

Code Key

Level / Variable	+2	+1	0	-1	-2
$x_1 \equiv D_c$ (mm)	10	8	6	4	2
$x_2 \equiv P_I$ (PSi)	200	170	140	110	80
$x_3 \equiv$ VS (OF/UF)	3/1	2.5/1	2/1	1.5/1	1/1

FIGURE 6



VARIABLE COORDINATE SYSTEM

CHAPTER 5

DATA ANALYSIS

5.1 TREATMENT OF RAW DATA

Due to the limitations imposed on sampling frequency by the analytic techniques employed and on run duration by the use of a monasaccharide carbohydrate source, few data were available for the determination of unit rate information. Interpolation techniques were investigated in an effort to find a suitable method for generating more data. Since only a discrete set of approximate values of empirical data was generated in the laboratory, and the degree of reliability of these data was not well established (Appendix No. II), it was not feasible to define an interpolation polynomial to fit the data exactly. Hildebrand (1956) has noted that such a polynomial could be represented by a curve which oscillates violently about the curve representing the true function. In addition, since the organic carbon concentration data were to be used for numerical differentiating, the effects of the deviation of the generated function from the true function would be magnified and result in unreliable data interpretation. It was, therefore, evident that raw data smoothing must precede an interpolation process.

5.1.1 Data Smoothing

In place of determining a polynomial approximation to a certain function by requiring that values of the approximation agree with the known approximate values to the true function, Hildebrand (1956) and Nielsen (1965) suggest that it is preferable to require that the approximation and the true function agree as well as possible over the domain specified by the raw data. To this end, the Gram modification of Legendre's Principle of Least-Squares was used to develop smoothing

formulae which put full and equal weight on primary data. In place of approximating the true function by a single, least-squares polynomial of specified degree over the entire range of data set, Hildebrand(1956) reports the desirability of replacing each datum by the value taken on by a least-squares polynomial of a degree relevant to a subrange of raw data centered at the point for which the entry is to be modified. This technique assumes that the true function can be approximated by some polynomial of specified degree over each subrange of data points but it also admits the possibility that a polynomial of fixed order may not be satisfactory over the entire domain.

Rather than artificially forcing the raw data to conform to an approximating polynomial of arbitrary order, a mathematical criterion was established for evaluating the best order of mathematical response based on the raw data. Using the proposition that "for equally spaced intervals of the independent variable, the n^{th} differences of a polynomial of the n^{th} degree are constant" (Nielsen (1965)) a forward difference table of the 5th rank was established for each set of primary data. The difference column exhibiting the smallest mean square based on the residual sum of squares about the column mean determined the mathematical degree of smoothing to be employed for each data set.

5.1.2 Interpolation Techniques

The Lagrangian technique of expressing an interpolation formula explicitly in terms of smoothed data was investigated to determine the amenability of the data to this type of analysis. Violent oscillations were observed in the generated functions for both smoothed and raw data, indicating the data to be ill-conditioned. This was ascribed in part to

the low degree of reliability of the raw data in an analytic sense and in part to the existence of a point of inflection in the estimated true function, both of which seriously reduce the power of Lagrangian analysis. In general, the degree of the generated Lagrangian Polynomial is one less than the number of data points and hence, this method artificially forces an order on an experimental curve, the mathematical accuracy of which is a function of the number of data points. Furthermore, the Lagranian method does not allow easy determination of the truncation error relevant to the result offered by interpolation based on a given number of ordinates, or easy determination of the number of ordinates needed to reduce the truncation error below prescribed limits. For these reasons, Lagrange Polynomial interpolation was not considered acceptable for this investigation.

Preliminary studies indicated Bessel's finite difference interpolation used in conjunction with a central difference table to be a realistic method of interpolation. Equally acceptable and more readily adaptable to this study was the use of the smoothed, least-squares approximation polynomial generated in the smoothing process. By selecting an n^{th} degree polynomial of the form

$$F(x) = a_o + a_1(x - x_o) + a_2(x - x_o)(x - x_1) \quad \ldots\ldots$$

$$+ a_n(x - x_o)\ldots\ldots(x - x_{n-1})$$

the unknown coefficients could be obtained from a forward difference table of the smoothed data. Interpolated data could then be obtained from the defining polynomial without distorting the polynomial of best fit.

The smoothing formulae used in this investigation are presented in Appendix No. III.

5.2 REVISED RAW DATA TREATMENT

It was found that this method of data treatment selected "best orders of fit" which did not result in a minimum residual sum of squares at the appropriate degrees of freedom for an assumed difference table variance distribution of $\sqrt{2}^{n}$ (where n = the difference rank). This suggested that an unknown source of variance was present. Rather than devising a search technique to provide a "best fit" for the variance, it was decided to treat the data by least-squares polynomials of increasing arithmetic order until the polynomial exhibiting the minimum residual mean square was determined. Terms in the generated expression accounting for sums of squares significant at the 95% level relative to the residual sum of squares for the appropriate degrees of freedom were retained to define the approximation to the "true" function.

CHAPTER 6

RESULTS AND INTERPRETATION

6.1 PRIMARY DATA

6.1.1 Specific Growth Rate

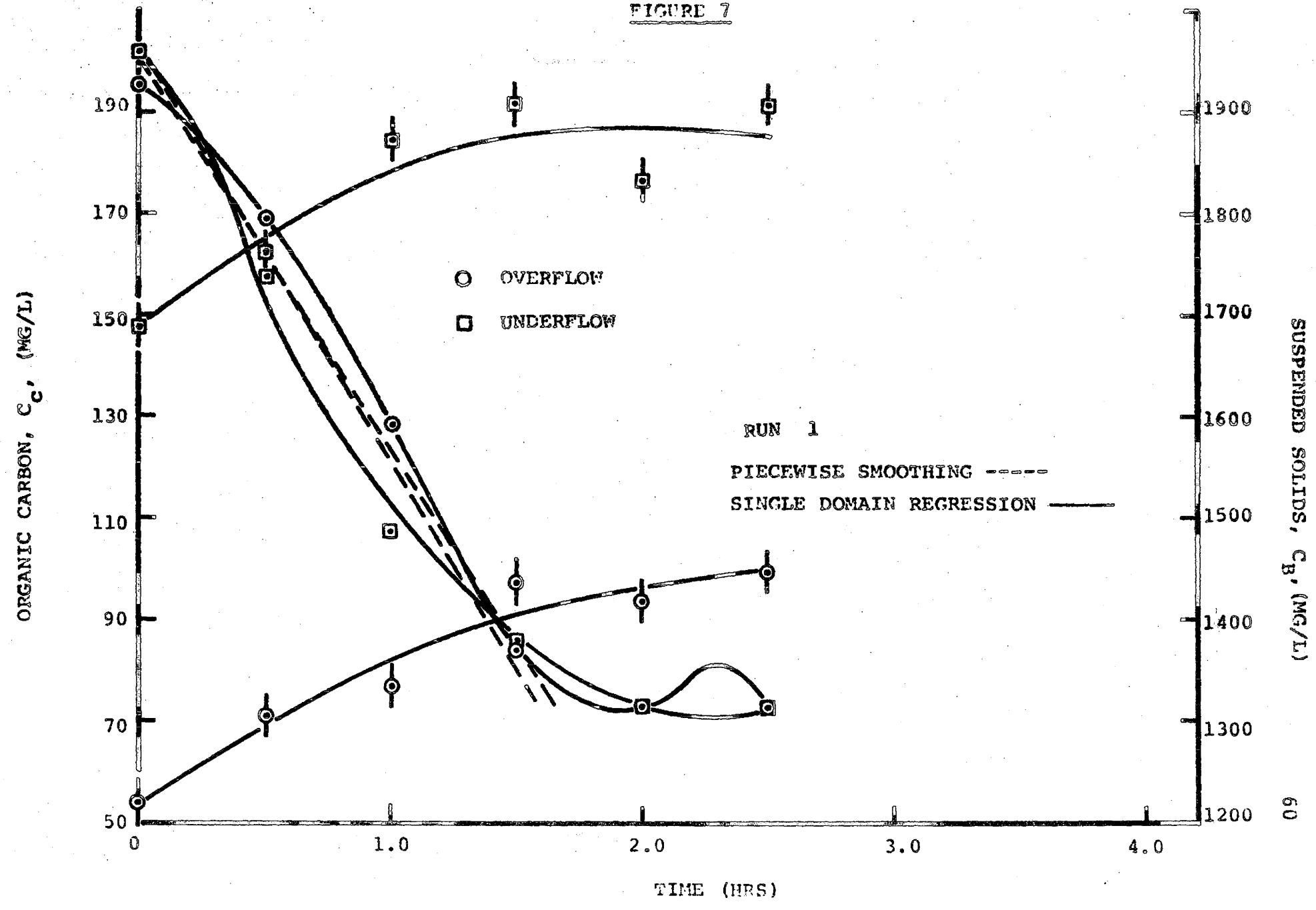
Since specific growth rate is defined to be the velocity of organic carbon removal per unit mass of micro-organisms $(\frac{1}{C_B}\frac{dC_c}{dt})$, it is evident that the magnitude of this quantity will be a function of the type and extent of data treatment used for the raw data mass concentrations of organic carbon and suspended solids.

With the exception of run No. 9, the raw data for the concentration of organic carbon indicated an arithmetic linear decrease with time to steady state levels. Within the limitation imposed by the frequency of sampling, these curves indicated a point of discontinuity between a constant velocity and a "null" velocity of carbon decrease, thereby suggesting a piecewise approach to data fitting. Intersecting arithmetic linear least-squares curves provided highly significant fits at the 95% confidence level and these smoothed data were used to calculate $\frac{dC_c}{dt}$, (Figures No. 7 through No. 24).

The raw carbon and solids data were also regressed in a non-piecewise fashion over the entire domain of each run. Least squares polynomials of increasing arithmetic order were fit to the data until the polynomial exhibiting the lowest residual mean square was determined. These fits were also highly significant at the 95% confidence level and were used to calculate a different set of specific growth rate values.

Since the regressed carbon data were differentiated for the evaluation

FIGURE 7


ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
190
170
150
130
110
90
70
50
1900
1800
1700
1600
1500
1400
1300
1200
0
1.0
2.0
3.0
4.0
OVERFLOW
UNDERFLOW
RUN 1
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——

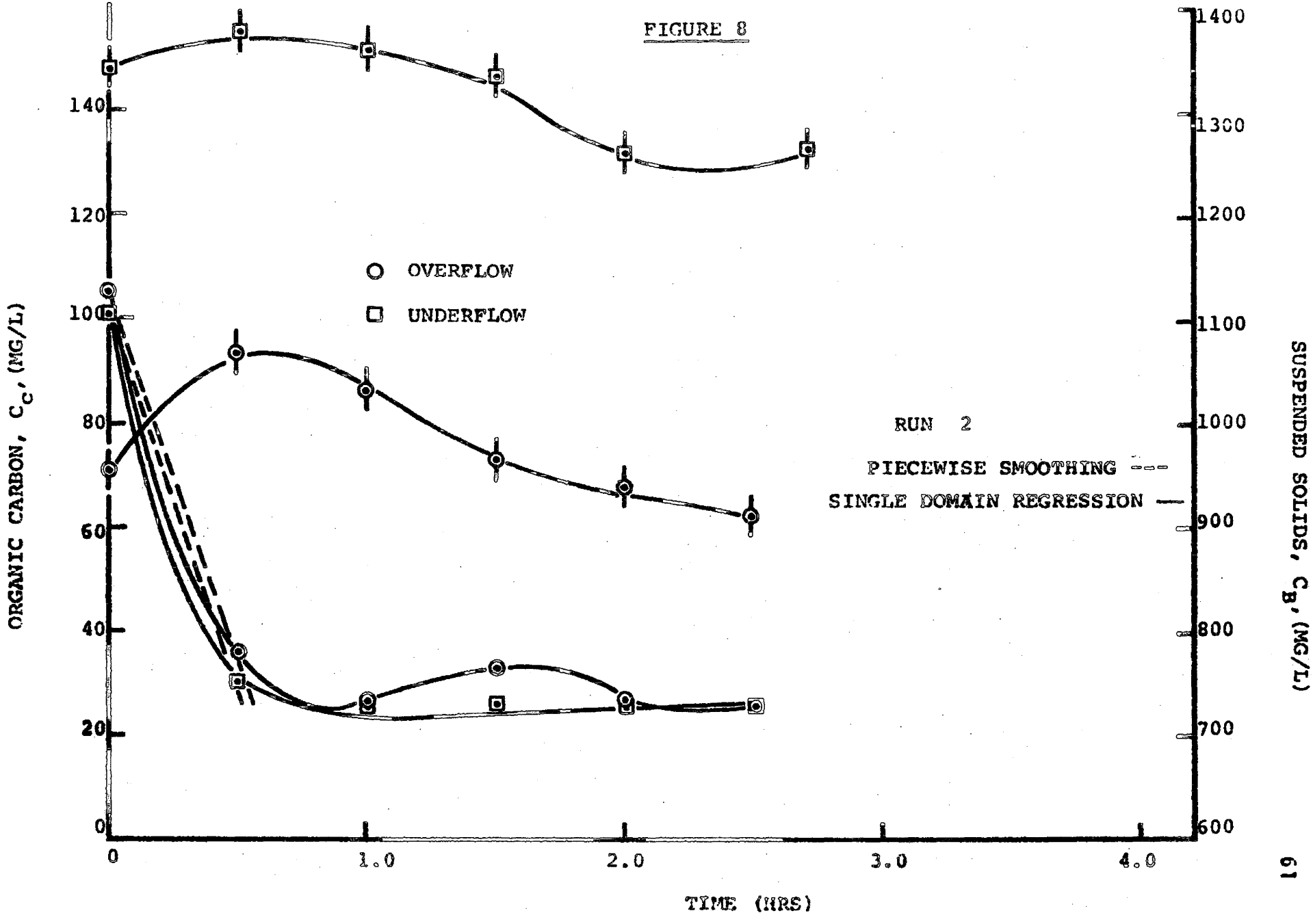


FIGURE 8
ORGANIC CARBON, C_C, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
OVERFLOW
UNDERFLOW
RUN 2
PIECEWISE SMOOTHING
SINGLE DOMAIN REGRESSION
0
20
40
60
80
100
120
140
600
700
800
900
1000
1100
1200
1300
1400
0
1.0
2.0
3.0
4.0

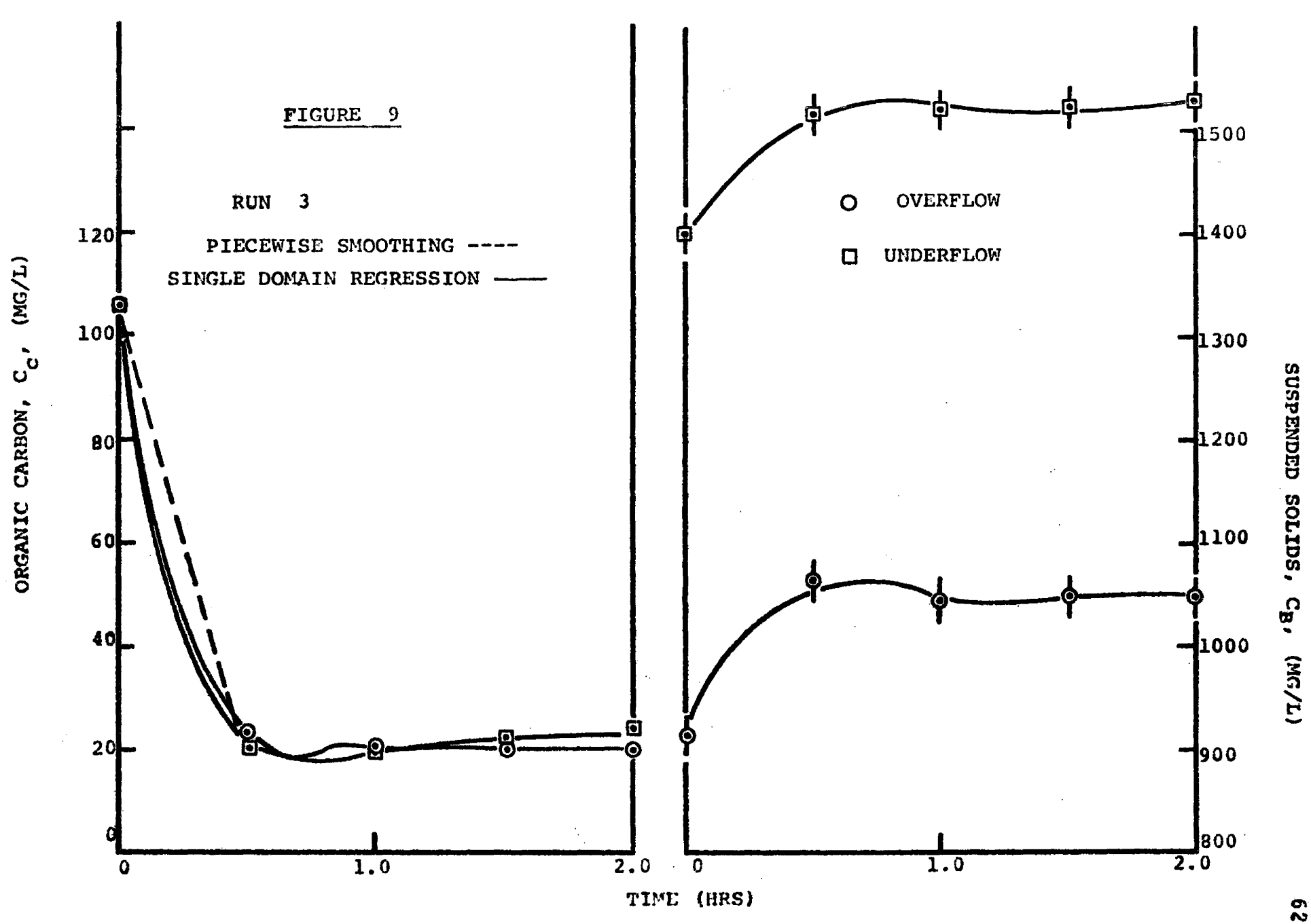


FIGURE 9
RUN 3
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
ORGANIC CARBON, C_C, (MG/L)
120
100
80
60
40
20
0
0
1.0
2.0
TIME (HRS)
OVERFLOW
UNDERFLOW
1500
1400
1300
1200
1100
1000
900
800
SUSPENDED SOLIDS, C_B, (MG/L)

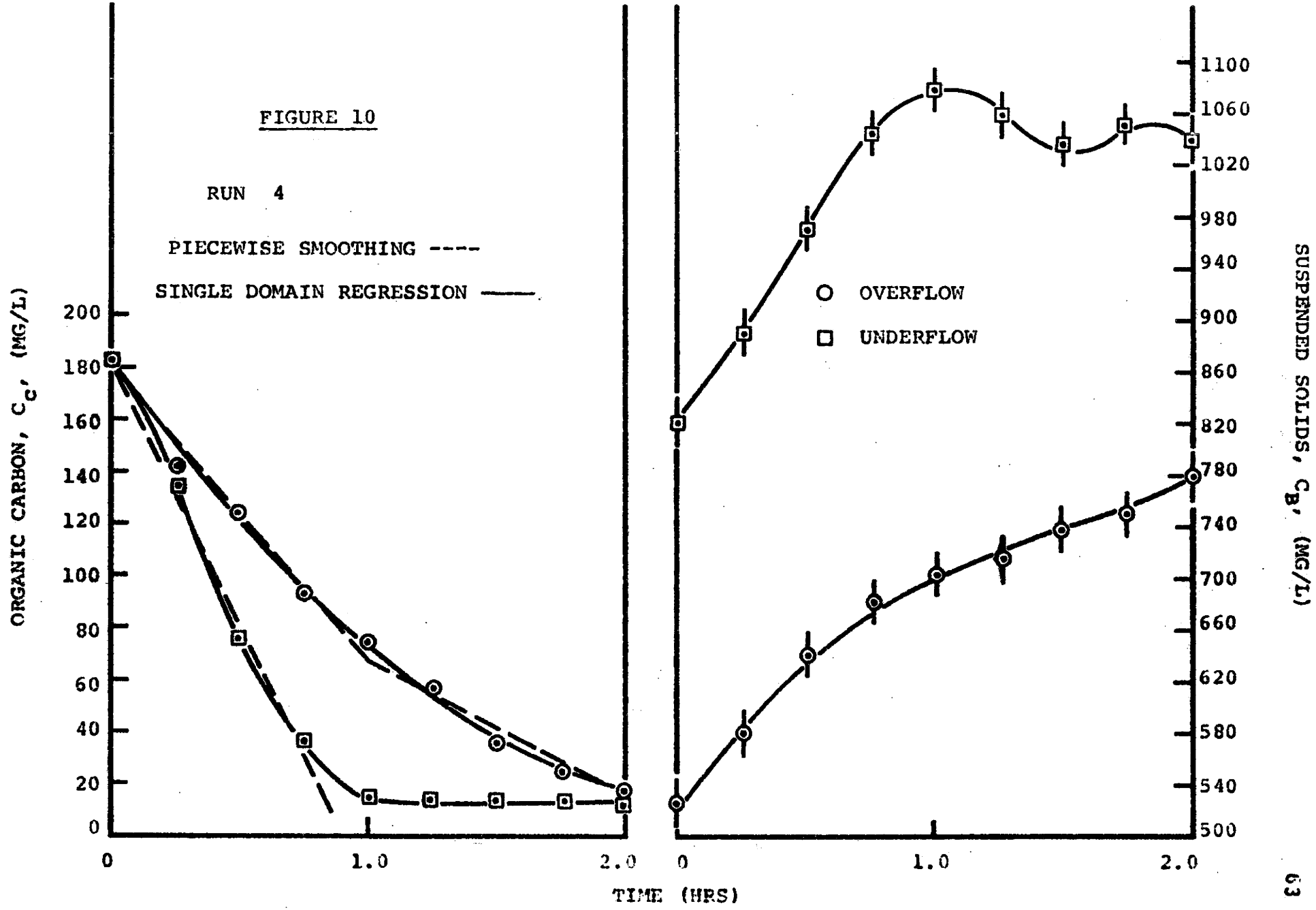
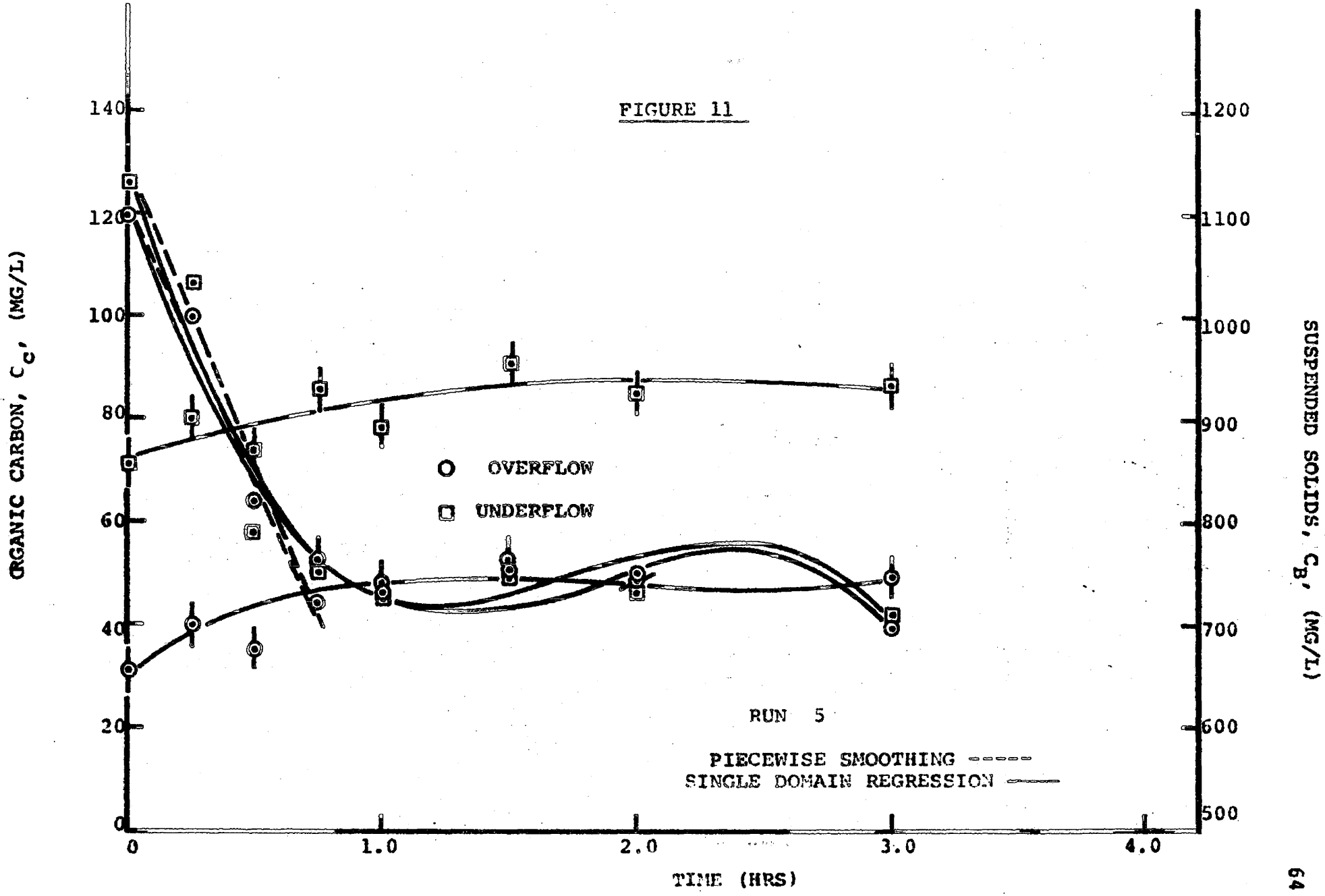


FIGURE 10
RUN 4
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
OVERFLOW
UNDERFLOW
0
20
40
60
80
100
120
140
160
180
200
500
540
580
620
660
700
740
780
820
860
900
940
980
1020
1060
1100
0
1.0
2.0

FIGURE 11


ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
0
1.0
2.0
3.0
4.0
0
20
40
60
80
100
120
140
500
600
700
800
900
1000
1100
1200
OVERFLOW
UNDERFLOW
RUN 5
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——

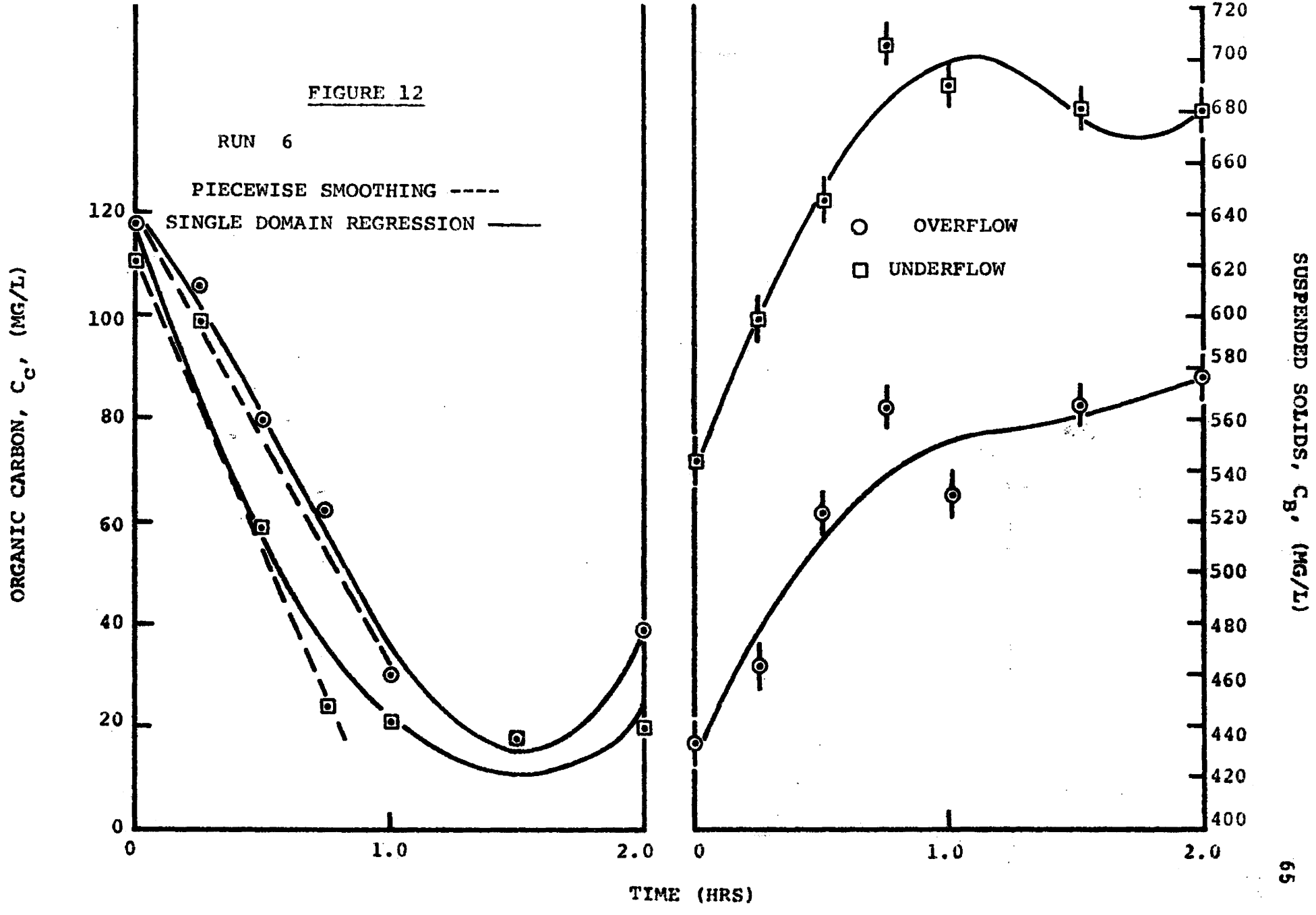


FIGURE 12
RUN 6
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
OVERFLOW
UNDERFLOW
ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_s, (MG/L)
TIME (HRS)
0
20
40
60
80
100
120
400
420
440
460
480
500
520
540
560
580
600
620
640
660
680
700
720
0
1.0
2.0

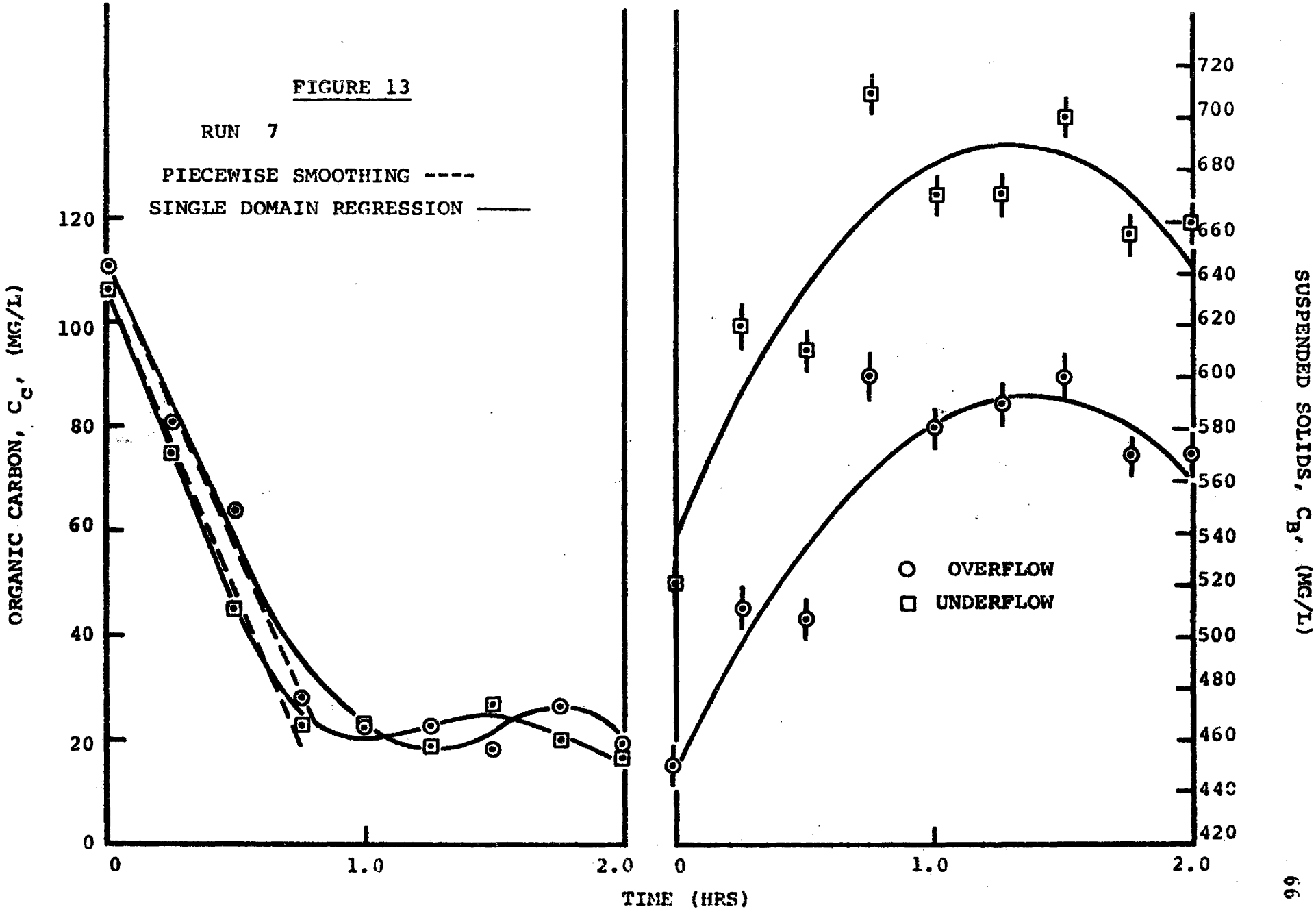


FIGURE 13
RUN 7
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_s, (MG/L)
TIME (HRS)
OVERFLOW
UNDERFLOW
0
20
40
60
80
100
120
420
440
460
480
500
520
540
560
580
600
620
640
660
680
700
720
0
1.0
2.0

FIGURE 14

RUN 8

PIECEWISE SMOOTHING ----

SINGLE DOMAIN REGRESSION ——

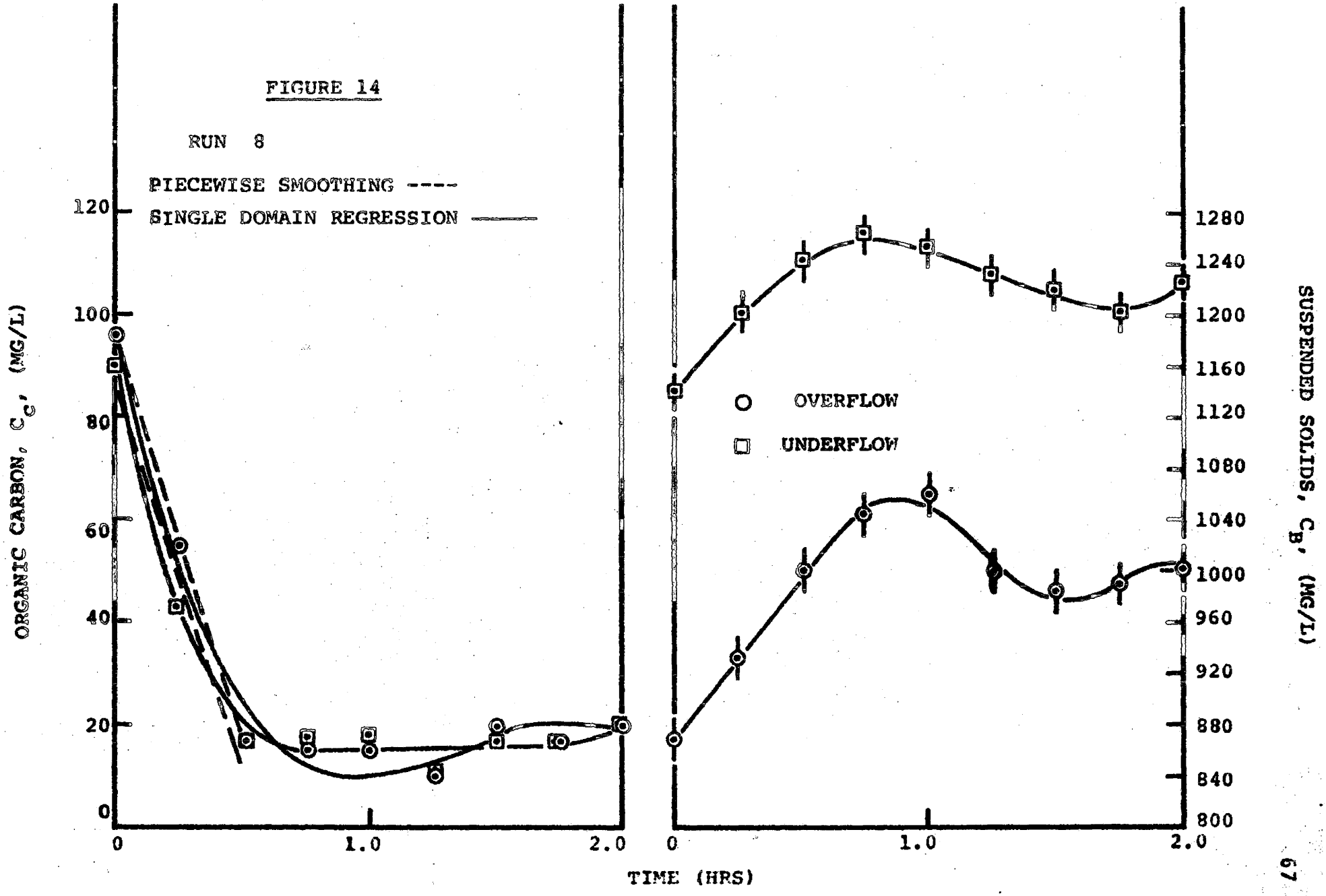


FIGURE 15

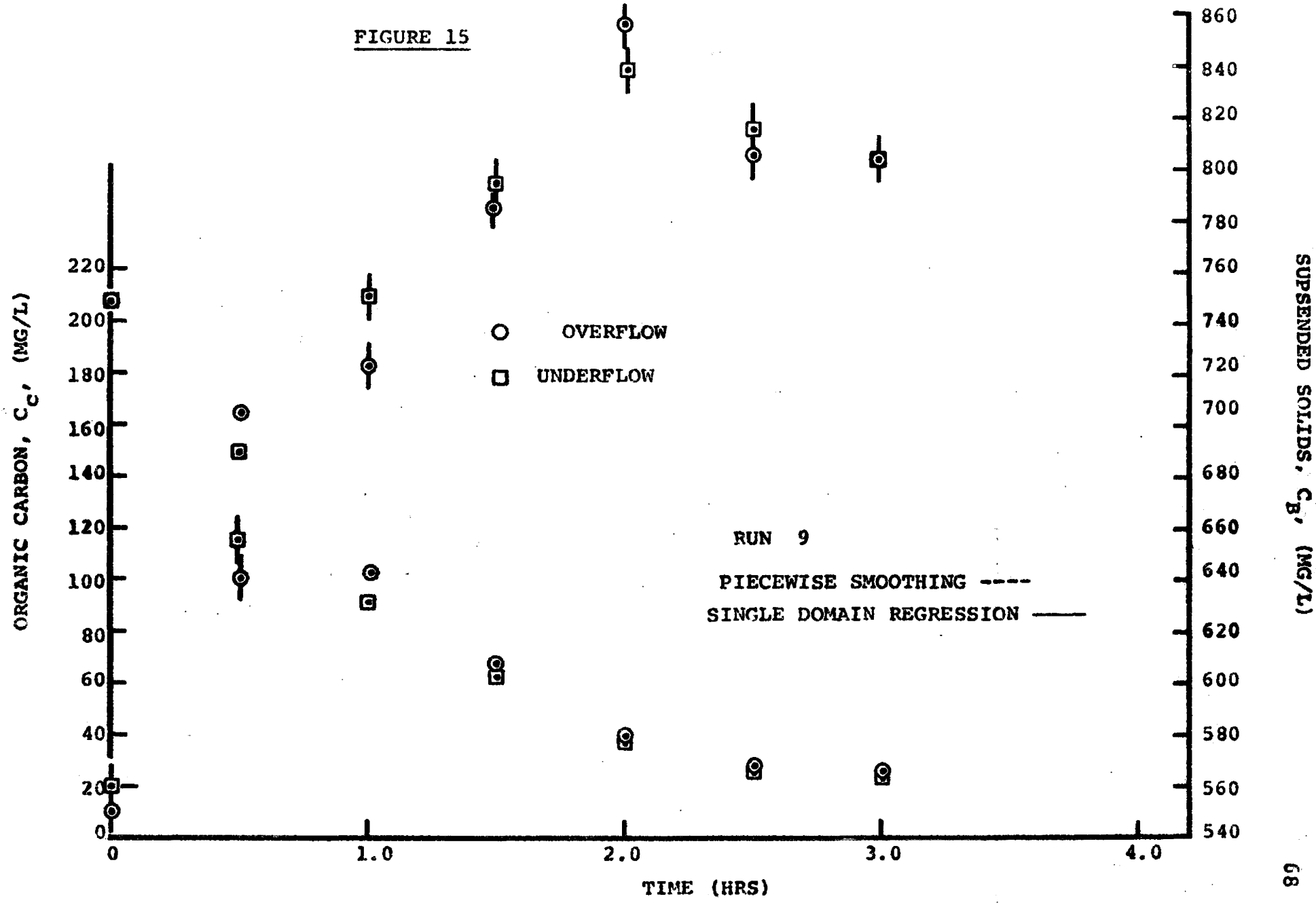
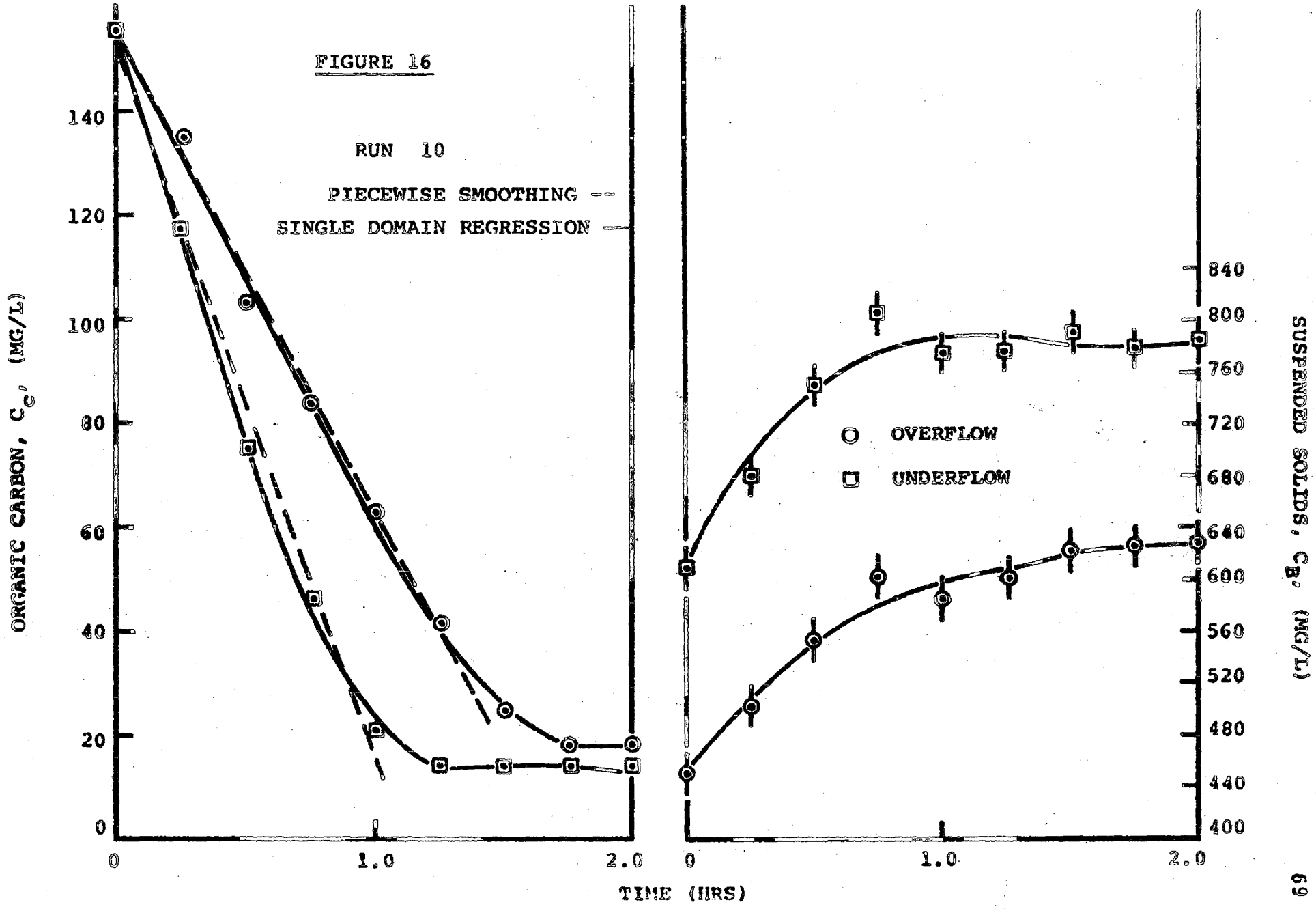

ORGANIC CARBON, C_C, (MG/L)
SUPSENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
OVERFLOW
UNDERFLOW
RUN 9
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
0
20
40
60
80
100
120
140
160
180
200
220
540
560
580
600
620
640
660
680
700
720
740
760
780
800
820
840
860
0
1.0
2.0
3.0
4.0

FIGURE 16


RUN 10
PIECEWISE SMOOTHING --
SINGLE DOMAIN REGRESSION —
ORGANIC CARBON, $C_{C'}$ (MG/L)
SUSPENDED SOLIDS, $C_{B'}$ (MG/L)
O OVERFLOW
□ UNDERFLOW
TIME (HRS)
0
20
40
60
80
100
120
140
400
440
480
520
560
600
640
680
720
760
800
840
0
1.0
2.0

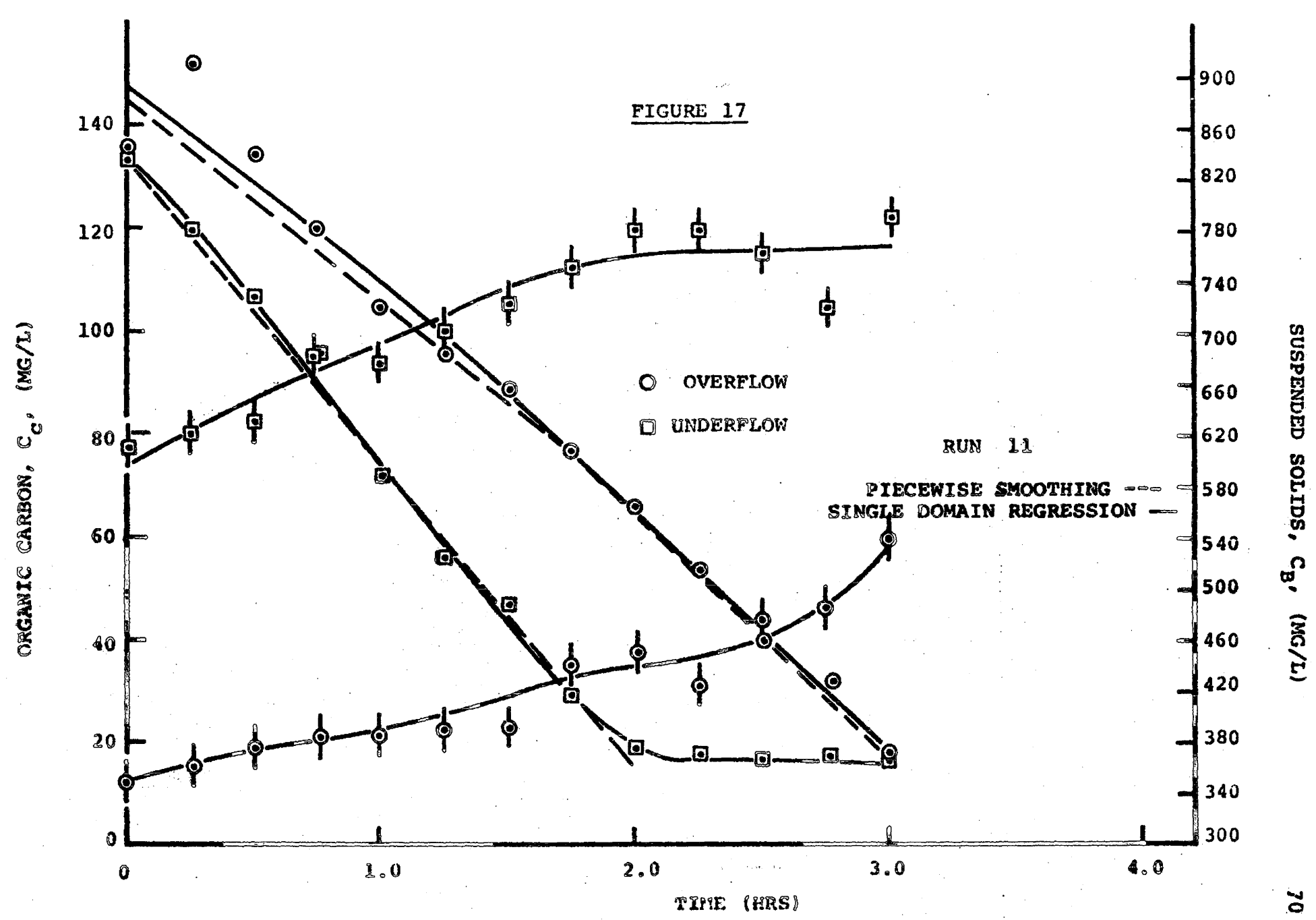


FIGURE 17
ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
OVERFLOW
UNDERFLOW
RUN 11
PIECEWISE SMOOTHING
SINGLE DOMAIN REGRESSION
0
20
40
60
80
100
120
140
300
340
380
420
460
500
540
580
620
660
700
740
780
820
860
900
0
1.0
2.0
3.0
4.0

FIGURE 18

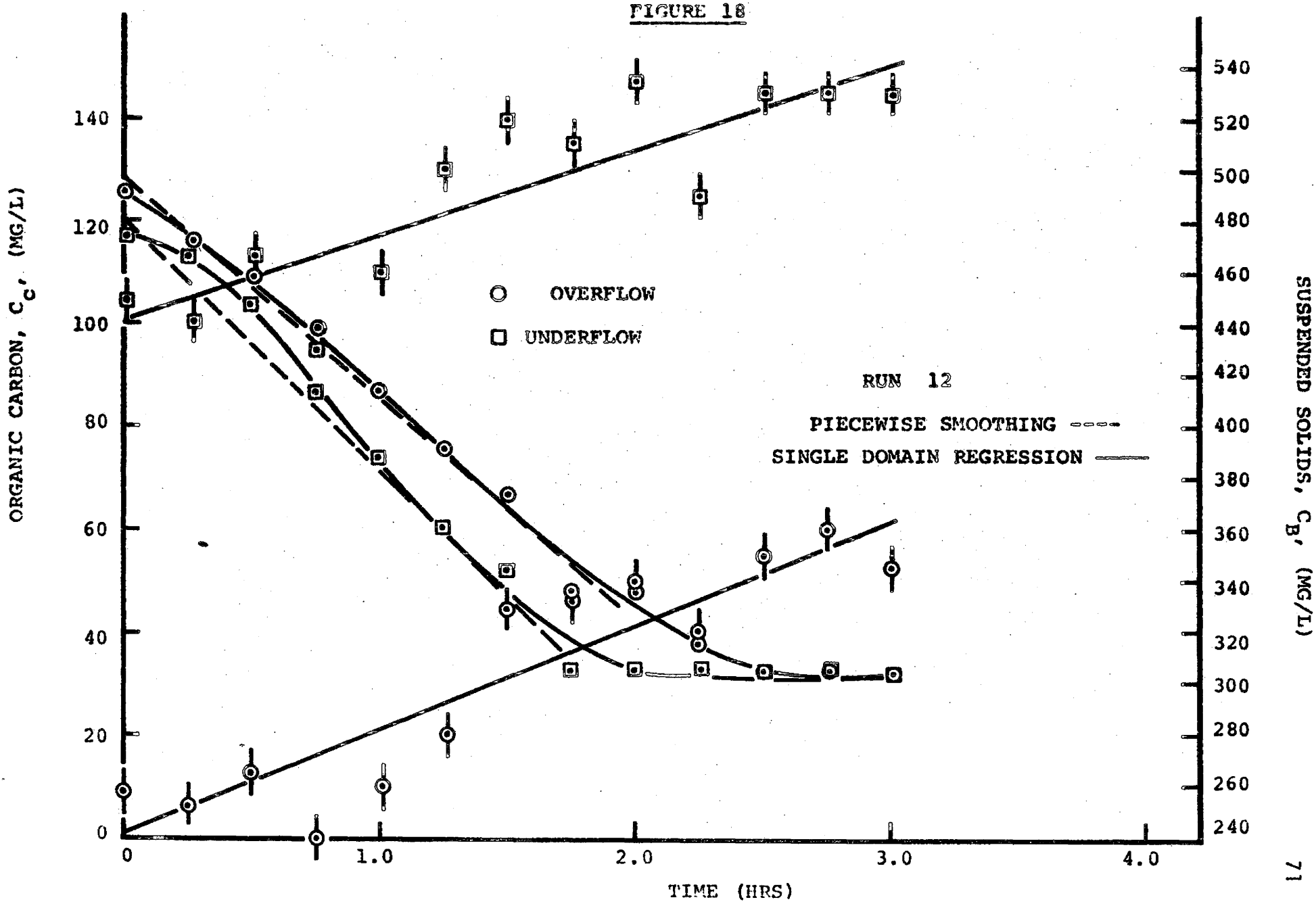

ORGANIC CARBON, $C_{c'}$ (MG/L)
SUSPENDED SOLIDS, $C_{B'}$ (MG/L)
TIME (HRS)
0
1.0
2.0
3.0
4.0
0
20
40
60
80
100
120
140
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
OVERFLOW
UNDERFLOW
RUN 12
PIECEWISE SMOOTHING
SINGLE DOMAIN REGRESSION

FIGURE 19

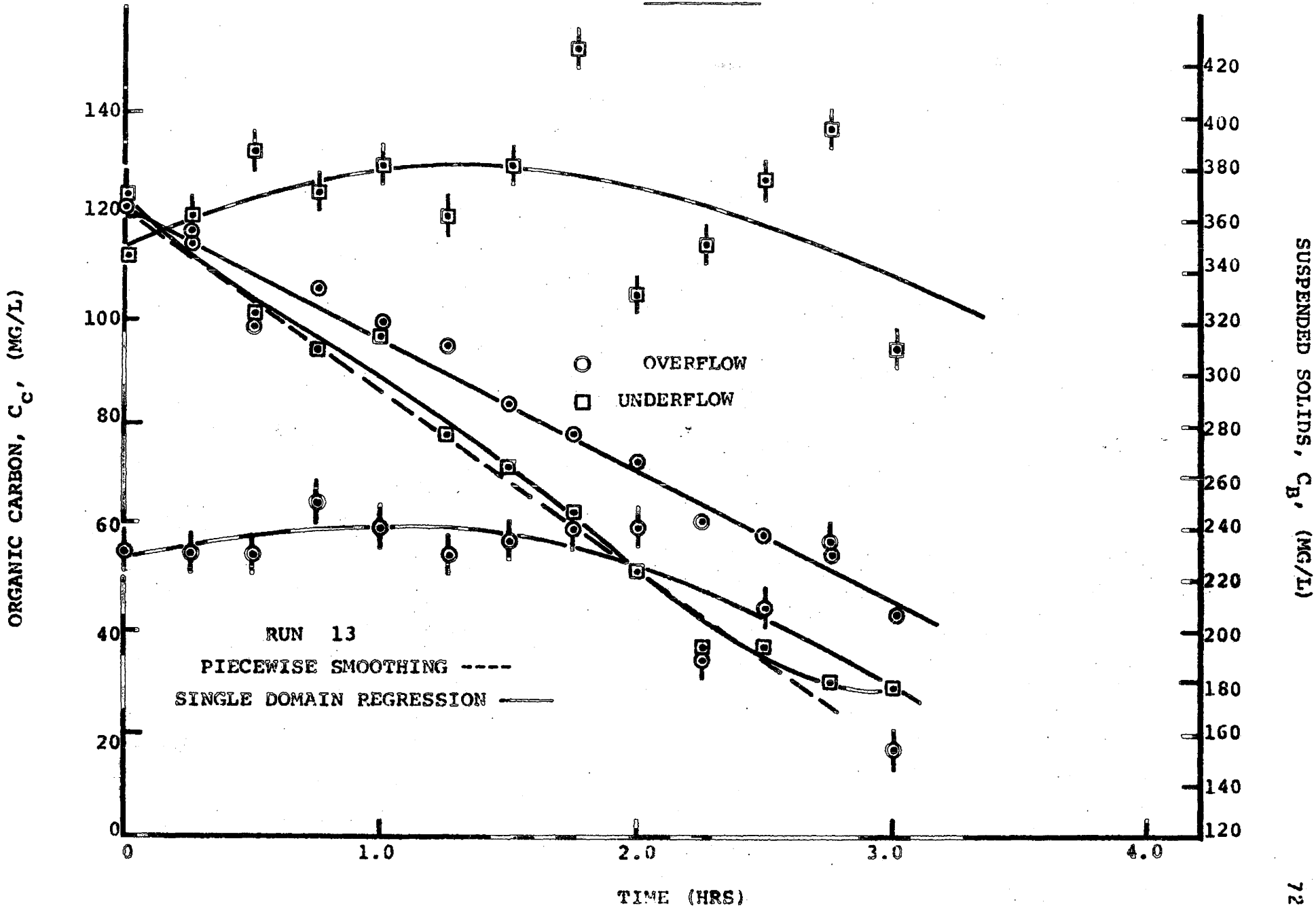

ORGANIC CARBON, C_C, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
0
1.0
2.0
3.0
4.0
0
20
40
60
80
100
120
140
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
OVERFLOW
UNDERFLOW
RUN 13
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——

FIGURE 20

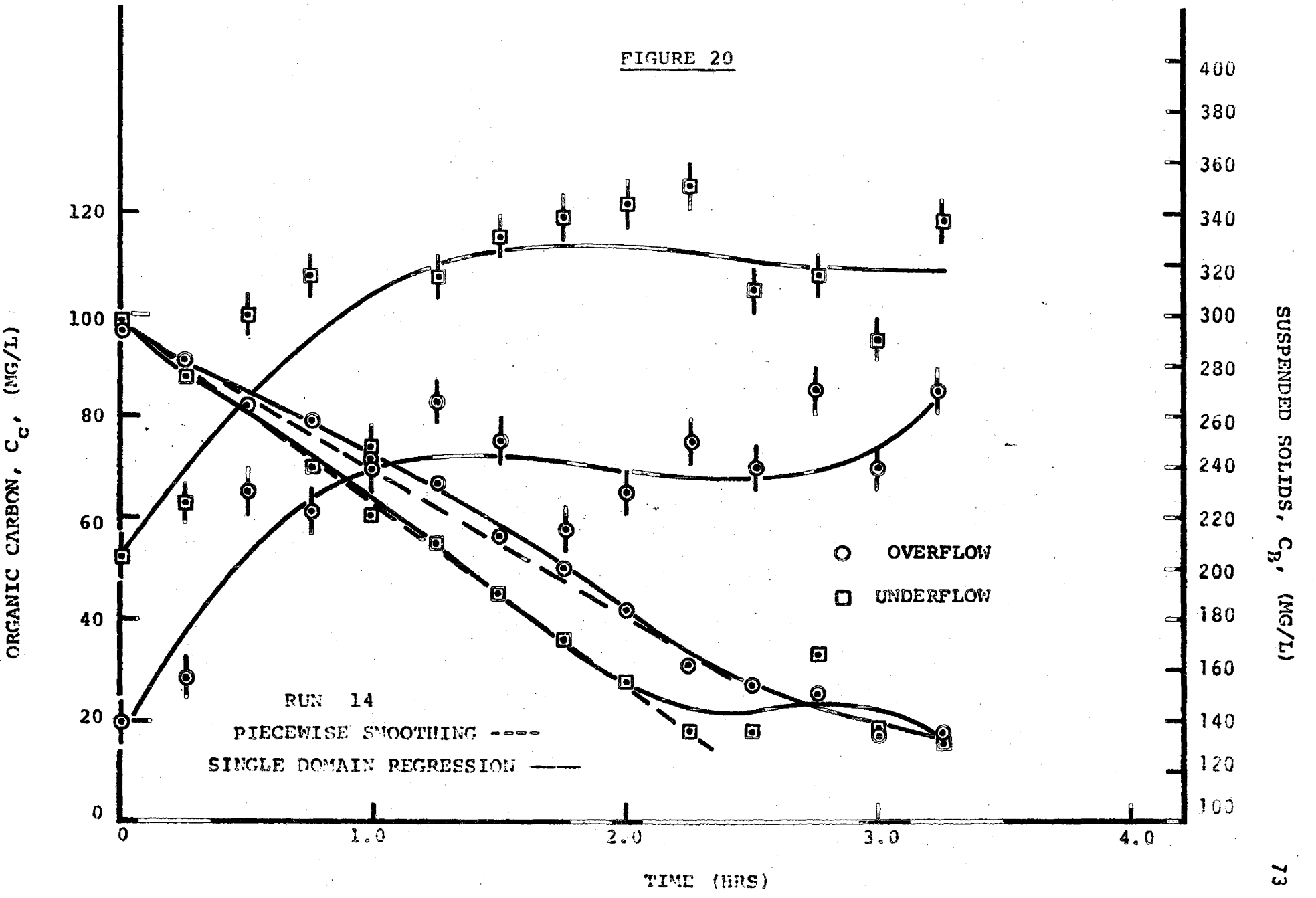

ORGANIC CARBON, C_C, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)
0
20
40
60
80
100
120
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
0
1.0
2.0
3.0
4.0
OVERFLOW
UNDERFLOW
RUN 14
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——

FIGURE 21

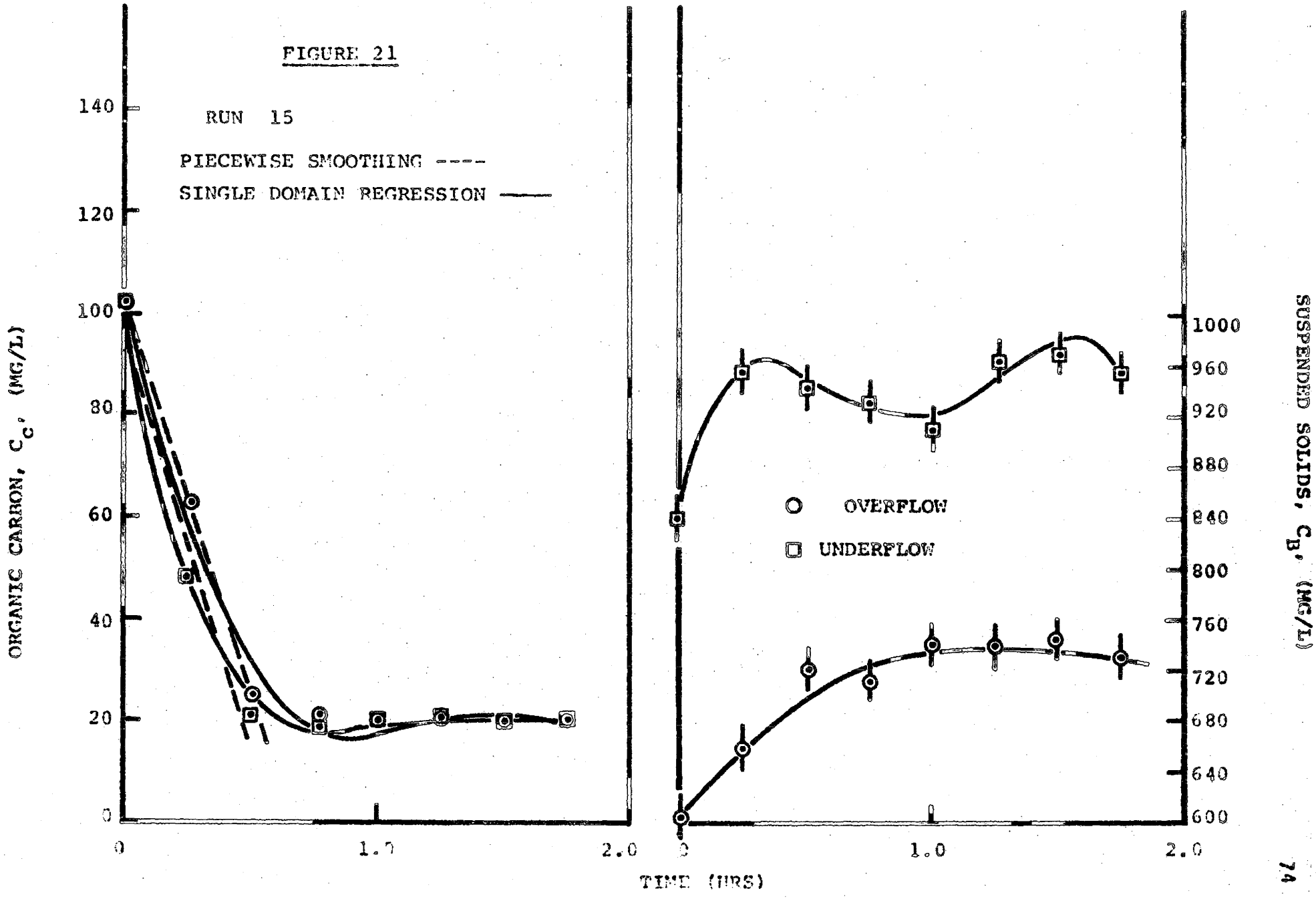
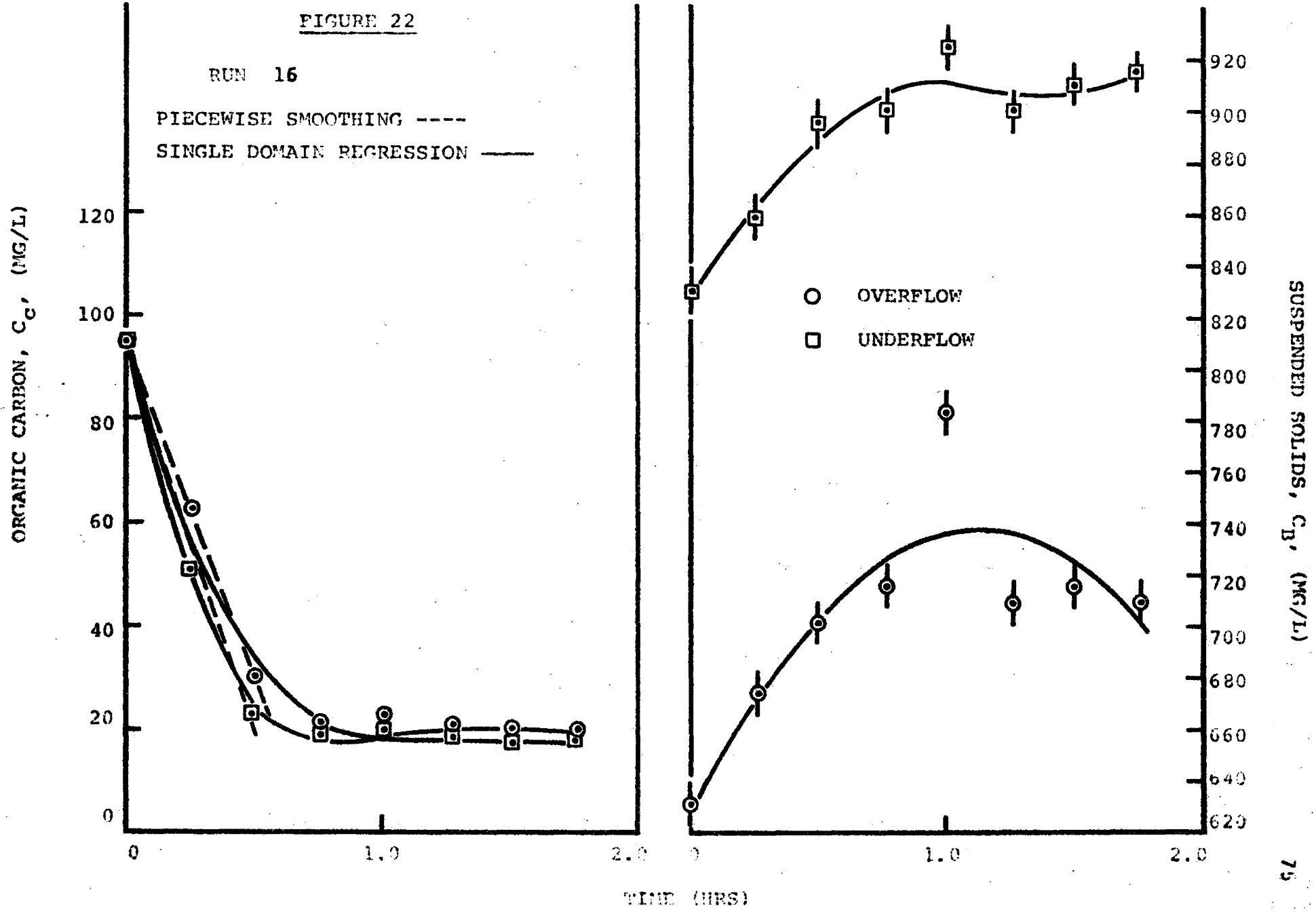

RUN 15
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
ORGANIC CARBON, C_C, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
OVERFLOW
UNDERFLOW
TIME (HRS)
0
20
40
60
80
100
120
140
600
640
680
720
760
800
840
880
920
960
1000
1.0
2.0

FIGURE 22

RUN 16

PIECEWISE SMOOTHING ----

SINGLE DOMAIN REGRESSION ——



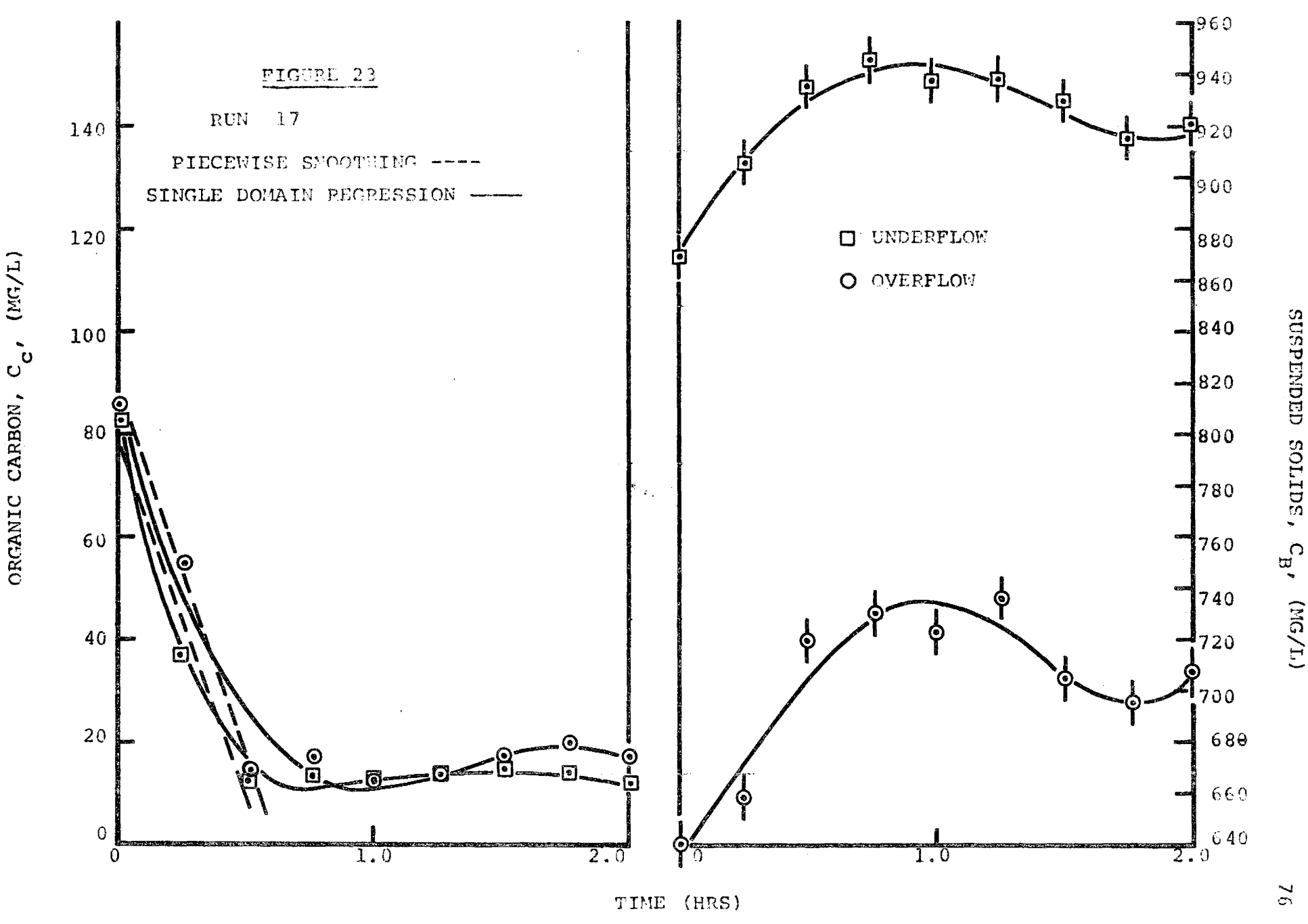


FIGURE 23
RUN 17
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
ORGANIC CARBON, C_c, (MG/L)
SUSPENDED SOLIDS, C_B, (MG/L)
UNDERFLOW
OVERFLOW
TIME (HRS)
0
20
40
60
80
100
120
140
640
660
680
700
720
740
760
780
800
820
840
860
880
900
920
940
960
0
1.0
2.0

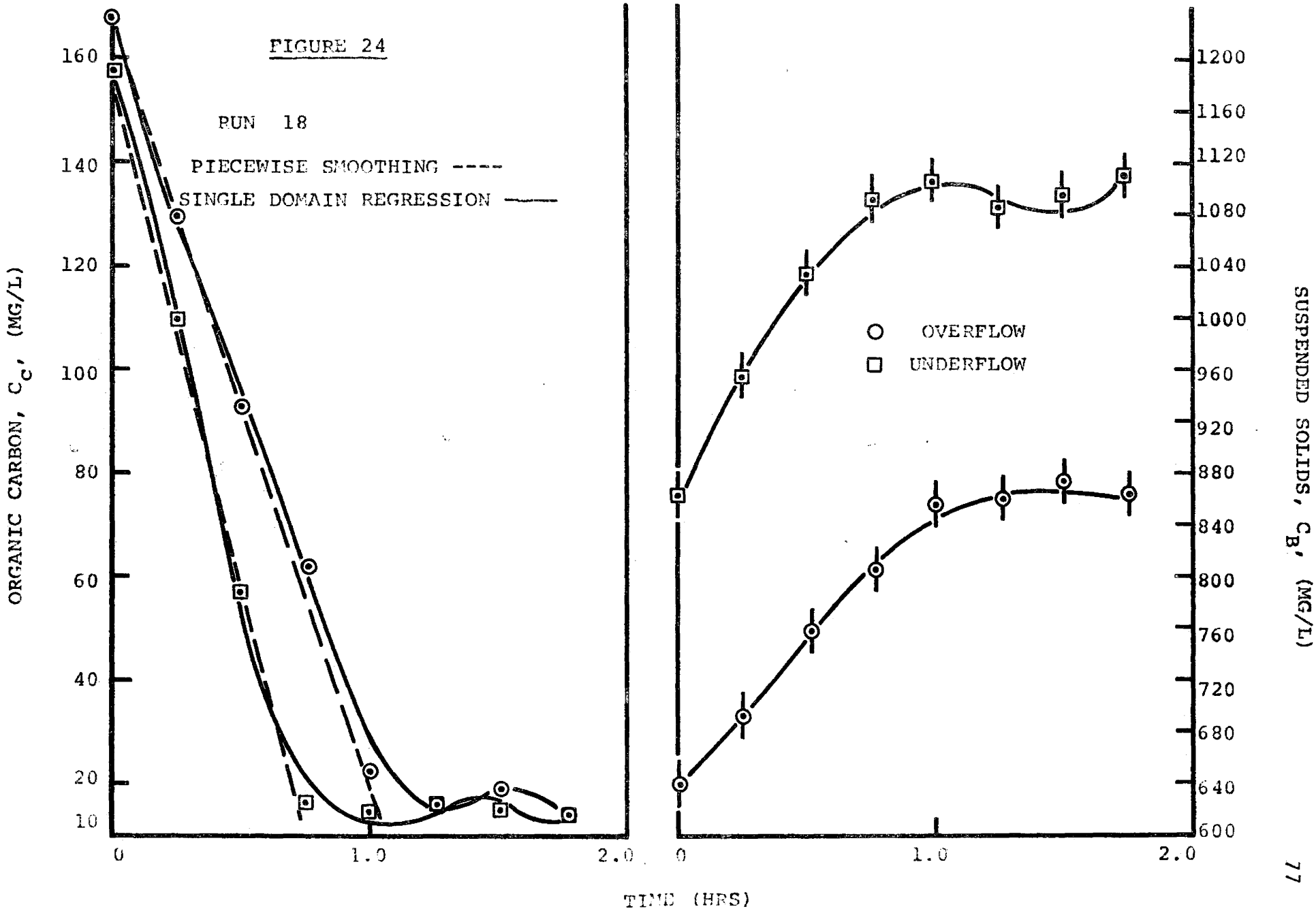


FIGURE 24
RUN 18
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——
ORGANIC CARBON, C_C, (MG/L)
160
140
120
100
80
60
40
20
10
0
1.0
2.0
OVERFLOW
UNDERFLOW
1200
1160
1120
1080
1040
1000
960
920
880
840
800
760
720
680
640
600
SUSPENDED SOLIDS, C_B, (MG/L)
TIME (HRS)

of unit rates, it is obvious that treatment of these data will be highly significant in defining any functional response of $\frac{1}{C_B}\frac{dC_c}{dt}$. Because carbon data has been traditionally expressed by a kinetic mechanism containing carbon concentration to some power, and because the rate of increase of suspended solids has been demonstrated to be a function of the velocity of carbon uptake, specific growth rate is usually plotted as a function of organic carbon concentration.

Figures No. 25 through No. 42 illustrate the dependence of $\frac{1}{C_B}\frac{dC_c}{dt}$ on C_c for which raw carbon data were fit in a piecewise fashion over the domain of each run. The slight dependence of unit rate on carbon is an expression of the variation in suspended solids levels over the course of each run. The constant velocity of carbon decrease to steady-state levels, coupled with the small increments in suspended solids relative to the initial levels, result in specific growth rates which are almost independent of the concentration of organic carbon above steady-state carbon values and which are zero below those levels. These curves resemble the two-phase kinetic mass transfer model proposed by Eckenfelder (1959).

Figures No.25 through No.42 illustrate the functional relationship of specific growth rate on organic carbon for raw data regressed into polynomials of best fit over the total domain of each run. These curves exhibit a strong dependence of specific growth rate on the concentration of organic carbon over the entire range of carbon levels prevalent throughout each test. Neglecting the drop-off in unit rate at the upper levels of organic carbon (which signify a slight lag in the initiation of carbon removal with time), these plots resemble the enzyme kinetic models of Michaelis-Menton, Monod (1949), Tessier (1936) and Schulze (1964) as well as the first and second order and second order autocatalytic kinetic models,

FIGURE 25

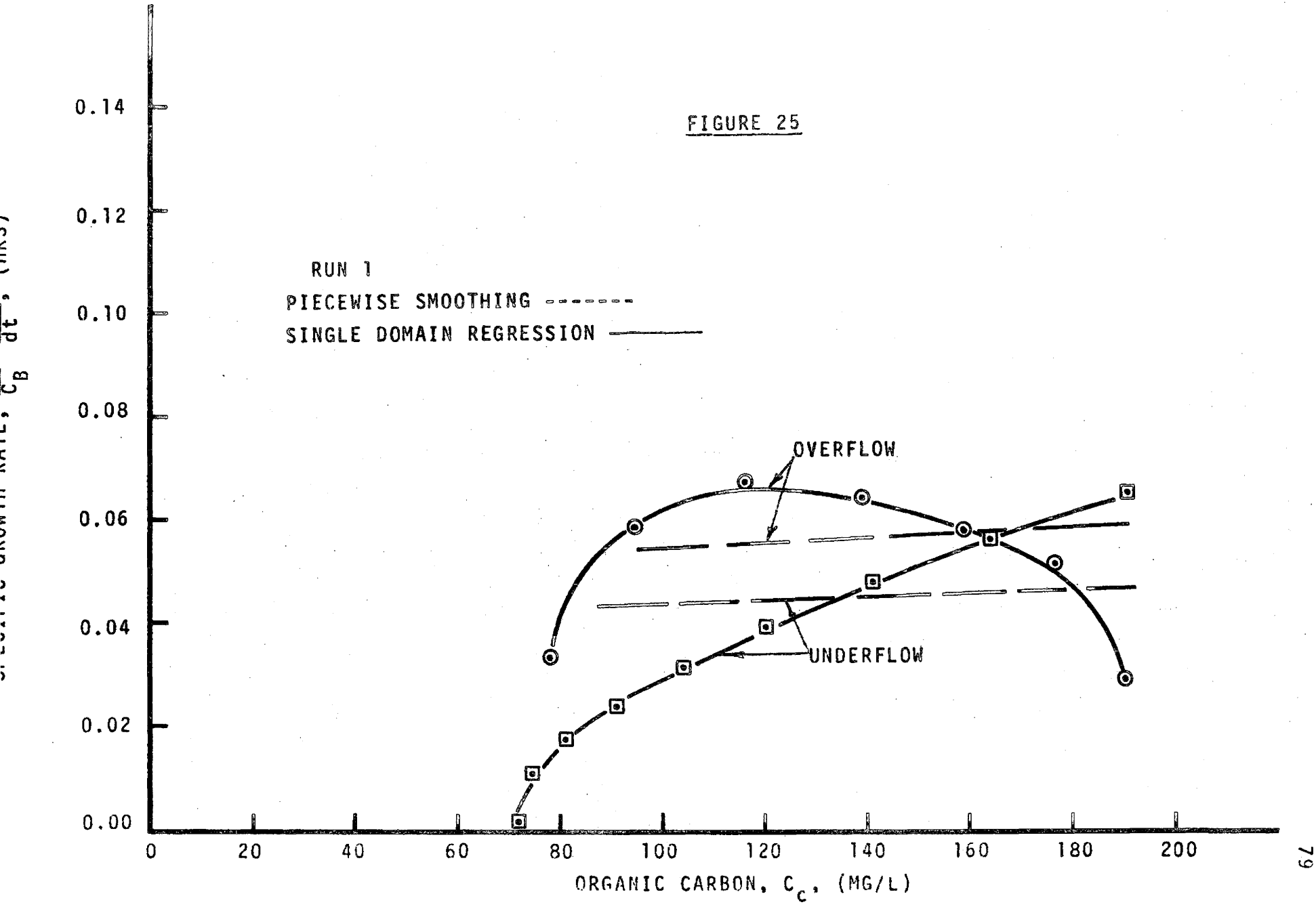

RUN 1
PIECEWISE SMOOTHING
SINGLE DOMAIN REGRESSION
OVERFLOW
UNDERFLOW
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
120
140
160
180
200
ORGANIC CARBON, C_c, (MG/L)

FIGURE 26

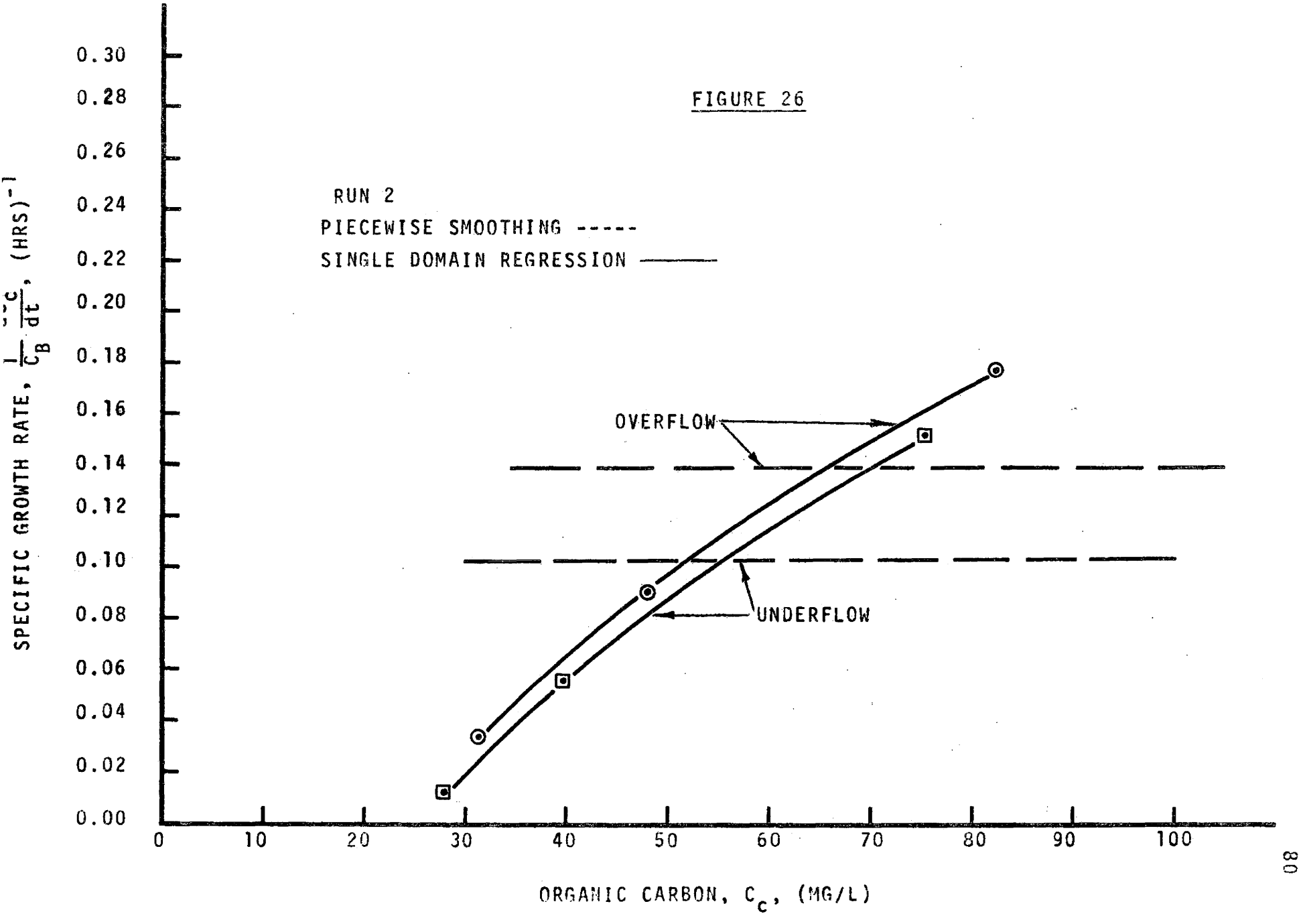

RUN 2
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
OVERFLOW
UNDERFLOW
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)

FIGURE 27

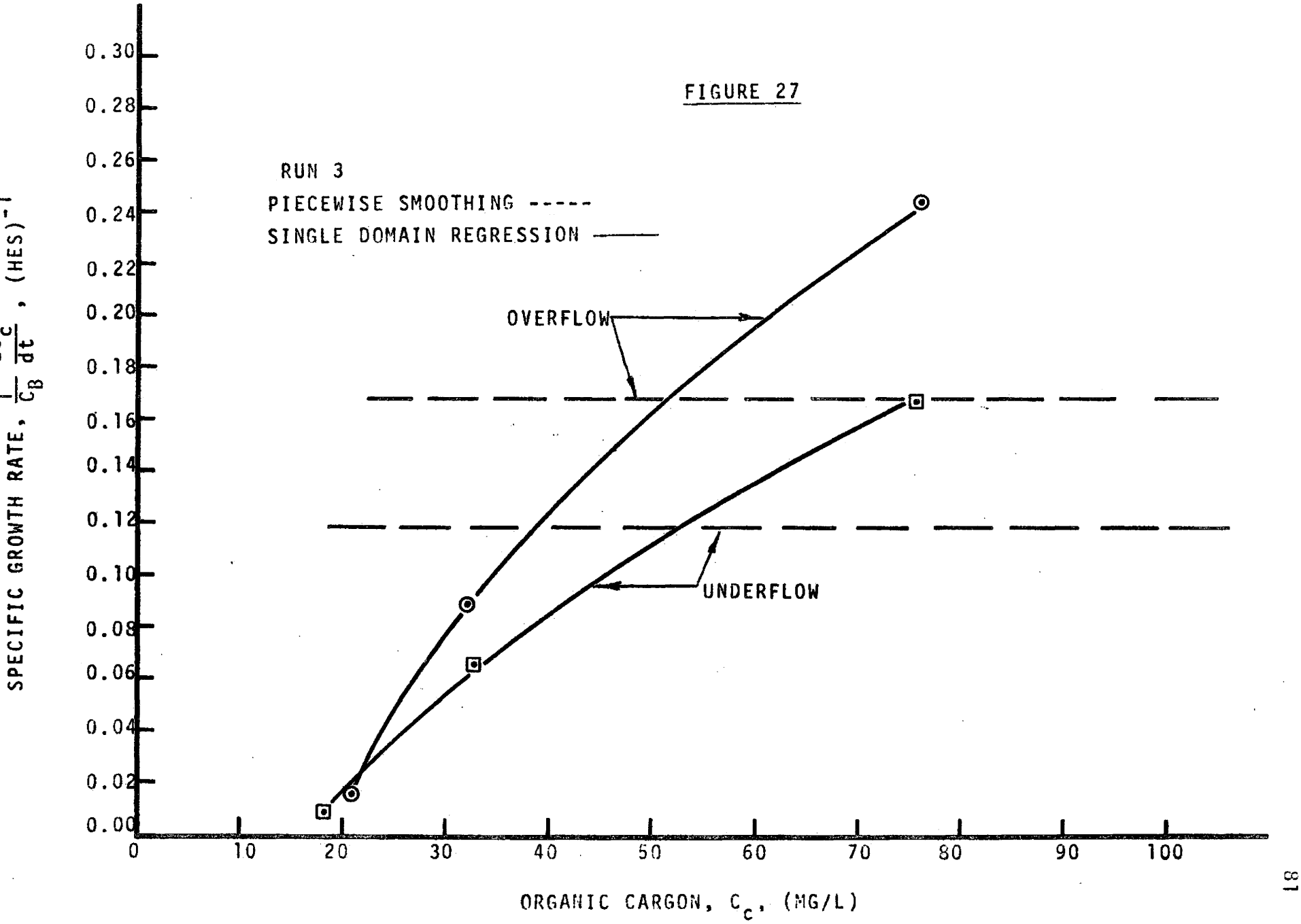

RUN 3
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
OVERFLOW
UNDERFLOW
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HES)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARGON, C_c, (MG/L)

FIGURE 28

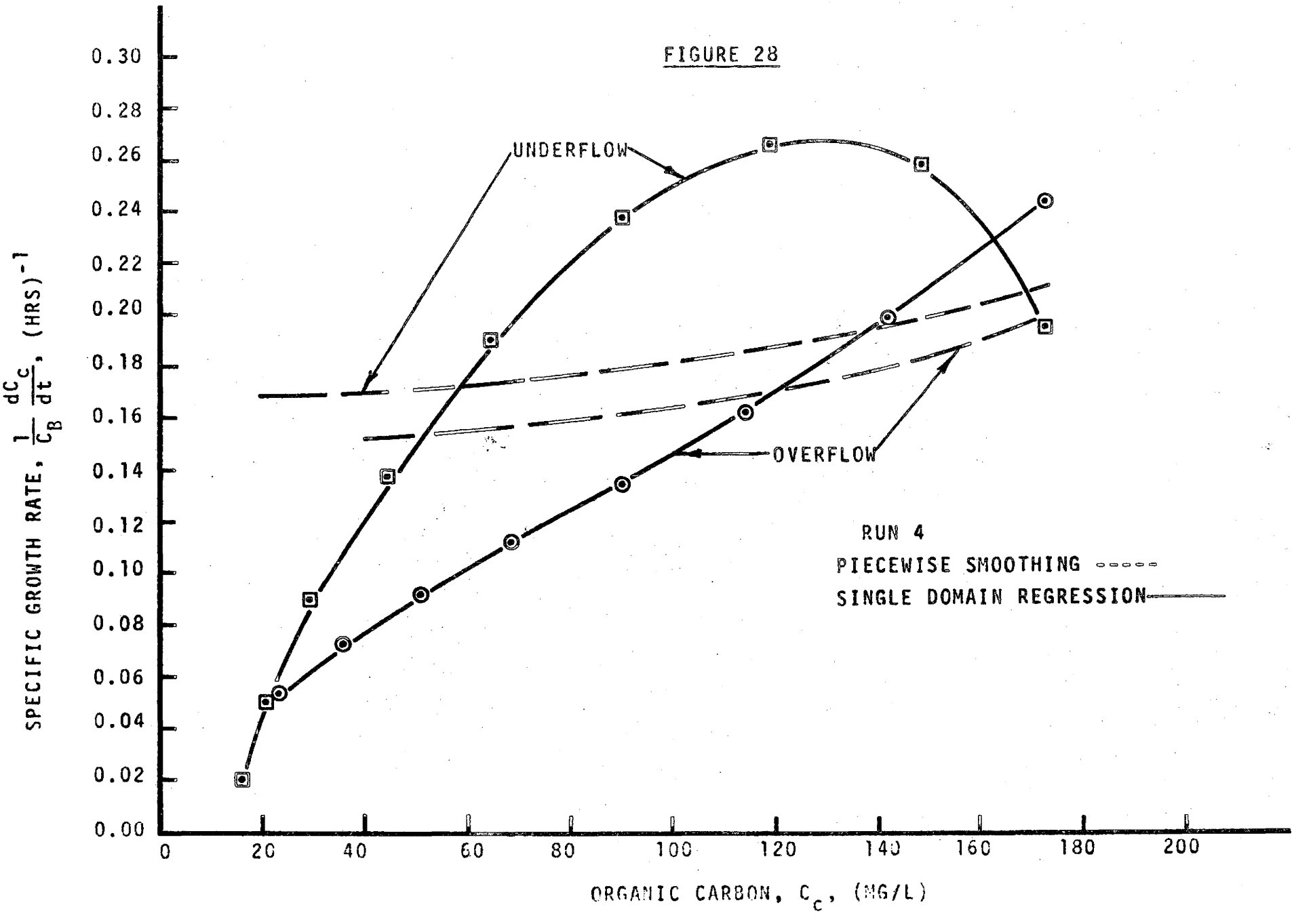

UNDERFLOW
OVERFLOW
RUN 4
PIECEWISE SMOOTHING
SINGLE DOMAIN REGRESSION
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
ORGANIC CARBON, C_c, (MG/L)
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
120
140
160
180
200

FIGURE 29

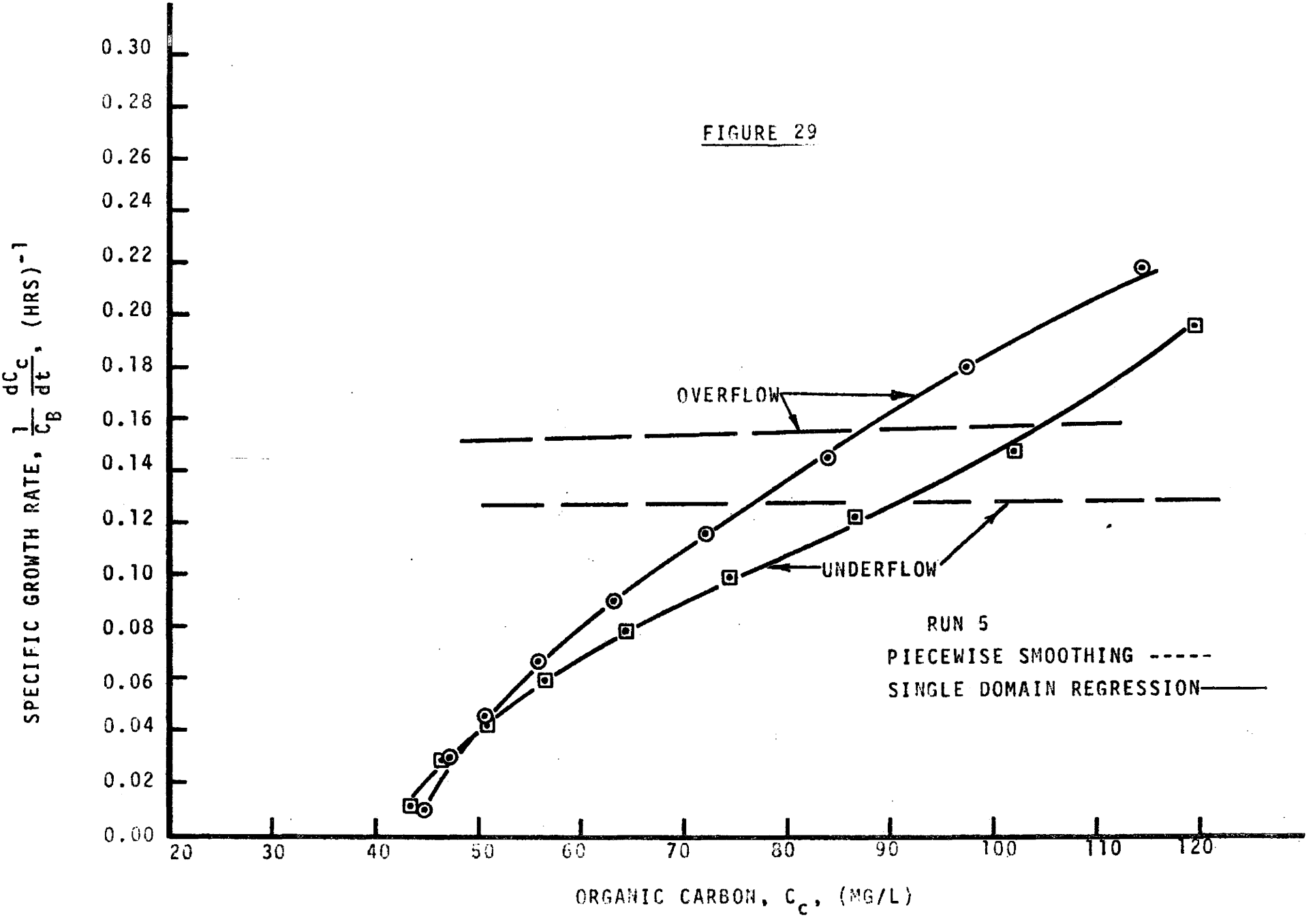

SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
20
30
40
50
60
70
80
90
100
110
120
ORGANIC CARBON, C_c, (MG/L)
OVERFLOW
UNDERFLOW
RUN 5
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION——

FIGURE 30

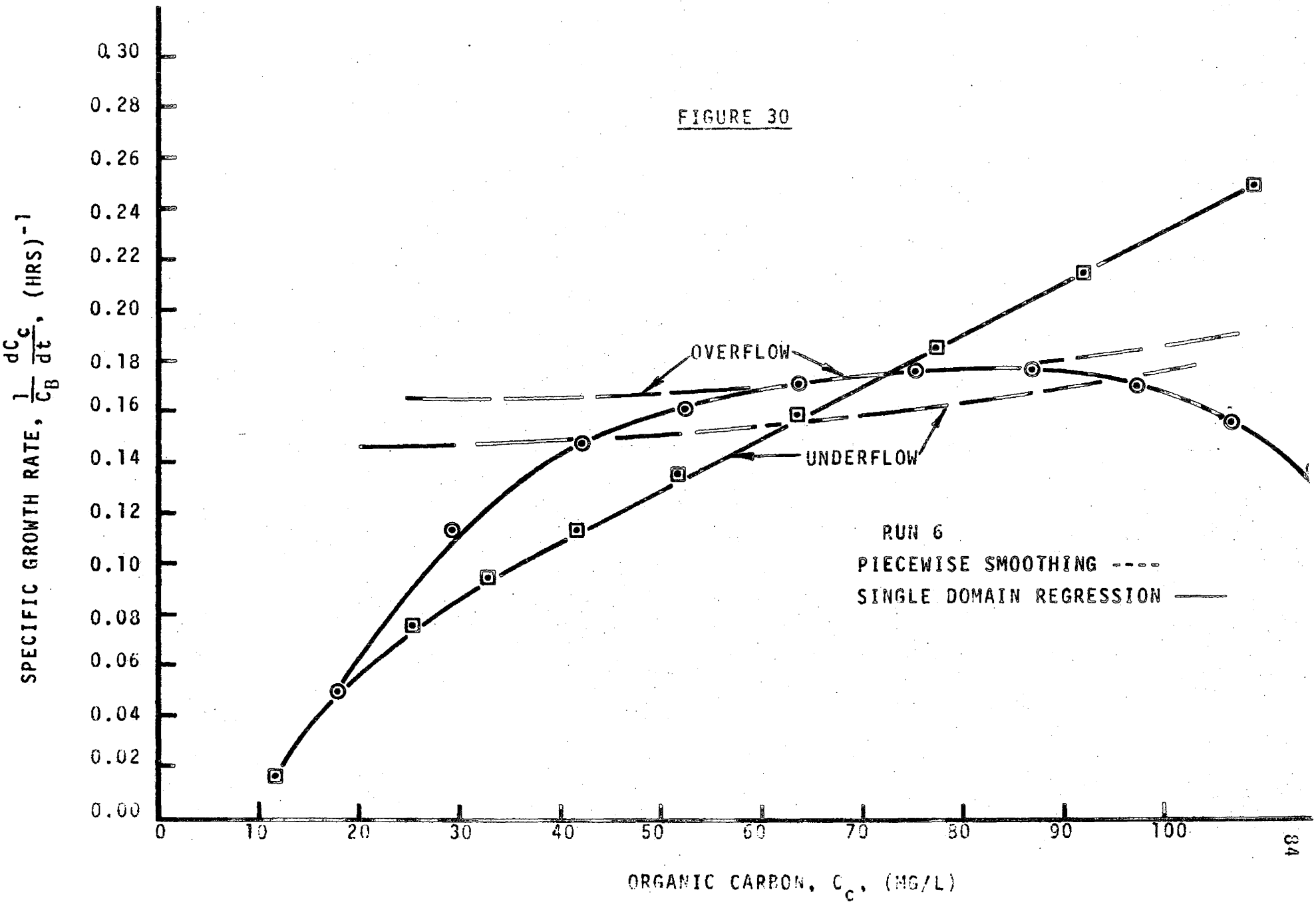

SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)
OVERFLOW
UNDERFLOW
RUN 6
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——

FIGURE 31

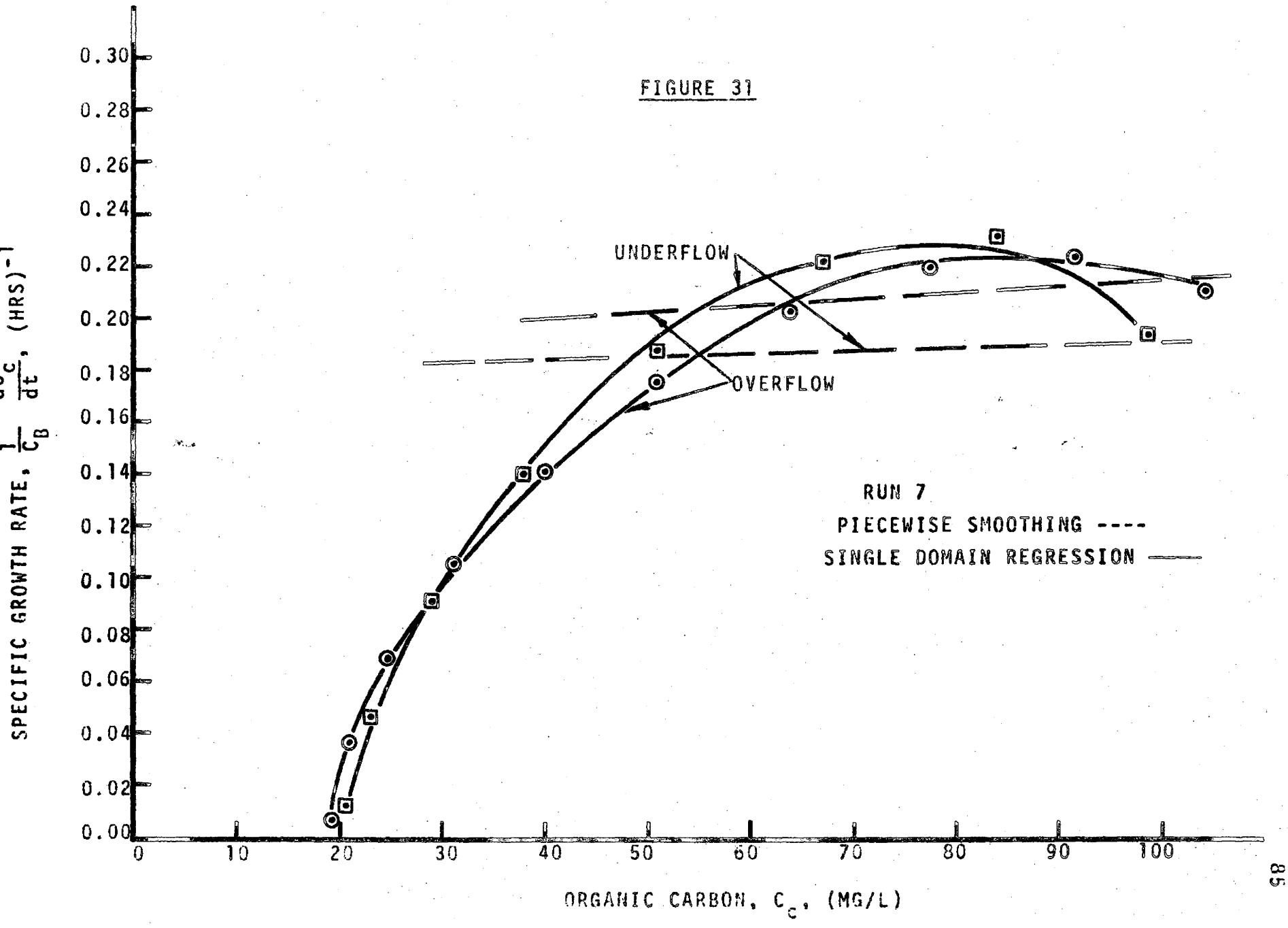

SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)
UNDERFLOW
OVERFLOW
RUN 7
PIECEWISE SMOOTHING ----
SINGLE DOMAIN REGRESSION ——

FIGURE 32

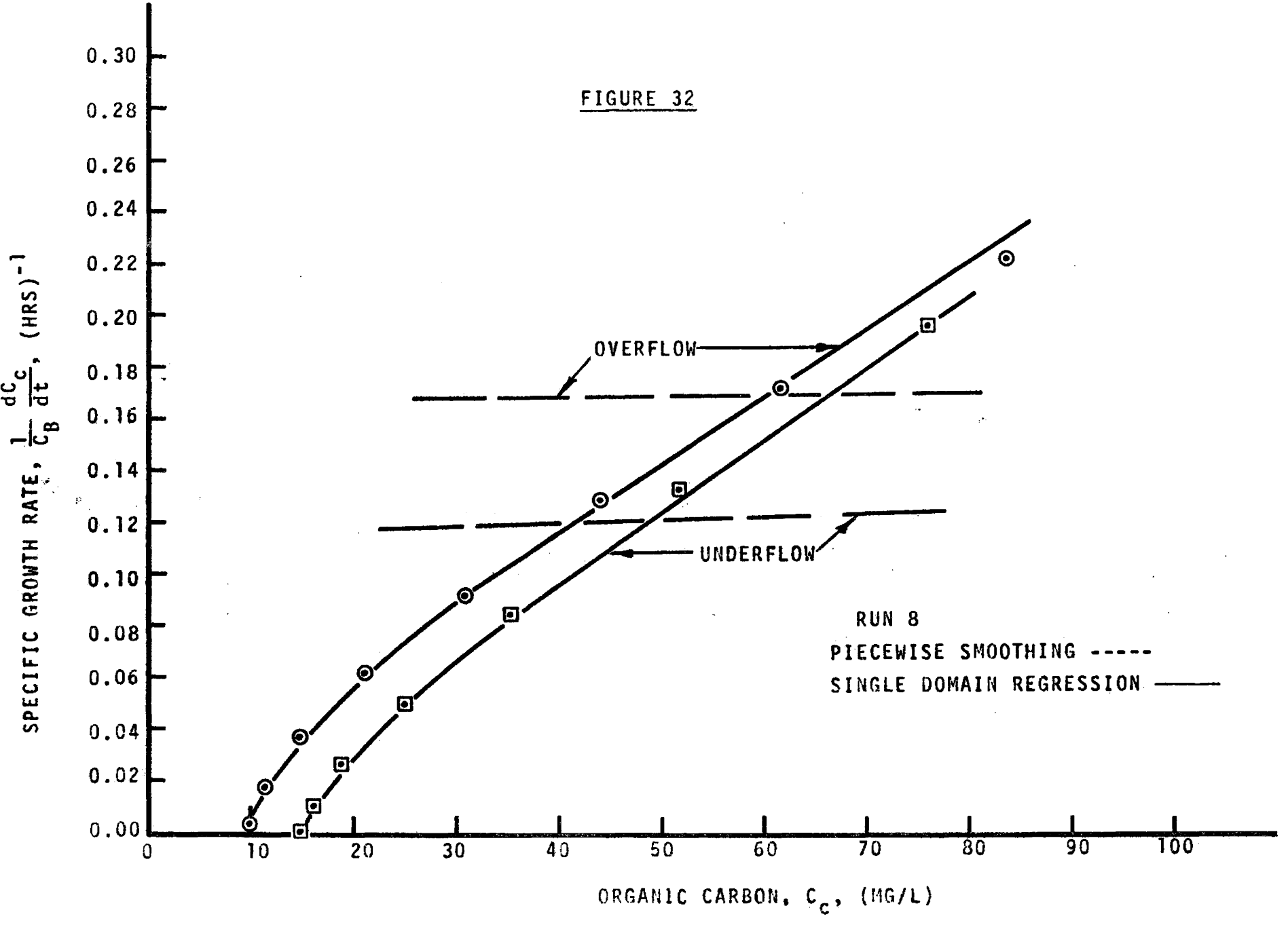

SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)
OVERFLOW
UNDERFLOW
RUN 8
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——

FIGURE 33

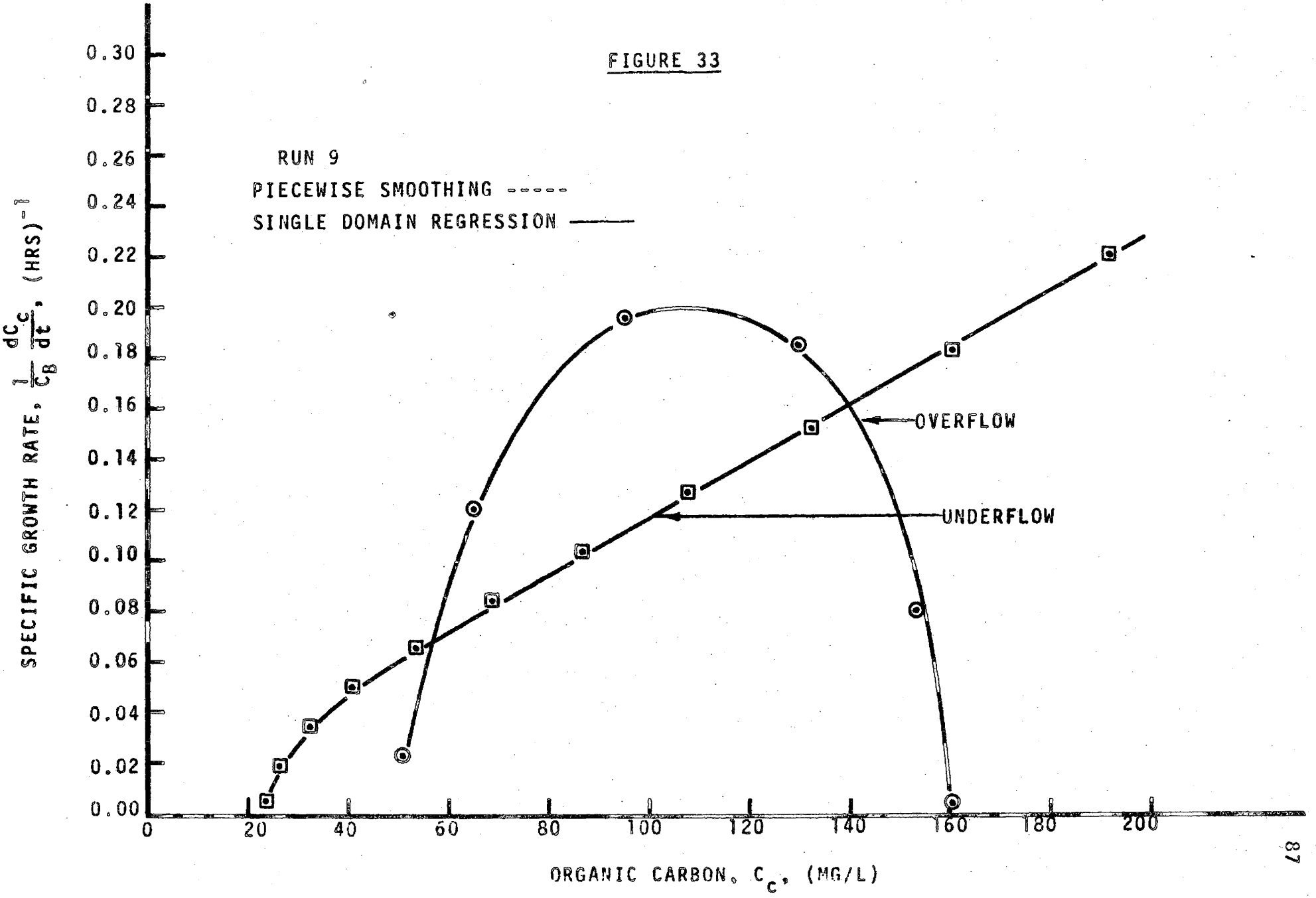

RUN 9
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
OVERFLOW
UNDERFLOW
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
120
140
160
180
200
ORGANIC CARBON, C_c, (MG/L)

FIGURE 34

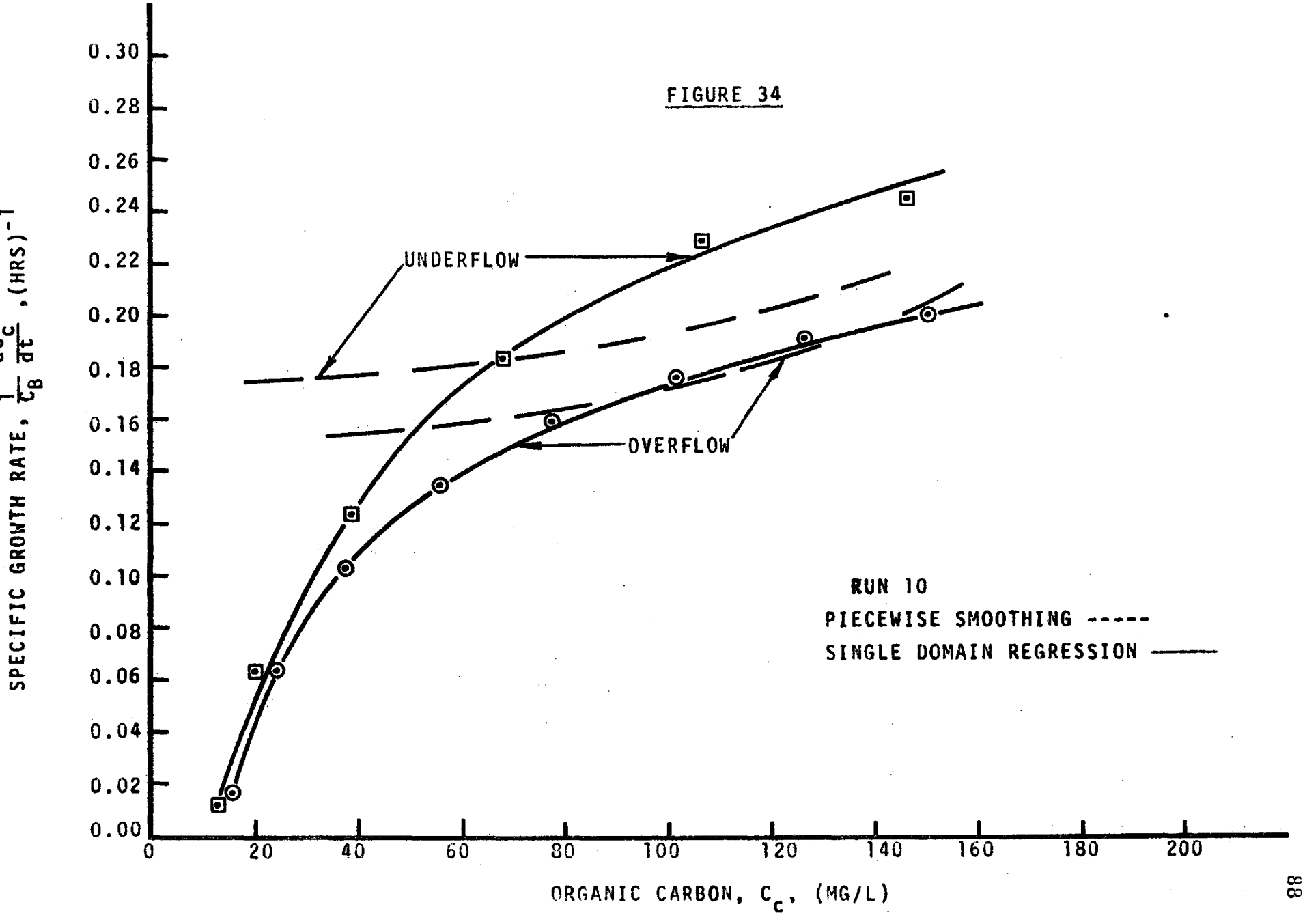

SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, (HRS)$^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
UNDERFLOW
OVERFLOW
RUN 10
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
0
20
40
60
80
100
120
140
160
180
200
ORGANIC CARBON, C_c, (MG/L)

FIGURE 35

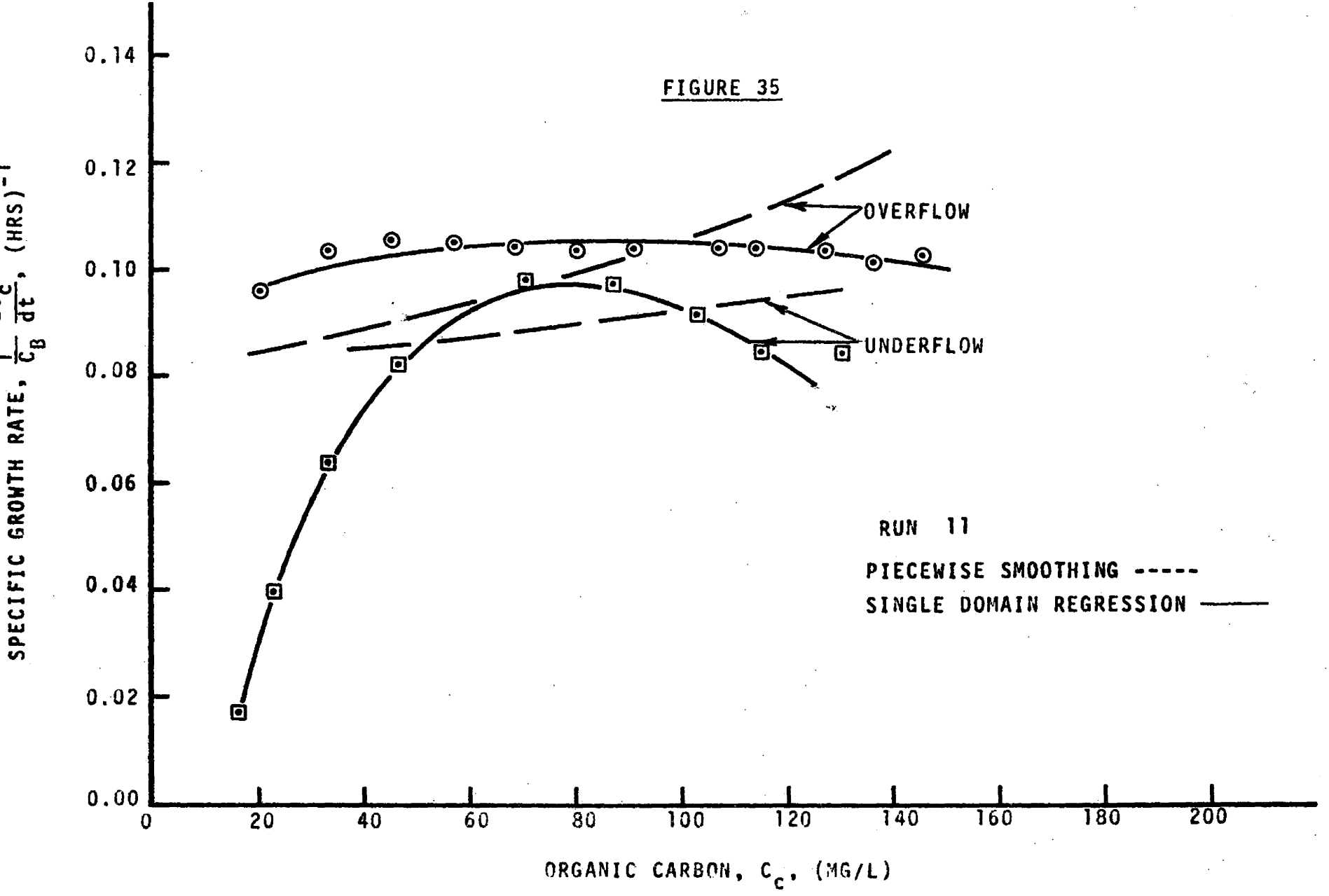

0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
120
140
160
180
200
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
ORGANIC CARBON, C_c, (MG/L)
OVERFLOW
UNDERFLOW
RUN 11
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——

FIGURE 36

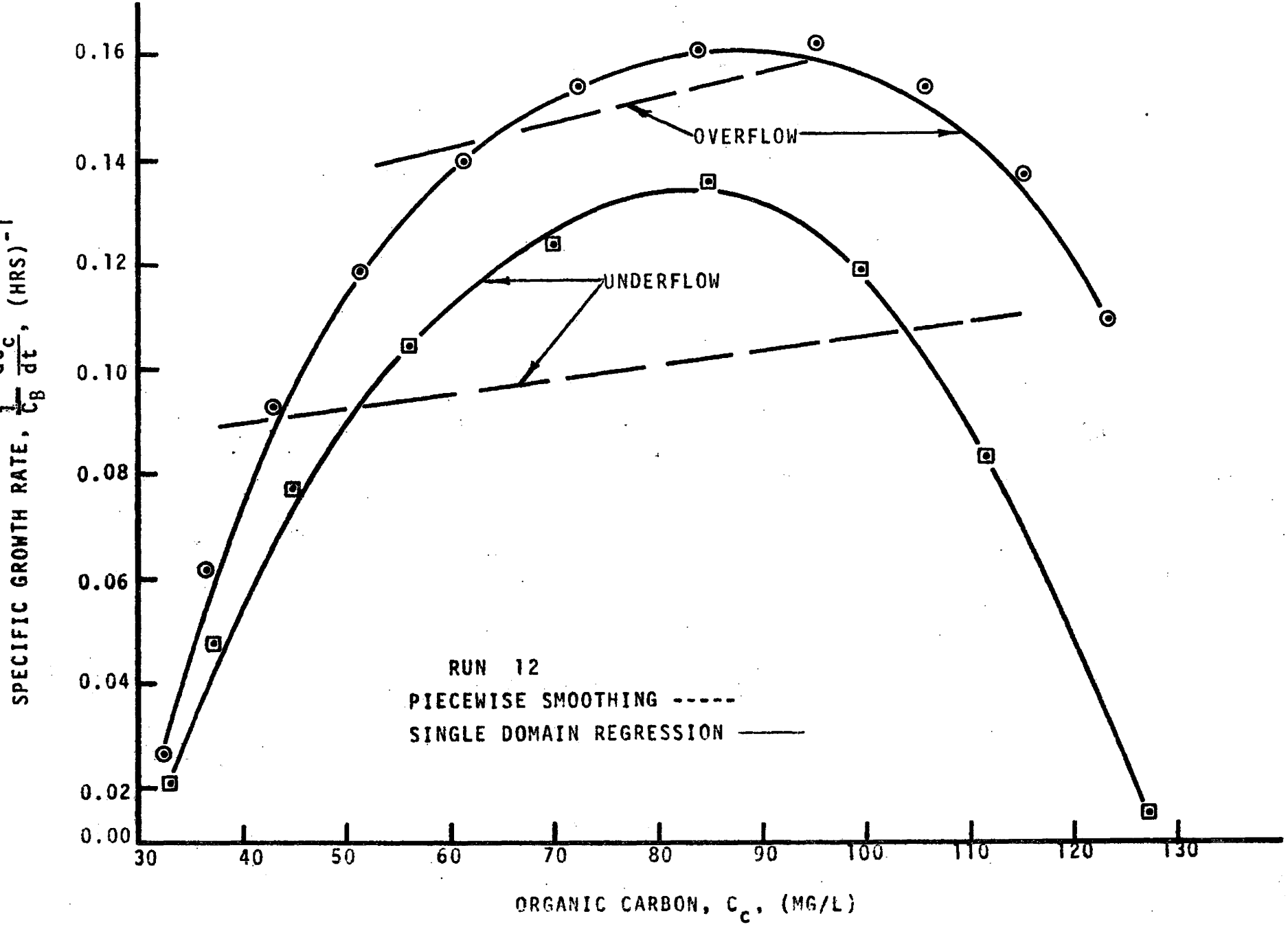

0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
OVERFLOW
UNDERFLOW
RUN 12
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
30
40
50
60
70
80
90
100
110
120
130
ORGANIC CARBON, C_c, (MG/L)

FIGURE 37

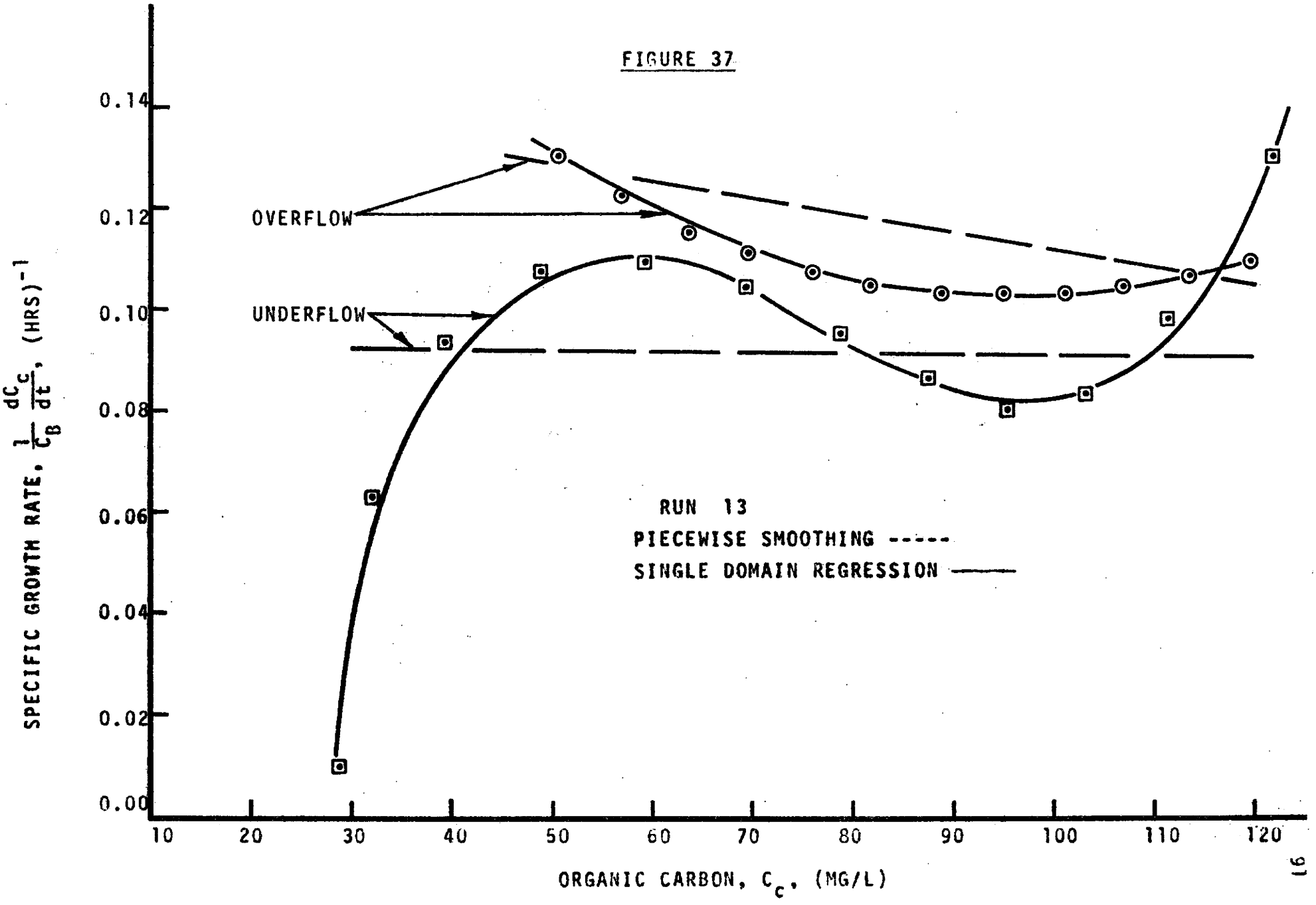

0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
OVERFLOW
UNDERFLOW
RUN 13
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
10
20
30
40
50
60
70
80
90
100
110
120
ORGANIC CARBON, C_c, (MG/L)

FIGURE 38

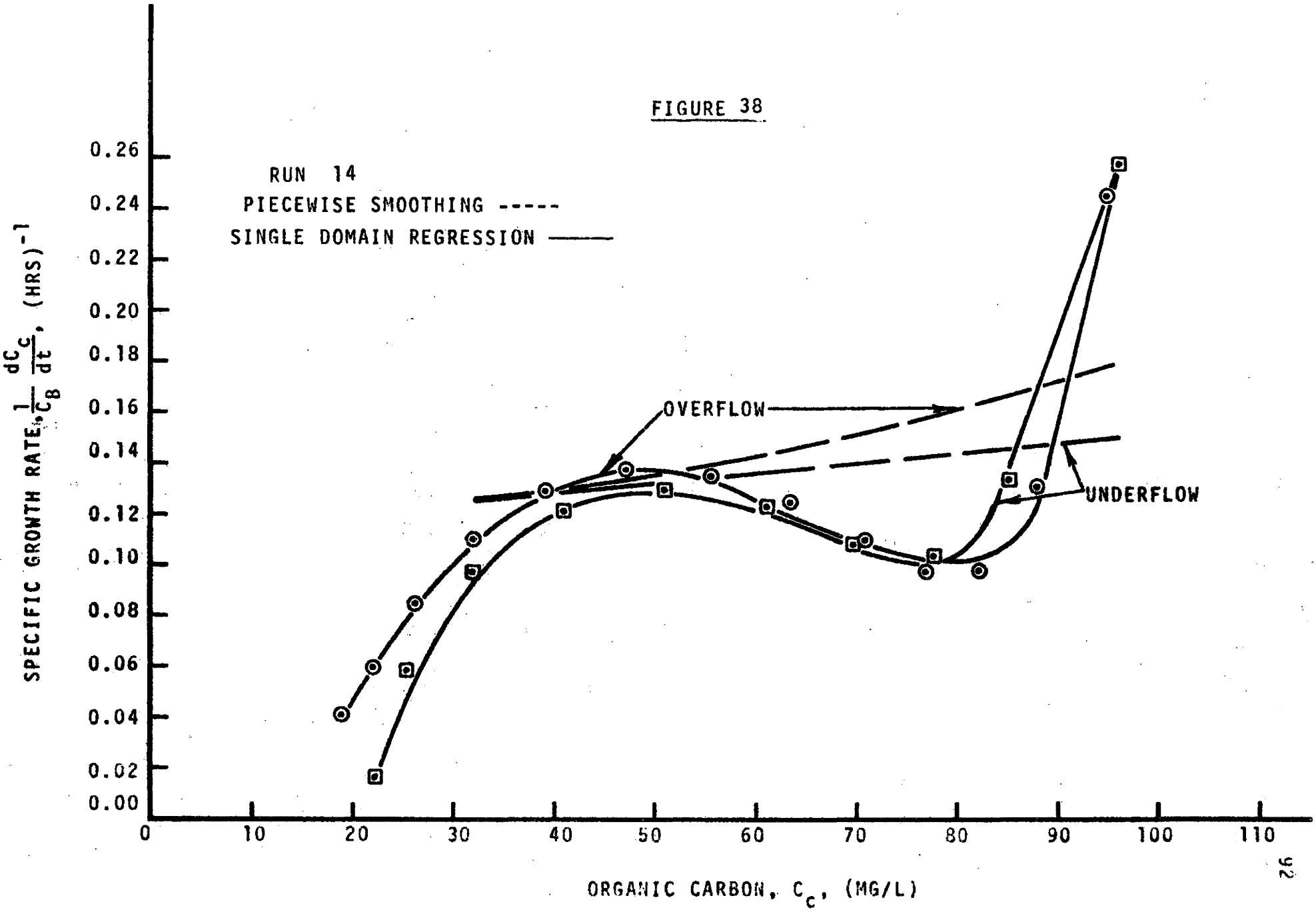
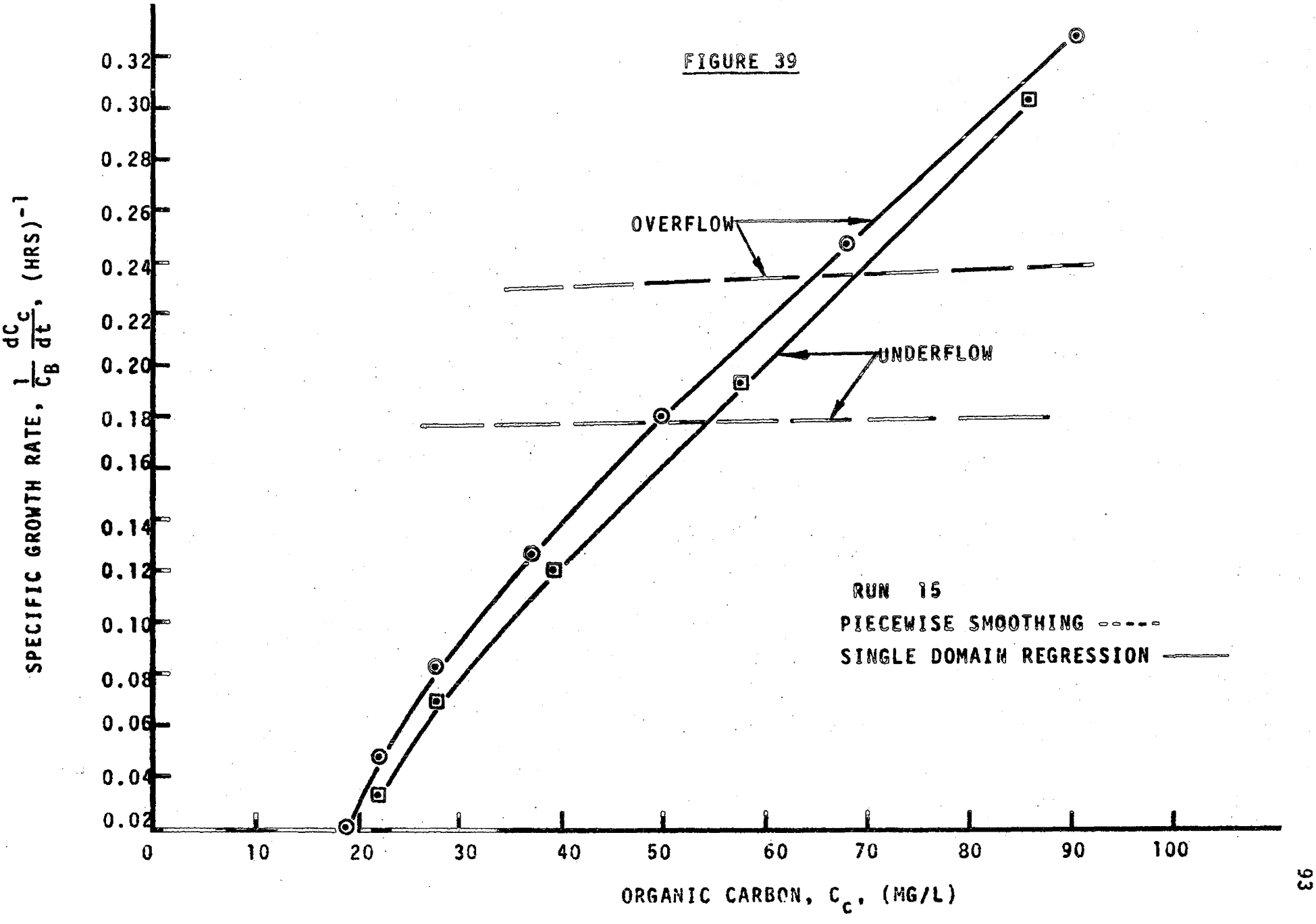

RUN 14
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
OVERFLOW
UNDERFLOW
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_C}{dt}$, $(HRS)^{-1}$
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100
110
ORGANIC CARBON, C_C, (MG/L)

FIGURE 39


SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
OVERFLOW
UNDERFLOW
RUN 15
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)

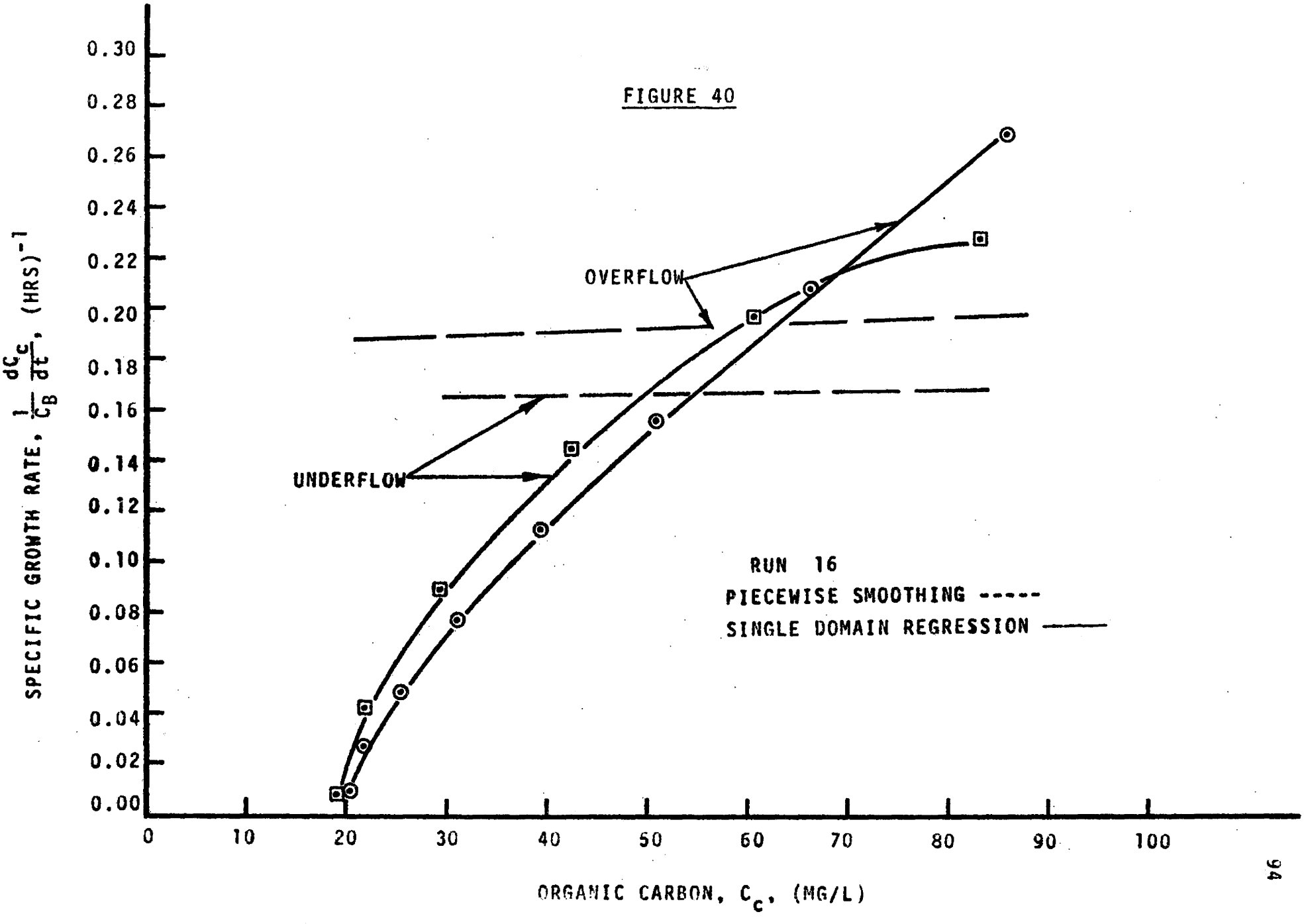


FIGURE 40
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
OVERFLOW
UNDERFLOW
RUN 16
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)

FIGURE 41

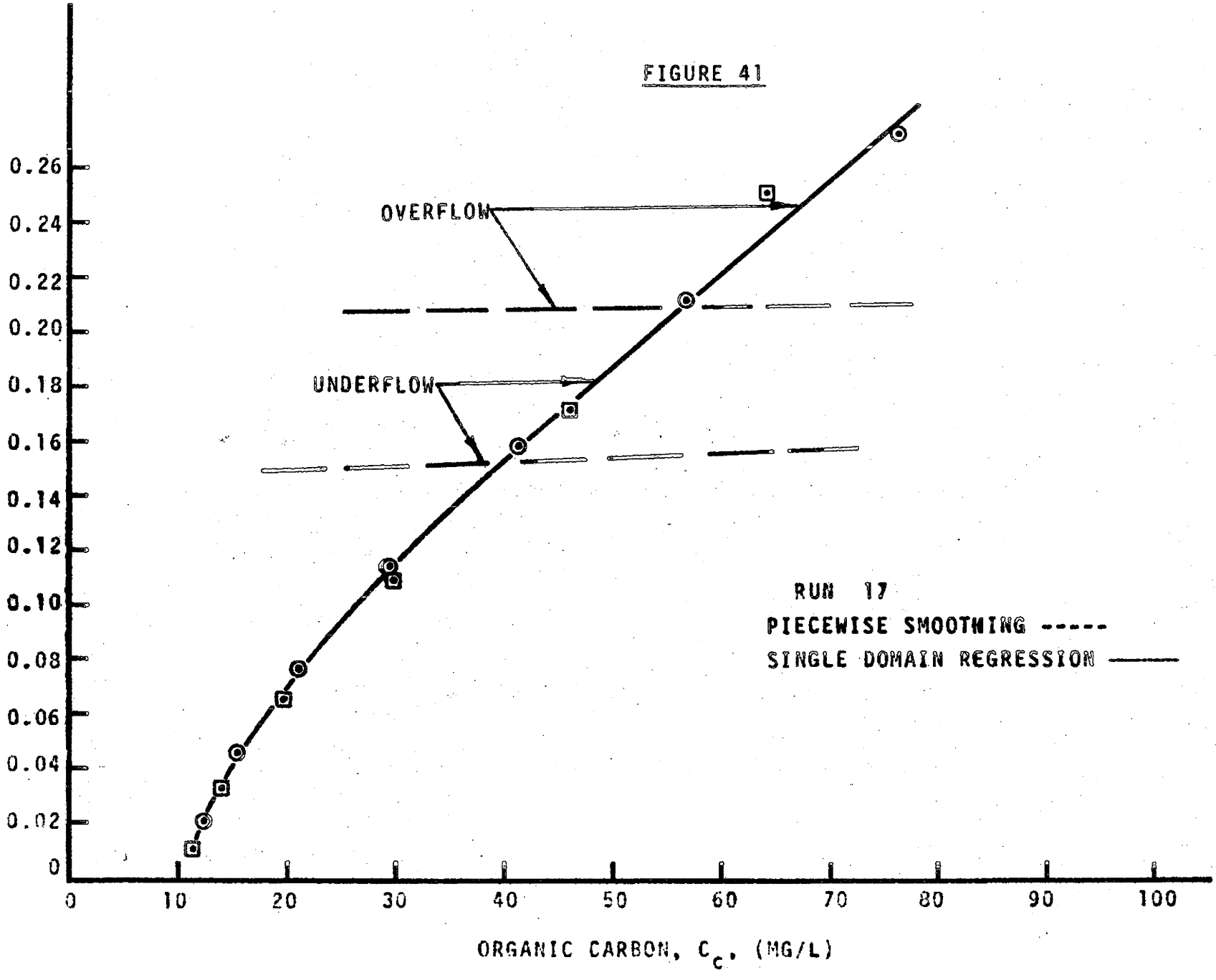
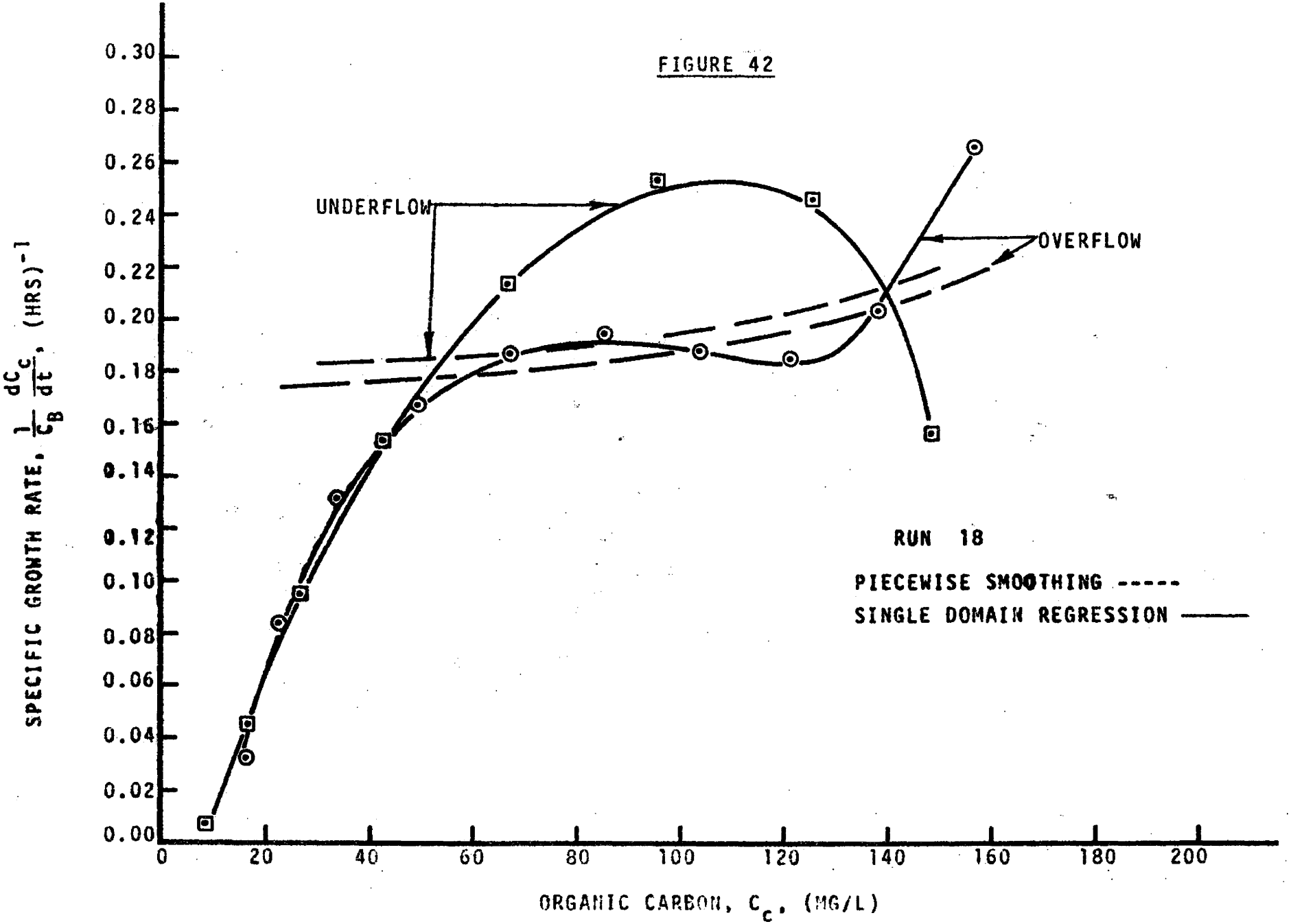

0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
OVERFLOW
UNDERFLOW
RUN 17
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
0
10
20
30
40
50
60
70
80
90
100
ORGANIC CARBON, C_c, (MG/L)

FIGURE 42


UNDERFLOW
OVERFLOW
RUN 18
PIECEWISE SMOOTHING -----
SINGLE DOMAIN REGRESSION ——
SPECIFIC GROWTH RATE, $\frac{1}{C_B}\frac{dC_c}{dt}$, $(HRS)^{-1}$
ORGANIC CARBON, C_c, (MG/L)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
0.26
0.28
0.30
0
20
40
60
80
100
120
140
160
180
200

prevalent in the literature. The mathematical model of Martin and Washington (1964) could also be easily fit to these curves.

Since both families of specific growth rate curves are derived from data which are significant at the 95% level, then specific growth rate may be argued to be both highly dependent on the mass concentration of organic carbon and virtually independent of the mass concentration of organic carbon.

It may be concluded that specific growth rate, within our ability to measure it, is a meaningless quantity for a batch process and its use as a justification for kinetic models or as a quantity for design must be questioned. It is evident that the functional form of specific growth rate is highly dependent on:

(1) the type of data fitting employed for carbon,

(2) the number of data entries in the "steady-state" region of carbon levels.

Since significantly different relationships between specific growth rate and organic carbon concentration were obtained when the range of data entries for each run was considered to consist of one and two domains, the question arises as to which method of treatment is "most justifiable".

When each data set was considered as a single domain, high order polynomials were observed to fit the raw data extremely well, several times resulting in residual mean squares not significant at the 99.9% level. It was felt, however, that only those coefficients accounting for a reduction in the total sum of squares significant at the 95% level (relative to the residual sum of squares) should be retained in the regression equations. This variation in treatment resulted in several regression lines whose residual

mean squares were significant at the 95% level. Relative to the method of piecewise fitting, this regression technique in general, represented a decrease in the goodness of fit.

Therefore, piecewise smoothing of the raw organic carbon data as a function of time was considered to be the most acceptable method of data treatment.

6.1.2 Organic Carbon

The change in mass concentration of organic carbon with time is presented in Figures 7-24 for the overflow and underflow reactors of each run.

For all but one of the experimental runs, the mass concentration of extracellular soluble organic carbon (hereafter defined as carbon) decreased at uniform rates to steady-state levels. Arithmetic linear removal characteristics with respect to time have been reported for several single aliphatic and aromatic organic compounds in mixed culture studies for which the extent of reaction has been indexed as COD or compound mass concentration: (Wilson (1967), Tischler and Eckenfelder (1968), Rao and Gaudy (1966), Krishnan and Gaudy (1966) and Banerji et al. (1968)).

This phenomenon, interpreted in a kinetic sense, indicates that the rate of carbon removal is independent of both the carbon concentration and the micro-organism concentration, since both of these quantities change with time.

The fact that the biological reaction system is independent of carbon concentration further suggests that mass transfer does not define rate limitation. The reaction constraint would appear to be exercised by the metabolic pathways of the micro-organisms.

The velocity of carbon removal is plotted against the initial mass concentration of micro-organisms for the overflow and underflow reactors of each run in Figure No. 43. Statistical Analysis (Appendix No. IV) indicated a linear correlation to be significant at the 99.9% level. A correlation coefficient "R" of 0.58126 was obtained for all the data considered as one set, indicating that the correlation line removed only 33.79% of the total sum of squares. Therefore a term of at least second order would be statistically significant at the 95% level in a regression equation.

The velocity of carbon disappearance appears to become less dependent on the mass concentration of micro-organisms above a suspended solids level of approximately 900 mg/l. Neglecting data entries above this value, a correlation significant at the 99.9% level was obtained, which removed approximately 73.1% of the total sum of squares. Although this represents an improvement in the fit, second order effects are still significant at the 95% level.

Grouping the data according to overflow and underflow fractions resulted in correlation lines which were significant at the 99.9% level and which defined residual sums of squares from the regression line which were not significant at the 95% level. (Figures No. 44 and No. 45). Linear regression on the grouped data including all entries indicated second order effects to be significant at the 95% level. Therefore it may be concluded that the velocities of carbon removal for the overflow and underflow fractions of this test were both linear functions of the initial mass concentration of micro-organisms to a suspended solids level of approximately 900 mg/l, and that above this level, the carbon removal rates exhibit only slight dependence on the solids value.

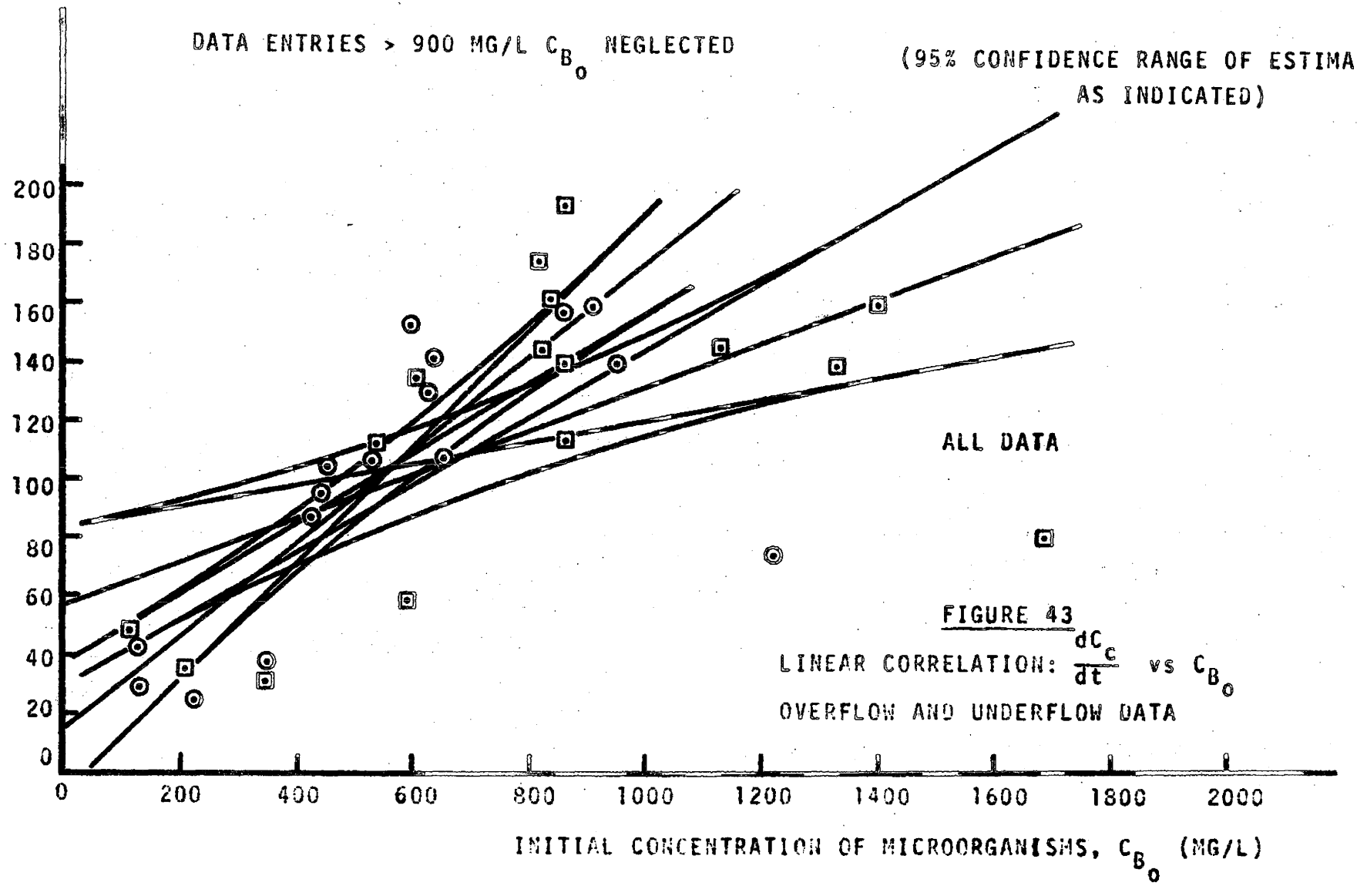


FIGURE 43

LINEAR CORRELATION: $\frac{dC_c}{dt}$ vs C_{B_o}

OVERFLOW AND UNDERFLOW DATA

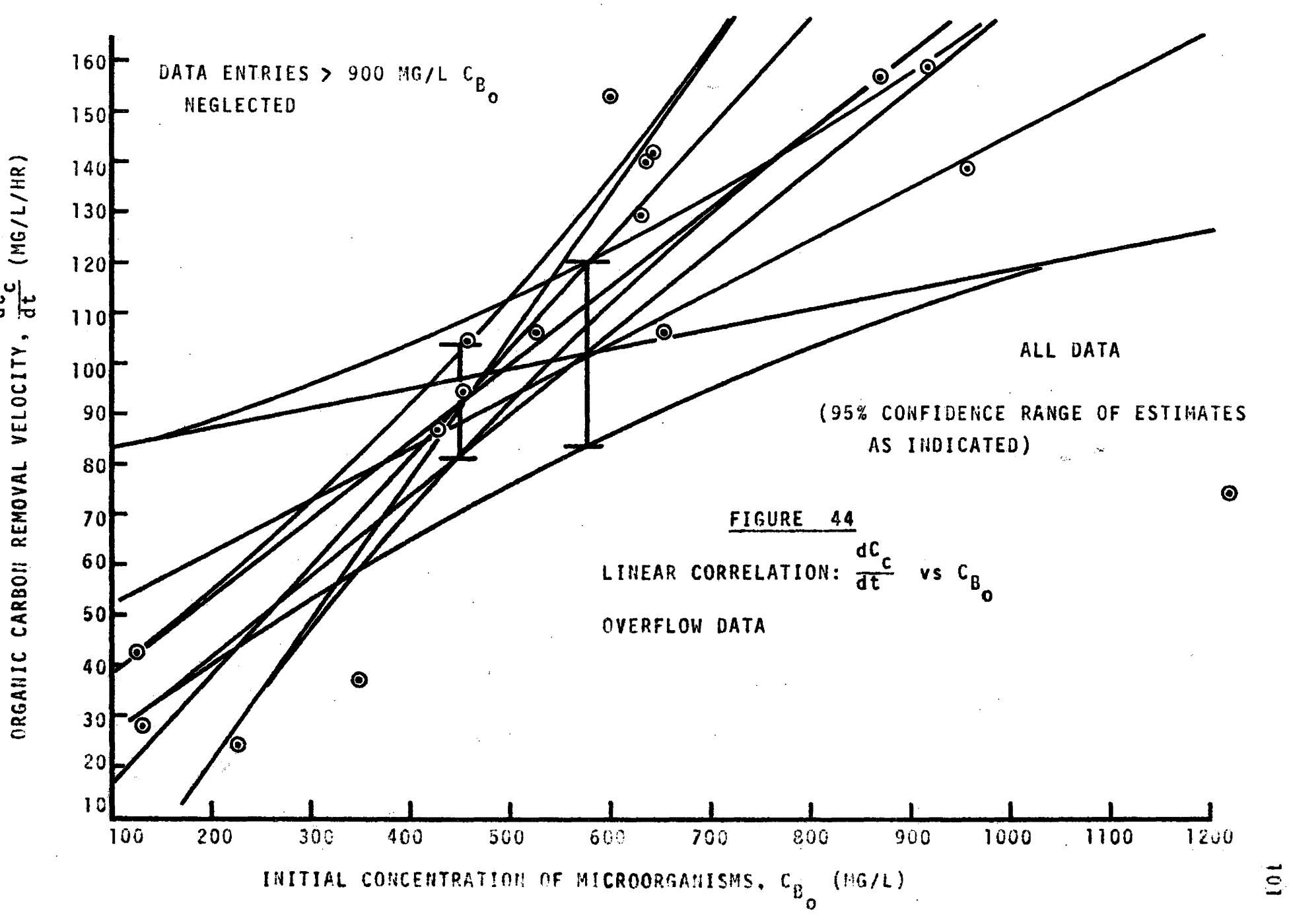


FIGURE 44

LINEAR CORRELATION: $\frac{dC_c}{dt}$ vs C_{B_o}

OVERFLOW DATA

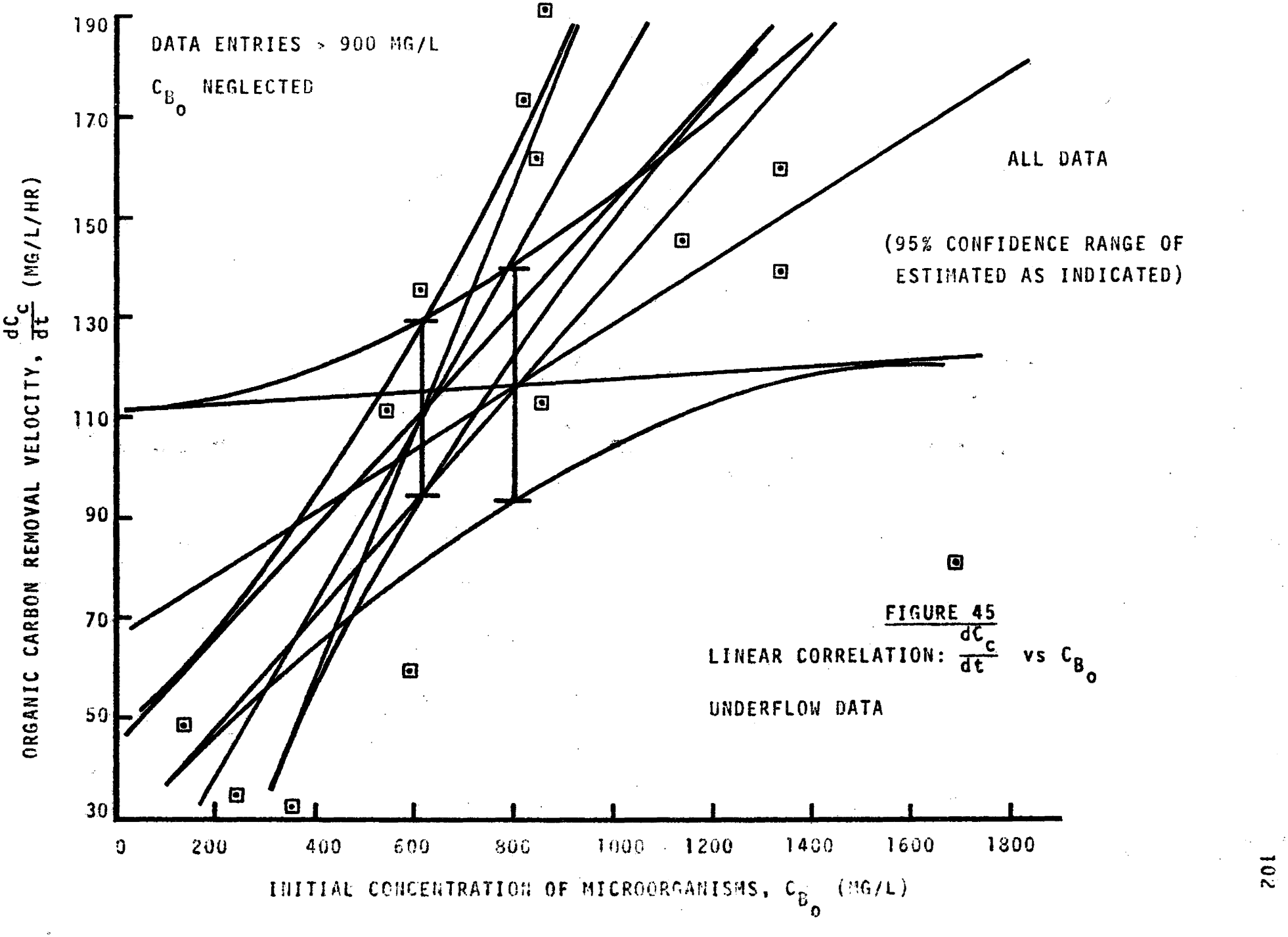


FIGURE 45

LINEAR CORRELATION: $\frac{dC_c}{dt}$ vs C_{B_o}

UNDERFLOW DATA

Linear relationships between these variables have been reported by Wilson (1967) for studies on batch oxygen uptake by mixed cultures growing on single substrates; by Tischler and Eckenfelder (1968) for studies on the batch removal rates of glucose, phenol and aniline as COD using mixed cultures; and by Banerji et al. (1968) for studies on the batch degradation rate of potato starch as total COD by acclimated mixed cultures of microorganisms. The fact that the dependence of $\frac{dC_c}{dt}$ on C_{B_o} decreases significantly at C_{B_o} levels greater than 900 mg/l suggests that a process constraint limited the uptake of organic carbon. Since oxygen and nutrient levels were maintained at values greater than those defining growth limitation and since limitation due to biological space would not be expressed at such a low suspended solids concentration, no fundamental explanation can be offered for this phenomenon. Four of the data entries at suspended solids levels greater than 900 mg/l (*) accrue from runs during which the frequency of sampling did not allow for the determination of sufficient information to accurately determine the rate of disappearance of carbon. Therefore, the critical value of 900 mg/l is most probably an expression of experimental error for the subject data entries. The same characteristic for glucose metabolism by acclimated mixed cultures has been reported at critical suspended solids levels of 1200 mg/l by Banerji et al. (1968) and 3500 mg/l by Wuhrmann (1956).

Below suspended solids mass concentration levels of 900 mg/l, the relationships between $\frac{dC_c}{dt}$ and C_{B_o} are:

(1) for the overflow population

$$\frac{dC_c}{dt} = 0.218106\ C_{B_o} - 5.23643$$

(2) for the underflow population

$$\frac{dC_c}{dt} = 0.17462\ C_{B_o} + 3.98624$$

Further analysis indicated that both slopes are significantly different from zero at the 95% level and that both slopes are not significantly different from each other at the 95% level. Therefore the two slopes were pooled to provide a better overall estimate. The resulting expressions are:

(1) for the overflow population

$$\frac{dC_c}{dt} = 0.1897361\ C_{B_o} - 5.23643$$

(2) for the underflow population

$$\frac{dC_c}{dt} = 0.1897361\ C_{B_o} + 3.98624$$

Microscopic examinations of the underflow and overflow fractions of the seed populations were performed to provide an approximate quantitative indication of the predominant species for each run. Filamentous bacteria, rod-shaped bacteria and spherical yeast cells were observed to vary in predominance throughout the duration of experimentation. Grouping the overflow and underflow data independently according to dominant microbial shape (Figures No.46 and No.47 and No.48) resulted in linear correlations between $\frac{dC_c}{dt}$ and C_{B_o} which were significant at the 95% level for the rod shaped overflow and underflow and for the spherical shaped overflow treatments. A linear correlation, significant at the 90% level, was obtained for the spherical shaped underflow treatment. These groupings exhibited no significant second order effects at the 95% level when data entries at solids levels greater than 900 mg/l were discarded; significant correlations could not be obtained when these data were retained. The filamentous shaped groupings did not exhibit significant correlation, probably

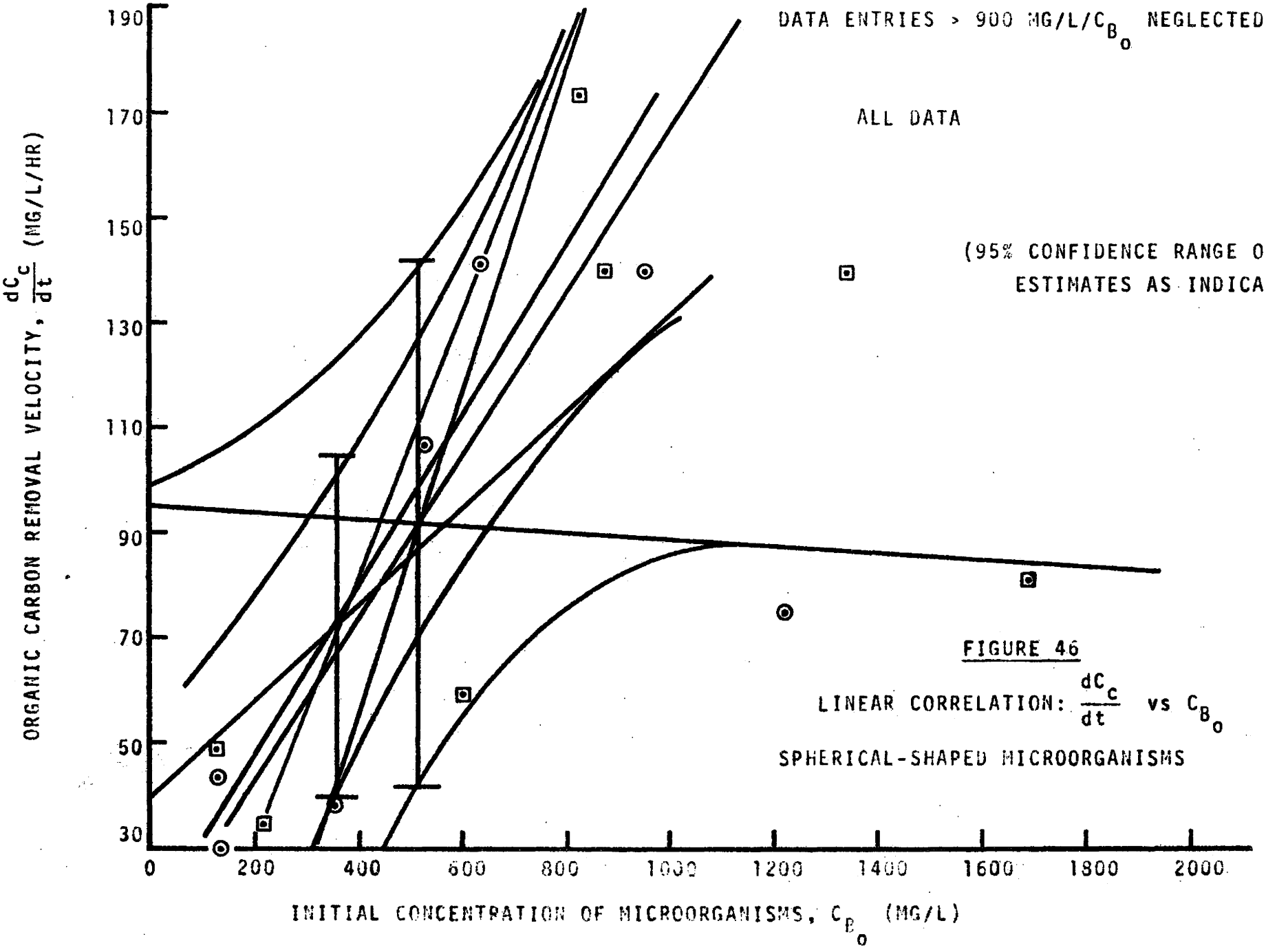


FIGURE 46

LINEAR CORRELATION: $\frac{dC_c}{dt}$ vs C_{B_o}

SPHERICAL-SHAPED MICROORGANISMS

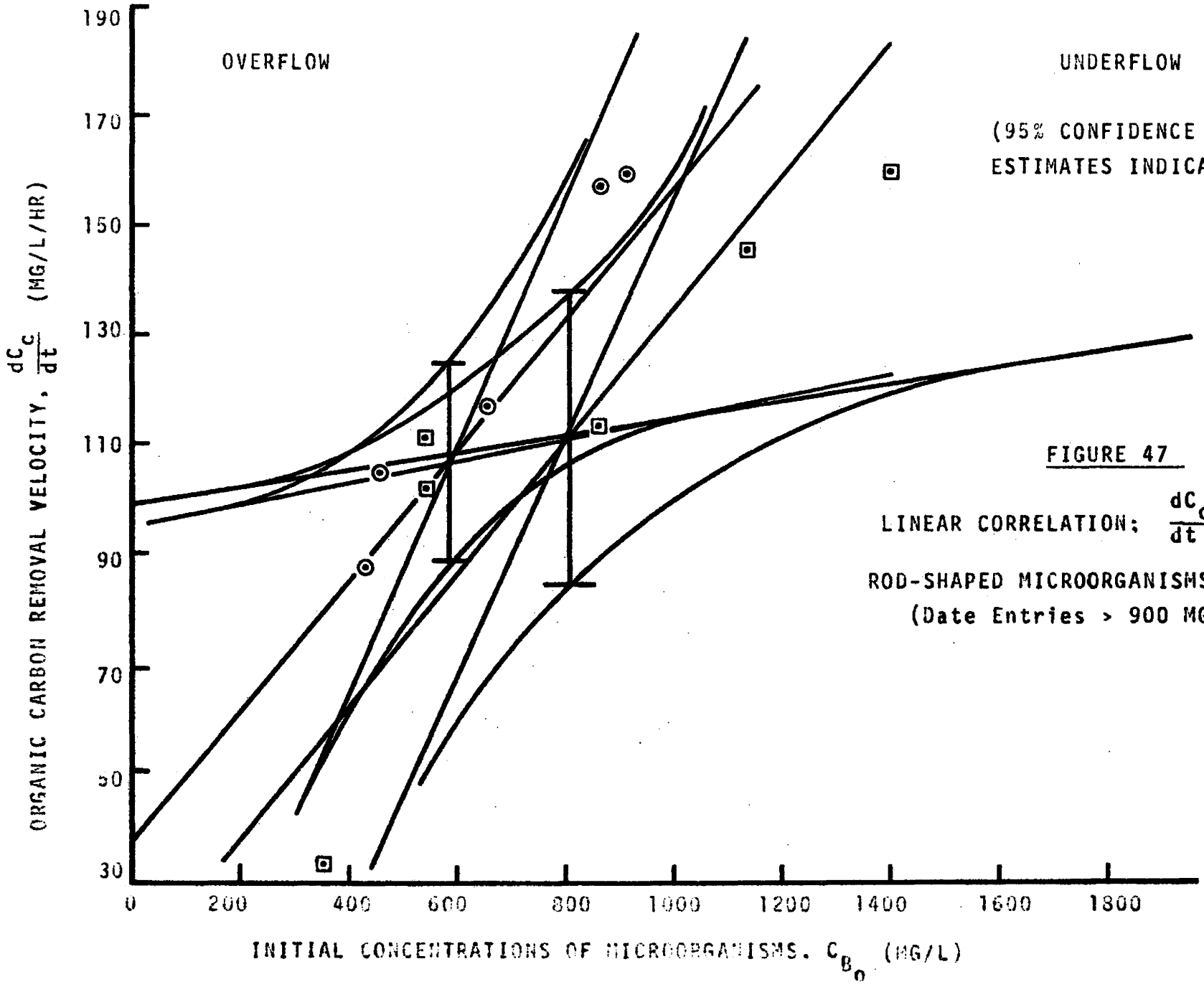


FIGURE 47

LINEAR CORRELATION; $\frac{dC_c}{dt}$ vs C_{B_o}

ROD-SHAPED MICROORGANISMS

(Date Entries > 900 MG/L/C_{B_o} Neglected

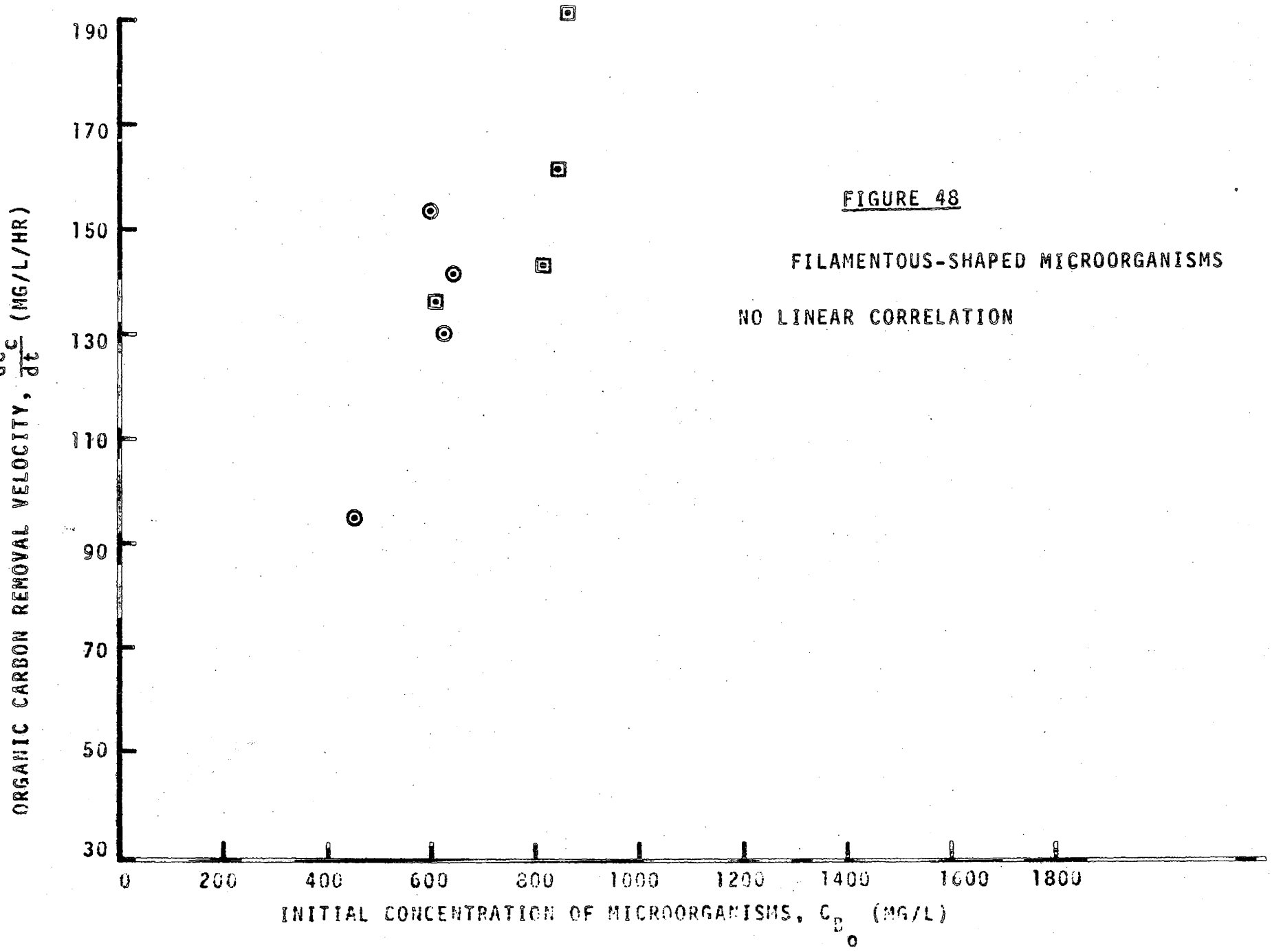


FIGURE 48

FILAMENTOUS-SHAPED MICROORGANISMS

NO LINEAR CORRELATION

due to the low number of initial degrees of freedom. All correlations exhibited slopes significantly different from zero.

Further testing based on the six groupings (Appendix No.IV) indicated that:

(1) the slopes of the six groupings were not significantly different, one from the other, therefore a pooled slope was determined,

(2) the vertical displacement between the 6 data sets was not significant,

(3) at the 95% significance level, a single line through all the data considered as 1 set gave as good a fit as individual lines through each data set.

Therefore, it is concluded that the rate of removal of carbon is a linear function of the initial concentration of micro-organisms below suspended solids levels of 900 mg/l. In this region, the best correlation is obtained if individual lines are drawn through the data grouped into overflow and underflow fractions:

$$\frac{dC_c}{dt} = 0.1897361\ C_{B_o} - 5.23643 \quad \text{(overflow)}$$

$$\frac{dC_c}{dt} = 0.1897361\ C_{B_o} + 3.98624 \quad \text{(underflow)}$$

Both lines have the same slope at the 95% confidence level, and there is no significant vertical displacement between the curves at the 95% confidence level. Thus the overflow and underflow fractions of the seed population exhibited the same carbon removal characteristics. The rate of increase of the carbon removal velocity with suspended solids was 0.1897361 ± 0.03904 mg/l carbon/hr/mg/l solids. Rao and Gaudy (1966) reported a range of

values from 0.286 to 0.698 mg/l glucose/hr/mg/l solids at 23.5°C and Banerji et al. (1968) report a value of 0.296 mg/l glucose/hr/mg/l solids. Above a mass concentration of micro-organisms of approximately 900 mg/l, the velocity of carbon removal appears to be independent of the suspended solids level. No fundamental significance is ascribed to this phenomenon.

From the data of mass concentration of carbon as a function of time, it is evident that steady-state carbon levels of approximately 20 mg/l (rather than zero) were observed for almost every run. This would suggest that the micro-organisms require a threshold level of carbon before it can be transported into the cells (mass transfer inhibition) or that residual organic carbon, probably tied up in compounds secreted by the micro-organisms during active metabolism, was present in the reactor in a form not readily available as an energy source to the cells. The latter explanation would seem to be more feasible and has been repoted by Rao and Gaudy (1966), Tischler and Eckenfelder (1968) and Gaudy, Komolrit and Bhatla (1963). For runs No. 1 and No. 5 , the carbon exhibited respective steady-state levels of approximately 80 mg/l and 45 mg/l. Since it was expected that dextrose would be readily used by the micro-organisms even at low concentrations, no fundamental explanation can be offered for these phenomena other than the possible inhibition of dextrose metabolism by some unexpected mechanism. Similar observations were reported by Rickard et al. (1965) but no explanation was offered.

The data of run Nos. 2 , 6 , 7 , indicate a significant increase in the carbon mass concentration at approximately one hour after the

initiation of "steady-state" conditions. This suggests that an organic substance(s), derived from dextrose metabolism was excreted into the medium (Gaudy, Komolrit and Bhatla (1963)).

6.1.3 Suspended Solids

The change in the mass concentration of suspended solids with time is presented in Figures 7-24 for the overflow and underflow reactors of each run.

Since the media compositions used in this investigation provided nutrient levels in excess of those defining growth limitation (Bennett (1967); McLean (1968)), the shape of the growth curve was assumed to be a function of the microbial population. Several investigations have defined the growth characteristic to be an expression of the physiological condition of the micro-organism population. The change in suspended solids with time has been ascribed to be a function of: the number of nucleii present per bacterium for the dominant species of a mixed culture by Lamanna and Mallette (1965); the degree of starvation of the acclimated culture inoculum by Clifton (1957) and Rao and Gaudy (1966); the duration of the phase of adjustment to a new environment by Lamanna and Mallette (1965); and the size of the seed inoculum by Rao and Gaudy (1966). The shapes of the growth characteristics observed in this study are ascribed in part to the large degree of scatter prevalent in the suspended solids data due to inherent errors in the membrane filter technique (Appendix No. II) and in part to the small increment in mass relative to the total population observed in most runs.

As noted previously, Kountz et al. (1959) and McKinney (1963) concluded that up to 25% of the suspended solids formed in the extended aeration process

may be inert. The data of Lamanna and Mallette (1965) reported for studies on the exponential growth of SALMONELLA PULLORUM in a non-synthetic nutrient broth, indicate that non-viable cells may comprise from approximately 8 to 25% of the total microbial population. Washington, Hetling and Rao (1964) studied the long term growth of mixed microbial cultures using a glucose carbon feed in semi-batch operation. They found that during the first eight months of operation, viable cells comprised from 45 to 60 percent by mass of the total population. Thereafter this level decreased to the range of 25 to 29 percent by mass. Martin et al. (1965) observed the growth of PSEUDOMONAS FLUORESCENS on glutamic acid in a continuous reactor. They observed a constant value of 56 percent viability as measured by organism reporduction.

Although a definitive determination of the true fraction of the biochemically active portion of a culture is questionable, the above findings indicate that a significant fraction of a micro-organism population may be inert relative to organic substrate. Therefore, it could be expected that the growth characteristics of the micro-organisms of this study were significantly damped by the presence of a large mass of inert solids relative to the total mass of solids.

The shapes of the smoothed growth curves were influenced to a large extent by the data entries after the occurrence of stead-state levels for soluble organic carbon. Lamanna and Mallette (1965) have noted that the so called "phase of microbial decline" is often so irregular as not to be fitted easily to some mathematical function. Thus, the smoothed growth curves may not be good approximations to the true growth curves.

From Figures No. 7 through No. 24 it can be seen that the increase in suspended solids to the point of substrate "exhaustion" (i.e. steady-state) was approximately the same for both overflow and underflow reactors.

This suggests that both the overflow and underflow fractions of the test slurry were the same. Microscopic examinations of the overflow and underflow populations for each run revealed no noticeable difference in the type or relative numbers of species present, suggesting that a preferential separation was not effected by the hydrocyclones used in this investigation.

Since the reciprocal of the suspended solids concentration was used to determine specific growth rate, and since the magnitudes of solids were large relative to the magnitudes of organic carbon velocities, the growth characteristic did not exercise a significant influence on the shape of the specific growth rate vs carbon response. The presence of a large number of inert solids could, however, significantly affect the magnitude of unit rate as a function of carbon.

6.2 DESIGN IMPLICATIONS

Within the scope of this experimental study, it has been concluded that the rate of decrease of soluble extracellular organic carbon appears to be independent of the organic carbon and a linear function of the concentration of micro-organisms. Since the concentration of suspended solids does not decrease as the reaction proceeds, then the velocity of carbon disappearance, interpreted in a kinetic sense, does not allow for discrimination between reactor sizes dictated by CSTR or PFTR designs limits (Levenspiel (1967)).

Since the rate of carbon decrease varies linearly with the concentration of suspended solids, then an optimum (i.e. minimum) reactor size would be defined when the concentration of micro-organisms is maximized.

This maximum level may be determined by:

(1) the increasing difficulty of transferring sufficient quantities of oxygen to the reactor (so that oxygen tension does not define rate limitation) as the concentration of suspended solids increases,

(2) the increasing difficulty of providing adequate solid-liquid phase separation in subsequent stages of the process as the concentration of suspended solids increases.

These factors should be balanced against the increased benefits that accrue from minimizing reactor volume.

Since the dependence of specific growth rate on organic carbon cannot be determined at the 95% confidence level, it is not feasible to use this quantity as a basis for reactor design.

This investigation has indicated that the velocity of substrate disappearance is independent of the suspended solids level as well as the organic carbon value for a single batch test. Therefore, it is advocated that a number of batch laboratory experiments be performed using acclimated mixed microbial cultures and the subject organic waste to define a relationship between the velocity of organic decrease and the concentration of suspended solids over a range for the latter which brackets the practical maximum level. Micro-organisms would then be removed from the process so that the practical maximum level is not exceeded. With this information it would be possible to determine an optimum reactor residence time knowing the influent and desired effluent organic concentration.

This procedure assumes that organic wastes met in practice will exhibit similar removal characteristics to the dextrose carbon source used

in this investigation. Recent studies on more complex wastes (Wilson (1967), Tischler and Ekenfelder (1968)) suggest that this is a reasonable assumption. For waste steams consisting of a variety of organic wastes, it would be necessary to determine if any mutual inhibition effects are present, in which case it may be feasible to investigate separate treatment facilities for these compounds if they originate from different sources or separation techniques if they originate from a common source.

Laboratory - Prototype Scale Up

By wasting a fraction of the test micro-organism population at frequent levels, and maintaining adequate quantities of trace nutrients, then a process of natural selection will determine the predominant microbial species in a batch study, thereby approximating prototype continuous operation. No laboratory-prototype scaling would be expected for the characteristics of the microbial population or the organic waste. Studies may be required to determine differences in laboratory-prototype mixing characteristics.

6.3 HYDROCYCLONE EVALUATION - CLASSIFICATION

The central composite statistical design employed in this investigation was analyzed (Appendix No. V) to determine the effects of the system variates (Hydrocyclone size; inlet pressure; volume split) on the biokinetic and separation response variates.

6.3.1 Biological Response

The facts that specific growth rate appears to be a meaningless quantity in an absolute sense and that organic carbon removal velocity appears to be a linear function of the concentration of suspended solids, suggest that a meaningful measure of the relative abilities of the overflow and underflow micro-organism fractions to remove organic carbon is not available. If the assumption is made that the specific growth rate responses of the overflow and underflow fractions are independent of the techniques of data treatment in a relative sense, then these quantities may be used in the statistical analysis. Since a single number representative of the difference between the two fractions was required at each treatment for the analysis, it was decided that:

(1) since the specific growth rate was almost independent of organic carbon concentration for primary data fitted in a piecewise fashion (Figures No.25 through No.42) then the difference between the mean values (overflow - underflow) of specific growth rate over the domanin of testing would provide the best estimate.

(2) since the specific growth rate was highly dependent on the organic carbon concentration for primary data fitted by the best possible polynomial for a single domain defined by each

run, then the ratio of areas (overflow/underflow) defined by the $\frac{1}{C_B}\frac{dC_c}{dt}$ vs C_c plot from the lower limit of C_c to the maximum value of $\frac{1}{C_B}\frac{dC_c}{dt}$ would provide the best estimate.

Difference in Means

The experimental design matrix is illustrated in Table No. 1 . To estimate the first order effects in the experimental region, a 2^3 facterial experiment comprising 12 tests including 4 replications at the centre point (to estimate the error) was performed. Applying the method illustrated in Appendix No. V the estimates of the coefficients of the response surface

$$y = \sum_{i=0}^{n} b_i x^i$$

were obtained.

An analysis of variance (Table No. 2) indicated that the lack of fit was not significant at the 95% level relative to experimental error. Since, however, none of the first order terms were significant at the 95% level relative to the residual mean square, it would appear that either the response was independent of the test variables or only second order terms (incorporated in the estimate b_o) were significant. Analysis of the first order model with interaction effects included (Table No. 3) indicated only the constant term of the response equation to be significant at the 95% level. The residual sum of squares obtained including interaction terms represented an improvement over that obtained for first order effects only (11.0% of total vs 28.7%). The lack of fit estimate again was not significant relative to the error estimate at the 95% level.

TABLE NO. 1

EXPERIMENTAL DESIGN MATRIX

(NOTE: TESTS PERFORMED IN RANDOM SEQUENCE)

Second Order

First Order and Interaction Effects

First Order

Trial	Factors and Coefficients											Response		
												Biological		
	x_o	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	x_1^2	x_2^2	x_3^2	Means	Ratios	Thickening
1	1	1	-1	1	-1	1	-1	-1	1	1	1	.01179	1.87772	7.12200
2	1	1	-1	-1	-1	-1	1	1	1	1	1	.03542	1.13223	8.46400
3	1	1	1	-1	1	-1	-1	-1	1	1	1	.05034	1.05000	10.483
4	1	1	1	1	1	1	1	1	1	1	1	-.00984	.62475	9.929
5	1	-1	1	-1	-1	1	-1	1	1	1	1	.02701	1.23678	6.205
6	1	-1	1	1	-1	-1	1	-1	1	1	1	.08140	1.16369	4.664
7	1	-1	-1	1	1	-1	-1	1	1	1	1	.02008	.95805	2.997
8	1	-1	-1	-1	1	1	1	-1	1	1	1	.04876	.75309	6.475
9	1	-2	0	0	0	0	0	0	4	0	0	0.0000	1.04108	.413
10	1	2	0	0	0	0	0	0	4	0	0	-.01241	.80466	7.083
11	1	0	2	0	0	0	0	0	0	4	0	.00588	1.19353	13.288
12	1	0	-2	0	0	0	0	0	0	4	0	.05005	1.25239	13.068
13	1	0	0	2	0	0	0	0	0	0	4	.02500	1.17678	8.322
14	1	0	0	-2	0	0	0	0	0	0	4	.01411	1.04113	9.462
15	1	0	0	0	0	0	0	0	0	0	0	.05820	1.07181	7.834
16	1	0	0	0	0	0	0	0	0	0	0	.02828	.98510	6.563
17	1	0	0	0	0	0	0	0	0	0	0	.05777	1.03206	7.313
18	1	0	0	0	0	0	0	0	0	0	0	-.00080	.84153	7.017

Second Order

First Order and First Order Plus Interaction Effects

Centre Point

TABLE NO. 2

ANALYSIS OF VARIANCE

FIRST ORDER MODEL

BIOLOGICAL -MEANS

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	.01390	1	.01390	17.51693*	17.85820*
B_1	.00100	1	.00100	1.26296	1.28757
B_2	.00013	1	.00013	.17010	.17341
B_3	.00042	1	.00042	.53175	.54211
ERROR	.00238	3	.00079	1.00000	1.01948
LACK OF FIT	.00385	5	.00077	.96942	.98831
RESIDUAL	.00623	8	.00078	.98089	1.00000

TOTAL SUM OF SQUARES = 0.02169

SUM OF SQUARES REMOVED BY MODEL $\doteq$ 71.3% OF TOTAL

Estimated Coefficients		Variance
B_o	.03403	6.6×10^{-5}
B_1	- .01119	9.9×10^{-5}
B_2	.00411	9.9×10^{-5}
B_3	- .00726	9.9×10^{-5}

ANALYSIS OF VARIANCE

FIRST ORDER MODEL AND INTERACTION

BIOLOGICAL - MEANS

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	.01390	1	.01390	17.51693*	23.16077*
B_1	.00100	1	.00100	1.26296	1.66988
B_2	.00013	1	.00013	.17010	.22490
B_3	.00042	1	.00042	.53175	.70308
B_{12}	.00027	1	.00027	.33740	.44611
B_{13}	.00150	1	.00150	1.88948	2.49826
B_{23}	.00027	1	.00027	0.34091	.45675
B_{123}	.00179	1	.00179	2.25405	2.98029
ERROR	.00238	3	.00079	1.00000	1.32219
LACK OF FIT	.00002	1	.00002	.02528	.03342
RESIDUAL	.00240	4	.00060	.75632	1.00000

TOTAL SS = .02169

SS REMOVED BY MODEL $\doteq$ 89% OF TOTAL

Estimated Coefficients		Variance
B_o	.03403	6.6×10^{-5}
B_1	- .01119	9.9×10^{-5}
B_2	.00411	9.9×10^{-5}
B_3	- .00726	9.9×10^{-5}
B_{12}	- .00578	9.9×10^{-5}
B_{13}	- .01369	9.9×10^{-5}
B_{23}	.00581	9.9×10^{-5}
B_{123}	.01495	9.9×10^{-5}

Second order effects were estimated by augmenting the 2^3 factorial design with 6 more treatments with coded levels of ± 2 for each of the 3 primary variates. The analysis of variance for this model (Table No. 4) again indicated an adequate fit but resulted in a response equation with only the constant term significant at the 95% level. The residual sum of squares represented approximately 18% of the total.

It is concluded that for the region of experimentation defined by the range in the predictor variates, the response was constant and independent of the predictor variates at the 95% confidence level; no information was generated as to the direction or the magnitude of changes in the predictor variates which would improve the response.

Therefore, for the materials and methods of this study, the overflow fraction of the test slurry removed organic carbon at a faster rate per mass of micro-organisms than did the underflow fraction. With 95% confidence, this difference in the specific growth rate was determined to be $0.03403\ (\text{Hrs})^{-1} \pm 0.01768$ at the 95% level.

It is concluded that the hydrocyclones used in this investigation provided a preferential classification, in a biokinetic sense, of a mixed population of micro-organisms.

Ratio of Areas

The experimental design matrix is defined in Table No.1 . Following the same procedure as before, an analysis of variance for the first order model indicated the lack of fit to be significant at the 95% level, suggesting that higher order terms are significant. Considering the first order model with interaction effects included (Table No.6) showed the mathematical fit to be significant at the 95% level. The only coefficients to be significant at the 95% level are:

TABLE NO. 4

ANALYSIS OF VARIANCE

SECOND ORDER MODEL

BIOLOGICAL - MEANS

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	.01340	1	.01942	24.47387*	**28.47085***
B_1	.00082	1	.00082	1.03009	1.19832
B_2	.00019	1	.00019	.24244	.28203
B_3	.00008	1	.00008	.10390	.12087
B_{12}	.00027	1	.00027	.33740	.39250
B_{13}	.00150	1	.00150	1.88948	2.19807
B_{23}	.00027	1	.00027	.34091	.39658
B_{123}	.00179	1	.00179	2.25405	2.62218
B_{11} B_{22} B_{33}	.00211	3	.00070	.8860	1.0294
ERROR	.00238	3	.00079	1.00000	1.16332
LACK OF FIT	.00239	4	.00060	.75432	.87751
RESIDUAL	.00477	7	.00068	.85961	1.00000

TOTAL SS = 0.02520

SS REMOVED BY MODEL = 82% OF TOTAL

Estimated Coefficients		Variance
B_o	.03955	1.85×10^{-4}
B_1	-.00715	5.00×10^{-5}
B_2	-.00347	5.00×10^{-5}
B_3	-.00227	5.00×10^{-5}
B_{12}	-.00578	9.9×10^{-5}
B_{13}	-.01369	9.9×10^{-5}
B_{23}	.00581	9.9×10^{-5}
B_{123}	-.01495	9.9×10^{-5}
B_{11}	-.00960	3.6×10^{-5}
B_{22}	-.00105	3.6×10^{-5}
B_{33}	-.00316	3.6×10^{-5}

TABLE NO. 5

ANALYSIS OF VARIANCE

FIRST ORDER MODEL

BIOLOGICAL - AREAS

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	13.49764	1	13.49764	1335.88391*	114.20248*
B_1	.04105	1	.04105	4.06318	.34735
B_2	.05214	1	.05214	5.16073	.44118
B_3	.02555	1	.02555	2.52877	.21618
ERROR	.03031	3	.01010	1.00000	.08549
LACK OF FIT	.91521	5	.18304	18.11601*	1.54871
RESIDUAL	.94552	8	.11819	11.69750	1.00000

TOTAL SS = 14.56191

SS REMOVED BY MODEL = 93.5% OF TOTAL

Estimated Coefficients		Variance
B_o	1.0605675	8.43×10^{-4}
B_1	.0716363	1.263×10^{-3}
B_2	- .0807338	1.263×10^{-3}
B_3	.0565138	1.263×10^{-3}

TABLE NO. 6

ANALYSIS OF VARIANCE

FIRST ORDER MODEL AND INTERACTION

BIOLOGICAL - AREAS

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	13.49764	1	13.49764	1335.88391*	808.70314*
B_1	.04105	1	.04105	4.06318	2.45973
B_2	.05214	1	.05214	5.16073	3.12415
B_3	.02555	1	.02555	2.52877	1.53084
B_{12}	.51234	1	.51234	50.70716*	30.69656*
B_{13}	.00444	1	.00444	.43898	.26574
B_{23}	.26237	1	.26237	25.96760*	15.71999*
B_{123}	.09961	1	.09961	9.85876	5.96819
ERROR	.03031	3	.01010	1.00000	.60537
LACK OF FIT	.03645	1	.03645	3.60754	2.18389
RESIDUAL	.06676	4	.01669	1.65188	1.00000

TOTAL SS = 14.56191

SS REMOVED BY MODEL ≙ 99.5% OF TOTAL

Estimated Coefficients		Variance
B_o	1.06057	8.4199×10^{-4}
B_1	.071636	1.263×10^{-3}
B_2	- .080734	"
B_3	.056514	"
B_{12}	- .25307	"
B_{13}	.023546	"
B_{23}	- .181099	"
B_{123}	- .111586	"

(1) the constant term

(2) the interaction between x_1 and x_2

(3) the interaction between x_2 and x_3

Therefore, all of the predictor variates must be retained in the analysis.

Proceding on to a second order model, the fit was found to be highly significant at the 95% level, the model accounting for 99.42 % of the total sum of squares. The lack of fit sum of squares and the error sum of squares were pooled to define a residual mean square with which to estimate the significance of the various coefficients.

The response surface was defined to be,

$$y = 0.9933 - 0.2531\, x_1x_2 - 0.1811\, x_2x_3 - 0.1226\, x_1^2 + 0.06276\, x_2^2 + 0.03426\, x_3^2$$

at the 95% confidence level.

Canonical transformation of the fitted equation (Appendix No. V) was performed to simplify the interpretation of the response surface. The canonical form of the second order model is:

$$Y - 0.9933 = 1.83398\, X_1^2 - 1.861915\, X_2^2 + 0.027934\, X_3^2$$

where X_i are the co-ordinate transformations of x_i.

It is noted that the coefficient of X_3^2 is small relative to the other coefficients, and is probably not significantly different from zero. The canonical response surface, neglecting X_3 dependence is mapped in Figure No.49 .

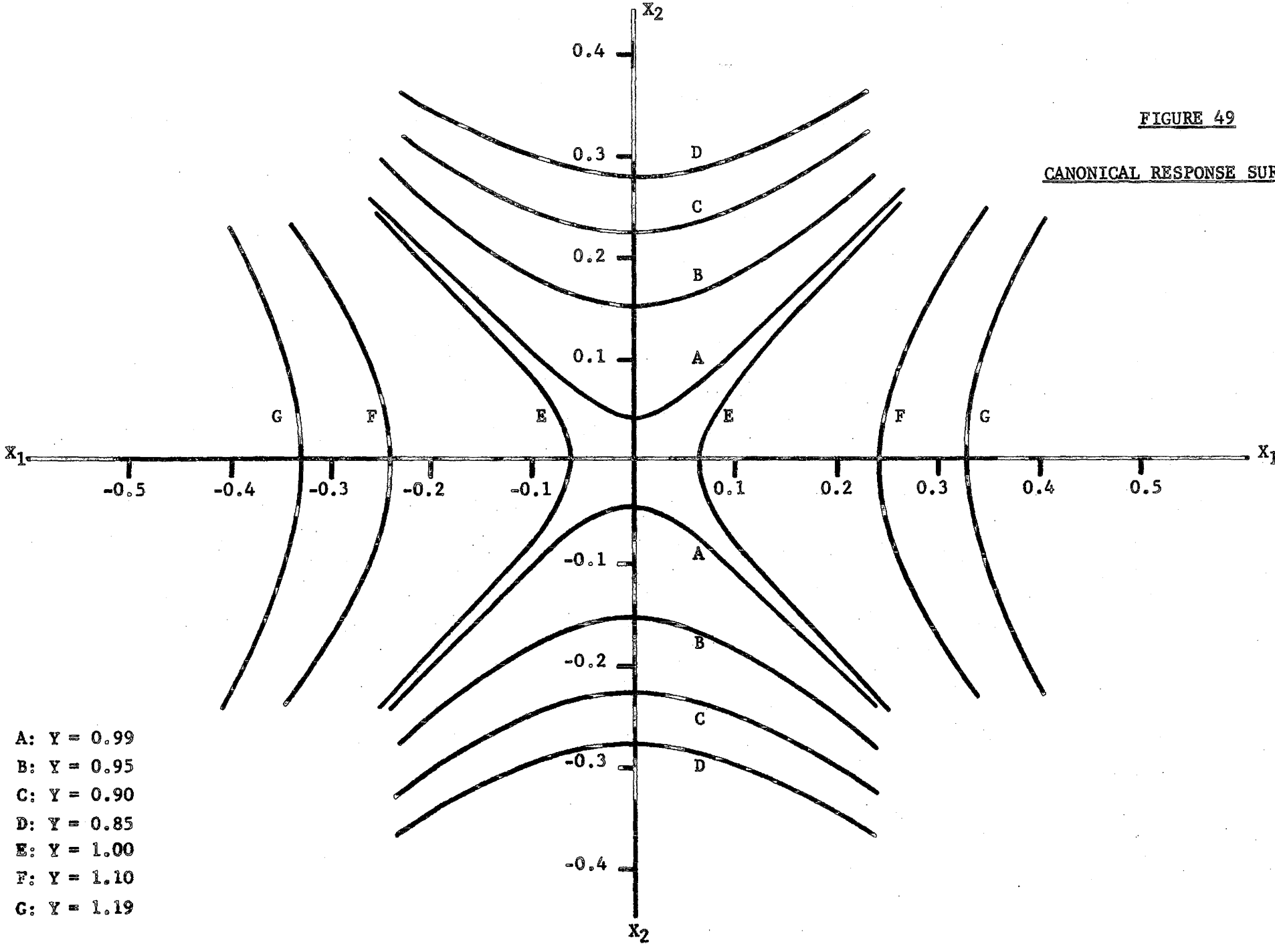


FIGURE 49

CANONICAL RESPONSE SURFACE

TABLE NO. 7

ANALYSIS OF VARIANCE

SECOND ORDER MODEL

BIOLOGICAL - AREAS

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	20.55762	1	19.10829	1891.17927	1069.61626
B_1	.00063	1	.00063	.06217	.03516
B_2	.03644	1	.03644	3.60671	2.03989
B_3	.03271	1	.03271	3.23713	1.83086
B_{12}	.51234	1	.51234	50.70716	28.67904
B_{13}	.00444	1	.00444	.43898	.24828
B_{23}	.26337	1	.26237	25.96760	14.68680
B_{123}	.09961	1	.09961	9.85876	5.57593
B_{11} B_{22} B_{33}	1.65378	3	0.51793	51.2800	28.99900
ERROR	.03031	3	.01010	1.00000	.56558
LACK OF FIT	.09474	4	.02369	2.34416	1.32581
RESIDUAL	.12505	7	.01786	1.76809	1.00000

TOTAL SS = 21.83599

SS REMOVED BY MODEL ≐ 99.994% OF TOTAL

Estimated Coefficients		Variance
B_o	.99334	2.3576×10^{-3}
B_1	.006265	6.315×10^{-4}
B_2	- .047724	"
B_3	.045213	"
B_{12}	- .253066	1.26299×10^{-3}
B_{13}	.023546	"
B_{23}	- .181099	"
B_{123}	- .111586	"
B_{11}	- .012259	4.631×10^{-4}
B_{22}	.06276	"
B_{33}	.03426	"

The region of experimentation has a large degree of curvature as indicated by the significant second order terms. The centre of the contours defining the response surface was found to coincide with the centre point of the experimental design due to the absence of first order effects signifacant at the 95% level. The centre of the design is a saddle point which indicates that either increasing the absolute value of X_1 for a given value of X_2 or decreasing the absolute value of X_2 for a given value of X_1 would improve the response. The response at the centre of the design was 0.9933 $\pm$ 0.1020 at the 95% confidence level.

The relative evaluation of the biological characteristics of the partitioned slurry is subject to question due to the poor estimators used to define the response.

6.4 HYDROCYCLONE EVALUATION - THICKENING

The Rietema - Tenbergen thickening efficiency for each run was used to define the response variate tested in the statistical design (Table No. 1); the experimental design natrix is illustrated in Table No. 1.

An analysis of variance on the first order model (Table No. 8) indicated that the fitted equation was significant at the 95% level and

TABLE NO. 8

ANALYSIS OF VARIANCE

FIRST ORDER MODEL

THICKENING

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	603.01870	1	603.01870	2121.68474	1029.35475
B_1	30.64271	1	30.64271	107.81450	52.30719
B_2	4.84072	1	4.84072	17.03177	8.26312
B_3	5.97715	1	5.97715	21.03025	10.20302
ERROR	.85265	3	.28422	1.00000	.48516
LACK OF FIT	3.83393	5	.76679	2.69789	1.30890
RESIDUAL	4.68658	8	.58582	2.06118	1.00000

TOTAL SS = 649.16585

SS REMOVED BY MODEL ≐ 99.278% OF TOTAL

Estimated Coefficients		Variance
B_o	7.08883	.023685
B_1	1.95712	.035527
B_2	0.77787	.035527
B3	- 0.86437	.035527

accounted for 99.278% of the total sum of squares. The lack of fit sum of squares and the error sum of squares were pooled to define a residual mean square with which to estimate the significance of the fitted coefficients. All terms in the first order model were found to be significant at the 95% level.

A check of the interaction effects (Table No.9) showed them to be not significant at 95%. A second order model exhibited a significant lack of fit at the 95% level. Therefore, the response surface was defined to be a plane (i.e. no curvature evident at the 95% confidence level).

The fitted coefficients indicate that separation efficiency is more dependent on hydrocyclone size than on inlet pressure or volume split. The response surface suggests that increasing hydrocyclone size and inlet pressure and decreasing volume split would increase thickening efficiency. The most promising magnitude and direction of change in the various predictor variates to increase the response can be estimated, (Table No. 11).

It is recommended that the first order experimental design be performed at successive points along this path, with each new experimental region being defined by the previous one.

When higher order terms become significant a second order design should be performed to describe what would hopefully be a response maxima.

TABLE NO. 9

ANALYSIS OF VARIANCE

FIRST ORDER MODEL AND INTERACTION

THICKENING

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	603.01870	1	603.01870	2121.68474	2666.89149
B_1	30.64271	1	30.64271	107.81450	135.51947
B_2	4.84072	1	4.84072	17.03177	21.40840
B_3	5.97715	1	5.97715	21.03025	26.43437
B_{12}	1.46976	1	1.46976	5.17124	6.50009
B_{13}	1.21914	1	1.21914	4.28947	5.39174
B_{23}	0.92820	1	0.92820	3.26583	4.10504
B_{123}	0.16503	1	0.16503	0.58063	0.72983
ERROR	0.85265	3	0.28422	1.00000	1.25697
LACK OF FIT	0.05180	1	0.05180	0.18226	0.22909
RESIDUAL	0.90445	4	0.22611	0.79556	1.00000

TOTAL SS = 649.16583

SS REMOVED BY MODEL ≙ 99.999% OF TOTAL

Estimated Coefficients		Variance
B_o	7.08883	0.023685
B_1	1.95712	0.035527
B_2	0.77787	0.035527
B_3	-0.86437	0.035527
B_{12}	0.42862	0.035527
B_{13}	0.39037	0.035527
B_{23}	0.34062	0.035527
B_{123}	-0.14363	0.035527

TABLE NO. 10

ANALYSIS OF VARIANCE

SECOND ORDER MODEL

THICKENING

Source	SS	DF	MS	F Test	
				Error	Residual
B_o	1038.74	1	937.73927	3299.37879	605.39705
B_1	52.55163	1	52.55163	184.89971	33.92691
B_2	2.77472	1	2.77472	9.76269	1.79134
B_3	5.28425	1	5.28425	18.59232	3.41147
B_{12}	1.46976	1	1.46976	5.17124	0.94886
B_{13}	1.21914	1	1.21914	4.28947	0.78707
B_{23}	.92820	1	0.92820	3.26583	0.59924
B_{123}	.16503	1	0.16503	0.58063	0.10654
B_{11} B_{22} B_{33}	91.659	3	30.553	107.4977	19.724
ERROR	.85265	3	.28422	1.00000	0.18349
LACK OF FIT	9.99011	4	2.49753	8.78740*	1.61238
RESIDUAL	10.84276	7	1.54897	5.44994	1.00000

TOTAL SS = 1205.634

SS REMOVED BY MODEL ≐ 99.983% OF TOTAL

Estimated Coefficients		Variance
B_0	6.84973	0.066317
B_1	1.81231	0.017764
B_2	0.41644	"
B_3	- 0.57469	"
B_{12}	0.42862	0.035527
B_{13}	0.39037	"
B_{23}	0.34062	"
B_{123}	- 0.14363	"
B_{11}	- 0.93894	"
B_{22}	1.41856	0.013027
B_{33}	0.34706	"

TABLE NO. 11

CALCULATION OF PATH OF STEEPEST ASCENT

THICKENING

		x_1 (Hydrocyclone size) (mm)	x_2 (Inlet Pressure) (psi)	x_3 (Volume split) (over/under)
Base Level		6	140	2.5
Unit		2	30	0.5
Estimated Slope		1.95712	0.77787	-0.86437
Unit x Slope		3.91424	23.3361	-0.43219
Change in Level for +5mm Change in x_1		5	29.8093	-0.55208
Series of possible Trials	1.	6	140	2.5
	2.	11	169.8093	1.94792
	3.	16	199.6186	1.39584
	4.	21	229.4279	0.84376
	5.	26	259.2372	0.29168

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS:

It is concluded that, for the materials and methods of this investigation:

(1) Reaction Kinetic validity cannot be claimed for a proposed mechanism of organic carbon removal by mixed cultures of microorganisms. The shape of the organic carbon vs. time characteristic is defined by curve fitting techniques.

(2) At the 95% confidence level, the velocity of organic carbon removal appeared to be independent of the mass concentrations of organic carbon and suspended solids for single batch tests.

(3) At the 95% confidence level, the velocity of organic carbon removal appeared to be a linear function of the initial mass concentration of suspended solids for the series of batch runs comprising this investigation.

(4) For the individual batch tests of this investigation, microbial "growth" was not evident from the organic carbon removal velocity. This phenomenon may be related to the absolute quantity of organic carbon relative to the initial microorganism concentration.

(5) The degree of dependence of specific growth rate on organic carbon is highly sensitive to the type of data treatment used for the organic carbon vs. time plot.

The use of specific growth rate to characterise a batch biological reactor system must be questioned.

(6) At the 95% confidence level, differences in the rate of removal of organic carbon could not be detected between the overflow and underflow fractions of the seed population, or between the predominant microbial shapes prevalent throughout the course of the investigation.

(7) Based on the Rietema-Tengbergen efficiency response, it appears that the thickening or separation efficiency would be improved by increasing hydrocyclone size and inlet pressure and decreasing volume split.

7.2. RECOMMENDATIONS

It is recommended that:

(1) Biological reactor design be based on a series of batch tests over a range of suspended solids levels bracketing the practical maximum level.

(2) The use of small size hydrocyclones be further investigated to define maxima for thickening efficiency. This would be especially useful for bench-scale, continuous reactor studies incorporating suspended solids recycling and wasting.

(3) A more accurate technique for determining the level of viable microorganisms be developed.

REFERENCES

(1) AGARDY, Franklin J., and KIADO, Michael L., "Effects of Refrigerated Storage on the Characteristics of Waste", Proc. 21st Industrial Waste Conference, Purdue University, 226, (1966).

(2) AGARDY, F. J., COLE, R. D., and PEARSON, E.A., "Kinetics and Activity Parameters of Anaerobic Fermentation Systems", 1st Annual Report, Sanitary Engineering Research Laboratory, University of California, Berkeley, California, (1963).

(3) ANDREWS, J. F., COLE, R. D., and PEARSON, E. A., "Kinetics and Characteristics of Multistage Methane Fermentation", Sanitary Engineering Research Laboratory Report No. 64-11, Univ. of Calif., Berkeley, Calif., (1964).

(4) ARDEN, E., and LOCKETT, W. T., "Experiments on the Oxidation of Sewage without the aid of Filters", J. Soc. Chem. Ind., 33, 523, (1914). Reprinted in J. Inst. Sew. Purif., 175, (1954).

(5) BANERJI, S. K., EWING, B. B., ENGELBRECHT, R. S., and SPEECE, R. E., "Kinetics of Removal of Starch in Activated Sludge Systems", J. WPCF, 40 (2), 161, (1968).

(6) BENNETT, John, "Batch and Continuous Biochemical Reactor Studies Using Mixed Microbial Cultures", M.Eng., Thesis, Dept. of Chemical Engineering, McMaster University, Hamilton, Ont. Canada, (1967).

(7) BERGMAN, W. E., ENGLE, C. J., MARWIL, S. J., and TEK, M. R., "3-Inch ID Cyclone Removes Solids from Drilling Fluids", World Oil, Drilling Section, April, 190, (1956).

(8) BOX, G. E. P., Chapter 11 in "Design and Analysis of Industrial Experiments", (Ed. by O. L. Davies), Oliver and Boyd, London, (1967).

(9) BRADLEY, D., "The Hydrocyclone", Pergamon Press Ltd., London, (1965).

(10) BRADLEY, D., and PULLING, D. J., "Flow Patterns in the Hydraulic Cyclone and Their Interpretation in Terms of Performance", Trans. Instn. Chem. Engrs., 37, 34, (1959).

(11) BUSCH, A. W., "Activated Sludge Kinetics and Effluent Quality", Proc. ASCE, J., Sanitary Engineering Division, SA 6, November, (1962).

(12) BUSCH, A. W., GRADY, L., RAO, T. S., and SWILLEY, E. L., "Short-Term Total Oxygen Demand Test", J. WPCF, 34(4), 354, (1962).

(13) CLIFTON, C. E., "Introduction to Bacterial Physiology", McGraw-Hill Book Co., New York, (1957).

(14) CONTOIS, D. E., "Kinetics of Bacterial Growth: Relation Between Population Density and Specific Growth Rate of Continuous Cultures", General Microbiology, 21, 40, (1959).

(15) DAHLSTROM, DONALD A., "Fundamentals and Applications of the Liquid, Cyclone", CEP Symposium Series, Mineral Engineering, 50(11), 41, (1954).

(16) DAVIES, OWEN L., "Design and Analysis of Industrial Experiments", 2nd Ed., Oliver and Boyd, London, (1967).

(17) DEAN, A. C. R., and HINSHELWOOD, SIR CYRIL, "Growth Function and Regulation in Bacterial Cells", Oxford at the Clarendon Press, London, (1966).

(18) DE GELDER, A. L., "Model Tests with Cyclones", Joint Symp. on Scaling -Up-1957: Instn. Chem. Engineers, 547, (1957).

(19) ECKENFELDER, W. Wesley, Jr., "Kinetic Relationships in the Bio-Oxidation of Sewage and Industrial Wastes", Proc. 14th Industrial Waste Conference, Purdue University, 495, (1959).

(20) ECKENFELDER, W. W., and O'CONNOR, D. J., "Biological Waste Treatment", Pergamon Press, New York, (1961).

(21) ELLISON, Hackler B., "Effects of Age and Storage Temperatures on Growth of Bacteria in Water Samples", J. AWWA, 24(6), 895, (1932).

(22) FAIR, G. M., and GEYER, J. C., "Water Supply and Waste Water Disposal", John Wiley and Sons, Inc., New York, (1954).

(23) FAIR, G. M., MOORE, E. W., and THOMAS, H. A., Jr., "The Natural Purification of River Muds and Pollutional Sediments", Sewage Works Journal, 13(2), 270, (1941).

(24) FITCH, E. B., and JOHNSON, E. C., "Operating Behaviour of Liquid - Solid Cyclones", Mining Engineering, March, 304, (1953).

(25) FOGARTY, WILLIAM J., and REEDER, Milton E., "BOD Retrieval through Frozen Storage", Public Works, March, 88, (1964).

(26) FONTEIN, F. J., VAN KOOY, J. G., and LENIGER, H. A., "The Influence of Some Variables Upon Hydrocyclone Performance", British Chemical Engineering, 7(6), 410, (1962).

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

(28) GARRETT, T. M., and SAWYER, C. N., "Kinetics of Removal of Soluble BOD by Activated Sludge", Proc. 7th Industrial Waste Conference, Purdue University, (1952).

(29) GAUDY, A. F., Jr., KOMOLRIT, K., and BHATLA, M. N., "Sequential Substrate Removal in Heterogeneous Populations", J. WPCF, 35(7), 903, (1963).

(30) GRAM, A. L., "Reaction Kinetics of Aerobic Biological Processes", Report No. 2, I.E.R. Series 90, Univ. of California, Berkeley, Calif., (1956).

(31) GRIEVES, R. B., MILBURY, W. F., and PIPES, W. O., "Mixing Model for Activated Sludge", Proc. 18th Industrial Waste Conference, Purdue University, 636, (1963).

(32) HAAS, P. A., NURMI, E. O., WHATLEY, M. E., and ENGEL, J. R., "Midget Hydrocyclones Remove Micron Particles", CEP, 53(4), 203, (1957).

(33) HELMERS, E. N., FRAME, J. D., GREENBERG, A. E., and SAWYER, C. N., "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, Part II", Sewage and Industrial Wastes, 23, 884, (1951).

(34) HETLING, Leo J., and WASHINGTON, D. R., "Kinetics of the Steady-State Bacterial Culture III. Growth Rate", Proc. 20th Industrial Waste Conference, Purdue University, 254, (1964).

(35) HERBERT, D., ELSWORTH, R., and TELLING, R. C., "The Continuous Culture of Bacteria: a Theoretical and Experimental Study", General Microbiology, 14, 601, (1956).

(36) HEUKELEKIAN, H., ORFORD, H.E., and MANGONELLI, R., "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process", Sewage and Industrial Wastes, 23, 945, (1951).

(37) HILDEBRAND, F. B., "Introduction to Numerical Analysis", McGraw-Hill Book Co., Toronto, (1956).

(38) HSIANG, Thomas C. H., "A Statistical Analysis of Hydrocyclone Parameters", M.Eng. Thesis, Dept. of Chemical Engineering, McMaster University, Hamilton, Ont., Canada, (1967).

(39) HUNTER, J. S., "Some Applications of Statistics to Experimentation", CEP Symposium Series, Computational and Mathematical Techniques, 56(31), (1960).

(40) HUNTER, W. G., and WU, S.M., "Statistical Experimental Design for Engineers", University Extension, The University of Wisconsin, Madison, Wisconsin, June 19-23, (1967).

(41) JOHNSON, Norman L. and Leone, FRED C., "Statistics and Experimental Design, Vol. II", John Wiley and Sons, Inc., New York, (1967).

(42) KATZ, William J., and ROHLICH, Gerard A., "A Study of the Equilibria and Kinetics of Adsorption by Activated Sludge", in "Biological Treatment of Sewage and Industrial Wastes", Vol. I, Ed. by McCabe and Eckenfelder, Reinhold Publishing Corp., New York, (1956).

(43) KESHAVAN, K., BEHN, V. C., and AMES, W. F., "Kinetics of Aerobic Removal of Organic Wastes", Proc. ASCE, J.,Sanitary Engineering Division, February, 99, (1964).

(44) KELSALL, D. F., "A Further Study of the Hydraulic Cyclone", Chem. Eng. Sci., 2, 254, (1953).

(45) KOUNTZ, R. R., and FORNEY, C., "Metabolic Energy Balances in a Total Oxidation Activated Sludge System", Sewage and Industrial Wastes, 31, 819, (1959).

(46) KRISHNAN, P., and GAUDY, A. F., Jr., "Mechanism and Kinetics of Substrate Utilization at High Biological Solids Concentrations", Proc. 21st Industrial Waste Conference, Purdue University, 495, (1966).

(47) LAMANNA, Carl, and MALLETTE, M. Frank, "Basic Bacteriology", The Williams and Wilkins Co., Baltimore, (1965).

(48) LEVENSPIEL, Octave, "Chemical Reaction Engineering", John Wiley and Sons, Inc., New York, (1967).

(49) LILGE, E. O., "Hydrocyclone Fundamentals", Trans. Inst. Min. and Met., 71, 285, (1962).

(50) MARTIN, E. J., and WASHINGTON, D. R., "Kinetics of the Steady-State Bacterial Culture I. Mathematical Model", Proc. 19th Industrial Waste Conference, Purdue University, 724, (1963).

(51) MARTIN, Edward J., and WASHINGTON, Donald R., "Kinetics of the Steady-State Bacterial Culture IV. Transfer Rates", Proc. 20th Industrial Waste Conference, Purdue University, 470, (1965).

(52) MATSCHKE, D. E., and DAHLSTROM, D. A., "Minature Hydrocyclones - Part II - Solid Elimination Efficiency", CEP, 55(1), 79, (1959).

(53) McCABE, Brother Joseph, AND ECKENFELDER, W. Wesley, Jr., "BOD Removal and Sludge Growth in the Activated Sludge Process", J.WPCF, 33(3), 258, (1961).

(54) McCABE, J., and ECKENFELDER, W. W., Jr., "Process Design of Biological Oxidation Systems for Industrial Waste Treatment", Waste Treatment, 156, Pergamon Press Ltd., London, (1960).

(55) McKINNEY, R. E., "Biological Oxidation of Organic Matter", in "Advances in Biological Waste Treatment", (Ed. by Eckenfelder and McCabe), 1, (1963).

(56) McLEAN, Howard A., "A Continuous Biochemical Reactor Study Using Mixed Microbial Cultures", M.Eng. Thesis, Depart. of Civil Engineering, McMaster University, Hamilton, Ont. Canada, (1968).

(57) MIDDLEBROOKS, E. Joe., and GARLAND, C. F., "Kinetics of Model and Field Extended - Aeration Wastewater Treatment Units", J. WPCF, 40(4), 586, (1968).

(58) MILLIPORE FILTER CORPORATION, TB-961, Bedford, Mass., (1961).

(59) MODER, J. J., and DAHLSTROM, D. A., "Fine-Size, Close-Specific-Gravity Solid Separation With the Liquid-Solid Cyclone", Chemical Engineering Progress, 48(2), 75, (1952).

(60) MONOD, J., "The Growth of Bacterial Cultures", Annual Review of Microbiology", 3, 371, (1949).

(61) MORGAN, C. E., and CLARKE, E. F., "Preserving Domestic Waste Samples by Freezing", Public Works, Nov., 73, (1964).

(62) MURPHY, K. L., "Biological Factors in Waste-Water Treatment", Proc. Canadian Institute on Pollution Control Conference, (1966).

(63) NIELSEN, Kaj J., "Methods in Numerical Analysis", the MacMillan Co., New York, (1965).

(64) NOVICK, A., "Growth of Bacteria", Annual Review of Microbiology, 9, 97, (1955).

(65) PELCZAR, Michael J., Jr., and REID, Roger D., "Microbiology", McGraw-Hill Book Co., Toronto, (1965).

(66) RAO, B. S., and GAUDY, A. F., Jr., "Effects of Sludge Concentration on Various Aspects of Biological Activity in Activated Sludge", J.WPCF, 38(5), 794, (1966).

(67) REYNOLDS, T. D., and YANG, J. T., "Model of the Completely Mixed Activated Sludge Process", Proc. 21st Industrial Waste Conference, Purdue University, 696, (1966).

(68) REVELLE, Charles S., LYNN, Walter R. and RIVERA, Mauricio A., "Bio-Oxidation Kinetics and Second-Order Equation Describing the BOD Reaction", J. WPCF, 37(12), 1679, (1965).

(69) RICKARD, M. D., and RILEY, W. H., "Carbon as a Parameter in Bacterial Systems, Growth Limitation and Substrate Utilizing Studies", Proc. 20th Industrial Waste Conference, Purdue University, 98, (1965).

(70) RIETEMA, K., and KRAJENBRI, H. J., "Theoretical Derivation of Tangential Velocity Profiles in a Flat Vortex Chamber - Influence of Turbulence and Wall Friction", Applied Sci.Research, 8 (2-3), 177, (1959).

(71) RIETEMA, K., "Performance and Design of Hydrocyclones
- I General Considerations
- II Pressure Drop in the Hydrocyclone
- III Separating Power of the Hydrocyclone
- IV Design of Hydrocyclones",
Chem. Eng. Sci., 15, 298-325, (1961).

(72) SCHAFFER, R. B., VAN HALL, C. E., McDERMOTT, G. N., BARTH, D., STENGER, V. A., SEBESTA, S. J., and GRIGGS, S. H., "Application of a Carbon Analyzer in Waste Treatment", J. WPCF, 37(11), 1545, (1965).

(73) SCHROEPFER, G. S., ROBINS, M. L., and SUSAG, R. H., "A Reappraisal of Deoxygenation Rates of Raw Sewage Effluents and Receiving Waters", J. WPCF, 32, 1212, (1960).

(74) SCHULZE, K. L., "The Activated Sludge Process as a Continuous Flow Culture", Water and Sewage Works, 111, 526, (1964).

(75) SETTER, L. R., CARPENTER, W. T., and WINSLOW, G. C., "Practical Application of Modified Sewage Aeration", Sewage Works Journal, 17, 669, (1945).

(76) SPICER, C., "The Theory of Bacterial Constant Growth Apparatus", Biometrics, 11, 225, (1955).

(77) "STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTE WATER" American Public Health Association, 12 Ed., New York, (1965).

(78) STANIER, Roger Y., DOUDOROFF, Michael and ADELBERG, Edward A., "The Microbial World", Prentice-Hall Inc., Englewood Cliffs, N.J., (1965).

(79) STEWART, M. J., "Reaction Kinetics and Operational Parameters of Continuous Flow Anaerobic Fermentation Processes." Sanitary Engineering Research Laboratory, Univ. of Calif.Berkeley,Calif.(1958).

(80) STREETER, N. W., and PHELPS, E. B., Public Health Bulletin 146, USPHS, (1925).

(81) TENBERGEN, H. J., and RIETEMA, K., "Efficiency of Phase Separation", in Cyclone in Industry, Elsevier Publishing Co., (1961).

(82) TESSIER, G., "Les Lois Quantitatives de la Croissance", Ann. Physiologie, 12, 527, (1936).

(83) TISCHLER, Lial F., and ECKENFELDER, W. Wesley, Jr.,"Linear Substrate Removal in the Activated Sludge Process", Fourth International Conference on Water Pollution Research, Prague, Pergamon Press Ltd., (1968).

(84) VOLK, William, "Applied Statistics for Engineers" McGraw-Hill Book Co., Toronto, (1958).

(85) WASHINGTON, D. R., HETLING, L. J., and RAO, S. S., "Long-Term Adaptation of Activated Sludge Organisms to Accumulated Sludge Mass", Proc. 19th Industrial Waste Conference, Purdue University, 655, (1964).

(86) WESTON, R. F., and ECKENFELDER, W. W., Jr., "Application of Biological Treatment to Industrial Wastes, Kinetics and Equilibria of Oxidative Treatment", Sewage and Industrial Waste, 27, 802, (1955).

(87) WILSON, I. S., "Some Problems in the Treatment of Effluents from the Manufacture of Organic Chemicals", Chemistry and Industry, 1278, (1967).

(88) WINNEBERGER, John N., AUSTIN, John N., and KLETT, Carol A., "Membrane Filter Weight Determinations", J. WPCF, 35(6), 807, (1963).

(89) WIRTS, J. J., and AUSFLUG, F. J., "Concentration of Activated Sludge", Sewage and Industrial Wastes, 23, 1219, (1951).

(90) WUHRMANN, K., "Factors Affecting Efficiency and Solids Production in the Activated Sludge Process", in "Biological Treatment of Sewage and Industrial Wastes", Vol. 1, (Ed. by McCabe and Eckenfelder), 49, Reinhold Publishing Corp., New York, (1956).

(91) WUHRMANN, K., "High Rate Activated Sludge Treatment and its Relation to Stream Sanitation", Sewage and Industrial Wastes, 26, 1,(1954).

(92) YOSHIOKA, N., and HOTTA, Y., "Liquid Cyclones as an Hydraulic Classifier", Chem. Eng., Japan, 19, 632, (1955).

(93) YOUNG, James C., and CLARK, John W., "Second Order Equation for BOD", Proc. ASCE, J. Sanitary Engineering Division, February, 43, (1965).

APPENDIX I

DESIGN OF HYDROCYCLONES

Theory

Theories on the separation in hydrocyclones have been developed based on the concept of the stability radius, at which centrifugal forces acting on the particle are balanced by the drag forces exerted by the radial flow. According to these theories, particles possessing stability radii smaller than the radius of the overflow exit migrate to the overflow, all other particles are discharged through the underflow. Rietema (1961) questions the validity of the stability radius concept, since it assumes that all particles attain their stability radius before being discharged. He presents an alternate theory which indicates that separation is a function of particle residence time and which admits that equilibrium conditions may not occur in the hydrocyclone.

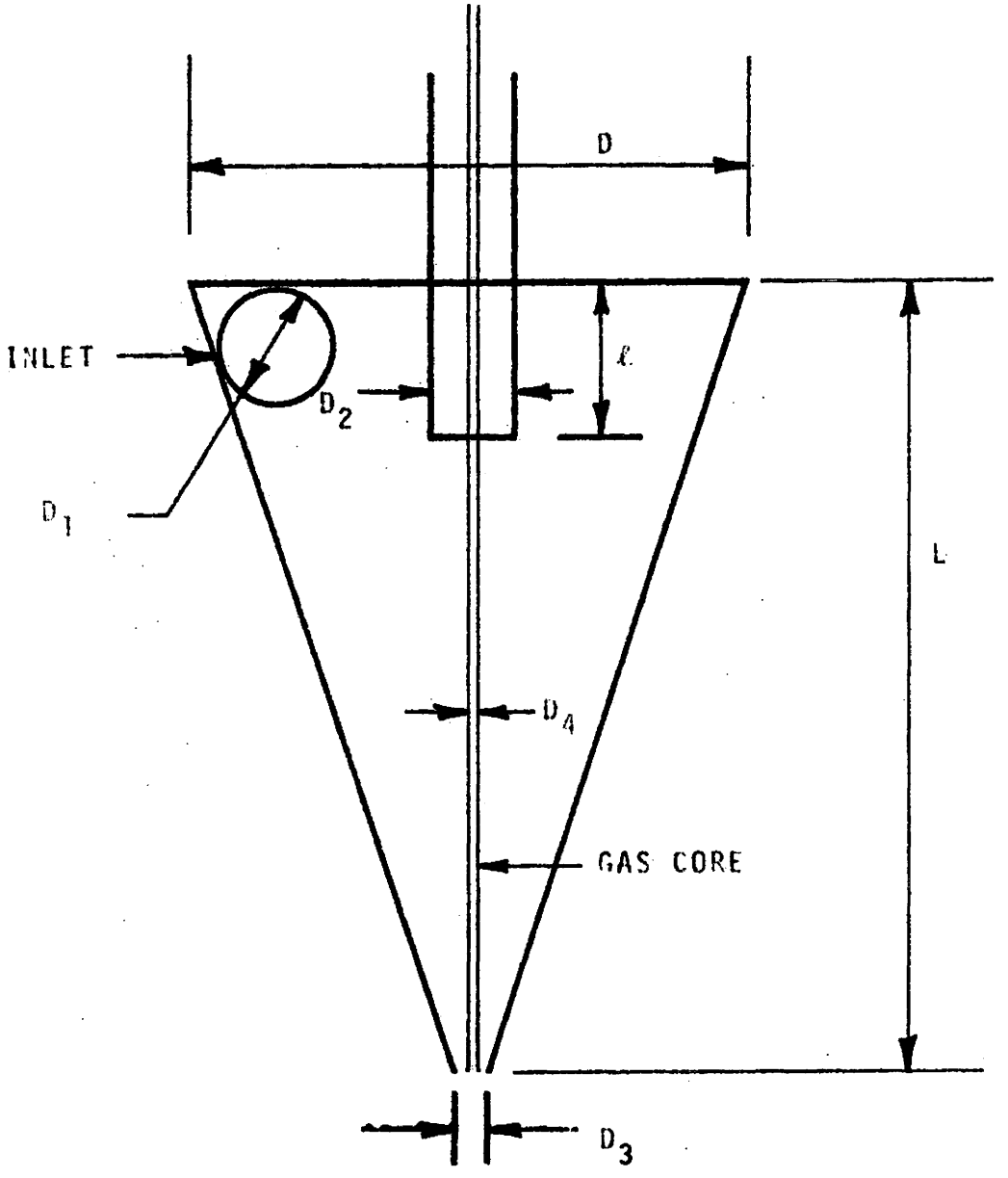
The residence time approach is based on a particle diameter, D_{p50}, which has an equal probability of migrating to either exit when injected along the center line of the inlet nozzle.

Referring to the following Figure and considering a particle which discharges through the underflow port, the radial distance covered by the particle relative to the carrier fluid (in residence time T) is R where $R = \frac{D}{2} - \frac{D_1}{2} - \frac{D_A}{2}$

therefore $$R = \int_0^T (V_{F_r} - V_{p_r})dt$$

where V_{F_r} = radial velocity component of the carrier fluid

FIGURE 50



D_4 = Diameter Gas Core
D = Diameter of Hydrocyclone at its base
D_1 = Inlet Diameter
D_2 = Overflow (Vortex Finder) Diameter
D_3 = Underflow Diameter
L = Length of Hydrocyclone
ℓ = Length of Vortex Finder

DEFINITION OF THE HYDROCYCLONE

V_{p_r} = radial velocity component of the particle

D_1 = diameter of inlet nozzle

D = diameter of hydrocyclone at its base

D_A = diameter of gas core

therefore $$\int_0^T V_{p_r}\, dt = \int_0^T V_{F_r}\, dt = R$$

By assuming that the axial and radial velocity components of the carrier fluid are constant

$$\int_0^T V_{F_r}\, dt = \int_0^T V_{F_r} \frac{dL}{V_{F_z}} = \frac{V_{F_r}}{V_{F_z}} L$$

where V_{F_z} = axial velocity component of the carrier fluid

L = length of cyclone

By assuming that the radial velocity component of the particle can be reasonably approximated by Stoke's law :

$$V_{p_r} = \frac{(D_{p50})^2 (\Delta\rho)\ (V_{p_\theta}{}^2)}{18\mu\quad (r\quad)}$$

where $\Delta\rho$ = density difference between particle and carrier fluid

V_{p_θ} = tangential component of the particle velocity

μ = dynamic liquid viscosity

r = radial distance

therefore $$\frac{(D_{p50})^2\ \Delta\rho}{18\mu} \int_0^T \frac{(V_{p_\theta})^2}{r}\, dt = \frac{V_{F_r}}{V_{F_z}} L = R$$

$$\frac{(D_{p50})^2\ \Delta\rho}{18\mu} \int_0^T \frac{(V_{p_\theta})^2}{r} \frac{dt}{dr}\, dr = \frac{V_{F_r}}{V_{F_z}} L = R$$

where $R = \frac{D}{2} - \frac{D_1}{2} - \frac{D_A}{2}$

By assuming that the axial velocity of the particle is equal to that of the fluid medium, and is constant, then for separation the radial velocity of the particle is constant.

Therefore $$\frac{dt}{dr} = \frac{L}{V_{p_z} R}$$

For a liquid cyclone operating with a gas core, the static pressure drop is equal to the centrifugal head

$$(\Delta P)_s = \int_R^{D_A/2} \frac{F(V_{F_\theta})^2}{r} \, dr$$

Therefore, the separation formula becomes

$$\frac{(D_{p50})^2 \cdot \Delta\rho \cdot L}{18\mu \cdot V_{F_z} \cdot R} \cdot \frac{(\Delta P)_s}{\rho_F} = \frac{V_{F_r}}{V_{F_z}} L - R$$

By assuming some relationship between the axial and inlet velocities of the carrier fluid:

$$V_{F_z} = C_1 V_{F_\theta} = C_1 \frac{4Q}{\pi D_1^2}$$

where C_1 = some undefined function

Q = total volumetric flowrate

Therefore: $$\frac{(D_{p50})^2 \Delta\rho}{\mu} L \frac{(\Delta P)_s}{\rho_F Q} = \frac{72C_1}{\pi} \frac{R}{D_1^2} \left(\frac{V_{F_r}}{V_{F_z}} L - R\right)$$

The righthand side of the above equation contains only cyclone dimensions and velocity ratios. Therefore, it is concluded that for a cyclone of specified shape the dimensionless group

$$\frac{72C_1}{\pi} \frac{R}{D_1^2} \left(\frac{V_{F_r}}{V_{F_z}} L - R\right)$$

will be constant.

Thus, the dimensionless group

$$\frac{(D_{p50})^2 \Delta\rho}{\mu} \; L \; \frac{(\Delta P)_s}{\rho_F g}$$

is a meaningful characteristic of a given hydrocyclone.

By assuming that the static pressure drop can be approximated by the pressure drop across the cyclone, the cyclone correlation number C_{y50} can be defined

$$C_{y50} = \frac{(D_{p50})^2 \Delta\rho}{\mu} \; L \; \frac{(\Delta P)_T}{\rho_F g}$$

This development has assumed that

(1) turbulence effects can be neglected

(2) gas core develops

(3) the cyclone has the shape of a cone

(4) there is no short circuiting, hindered discharge or recirculation.

For optimum hydrocyclone design, the cyclone correlation number should be minimized since this

(1) minimizes the total pressure drop required

(2) maximizes the capacity of the cyclone

(3) maximizes the smallness of the particle which can be separated

Using a suspension of quartz fines in water, Rietema (1961) investigated the effects of hydrocyclone geometry on C_{y50}. He observed an optimum value of 3.50 for C_{y50} corresponding to hydrocyclone shapes defined by

$$\frac{L}{D} = 5; \quad \frac{D_1}{D} = 0.28; \quad \frac{D_2}{D} = 0.34; \quad \frac{l}{D} = 0.4$$

where D_2 = overflow diameter

l = vortex finder length.

Knowing the available pressure drop, the 50 per cent separation diameter and the physical properties of the solid and liquid phases, it is possible to design a hydrocyclone in accordance with the optimum shape defined by Rietema.

The inlet Reynolds number can be determined for optimum cyclone geometry

$$Re_{inlet} = \frac{4\rho_F Q}{\pi D_i \mu}$$

From C_{y50} it follows that

$$\rho_F Q = \frac{(D_{p50})^2 \, \Delta\rho \; L(P)_T}{\mu \, C_{y50}}$$

for $C_{y50} = 3.50$

$$Re_{inlet} = 6.5 \, \frac{(D_{p50})^2 \, \Delta\rho \; (\Delta P)_T}{\mu^2}$$

Rietema (1961) has correlated $\frac{Re_{inlet}}{6.5}$ and the dimensionless groups

$$D \left(\frac{\rho_F}{\mu}\right) \sqrt{\frac{(\Delta P)_T}{\rho_F}} \quad \text{and} \quad Q\left(\frac{\rho_F}{\mu}\right)^2 \sqrt{\frac{(\Delta P)_T}{\rho_F}}$$

from which the hydrocyclone diameter D and the hydrocyclone capacity Q can be determined.

For optimum separation, the hydrocyclone dimensions, being fixed ratios of D, can be calculated.

Design

Using the data of Lamanna and Mallette (1965), the physical design properties of the microbial slurry were selected to be:

$$D_{p50} = 1 \mu$$

$$\rho_p = 1.10 \text{ gms/cc}$$

From the performance characteristics of the pump, the available pressure drop was selected to be 140 psi.

Therefore $$\frac{(D_{p50})^2 \, \Delta\rho \, \Delta P_T}{\mu^2} \geq 10^2$$

where $D_{p50} = 1 \mu = 3.281 \times 10^{-6}$ Ft.

$\Delta\rho = (1.10 - 1.00) = 0.1$ gm/cc $= 6.234$ lbm/ft^3

$\Delta P_T = 140$ lbf/ft^2 $= 2.018 \times 10^4$ lbf/ft^2

$\mu = 6.72 \times 10^{-7}$ lbf/(ft.-sec.) at 20°C

Therefore

$D < 1$ mm.

APPENDIX II

DATA RELIABILITY

BIOCHEMICAL TECHNIQUES

Suspended Solids Determinations

The determination of suspended solids concentration by the membrane filter technique has several inherent errors which could seriously influence data interpretation.

Busch et al (1962) noted that fresh membrane filters contain soluble materials which may be removed when accepting a sample, and result in non-representative tare values. Tests by the Millipore Filter Corporation (1961) indicate that as much as 2.5% of the membrane weight may be lost by boiling in distilled water. Winneberger et al (1963) report similar findings and advocate minimizing this source of error by pre-soaking fresh filters in distilled water for 1 hour agitated at .5 minute intervals.

Preliminary testing in the course of this investigation indicated that distilled water filter washes of 100 ml aliquots, passed under vacuum, resulted in steady-state tare values. Therefore, a 100 ml distilled water charge was applied to each fresh filter prior to use during this study.

Winneberger et al (1963) studied the significance of filter cake hydroscopicity on suspended solids determinations. After desication for 24 hours spent filters were placed on an analytical balance and changes in weight were observed as a function of time. Their results show that a rapid increase in weight occurred during the period from 0.5 to 4.0 minutes. In this investigation, this error was minimized by using a standardization time between removal from the desicator and weighing less than 0.5 minutes. Since a desicated atmosphere was

maintained in the balance, filter cake hydroscopicity was not considered a major source of error.

It has been reported that after rapid drying over a 4 hour interval, spent filters lose weight at a slow uniform rate over a 4 day dessication period (Winneberger et al (1963)). This error, minimized by using a desiccation treatment of 25 hours, was considered insignificant in this study.

Winneberger et al (1963) also studied the effect of oven drying on solids determinations. Ten desiccated spent filters, each with a residue of approximately 8.50 mg. were weighed, then placed inside a forced air oven at 103°C. At regular intervals the filters were removed, cooled in desiccators, reweighed and returned to the oven.

The authors observed a permanent mean weight loss of 1.01 mg. after one hour of oven drying. This was ascribed to volatization of the suspended solids cake at the oven temperature. McLean (1968) using 1 hour oven drying at 103°C observed a weight decrease of 0.81 per cent, plus or minus 0.63 per cent at a 99 per cent confidence level for residue weights of from 0.5 to 0.30 mg. (after accounting for a weight decrease in the filter paper). No provision was made for measuring or minimizing this source of error in this investigation.

A significant source of error was expected to be induced by the exposure of each filter paper and its foil dish to the laboratory environment for approximately 15 minutes during testing. Contamination by particulate matter settling from the air onto the media and adhering to the foil dish from contact with unclean surfaces could not be controlled. To minimize these phenomena, a control filter was assigned to each group of six filters and its change in weight was used to correct

for environmental contamination.

Organic Carbon-Determinations

Schaffer et al (1963) reported that organic carbon measurements can be effected by the Beckman IR 315 infrared analyzer with a sensitivity of 0.21 mg/l for a standard solution of 1 mg/l organic carbon, if 4 determinations are made and averaged. These results were obtained using high gain values indicating that the instrument noise to signal ratio did not decrease sensitivity, or accuracy. Since lower gain values were used throughout this investigation, decreases in accuracy were ascribed to operator techniques. Since calibration curves were made for each test run using standardized operator techniques, significant errors were not expected for organic carbon determinations. Sample contamination through contact with glassware remained a possibility but its effects were presumed to be insignificant.

Organic determination by the combustion infrared method are reported to be singularly free of interferences (Richard et al (1965)). This technique, however, exhibits a positive response to dissolved carbon dioxide, carbonates and bicarbonates. The sample preparation employed in this study, (pH adjustment followed by a helium surge) has been shown to be completely effective in removing inorganic forms of carbon and representative organic carbon determinations were assumed (Richard et al (1965); Schaffer et al (1963)).

APPENDIX III

SMOOTHING FORMULAE

In place of approximating the data by a single least-squares polynomial of specified degree over the entire range of values, each data entry was replaced by a least-squares polynomial of "best order" relevant to a subrange of points centered at the point for which the entry was to be modified.

In general, the data of each subrange were fit by a polynomial of the form

$$y(x) = b_0P_0 + b_1P_1(x) + b_2P_2(x) + \ldots + b_nP_n(x)$$

where $P_i(x)$ is an i^{th} degree polynomial in x. By the principle of least-squares, the squares of the residuals were to be minimized.

$$\sum_{j=0}^{n}{}' \ (b_0P_0 + b_1P_1 + \ldots + b_nP_n - y_j^2) = R^2(b_i)$$

If $\Sigma' P_i P_j = 0$ for $i \neq j$, the normal equations reduce to the set

$$\Sigma'(P_n^2 b_n) - \Sigma'(P_n y_j) = 0$$

and thus the coefficients of the fitted polynomial can be expressed:

$$b_i = \frac{\Sigma' P_i y_i}{\Sigma' P_i^2} \qquad \text{(Nielsen (1964))}$$

A set of polynomials meeting this criterion are the orthogonal polynomials. The following set of orthogonal polynomials was selected (Nielsen (1964))

$$P_0(x) = 1$$

$$P_1(x) = x - \bar{x}$$

$$P_{i+1}(x) = P_1P_i - \frac{i^2(m^2 - i^2)}{4(4i^2 - 1)} P_{i-1}$$

where m = the number of given data for the subrange (odd)

and $\bar{x} = \frac{(m - 1)}{2} + 1$

The data entries can be replaced by "smoothed" values defined by:

$$y(x) = \sum_{i=1}^{m}{}' \; C_i y_i$$

where $C_i = \sum_{j=1}^{m-1}{}' \frac{1}{S_j} P_j(i) P_j(x)$

and $S_j = \sum_{i=1}^{m}{}' P_i^2(x_j)$ $(i = 0,1,2, \ldots n_{m-1})$

In this fashion, the following least-squares approximation polynomials were developed to smooth the raw data. Superscript 1 denotes the latest datum modification.

First Order 3 Point Least-Squares Approximation

$$y_1' = \frac{1}{6}[5y_1 + 2y_2 - y_3]$$
$$y_i' = \frac{1}{3}[y_{i-1} + y_i + y_{i+1}]$$
$$y_m' = \frac{1}{6}[-y_{m-2} + 2y_{m-1} + 5y_m]$$

First Order 5 Point Least-Squares Approximation

$$y_1' = \frac{1}{5}[3y_1 + 2y_2 + y_3 - y_5]$$
$$y_2' = \frac{1}{10}[4y_1 + 3y_2 + 2y_3 + y_4]$$
$$y_i' = \frac{1}{5}[y_{i-2} + y_{i-1} + y_i + y_{i+1} + y_{i+2}]$$
$$y_{m-1}' = \frac{1}{10}[y_{m-3} + 2y_{m-2} + 3y_{m-1} + 4y_m]$$
$$y_m' = \frac{1}{5}[-y_{m-4} + y_{m-2} + 2y_{m-1} + 3y_m]$$

Second Order 5 Point Least-Squares Approximation

$$y_1' = \frac{1}{35}[31y_1 + 9y_2 - 3y_3 - 5y_4 + 3y_5]$$
$$y_2' = \frac{1}{35}[9y_1 + 13y_2 + 12y_3 + 6y_4 - 5y_5]$$
$$y_i' = \frac{1}{35}[-3y_{i-2} + 12y_{i-1} + 17y_i + 12y_{i+1} - 3y_{i+2}]$$
$$y_{m-1}' = \frac{1}{35}[-5y_{m-4} + 6y_{m-3} + 12y_{m-2} + 13y_{m-1} + 9y_m]$$
$$y_m' = \frac{1}{35}[3y_{m-4} - 5y_{m-3} - 3y_{m-2} + 9y_{m-1} + 31y_m]$$

Third Order 5 Point Least-Squares Approximation

$$y'_1 = \frac{1}{70}[69y_1 + 4y_2 - 6y_3 + 4y_4 - y_5]$$

$$y'_2 = \frac{1}{35}[2y_1 + 27y_2 + 12y_3 - 8y_4 + 2y_5]$$

$$y'_i = \frac{1}{35}[-3y_{i-2} + 12y_{i-1} + 17y_i + 12y_{i+1} - 3y_{i+2}]$$

$$y'_{m-1} = \frac{1}{35}[2y_{m-4} - 8y_{m-3} + 12y_{m-2} + 27y_{m-1} + 2y_m]$$

$$y'_m = \frac{1}{70}[-y_{m-4} + 4y_{m-3} - 6y_{m-2} + 4y_{m-1} + 69y_m]$$

Fourth Order 5 Point Least-Squares Approximation

$$y'_1 = \frac{1}{924}[912y_1 + 50y_2 - 70y_3 + 20y_4 + 40y_5 - 38y_6 + 10y_7]$$

$$y'_2 = \frac{1}{924}[50y_1 + 712y_2 + 310y_3 - 120y_4 - 130y_5 + 140y_6 - 38y_7]$$

$$y'_3 = \frac{1}{924}[-70y_1 + 310y_2 + 424y_3 + 300y_4 + 50y_5 - 130y_6 + 40y_7]$$

$$y'_i = \frac{1}{924}[20y_{i-3} - 120y_{i-2} + 300y_{i-1} + 525y_i + 300y_{i+1} - 120y_{i+2} + 20y_{i+3}]$$

$$y'_{m-2} = \frac{1}{924}[40y_{m-6} - 130y_{m-5} + 50y_{m-4} + 300y_{m-3} + 424y_{m-2} + 310y_{m-1} - 70y_m]$$

$$y'_{m-1} = \frac{1}{924}[-38y_{m-6} + 140y_{m-5} - 130y_{m-4} - 120y_{m-3} + 310y_{m-2} + 712y_{m-1} + 50y_m]$$

$$y'_m = \frac{1}{924}[10y_{m-6} - 38y_{m-5} + 40y_{m-4} + 20y_{m-3} - 70y_{m-2} + 50y_{m-1} + 912y_m]$$

Fifth Order 7 Point Least-Squares Approximation

$$y'_1 = \frac{1}{924}[923y_1 + 6y_2 - 15y_3 + 20y_4 - 15y_5 + 6y_6 - y_7]$$

$$y'_2 = \frac{1}{154}[y_1 + 148y_2 + 15y_3 - 20y_4 + 15y_5 - 6y_6 + y_7]$$

$$y'_3 = \frac{1}{308}[-5y_1 + 30y_2 + 233y_3 + 100y_4 - 75y_5 + 30y_6 - 5y_7]$$

$$y'_i = \frac{1}{231}[5y_{i-3} - 30y_{i-2} + 75y_{i-1} + 131y_i + 75y_{i+1} - 30y_{i+2} + 5y_{i+3}]$$

$$y'_{m-2} = \frac{1}{308}[-5y_{m-6} + 30y_{m-5} + 75y_{m-4} + 100y_{m-3} + 223y_{m-2} - 30y_{m-1} - 5y_m]$$

$$y'_{m-1} = \frac{1}{154}[y_{m-6} - 6y_{m-5} + 15y_{m-4} - 20y_{m-3} + 15y_{m-2} + 148y_{m-1} + y_m]$$

$$y'_m = \frac{1}{924}[-y_{m-6} + 6y_{m-5} - 15y_{m-4} + 20y_{m-3} - 15y_{m-2} + 6y_{m-1} + 923y_m]$$

```
C
C     ANALYSIS OF BATCH DATA
C     T.E.POLLOCK
C
C     PROGRAM DETERMINES BEST MATHEMATICAL CORRELATION ORDER (5TH ORDER
C     MAX) FROM DIFFERENCE TABLE OF RAW DATA, SMOOTHS DATA USING LEAST-
C     SQUARES OF APPROPRIATE ORDER. DETERMINES COEFFICIENTS OF POLYNOM-
C     IAL OF BEST FIT FROM DIFFERENCE TABLE OF SMOOTHED DATA , AND INT-
C     ERPOLATES AT MID INTERVALS OF INDEPENDENT VARIABLE
C
C     PROGRAM REQUIRES DATA WITH EQUAL SPACING OF INDEPENDENT VARIABLE
C     WITH ONLY ONE DEPENDENT VARIABLE
C
C     DEFINITIONS
C        DIA=HYDROCYCLONE DIAMETER
C        VS=VOLUME SPLIT
C        PI=INLET PRESSURE(PSI)
C        PO=OVERFLOW PRESSURE(PSI)
C        PU=UNDERFLOW PRESSURE(PSI)
C        QI=INFLUENT FLOWRATE
C        QO=OVERFLOW FLOWRATE
C        QU=UNDERFLOW FLOWRATE
C        T=TIME
C        TT=INTERPOLATED AND SMOOTHED TIME
C        CO=OVERFLOW REACTOR CARBON CONC
C        CU=UNDERFLOW REACTOR CARBON CONC
C        ZMO=OVERFLOW REACTOR SOLIDS CONC
C        ZMU=UNDERFLOW REACTOR SOLIDS CONC
C        XX=INTERPOLATION ABSCISSA
C        YY=INTERPOLATION ORDINATE
C        NDATA=NO. DATA SETS
C        NPTS=NO POINTS IN EACH DATA SET
C        NINTRP=NO INTERPOLATION POINTS DESIRED
C        Z=INTERPOLATED AND SMOOTHED VALUE OF INDEPENDENT VARIABLE
C        ZZ=INTERPOLATED AND SMOOTHED VALUE OF DEPENDENT VARIABLE
C        X=INDEPENDENT VARIABLE
C        Y=DEPENDENT VARIABLE
C        L=COUNTER
C        SGR=SPECIFIC GROWTH RATE
C        FDIF1Y=FIRST FORWARD DIFFERENCE
C        FDIF2Y=SECOND FORWARD DIFFERENCE
C        FDIF3Y=THIRD FORWARD DIFFERENCE
C        S=SMOOTHED VALUE OF DEPENDENT VARIABLE
C
      DIMENSION T(99),CO(99),CU(99),ZMO(99),ZMU(99),XX(99),Y(99),X(99),
     1Z(99),ZZ(99),SOLIDS(99),CARBON(99),DCDT(99),SGR(99),FDIF1Y(99),
     1FDIF2Y(99),FDIF3Y(99),FD(99),SD(99),TD(99),S(99),TT(99),YY(99)
      DIMENSION FDIF4Y(99),FDIF5Y(99),QD(99),CD(99),DIFF(99)
      COMMON T,TT,CO,CU,ZMO,ZMU,XX,NDATA,NPTS,NINTRP,Y,X,ZZ,Z,NT
      LL=1
   99 CONTINUE
      WRITE(6,16)
C
C     SYSTEM DEFINITION
C
C
C     DATA INPUT
C
      READ(5,44)NRUN
```

```
      READ(5,44) NPTS
      NINTRP=NPTS-1
      NDATA=18
      WRITE(6,38)NRUN
      READ(5,2)(T(K),K=1,NPTS)
      READ(5,3)(CO(K),K=1,NPTS)
      READ(5,4)(CU(K),K=1,NPTS)
      READ(5,5)(ZMO(K),K=1,NPTS)
      READ(5,6)(ZMU(K),K=1,NPTS)
      READ(5,7)(XX(K),K=1,NINTRP)
      MERV=2*NDATA
C
C     OVERFLOW CARBON VS TIME DATA
C
      WRITE(6,8)
      DO 100 I=1,NPTS
      Y(I)=CO(I)
  100 X(I)=T(I)
      CALL SMOOTH
      DO 101 I=1,NT
      CO(I)=ZZ(I)
      TT(I)=Z(I)
  101 CONTINUE
C
C      UNDERFLOW CARBON VS TIME DATA
C
      WRITE(6,9)
      DO 102 I=1,NPTS
      Y(I)=CU(I)
  102 X(I)=T(I)
      CALL SMOOTH
      DO 103 I=1,NT
      CU(I)=ZZ(I)
      TT(I)=Z(I)
  103 CONTINUE
C
C     OVERFLOW SOLIDS VS TIME DATA
C
      WRITE(6,10)
      DO 104 I=1,NPTS
      Y(I)=ZMO(I)
  104 X(I)=T(I)
      CALL SMOOTH
      DO 105 I=1,NT
      ZMO(I)=ZZ(I)
      TT(I)=Z(I)
  105 CONTINUE
C
C     UNDERFLOW SOLIDS VS TIME DATA
C
      WRITE(6,11)
      DO 106 I=1,NPTS
      Y(I)=ZMU(I)
  106 X(I)=T(I)
      CALL SMOOTH
      DO 107 I=1,NT
      ZMU(I)=ZZ(I)
      TT(I)=Z(I)
```

```
  107 CONTINUE
C
C     CALCULATION OF SPECIFIC GROWTH RATE USING A LINEAR APPROXIMATION
C
      L=0
      WRITE(6,12)
      WRITE(6,13)
      WRITE(6,14)
      NS=NT-1
  110 CONTINUE
      DO 108 I=1,NS
      SOLIDS(I)=(ZMO(I)+ZMO(I+1))/2.
      CARBON(I)=(CO(I)+CO(I+1))/2.
      DCDT(I)=(CO(I)-CO(I+1))/(TT(I+1)-TT(I))
      SGR(I)=(1./SOLIDS(I))*DCDT(I)
      WRITE(6,36)TT(I),SGR(I),CARBON(I)
  108 CONTINUE
      IF(L.GT.0) GO TO 111
      DO 109 I=1,NT
      ZMO(I)=ZMU(I)
      CO(I)=CU(I)
  109 CONTINUE
      WRITE(6,15)
      L=L+1
      GO TO 110
  111 IF(LL.GT.MERV) STOP
      LL=LL+1
      GO TO 99
C
C     FORMAT STATEMENTS
C
    1 FORMAT(3I3)
    2 FORMAT(6F10.5)
    3 FORMAT(6F10.5)
    4 FORMAT(6F10.5)
    5 FORMAT(6F10.5)
    6 FORMAT(6F10.5)
    7 FORMAT(5F10.5)
    8 FORMAT(1H1,29H OVERFLOW CARBON VS TIME DATA  )
    9 FORMAT(1H1,30H UNDERFLOW CARBON VS TIME DATA  )
   10 FORMAT(1H1,29H OVERFLOW SOLIDS VS TIME DATA  )
   11 FORMAT(1H1,30H UNDERFLOW SOLIDS VS TIME DATA  )
   12 FORMAT(1H1,50H SPECIFIC GROWTH RATE USING A LINEAR APPROXIMATION
   13 FORMAT(1H0,23H OVERFLOW BATCH REACTOR  )
   14 FORMAT(1H0,10H TIME(HRS),10X,28H SPECIFIC GROWTH RATE(HRS-1)  )
   15 FORMAT(1H0,24H UNDERFLOW BATCH REACTOR  )
   16 FORMAT(1H1,42H HYDROCYCLONE BATCH BIOLOGICAL PERFORMANCE  )
   17 FORMAT(8F10.5)
   18 FORMAT(1H0,5H DIA=,F10.5,4H VS=,F10.5,4H PI=,F10.5,4H PO=,F10.5,4
     1 PU=,F10.5,4H QI=,F10.5,4H QO=,F10.5,4H QU=,F10.5)
   19 FORMAT(3H MM,33X,4H PSI,10X,4H PSI,10X,4H PSI,8X,6H L/MIN,8X,6H L
     1MIN,8X,6H L/MIN  )
   36 FORMAT(1H0,F10.5,15X,F10.5,24X,F10.3)
   38 FORMAT(1H0,7H SET NO ,I3)
   44 FORMAT(I3)
      END
$IBFTC SMOOTH
      SUBROUTINE SMOOTH
```

```
      DIMENSION T(99),CO(99),CU(99),ZMO(99),ZMU(99),XX(99),Y(99),X(99),
     1Z(99),ZZ(99),SOLIDS(99),CARBON(99),DCDT(99),SGR(99),FDIF1Y(99),
     1FDIF2Y(99),FDIF3Y(99),FD(99),SD(99),TD(99),5(99),TT(99),YY(99)
      DIMENSION FDIF4Y(99),FDIF5Y(99),QD(99),CD(99),DIFF(99)
      COMMON T,TT,CO,CU,ZMO,ZMU,XX,NDATA,NPTS,NINTRP,Y,X,ZZ,Z,NT
C
C     ESTABLISH DIFFERENCE TABLE OF RAW DATA
C
C
      WRITE(6,20)
      WRITE(6,21)
      WRITE(6,22)
      DO 200 I=1,NPTS
      FDIF1Y(I)=0.
      FDIF2Y(I)=0.
      FDIF3Y(I)=0.
      FDIF4Y(I)=0.
      FDIF5Y(I)=0.
  200 CONTINUE
      FDSUM=0.
      SDSUM=0.
      TDSUM=0.
      QDSUM=0.
      CDSUM=0.
      NN=NPTS-1
      NNN=NN-1
      NNNN=NNN-1
      N5=NNNN-1
      N6=N5-1
      DO 201 I=1,NN
      FDIF1Y(I)=Y(I+1)-Y(I)
      FDIF1Y(I)=FDIF1Y(I)/SQRT(2.)
      FDSUM=FDSUM+FDIF1Y(I)
  201 CONTINUE
      IF(NPTS.LE.6) GO TO 700
      DO 202 I=1,NNN
      FDIF2Y(I)=Y(I+2)-2.*Y(I+1)+Y(I)
      FDIF2Y(I)=FDIF2Y(I)/2.
      SDSUM=SDSUM+FDIF2Y(I)
  202 CONTINUE
      DO 203 I=1,NNNN
      FDIF3Y(I)=Y(I+3)-3.*Y(I+2)+3.*Y(I+1)-Y(I)
      FDIF3Y(I)=FDIF3Y(I)/(2.*SQRT(2.))
      TDSUM=TDSUM+FDIF3Y(I)
  203 CONTINUE
      DO 303 I=1,N5
      FDIF4Y(I)=Y(I+4)-4.*Y(I+3)+6.*Y(I+2)-4.*Y(I+1)+Y(I)
      FDIF4Y(I)=FDIF4Y(I)/4.
      QDSUM=QDSUM+FDIF4Y(I)
  303 CONTINUE
      DO 304 I=1,N6
      FDIF5Y(I)=Y(I+5)-5.*Y(I+4)+10.*Y(I+3)-10.*Y(I+2)+5.*Y(I+1)-Y(I)
      FDIF5Y(I)=FDIF5Y(I)/(4.*SQRT(2.))
      CDSUM=CDSUM+FDIF5Y(I)
  304 CONTINUE
      DO 204 I=1,NPTS
      WRITE(6,23)X(I),Y(I),FDIF1Y(I),FDIF2Y(I),FDIF3Y(I),FDIF4Y(I),FDIF5
     1Y(I)
  204 CONTINUE
```

```
C
C       ESTIMATION OF ORDER FROM DIFFERENCE TABLE
C
        FTOTAL=0.
        STOTAL=0.
        TTOTAL=0.
        QTOTAL=0.
        CTOTAL=0.
        FDAVG=FDSUM/FLOAT(NN)
        SDAVG=SDSUM/FLOAT(NNN)
        TDAVG=TDSUM/FLOAT(NNNN)
        QDAVG=QDSUM/FLOAT(N5)
        CDAVG=CDSUM/FLOAT(N6)
        DO 205 I=1,NN
        FD(I)=(FDAVG-FDIF1Y(I))**2
        FTOTAL=FTOTAL+FD(I)
  205   CONTINUE
        DIFF(1)=FTOTAL/FLOAT(NN-1)
        DO 206 I=1,NNN
        SD(I)=(SDAVG-FDIF2Y(I))**2
        STOTAL=STOTAL+SD(I)
  206   CONTINUE
        DIFF(2)=STOTAL/FLOAT(NNN-1)
        DO 207 I=1,NNNN
        TD(I)=(TDAVG-FDIF3Y(I))**2
        TTOTAL=TTOTAL+SD(I)
  207   CONTINUE
        DIFF(3)=TTOTAL/FLOAT(NNNN-1)
        DO 305 I=1,N5
        QD(I)=(QDAVG-FDIF4Y(I))**2
        QTOTAL=QTOTAL+QD(I)
  305   CONTINUE
        DIFF(4)=QTOTAL/FLOAT(N5-1)
        DO 306 I=1,N6
        CD(I)=(CDAVG-FDIF5Y(I))**2
        CTOTAL=CTOTAL+CD(I)
  306   CONTINUE
        DIFF(5)=CTOTAL/FLOAT(N6-1)
        IF(NPTS.LE.3) NORD=1
        IF(NPTS.EQ.4) NORD=2
        IF(NPTS.EQ.5) NORD=3
        IF(NPTS.EQ.6) NORD=4
        IF(NPTS.GT.6) NORD=5
        DO 307 I=1,NORD
        K=0
        DO 308 J=1,NORD
        IF(DIFF(I).LE.DIFF(J)) K=K+1
  308   CONTINUE
        IF(K.EQ.NORD) GO TO 309
  307   CONTINUE
        WRITE(6,39)
  309   ORDER=FLOAT(I)
C
C       SMOOTHING OF RAW DATA AND INTERPOLATION
C
        NP=NPTS
        NPP=NPTS-2
        NTT=NPTS
```

```
      NNT=NPTS-3
      IF(ORDER.EQ.2.) GO TO 210
      IF(ORDER.EQ.3.) GO TO 211
      IF(ORDER.EQ.4.) GO TO 310
      IF(ORDER.EQ.5.) GO TO 311
      WRITE(6,24)
  700 ORDER=1.
      MARY=NPTS-1
C
C     LEAST SQUARES 3-POINT LINEAR SMOOTHING
C
      DO 701 I=1,1000
      S(1)=(1./6.)*(5.*Y(1)+2.*Y(2)-1.*Y(3))
      DO 702 K=1,MARY
      IF(K.EQ.1) K=2
      S(K)=(1./3.)*(Y(K-1)+Y(K)+Y(K+1))
  702 CONTINUE
      S(NPTS)=(1./6.)*(-Y(NPTS-2)+2.*Y(NPTS-1)+5.*Y(NPTS))
      DO 703 J=1,NPTS
  703 Y(J)=S(J)
  701 CONTINUE
      IF(NPTS.LE.6) GO TO 800
      GO TO 215
  210 CONTINUE
      WRITE(6,25)
C
C     LEAST SQUARES 5-POINT QUADRATIC SMOOTHING
C
      DO 250 I=1,1000
      S(1)=(1./35.)*(31.*Y(1)+9.*Y(2)-3.*Y(3)-5.*Y(4)+3.*Y(5))
      S(2)=(1./35.)*(9.*Y(1)+13.*Y(2)+12.*Y(3)+6.*Y(4)-5.*Y(5))
      DO 251 K=3,NPP
  251 S(K)=(1./35.)*(-3.*Y(K-2)+12.*Y(K-1)+17.*Y(K)+12.*Y(K+1)+3.*Y(K+2)
     1)
      S(NP-1)=(1./35.)*(-5.*Y(NP-4)+6.*Y(NP-3)+12.*Y(NP-2)+13.*Y(NP-1)+9
     1.*Y(NP))
      S(NP)=(1./35.)*(3.*Y(NP-4)-5.*Y(NP-3)-3.*Y(NP-2)+9.*Y(NP-1)+31.*Y(
     1NP))
      DO 252 J=1,NP
  252 Y(J)=S(J)
  250 CONTINUE
      GO TO 215
  211 CONTINUE
      WRITE(6,26)
C
C     LEAST SQUARES 5-POINT CUBIC SMOOTHING
C
      DO 216 I=1,1000
      S(1)=(1./70.)*(69.*Y(1)+4.*Y(2)-6.*Y(3)+4.*Y(4)-Y(5))
      S(2)=(1./35.)*(2.*Y(1)+27.*Y(2)+12.*Y(3)-8.*Y(4)+2.*Y(5))
      DO 217 K=3,NPP
  217 S(K)=(1./35.)*(-3.*Y(K-2)+12.*Y(K-1)+17.*Y(K)+12.*Y(K+1)-3.*Y(K+2)
     1)
      S(NP-1)=(1./35.)*(2.*Y(NP-4)-8.*Y(NP-3)+12.*Y(NP-2)+27.*Y(NP-1)+2.
     1*Y(NP))
      S(NP)=(1./70.)*(-Y(NP-4)+4.*Y(NP-3)-6.*Y(NP-2)+4.*Y(NP-1)+69.*Y(NP
     1))
      DO 218 J=1,NP
```

```
  218 Y(J)=S(J)
  216 CONTINUE
      GO TO 215
  310 CONTINUE
      WRITE(6,40)
C
C     LEAST SQUARES 7-POINT FOURTH ORDER SMOOTHING
C
      DO 312 I=1,1000
      S(1)=(1./924.)*(912.*Y(1)+50.*Y(2)-70.*Y(3)+20.*Y(4)+40.*Y(5)-38.*
     1Y(6)+10.*Y(7))
      S(2)=(1./924.)*(50.*Y(1)+712.*Y(2)+310.*Y(3)-120.*Y(4)-130.*Y(5)+1
     140.*Y(6)-38.*Y(7))
      S(3)=(1./924.)*(-70.*Y(1)+310.*Y(2)+424.*Y(3)+300.*Y(4)+50.*Y(5)-1
     130.*Y(6)+40.*Y(7))
      DO 313 K=4,NNT
  313 S(K)=(1./924.)*(20.*Y(K-3)-120.*Y(K-2)+300.*Y(K-1)+524.*Y(K)+300.*
     1Y(K+1)-120.*Y(K+2)+20.*Y(K+3))
      S(NTT-2)=(1./924.)*(40.*Y(NTT-6)-130.*Y(NTT-5)+50.*Y(NTT-4)+300.*Y
     1(NTT-3)+424.*Y(NTT-2)+310.*Y(NTT-1)-70.*Y(NTT))
      S(NTT-1)=(1./924.)*(-38.*Y(NTT-6)+140.*Y(NTT-5)-130.*Y(NTT-4)-120.
     1*Y(NTT-3)+310.*Y(NTT-2)+712.*Y(NTT-1)+50.*Y(NTT))
      S(NTT)=(1./924.)*(10.*Y(NTT-6)-38.*Y(NTT-5)+40.*Y(NTT-4)+20.*Y(NTT
     1-3)-70.*Y(NTT-2)+50.*Y(NTT-1)+912.*Y(NTT))
      DO 400 J=1,NP
  400 Y(J)=S(J)
  312 CONTINUE
      GO TO 215
  311 CONTINUE
      WRITE(6,41)
C
C     LEAST SQUARES 7-POINT FIFTH ORDER SMOOTHING
C
      DO 314 I=1,1000
      S(1)=(1./924.)*(923.*Y(1)+6.*Y(2)-15.*Y(3)+20.*Y(4)-15.*Y(5)+6.*Y(
     16)-1.*Y(7))
      S(2)=(1./154.)*(1.*Y(1)+148.*Y(2)+15.*Y(3)-20.*Y(4)+15.*Y(5)-6.*Y(
     16)+1.*Y(7))
      S(3)=(1./308.)*(-5.*Y(1)+30.*Y(2)+233.*Y(3)+100.*Y(4)-75.*Y(5)+30.
     1*Y(6)-5.*Y(7))
      DO 315 K=4,NNT
  315 S(K)=(1./231.)*(5.*Y(K-3)-30.*Y(K-2)+75.*Y(K-1)+131.*Y(K)+75.*Y(K+
     11)-30.*Y(K+2)+5.*Y(K+3))
      S(NTT-2)=(1./308.)*(-5.*Y(NTT-6)+30.*Y(NTT-5)-75.*Y(NTT-4)+100.*Y
     1(NTT-3)+233.*Y(NTT-2)+30.*Y(NTT-1)-5.*Y(NTT))
      S(NTT-1)=(1./154.)*(1.*Y(NTT-6)-6.*Y(NTT-5)+15.*Y(NTT-4)-20.*Y(NTT
     1-3)+15.*Y(NTT-2)+148.*Y(NTT-1)+1.*Y(NTT))
      S(NTT)=(1./924.)*(-1.*Y(NTT-6)+6.*Y(NTT-5)-15.*Y(NTT-4)+20.*Y(NTT-
     13)-15.*Y(NTT-2)+6.*Y(NTT-1)+923.*Y(NTT))
      DO 401 J=1,NP
  401 Y(J)=S(J)
  314 CONTINUE
C
C     ESTABLISH DIFFERENCE TABLE OF SMOOTHED DATA
C
  215 CONTINUE
  800 CONTINUE
      DO 219 K=1,NN
```

```
      FDIF1Y(K)=Y(K+1)-Y(K)
  219 CONTINUE
      IF(NPTS.LE.6) GO TO 900
      DO 220 K=1,NNN
      FDIF2Y(K)=Y(K+2)-2.*Y(K+1)+Y(K)
  220 CONTINUE
      DO 221 K=1,NNNN
      FDIF3Y(K)=Y(K+3)-3.*Y(K+2)+3.*Y(K+1)-Y(K)
  221 CONTINUE
      DO 316 K=1,N5
      FDIF4Y(K)=Y(K+4)-4.*Y(K+3)+6.*Y(K+2)-4.*Y(K+1)+Y(K)
  316 CONTINUE
      DO 317 K=1,N6
      FDIF5Y(K)=Y(K+5)-5.*Y(K+4)+10.*Y(K+3)-10.*Y(K+2)+5.*Y(K+1)-Y(K)
  317 CONTINUE
C
C     INTERPOLATION USING POLYNOMIAL OF BEST FIT
C
      IF(ORDER.EQ.2.) GO TO 222
      IF(ORDER.EQ.3.) GO TO 223
      IF(ORDER.EQ.4.) GO TO 318
      IF(ORDER.EQ.5.) GO TO 319
  900 CONTINUE
C
C     LINEAR INTERPOLATION
C
      I=2
      AH=X(I)-X(I-1)
      AO=Y(I-1)
      A1=FDIF1Y(I-1)/AH
      BOLIN=AO-A1*X(I-1)
      B1LIN=A1
      DO 300 L=1,NINTRP
      YY(L)=BOLIN+B1LIN*XX(L)
  300 CONTINUE
      WRITE(6,34)BOLIN,B1LIN
      GO TO 226
  222 CONTINUE
C
C     QUADRATIC INTERPOLATION
C
      I=2
      AH=X(I)-X(I-1)
      AO=Y(I-1)
      A1=FDIF1Y(I-1)/AH
      A2=FDIF2Y(I-1)/(2.*AH**2)
      BOQUAD=AO-A1*X(I-1)+A2*X(I-1)*X(I)
      B1QUAD=A1-A2*(X(I-1)+X(I))
      B2QUAD=A2
      DO 301 L=1,NINTRP
      YY(L)=BOQUAD+XX(L)*(B1QUAD+B2QUAD*XX(L))
  301 CONTINUE
      WRITE(6,35)BOQUAD,B1QUAD,B2QUAD
      GO TO 226
  223 CONTINUE
C
C     CUBIC INTERPOLATION
C
```

```
      I=2
      AH=X(I)-X(I-1)
      AO=Y(I-1)
      A1=FDIF1Y(I-1)/AH
      A2=FDIF2Y(I-1)/(2.*AH**2)
      A3=FDIF3Y(I-1)/(3.*2.*AH**3)
      BOCUBE=AO-A1*X(I-1)+A2*X(I-1)*X(I)-A3*X(I-1)*X(I)*X(I+1)
      B1CUBE=A1-A2*(X(I-1)+X(I))+A3*(X(I-1)*X(I)+X(I-1)*X(I+1)+X(I)*X(I+
     11))
      B2CUBE=A2-A3*(X(I-1)+X(I)+X(I+1))
      B3CUBE=A3
      DO 302 L=1,NINTRP
      YY(L)=BOCUBE+XX(L)*(B1CUBE+XX(L)*(B2CUBE+B3CUBE*XX(L)))
  302 CONTINUE
      WRITE(6,37)BOCUBE,B1CUBE,B2CUBE,B3CUBE
      GO TO 226
  318 CONTINUE
C
C     FOURTH ORDER INTERPOLATION
C
      I=2
      AH=X(I)-X(I-1)
      AO=Y(I-1)
      A1=FDIF1Y(I-1)/AH
      A2=FDIF2Y(I-1)/(2.*AH**2)
      A3=FDIF3Y(I-1)/(3.*2.*AH**3)
      A4=FDIF4Y(I-1)/(4.*3.*2.*AH**4)
      BOFRTH=AO-A1*X(I-1)+A2*X(I-1)*X(I)-A3*X(I-1)*X(I)*X(I+1)+A4*X(I-1)
     1*X(I)*X(I+1)*X(I+2)
      B1FRTH=A1-A2*(X(I-1)+X(I))+A3*(X(I-1)*X(I)+X(I-1)*X(I+1)+X(I+1)*X(
     1I))-A4*(X(I-1)*X(I)*X(I+1)+X(I-1)*X(I)*X(I+2)+X(I-1)*X(I+1)*X(I+2)
     1+X(I)*X(I+1)*X(I+2))
      B2FRTH=A2-A3*(X(I-1)+X(I)+X(I+1))+A4*(X(I-1)*X(I)+X(I-1)*X(I+1)+X(
     1I-1)*X(I+2)+X(I)*X(I+1)+X(I)*X(I+2)+X(I+1)*X(I+2))
      B3FRTH=A3-A4*(X(I-1)+X(I)+X(I+1)+X(I+2))
      B4FRTH=A4
      DO 320 L=1,NINTRP
      YY(L)=BOFRTH+XX(L)*(B1FRTH+XX(L)*(B2FRTH+B3FRTH*XX(L)+B4FRTH*XX(L)
     1*XX(L)))
  320 CONTINUE
      WRITE(6,42) BOFRTH,B1FRTH,B2FRTH,B3FRTH,B4FRTH
      GO TO 226
  319 CONTINUE
C
C     FIFTH ORDER INTERPOLATION
C
      I=2
      AH=X(I)-X(I-1)
      AO=Y(I-1)
      A1=FDIF1Y(I-1)/AH
      A2=FDIF2Y(I-1)/(2.*AH**2)
      A3=FDIF3Y(I-1)/(3.*2.*AH**3)
      A4=FDIF4Y(I-1)/(4.*3.*2.*AH**4)
      A5=FDIF5Y(I-1)/(5.*4.*3.*2.*AH**5)
      BOFITH=AO-A1*X(I-1)+A2*X(I-1)*X(I)-A3*X(I-1)*X(I)*X(I+1)+A4*X(I-1)
     1*X(I)*X(I+1)*X(I+2)-X(I-1)*X(I)*X(I+1)*X(I+2)*X(I+3)*A5
      B1FITH=A1-A2*(X(I-1)+X(I))+A3*(X(I-1)*X(I)+X(I-1)*X(I+1)+X(I+1)*X(
     1I))-A4*(X(I-1)*X(I)*X(I+1)+X(I-1)*X(I)*X(I+2)+X(I-1)*X(I+1)*X(I+2)
```

```
     1)+A5*(X(I-1)*X(I)*X(I+1)*X(I+2)+X(I-1)*X(I+1)*X(I+2)*X(I+3)+X(I)*X
     1(I+1)*X(I+2)*X(I+3)+X(I-1)*X(I)*X(I+1)*X(I+3)+X(I-1)*X(I)*X(I+2)*X
     1(I+3))-A4*X(I)*X(I+1)*X(I+2)
      B2FITH=A2-A3*(X(I-1)+X(I)+X(I+1))+A4*(X(I-1)*X(I)+X(I-1)*X(I+1)+X(
     1I-1)*X(I+2)+X(I)*X(I+1)+X(I)*X(I+2)+X(I+1)*X(I+2))-A5*(X(I-1)*X(I+
     11)*X(I+2)+X(I)*X(I+1)*X(I+2)+X(I-1)*X(I)*X(I+1)+X(I-1)*X(I)*X(I+2)
     1+X(I+1)*X(I+2)*X(I+3)+X(I-1)*X(I+1)*X(I+3)+X(I-1)*X(I+2)*X(I+3)+X(
     11)*X(I+1)*X(I+3)+X(I)*X(I+2)*X(I+3)+X(I-1)*X(I)*X(I+3))
      B3FITH=A3-A4*(X(I-1)+X(I)+X(I+1)+X(I+2))+A5*(X(I+1)*X(I+2)+X(I-1)*
     1X(I+1)+X(I-1)*X(I+2)+X(I)*X(I+1)+X(I)*X(I+2)+X(I-1)*X(I)+X(I+1)*X(
     1I+3)+X(I-1)*X(I+3)+X(I)*X(I+3)+X(I+2)*X(I+3))
      B4FITH=A4-A5*(X(I+1)+X(I+2)+X(I-1)+X(I)+X(I+3))
      B5FITH=A5
      DO 323 L=1,NINTRP
      YY(L)=B0FITH+XX(L)*(B1FITH+XX(L)*(B2FITH+XX(L)*(B3FITH+XX(L)*(B4FI
     1ITH+B5FITH*XX(L)))))
  323 CONTINUE
      WRITE(6,43) B0FITH,B1FITH,B2FITH,B3FITH,B4FITH,B5FITH
  226 CONTINUE
      NT=NPTS+NINTRP
      DO 233 K=1,NPTS
      I=2*K-1
      ZZ(I)=Y(K)
  233 Z(I)=T(K)
      DO 231 K=1,NINTRP
      I=2*K
      ZZ(I)=YY(K)
  231 Z(I)=XX(K)
      WRITE(6,27)
      WRITE(6,28)
      WRITE(6,29)
      DO 232 I=1,NPTS
      WRITE(6,30)T(I),Y(I),FDIF1Y(I),FDIF2Y(I),FDIF3Y(I),FDIF4Y(I),FDIF5
     1Y(I)
  232 CONTINUE
      WRITE(6,31)
      WRITE(6,32)
      DO 234 I=1,NT
      WRITE(6,33)Z(I),ZZ(I)
  234 CONTINUE
C
C     FORMAT STATEMENTS
C
   20 FORMAT(1HO,13H PRIMARY DATA)
   21 FORMAT(1HO,17H DIFFERENCE TABLE  )
   22 FORMAT(1HO,10H TIME(HRS),3X,11H CONC(MG/L),1X,11H FIRST DIFF,2X,12
     1H SECOND DIFF,2X,11H THIRD DIFF,2X,12H FOURTH DIFF,2X,11H FIFTH DI
     1FF)
   23 FORMAT(1HO,F9.3,3X,F10.3,3X,F10.3,3X,F10.3,3X,F10.3,3X,F10.3,3X,F1
     10.3)
   24 FORMAT(1HO,24H FIRST ORDER CORRELATION  )
   25 FORMAT(1HO,25H SECOND ORDER CORRELATION  )
   26 FORMAT(1HO,24H THIRD ORDER CORRELATION  )
   27 FORMAT(1H1,14H SMOOTHED DATA  )
   28 FORMAT(1HO,17H DIFFERENCE TABLE  )
   29 FORMAT(1HO,10H TIME(HRS),3X,11H CONC(MG/L),1X,11H FIRST DIFF,2X,12
     1H SECOND DIFF,2X,11H THIRD DIFF,2X,12H FOURTH DIFF,2X,11H FIFTH DI
     1FF)
```

```
 30 FORMAT(1H0,F9.3,3X,F10.3,3X,F10.3,3X,F10.3,3X,F10.3,3X,F10.3,3X,F1
   10.3)
 31 FORMAT(1H0,31H SMOOTHED AND INTERPOLATED DATA  )
 32 FORMAT(1H0,10H TIME(HRS),3X,11H CONC(MG/L)  )
 33 FORMAT(1H0,F10.3,4X,F10.3)
 34 FORMAT(1H0,7H B0LIN=,F10.3,3X,7H B1LIN=,F10.3)
 35 FORMAT(1H0,8H B0QUAD=,F10.3,3X,8H B1QUAD=,F10.3,3X,8H B2QUAD=,F10.
   13)
 37 FORMAT(1H0,8H B0CUBE=,F10.3,8H B1CUBE=,F10.3,8H B2CUBE=,F10.3,8H B
   13CUBE=,F10.3)
 39 FORMAT(1H1,21H ORDER NOT DETERMINED  )
 40 FORMAT(1H0,25H FOURTH ORDER CORRELATION )
 41 FORMAT(1H0,24H FIFTH ORDER CORRELATION  )
 42 FORMAT(1H0,8H B0FRTH=,F10.3,8H B1FRTH=,F10.3,8H B2FRTH=,F10.3,8H B
   13FRTH=,F10.3,8H B4FRTH=,F10.3)
 43 FORMAT(1H0,8H B0FITH=,F10.3,8H B1FITH=,F10.3,8H B2FITH=,F10.3,8H B
   13FITH=,F10.3,8H B4FITH=,F10.3,8H F5FITH=,F10.3)
    RETURN
    END
```

CD TOT 0600

APPENDIX IV

CORRELATION - REGRESSION ANALYSIS OF $\frac{dC_c}{dt}$ vs C_{B_o}

(Volk (1958))

DEFINITIONS

R	correlation coefficient
D.F.	degree of freedom
b	slope
$S^2(\zeta)$	variance of ζ
$S(\zeta)$	standard deviation of ζ
y	dependent variable
x	independent variable
$\bar{y}$	mean of y
$\bar{x}$	mean of x
$\hat{y}$	estimate of y (least-squares)

FORMULAE

Least-Squares Fitting

$$\Sigma' y^2 = \Sigma(y - \bar{y})^2 = \Sigma y^2 - \bar{y}\,\Sigma xy$$

$$\Sigma' x^2 = \Sigma(x - \bar{x})^2 = \Sigma x^2 - \bar{x}\,\Sigma x$$

$$\Sigma' xy = \Sigma xy - \bar{x}\,\Sigma y$$

$$\bar{y} = \frac{\Sigma y}{N} \quad \text{where N = \# Data}$$

$$\bar{x} = \frac{\Sigma x}{N}$$

$$\Sigma' \hat{y}^2 = \Sigma(y - \hat{y})^2$$

$$R = \left[\frac{\Sigma' y^2 - \Sigma' \hat{y}^2}{\Sigma' y^2}\right]^{1/2}$$

$$F = \frac{R^2(N - 2)}{(1 - R^2)}$$

$$t = \frac{R\sqrt{N - 2}}{\sqrt{1 - R^2}}$$

Confidence Limits of Slope and Least Squares Line

$$s^2(\hat{y}) = \frac{\Sigma(y - \hat{y})^2}{N - 2}$$

$$s^2(\bar{y}) = \frac{s^2(\hat{y})}{N}$$

$$s^2(b) = \frac{s^2(\hat{y})}{\Sigma^1 x^2}$$

$$s^2(x) = \frac{s^2(x)}{N}$$

Comparison of Two Slopes

$$s^2(\hat{y})_{pooled} = \frac{(N_1 - 2)s^2(\hat{y}_1) + (N_2 - 2)s^2(\hat{y}_2)}{(N_1 - 2) + (N_2 - 2)}$$

$$t = \frac{b_1 - b_2}{s(\hat{y})_{pooled}\left[\frac{1}{\Sigma^1 x_1^2} + \frac{1}{\Sigma^1 x_2^2}\right]^{1/2}}$$

$$b_{pooled} = \frac{\Sigma^1 x_1 y_1 + \Sigma^1 x_2 y_2}{\Sigma^1 x_1^2 + \Sigma^1 x_2^2}$$

Comparison of Several Lines

$$\Sigma^1 c^2 = \frac{[\Sigma^1(xy)]^2}{\Sigma^1 x^2}$$

$$\Sigma^1 \zeta^2 = \frac{(\Sigma\zeta_1)^2}{n_1} + \frac{(\Sigma\zeta_2)^2}{n_2} + \cdots + \frac{(\Sigma\zeta_k)^2}{n_k} - \frac{(\Sigma\zeta_T)^2}{N}$$

for k data sets and $N = \sum_{i=1}^{k} n_i$

$$\zeta_T = \sum_{i=1}^{k} \zeta_i$$

$$\Sigma\bar{\zeta}\bar{\beta} = \frac{\Sigma\zeta_1\Sigma\beta_1}{n_1} + \frac{\Sigma\zeta_2\Sigma\beta_2}{n_2} + \text{---} + \frac{\Sigma\zeta_k\Sigma\ \beta_k}{n_k} - \frac{\Sigma\zeta\Sigma\beta}{N}$$

$$b_{pooled} = \frac{\sum_{sets}(\Sigma^1 xy)}{\sum_{sets}(\Sigma^1 x^2)}$$

TABLE NO. 12

LINEAR LEAST-SQUARES CORRELATIONS

$\frac{dC_c}{dt}$ vs C_{B_o}

Correlation	Mean of Dependent Variables			Slope of Regression Line			Correlation Coefficient		
	Level	D.F.	± 95% Limits	Level	D.F.	± 95% Limits	R	R^2 (Fraction SS Removed)	R Significant at 95%
All Data	109.438	32	13.8229	.07401	32	.03740	.58125	.33786	Yes
All Data <900 Solids	105.868	26	10.1567	.15952	26	.03904	.85487	.73080	Yes
All Overflow Data	102.274	15	18.729	.10262	15	.06514	.65498	.42899	Yes
All Underflow Data	116.602	15	22.5230	.06123	15	.05539	.51968	.27007	Yes
Overflow Data <900 Solids	92.7315	11	11.417	.21810	11	.062147	.91887	.84437	Yes
Underflow Data <900 Solids	111.908	11	17.5797	.17462	11	.06985	.85644	.73350	Yes
All Spherical Shaped Overflow Data	82.0809	5	41.0581	.07005	5	.10796	.59798	.35790	No
All Spherical Shaped Underflow Data	97.1100	5	49.9154	.04660	5	.09453	.49307	.24312	No
All Rod Shaped Overflow Data	107.100	4	18.087	.17710	4	.07314	.95847	.91866	Yes
All Rod Shaped Underflow Data	111.214	4	26.7654	.09632	4	.07257	.87890	.77239	Yes
All Filamentous Overflow Data	130.371	2	34.5247	.24384	2	.44643	.85685	.73418	No
All Filamentous Underflow Data	158.793	2	48.0723	.14041	2	.45923	.68113	.46394	No
Spherical Shaped Overflow Data <900 Solids	71.8846	3	32.3208	.20009	3	.15755	.91914	.84481	Yes
Spherical Shaped Underflow Data <900 Solids	91.6682	3	49.952	.15512	3	.16157	.86991	.70477	No

TABLE NO. 13
STATISTICAL ANALYSIS
FOR LINEAR CORRELATION

$\frac{dC_e}{dt}$ vs C_{B_o}

Correlation	Mean of Independent Variable	Intercept	F Ratio	t Statistic	Variance of Mean	Variance of Slope	Variance of Estimate
All Data	688.252	58.4979	16.3281	4.04080	45.8234	33.55×10^{-5}	15.58×10^{2}
All Data < 900 Solids	567.218	15.3805	70.5837	8.40141	24.4038	36.056×10^{-5}	68.331×10^{1}
All Overflow Data	576.298	43.1285	11.2696	3.35702	77.2545	93.463×10^{-5}	13.133×10^{2}
All Underflow Data	820.205	67.6011	5.55005	2.35585	111.777	67.562×10^{-5}	19.002×10^{2}
Overflow Data < 900 Solids	449.175	- 5.23643	59.6664	7.7244	26.9084	79.7272×10^{-5}	34.981×10^{1}
Underflow Data < 900 Solids	618.034	3.98624	30.2752	5.50229	63.7947	10.072×10^{-4}	82.9331×10^{1}
All Spherical Shaped Overflow Data	563.244	42.6223	2.78317	1.66828	255.032	17.634×10^{-4}	17.8523×10^{2}
All Spherical Shaped Underflow Data	804.819	59.6051	1.60607	1.26731	376.936	13.521×10^{-4}	26.3855×10^{2}
All Rod Shaped Overflow Data	589.755	2.65104	45.1775	6.72142	42.4191	69.4293×10^{-5}	25.4514×10^{1}
All Rod Shaped Underflow Data	805.370	33.6365	13.5742	3.68432	92.9631	68.344×10^{-5}	55.7778×10^{1}
All Filamentous Overflow Data	578.958	-13.815	5.52399	2.35032	64.3752	10.7638×10^{-3}	25.7501×10^{1}
All Filamentous Underflow Data	784.384	48.6574	1.73089	1.31563	124.809	11.3901×10^{-3}	49.9237×10^{1}
Spherical Shaped Overflow Data < 900 Solids	353.856	1.08170	16.3310	4.04117	103.173	24.5153×10^{-4}	51.5863×10^{1}
Spherical Shaped Underflow Data < 900 Solids	520.741	10.8870	9.33328	3.05504	246.438	25.7836×10^{-4}	12.3219×10^{2}

TABLE NO. 14

SLOPES SIGNIFICANTLY DIFFERENT FROM ZERO

CORRELATION	(b) Slope	s(b) STD. Dev. Slope	D.F.	SIGNIFICANT $t = \frac{b}{s(b)}$ at
All Data	0.07401	.0183165	32	4.0406, 99.9%
All Data < 900 Solids	0.15952	.0189883	26	8.401, 99.9%
All Overflow	0.102629	.0305716	15	3.357, 99%
All Underflow	0.0612349	.0259926	15	2.3559, 95%
Overflow < 900 Solids	0.2818106	.0282360	11	7.72439, 99.9%
Underflow < 900 Solids	0.174620	.0317359	11	5.50228, 99.9%
All Spherical Over	0.0700560	.0419928	5	1.668286, 80%
All Spherical Under	0.0466004	.0367712	5	1.26731, 70%
All Rod Over	0.177106	.0263494	4	6.7214, 99%
All Rod Under	0.0963256	.0261447	4	3.6843, 95%
All Fil. Over	0.243842	.103749	2	2.35031, 80%
All Fil. Under	0.140416	.106724	2	1.31564, 60%
Spherical Over < 900	0.200090	.0495129	3	4.04117, 95%
Spherical Under < 900	0.155127	.0507775	3	3.05503, 90%

TABLE NO. 15

SIGNIFICANT DIFFERENCE BETWEEN TWO SLOPES

CORRELATIONS UNDER TEST	$S^2(\hat{y}_1) + S^2(\hat{y}_2)$	$S^2(\hat{y})_p$	$b_1 - b_2$	$\Sigma^1 x_1^2$	$\Sigma^1 x_2^2$	t	DF	SIGNIFICANT AT 95%
(1) All Overflow (2) All Underflow	3213.55	1606.775	.04139410	1.40519×10^6	2.81256×10^6	1.03265	30	NO
(1) Overflow (2) Underflow (<900 solids)	1179.140	589.570	.0434860	4.38757×10^5	8.23428×10^5	0.95811	26	NO
Spherical (1) Over (2) Under	4423.78	2211.890	.0234556	1.01238×10^6	1.95142×10^6	0.40718	10	NO
Rod (1) Over (2) Under	812.292	406.146	.080780	3.66581×10^5	8.16005×10^5	1.81380	8	NO
Fil. (1) Over (2) Under	756.738	378.369	.1034320	2.39229×10^4	4.38310×10^4	.066489	4	NO
Spherical < 900 solids (1) Over (2) Under	1748.053	874.027	.044963	2.10425×10^5	4.77897×10^5	.58309	6	NO

TABLE NO. 16

POOLED SLOPE CALCULATIONS

Correlation Under Test	$\Sigma' x_1 y_1$	$\Sigma' x_2 y_2$	b_p	s(b)	DF	$b_p \pm$ at 95%	t test on slopes		
							t	Diff. from zero	At
All Data (1) Over (2) Under	1.44214 x 10^5	1.72227 x 10^5	7.502602 x 10^{-2}	4.00852 x 10^{-2}	30	8.18539 x 10^{-2}	1.87166		90%
<900 solids (1) Over (2) Under	9.56956 x 10^4	1.43787 x 10^5	1.807361 x 10^{-1}	4.53875 x 10^{-2}	26	9.33167 x 10^{-2}	4.18036		99.9%
Spherical (1) Over (2) Under	7.09232 x 10^4	9.09368 x 10^4	5.461232 x 10^{-2}	5.76055 x 10^{-2}	10	1.28345 x 10^{-1}	0.94804		60%
Rod (1) Over (2) Under	6.49235 x 10^4	7.86022 x 10^4	1.213658 x 10^{-1}	4.45365 x 10^{-2}	8	1.027012 x 10^{-1}	2.72509		95%
Fil. (1) Over (2) Under	5.83342 x 10^3	6.15431 x 10^3	1.769300 x 10^{-1}	1.563623	4	4.34061	.1131541	NO	-
Spherical < 900 solids (1) Over (2) Under	4.21039 x 10^4	7.41350 x 10^4	1.68873 x 10^{-1}	7.71118 x 10^{-2}	6	1.88693 x 10^{-1}	2.189976		90%

TABLE NO. 17

ANALYSIS OF SIX CURVES COLLECTIVELY

AND INDIVIDUALLY

(Data > 900 solids discarded)

SUM OF SQUARES

Correlation	$\Sigma^1 x^2$	$\Sigma^1 y^2$	$\Sigma^1 xy$	$\Sigma^1 c^2$	$\Sigma^1 y^2$	Slope
Total	1.89515×10^6	6.59964×10^4	3.02330×10^5	4.82304×10^4	1.77660×10^4	1.59529×10^{-1}
Means	5.07724×10^5	2.09225×10^4	8.60358×10^5	1.45791×10^4	6.34346×10^3	1.69454×10^{-1}
Difference	1.38742×10^6	4.50739×10^4	2.16295×10^5	3.37196×10^3	1.13542×10^4	1.55897×10^{-1} *
Set No.						
1	2.10425×10^5	9.97215×10^3	4.21039×10^4	8.42456×10^3	1.54759×10^3	2.00090×10^{-1}
2	4.77897×10^5	1.51969×10^4	7.41350×10^4	1.15004×10^4	3.69657×10^3	1.55127×10^{-1}
3	2.39640×10^5	9.15829×10^3	4.42770×10^4	8.18083×10^3	9.77463×10^2	1.84765×10^{-1}
4	3.91704×10^5	6.94647×10^3	4.37910×10^4	4.89565×10^3	2.05082×10^3	1.111796×10^{-1}
5	2.39229×10^4	1.93743×10^3	5.83342×10^3	1.42243×10^3	5.15001×10^2	2.43842×10^{-1}
6	4.38310×10^4	1.86260×10^3	6.15431×10^3	8.64126×10^2	9.98474×10^2	1.40410×10^{-1}
Sum	1.38742×10^6	4.50739×10^4	2.16295×10^5	3.52880×10^4	9.78592×10^3	

* Pooled Slope

ANALYSIS OF VARIANCE

1 Test the relative significance of using least-squares lines through each data set and of using a correlation line through each data set with a pooled slope.

Sum of Squares with Pooled Slope = 1.13542×10^4

D.F. = 21

Sum of Squares with Least Squares Slope = 9.78592×10^3

D.F. = 16

$$F = \frac{[1.13542 \times 10^4 - 9.78592 \times 10^3]/5}{9.78592/16}$$

= 0.512835 at 5, 16 D.F.

$F_{0.05,5,16} = 2.85$ ∴ NS at 95%

∴ (1) use pooled slope (0.155897)

(2) use 1.13452×10^4 as error estimate

2 Determine if there is a significant vertical displacement between the curves.

Sum of Squares due to Error Estimate = 1.13542×10^4

D.F. = 21

Sum of Squares due to Vertical Displacement = (total SS - mean SS - error SS)

at 1 D.F.

$= 6.83141 \times 10^1$

$$F = \frac{6.83141 \times 10^1}{1.13542 \times 10^4/21} = 0.126349 \text{ at } 1, 21 \text{ D.F.}$$

$F_{0.05,1,21} = 4.32$

Therefore, at 95% there is no significant vertical displacement between the curves.

3 Test the relative significance of using a single least-squares line through all the data considered as one set and of using individual lines with a common (pooled) slope through each of the sets.

Sum of Squares for One Line Through All Data As One Set

$= 1.7766 \times 10^4$

D.F. = 26

Sum of Squares for Pooled Slope Line Through Each Set

($\equiv$ error) $= 1.13542 \times 10^4$

D.F. = 21

$$F = \frac{[1.7766 \times 10^4 - 1.13542 \times 10^4]/5}{1.13542 \times 10^4/21}$$

$= 2.37177$ at 5, 21 D.F.

$F_{0.05,5,21} = 2.68$

Therefore, at 95% level can use a single correlation line.

4 Test to see if the overall slope is significantly different from zero

$$t = \frac{b - 0}{s(b)} = 2.056, \text{ sig. at 95\%}$$

Therefore, slope is different from zero at the 95% level.

```
C      T.E.POLLOCK                          JUNE 1969
C      REGRESSION-CORRELATION ANALYSIS
C      PROGRAM ACCEPTS N SETS OF DATA FOR WHICH LINEAR LEAST SQUARES LINE
C      -S ARE TO BE FIT TO EACH SET AND CALCS FOR EACH SET=
C              1 LEAST SQUARES LINE
C              2 SIGNIFICANCE OF LINEAR CORRELATION
C              3 F RATIO AND T STATISTIC
C              4 CONFIDENCE LIMITS OF SLOPE
C              5 CONFIDENCE LIMITS OF LEAST SQUARES LINE
C      CONSIDERING THE N SETS OF DATA TO BE ONE SET,PROGRAM DETERMINES
C      RELATIVE SIGNIFICANCE OF=
C              1 SINGLE CORRELATION LINE VS INDIVIDUAL CORRELATION LINE
C                FOR EACH OF THE N SETS
C              2 POOLED SLOPE FOR EACH OF THE N SETS VS INDIVIDUAL SLOPES
C                FOR EACH OF THE N SETS
C              3 DISPLACEMENT OF THE INDEPENDENT VARIABLE OVER THE N SETS
C      DEFINITIONS
C         X=INDEPENDENT VARIABLE
C         Y=DEPENDENT VARIABLE
C         SUMY=SUM OF Y
C         SUMX=SUM OF X
C         YS=SQUARE OF Y
C         XS=SQUARE OF X
C         SUMYS=SUM OF YS
C         SUMXS=SUM OF XS
C         YMEAN=MEAN OF Y VALUES
C         XMEAN=MEAN OF X VALUES
C         R=CORRELATION COEFFICIENT
C         SLOPE=SLOPE OF LEAST SQUARES LINE
C         TRCPT=INTERCEPT OF LEAST SQUARES LINE
C         F=FRATIO STATISTIC
C         T=T STATISTIC
C         YLS=LEAST SQUARE VALUE OF Y
C         SSY=SUM OF SQUARES OF Y RELATIVE TO YMEAN
C         VARYM=VARIANCE OF YMEAN
C         VARSLP=VARIANCE OF SLOPE
C         VARYLS=VARIANCE OF FITTED LINE
C         M=NO. OF DATA PTS IN EACH SET
C         N=NO.OF DATA SETS
C         DSY=SQUARED DEVIATION OF Y FROM YLS
C         SDYLS=STD. DEVIATION OF Y RELATIVE TO YLS
C         SDSLP=STD. DEVIATION OF SLOPE
C         SUM1CS=SUM OF SQUARES OF DEVIATION REMOVED BY THE CORRELATION
C         SUM1LS=SUM OF SQUARES OF DEVIATION OF Y FROM YLS
      DIMENSION Y(10,150),X(10,150),SUMY(10),SUMX(10),YS(10,150),XS(10,1
     150),SUMYS(10),SUMXS(10),YMEAN(10),XMEAN(10),XY(10,150),SUMXY(10),S
     1UM1YS(10),SUM1XS(10),SUM1XY(10),R(10),SLOPE(10),TRCPT(10),F(10),
     1    SSY(10),SSX(10),T(6),DSY(10,150),VARYM(10),VARSLP(10),VARLS(10
     1),SUM1CS(10),SUM1LS(10),M(10),SDYM(10),SDSLP(10),YLS(10,150),VARXM
     1(10)
C
C      DATA INPUT
C
      N=2
      NSETS=2
      DO 1001 KKK=1,NSETS
      IF(KKK.GE.9) N=6
      READ(5,1)(M(I),I=1,N)
      DO 1000 I=1,N
```

```
      MO=M(I)
      DO 100 J=1,MO
      READ(5,2) X(I,J),Y(I,J)
  100 CONTINUE
C
C     LEAST SQUARES ANALYSIS
C
      SUMY(I)=0.
      SUMX(I)=0.
      SUMYS(I)=0.
      SUMXS(I)=0.
      SUMXY(I)=0.
      DO 101 J=1,MO
      SUMY(I)=SUMY(I)+Y(I,J)
      SUMX(I)=SUMX(I)+X(I,J)
      YS(I,J)=Y(I,J)**2
      XS(I,J)=X(I,J)**2
      SUMYS(I)=SUMYS(I)+YS(I,J)
      SUMXS(I)=SUMXS(I)+XS(I,J)
      XY(I,J)=X(I,J)*Y(I,J)
      SUMXY(I)=SUMXY(I)+XY(I,J)
  101 CONTINUE
      YMEAN(I)=SUMY(I)/FLOAT(M(I))
      XMEAN(I)=SUMX(I)/FLOAT(M(I))
      SUM1YS(I)=SUMYS(I)-YMEAN(I)*SUMY(I)
      SUM1XS(I)=SUMXS(I)-XMEAN(I)*SUMX(I)
      SUM1XY(I)=SUMXY(I)-XMEAN(I)*SUMY(I)
      SLOPE(I)=SUM1XY(I)/SUM1XS(I)
      TRCPT(I)=YMEAN(I)-SLOPE(I)*XMEAN(I)
      R(I)=SUM1XY(I)/SQRT(SUM1XS(I)*SUM1YS(I))
      SUM1CS(I)=(SUM1XY(I))**2/SUM1XS(I)
      F(I)=((R(I)**2)*FLOAT(M(I)-2))/(1.-R(I)**2)
      T(I)=(R(I)*SQRT(FLOAT(M(I)-2)))/SQRT(1.-R(I)**2)
      WRITE(6,3)
      WRITE(6,7)
      WRITE(6,4)I
      WRITE(6,5)
      DO 102 J=1,MO
      WRITE(6,6) X(I,J),Y(I,J)
  102 CONTINUE
      WRITE(6,8)
      DO 103 J=1,MO
      WRITE(6,9)Y(I,J),X(I,J),YS(I,J),XS(I,J),XY(I,J)
  103 CONTINUE
      WRITE(6,10)
      WRITE(6,9)SUMY(I),SUMX(I),SUMYS(I),SUMXS(I),SUMXY(I)
      WRITE(6,11) YMEAN(I)
      WRITE(6,12)XMEAN(I)
      WRITE(6,13) SLOPE(I)
      WRITE(6,14)TRCPT(I)
      WRITE(6,15)R(I)
      WRITE(6,16)F(I)
      WRITE(6,17)T(I)
      WRITE(6,18)
      WRITE(6,37)
      DO 104 J=1,MO
      YLS(I,J)=TRCPT(I)+SLOPE(I)*X(I,J)
      WRITE(6,19)X(I,J),YLS(I,J)
```

```
  104 CONTINUE
      SUM1LS(I)=SUM1YS(I)-SUM1CS(I)
C
C     CONFIDENCE LIMITS OF SLOPE + LEAST SQUARES LINE
C
      SSY(I)=0.
      DO 105 J=1,MO
      DSY(I,J)=(Y(I,J)-YLS(I,J))**2
      SSY(I)=SSY(I)+DSY(I,J)
  105 CONTINUE
      VARLS(I)=SSY(I)/FLOAT(M(I)-2)
      VARYM(I)=VARLS(I)/FLOAT(M(I))
      VARSLP(I)=VARLS(I)/SUM1XS(I)
      SDYM(I)=SQRT(VARYM(I))
      SDSLP(I)=SQRT(VARSLP(I))
      WRITE(6,20)
      WRITE(6,21) VARLS(I)
      WRITE(6,22)VARYM(I),SDYM(I)
      WRITE(6,23)VARSLP(I),SDSLP(I)
 1000 CONTINUE
C
C     ESTIMATE OF RELATIVE SIGNIFICANCE OF LINEAR CORRELATIONS BETWEEN
C     SETS OF DATA TAKEN INDIVIDUALLY AND COLLECTIVELY
C
      WRITE(6,24)
C
C     SUM OF SQUARES FOR COMPARISON OF SEVERAL LINEAR CORRELATIONS
C
      XSUM=0.
      YSUM=0.
      XYSUM=0.
      CSUM=0.
      YLSUM=0.
      DO 106 I=1,N
      XSUM=XSUM+SUM1XS(I)
      YSUM=YSUM+SUM1YS(I)
      XYSUM=XYSUM+SUM1XY(I)
      CSUM=CSUM+SUM1CS(I)
      YLSUM=YLSUM+SUM1LS(I)
  106 CONTINUE
C
C     SUM OF SQUARES FOR TOTAL
C
      MM=0
      DO 107 I=1,N
      MM=MM+M(I)
  107 CONTINUE
      TSUMX=0.
      TSUMY=0.
      TSUMYS=0.
      TSUMXS=0.
      TSUMXY=0.
      DO 108 I=1,N
      MU=M(I)
      DO 108 J=1,MU
      TSUMX=TSUMX+X(I,J)
      TSUMY=TSUMY+Y(I,J)
      TSUMYS=TSUMYS+(Y(I,J))**2
```

```
      TSUMXS=TSUMXS+(X(I,J))**2
      TSUMXY=TSUMXY+X(I,J)*Y(I,J)
  108 CONTINUE
      TYMEAN=TSUMY/FLOAT(MM)
      TXMEAN=TSUMX/FLOAT(MM)
      TSUM1X=TSUMXS-TXMEAN*TSUMX
      TSUM1Y=TSUMYS-TYMEAN*TSUMY
      TSUM1B=TSUMXY-TXMEAN*TSUMY
      TSUM1C=(TSUM1B)**2/TSUM1X
      TSUM1L=TSUM1Y-TSUM1C
      TSLOPE=TSUM1B/TSUM1X
      TTRCPT=TYMEAN-TSLOPE*TXMEAN
      TR=TSUM1B/SQRT(TSUM1X*TSUM1Y)
      TF=(TR**2)*FLOAT(MM-2)/(1.-TR**2)
      TT=TR*SQRT(FLOAT(MM-2))/SQRT(1.-TR**2)
C
C     SUMS OF SQUARES FOR MEANS
C
      ZSUMXS=0.
      ZSUMYS=0.
      ZSUMXY=0.
      DO 109 I=1,N
      ZSUMXS=ZSUMXS+(SUMX(I))**2/FLOAT(M(I))
      ZSUMYS=ZSUMYS+SUMY(I)**2/FLOAT(M(I))
      ZSUMXY=ZSUMXY+SUMX(I)*SUMY(I)/FLOAT(M(I))
  109 CONTINUE
      ZSUMXS=ZSUMXS-TSUMX**2/FLOAT(MM)
      ZSUMYS=ZSUMYS-TSUMY**2/FLOAT(MM)
      ZSUMXY=ZSUMXY-TSUMX*TSUMY/FLOAT(MM)
      ZSUMCS=ZSUMXY**2/ZSUMXS
      ZSUMLS=ZSUMYS-ZSUMCS
      ZSLOPE=ZSUMXY/ZSUMXS
      DIFFXW=TSUM1X-ZSUMXS
      DIFFYW=TSUM1Y-ZSUMYS
      DIFFBW=TSUM1B-ZSUMXY
      DIFFCW=DIFFBW**2/DIFFXW
      DIFFLW=DIFFYW-DIFFCW
      PSLOPE=XYSUM/XSUM
      WRITE(6,25)
      WRITE(6,27)
      WRITE(6,26)TSUM1X,TSUM1Y,TSUM1B,TSUM1C,TSUM1L,TSLOPE
      WRITE(6,28)ZSUMXS,ZSUMYS,ZSUMXY,ZSUMCS,ZSUMLS,ZSLOPE
      WRITE(6,29)DIFFXW,DIFFYW,DIFFBW,DIFFCW,DIFFLW,PSLOPE
      DO 110 I=1,N
      WRITE(6,30)I,SUM1XS(I),SUM1YS(I),SUM1XY(I),SUM1CS(I),SUM1LS(I),SLO
     1PE(I)
  110 CONTINUE
      WRITE(6,31)XSUM,YSUM,XYSUM,CSUM,YLSUM
C
C     ANALYSIS OF VARIANCE
C
      WRITE(6,32)
      WRITE(6,33)
      A=ZSUMLS
      B=TSUM1L-ZSUMLS-DIFFLW
      C=DIFFLW-YLSUM
      D=YLSUM
      WRITE(6,34)A,B,C,D
```

```
      WRITE(6,35)
      AA=ZSUMLS
      BB=TSUM1L-ZSUMLS-DIFFLW
      CC=DIFFLW
      WRITE(6,36)AA,BB,CC
 1001 CONTINUE
C
C     FORMAT STATEMENTS
C
    1 FORMAT(6I4)
    2 FORMAT(2F10.5)
    3 FORMAT(1H0,44H LEAST SQUARES ANALYSIS OF INDIVIDUAL CURVES   )
    4 FORMAT(1H0,12H DATA SET NO  ,2X,I3)
    5 FORMAT(1H0,5H BUGS  ,15X,7H CARBON  )
    6 FORMAT(1H ,F10.4,5X,F10.4)
    7 FORMAT(1H0,11H ECHO CHECK  )
    8 FORMAT(1H0,15X,2H Y,9X,2H X,9X,3H YS,8X,3H XS,8X,3H XY )
    9 FORMAT(1H ,10X,1PE11.4,5X,1PE11.4,5X,1PE11.4,5X,1PE11.4,5X,1PE11.4
     1)
   10 FORMAT(1H0,4H SUM  )
   11 FORMAT(1H0,17H MEAN VALUE OF Y= ,1PE11.4)
   12 FORMAT(1H0,17H MEAN VALUE OF X=  ,1PE11.4)
   13 FORMAT(1H0,21H LEAST SQUARES SLOPE=  ,1PE11.4)
   14 FORMAT(1H0,25H LEAST SQUARES INTERCEPT=  ,1PE11.4)
   15 FORMAT(1H0,19H CORRELATION COEFF=  ,1PE11.4)
   16 FORMAT(1H0,9H F RATIO=  ,1PE11.4)
   17 FORMAT(1H0,13H T STATISTIC=  ,1PE11.4)
   18 FORMAT(1H0,12H FITTED LINE  )
   19 FORMAT(1H ,1PE11.4,5X,1PE11.4)
   20 FORMAT(1H0,50H CONFIDENCE LIMITS OF SLOPE AND LEAST SQUARES LINE
     1  )
   21 FORMAT(1H0,22H VARIANCE OF ESTIMATE=  ,5X,1PE11.4)
   22 FORMAT(1H0,18H VARIANCE OF MEAN=  ,5X,1PE11.4,5X,9H STD DEV=  ,5X,
     11PE11.4)
   23 FORMAT(1H0,19H VARIANCE OF SLOPE=  ,5X,1PE11.4,5X,9H STD DEV=  ,5X
     1,1PE11.4)
   24 FORMAT(1H0,116H ESTIMATE OF RELATIVE SIGNIFICANCE OF LINEAR CORREL
     1ATIONS BETWEEN N SETS OF DATA TAKEN INDIVIDUALLY AND COLLECTIVELY
     1       )
   25 FORMAT(1H0,16H SUMS OF SQUARES  )
   26 FORMAT(1H0,6H TOTAL  ,4X,1PE11.4,1X,1PE11.4,1X,1PE11.4,1X,1PE11.4,
     11X,1PE11.4,1X,1PE11.4)
   27 FORMAT(12X,7H SIGXSQ,3X,7H SIGYSQ,3X,6H SIGXY,4X,7H SIGCSQ,3X,7H S
     1IGYLS,4X,6H SLOPE )
   28 FORMAT(1H0,6H MEANS  ,4X,1PE11.4,1X,1PE11.4,1X,1PE13.4,1X,1PE11.4,
     11X,1PE11.4,1X,1PE13.4)
   29 FORMAT(1H0,7H DIFFCS ,4X,1PE11.4,1X,1PE11.4,1X,1PE11.4,1X,1PE11.4,
     11X,1PE11.4,1X,1PE11.4)
   30 FORMAT(1H ,4H SET,1X,I3,2X,1PE11.4,1X,1PE11.4,1X,1PE11.4,1X,1PE11.
     14,1X,1PE11.4,1X,1PE11.4)
   31 FORMAT(1H ,4H SUM,6X,1PE11.4,1X,1PE11.4,1X,1PE11.4,1X,1PE11.4,1X,1
     1PE11.4,1X,1PE11.4)
   32 FORMAT(1H0,21H ANALYSIS OF VARIANCE  )
   33 FORMAT(1H0,18H INDIVIDUAL SLOPES  )
   34 FORMAT(1H ,7H MEANS= ,1X,1PE11.4/ 26H MEANS SLOPE-POOLED SLOPE  ,1
     1X,1PE11.4/ 16H BETWEEN SLOPES=  ,1X,1PE11.4/ 7H ERROR=  ,1X,1PE11.
     14)
   35 FORMAT(1H0,13H POOLED SLOPE  )
```

```
36 FORMAT(1H0,7H MEANS=,1X,1PE11.4/ 6H DIFF=   ,1X,1PE11.4/ 7H ERROR=
  1   ,1X,1PE11.4)
37 FORMAT(1H0,5X,2H X,9X,4H YLS)
   STOP
   END
```

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APPENDIX V

ANALYSIS OF CENTRAL COMPOSITE STATISTICAL DESIGN

(Johnson & Leone (1967)
Davies (1967)
Wu (1963))

DEFINITIONS

$[X]$	matrix of coefficient factors
$[Y]$	response matrix
$[B]$	matrix of fitted coefficients
$S^2(\bar{y})$	estimated variance of centre point
$S^2(B)$	estimated variance of fitted coefficients
$[\hat{Y}]$	matrix of estimated response from model
$[SS]$	matrix of sum of squares accounted for by fitted coefficients

FORMULAE

$$[B] = \left[[X]^1[X]\right]^{-1}[X]^1[Y]$$

$$[S^2(B)] = \left[[X]^1[X]\right]^{-1} S^2(\bar{Y})$$

$$[\hat{Y}] = [X]\,[B]$$

$$[SS] = [B]\,[X]^1\,[Y]$$

CANONICAL TRANSFORMATION

(Davies (1967))

Fitted equation:

$$y = 0.9933 - 0.2531\, x_1x_2 - 0.1811\, x_2x_3 - 0.1226\, x_1^2 + 0.06276\, x_2^2 + 0.03426\, x_3^2 \qquad (V\text{-}1)$$

Determination of Centre of System

The position of the centre of the system of contours represented by equation (V-1) is found by solving the following set of equations in x_1, x_2, x_3:

$$\left.\begin{aligned} \frac{\partial y}{\partial x_1} &= 0 = -0.2531\ x_2 - 0.2452\ x_1 \\ \frac{\partial y}{\partial x_2} &= 0 = -0.2531\ x_1 - 0.1811\ x_3 + 0.12552\ x_2 \\ \frac{\partial y}{\partial x_3} &= 0 = -0.1811\ x_2 + 0.06852\ x_3 \end{aligned}\right\} \quad \text{(V-2)}$$

yielding centre co-ordinates x_{is} of (0,0,0).

Therefore, the centre of the system coincided with the centre of the experimental design at the 95% level where first order effects are not significant.

Therefore, $Y_s = 0.9933$

where $Y_s = y(0,0,0)$

Canonical Form of Second Degree Equation

$$Y - Y_s = B_{11}\ X_1^2 + B_{22}\ X_2^2 + B_{33}\ X_3^2$$

where X_i are the transformed x_i and B_{ii} are the solutions to

$$\begin{vmatrix} b_{11} - B_{ii} & \frac{1}{2} b_{12} & \frac{1}{2} b_{13} \\ \frac{1}{2} b_{12} & b_{22} - B_{ii} & \frac{1}{2} b_{23} \\ \frac{1}{2} b_{13} & \frac{1}{2} b_{23} & b_{33} - B_{ii} \end{vmatrix} = 0 \quad \text{(V-3)}$$

$B_{11} = 1.83398$

$B_{22} = -1.861915$

$B_{33} = 0.02793406$

Determination of Axes of Canonical Equation

The orthogonal transformation which changes equation (V-1) to its canonical form is

$$X_i = \sum_{j=1}^{3} m_{ij} (x_j - x_{js})$$

where the m_{ij} are determined from the set of equations

$$\sum_{k=1}^{3} \left[\sum_{j=1}^{3} \varsigma_{kj} \cdot m_{ij} = 0 \right] \qquad \text{for } i = 1,2,3$$

where ς_{kj} are the elements of the determinant (V-3) with the values B_{ij} included yielding:

$$[m] = \begin{bmatrix} 0.33143 & -5.12626 & 99.865182 \\ 0.596953 & 4.718997 & 99.880 \\ -4.13068 \times 10^{-2} & 4.91535 \times 10^{-2} & 7.66577 \times 10 \end{bmatrix}$$

Canonical Form

$$Y - 0.9933 = 1.83398\, X_1^2 - 1.861915\, X_2^2 + 0.027934\, X_3^2$$

where $X_1 = 0.33143\, x_1 - 5.12626\, x_2 + 99.86518\, x_3$

$X_2 = 0.59695\, x_1 + 4.71900\, x_2 + 99.880\, x_3$

$X_3 = -4.13068 \times 10^{-2} x_1 + 4.91535 \times 10^{-2} x_2 + 7.66577 \times 10^{-2} x_3$

```
C
C     T.E.POLLOCK
C
C     PROGRAM DETERMINES FIRST AND SECOND ORDER RESPONSE SURFACE TO
C     A CENTRAL COMPOSITE STATISTICAL DESIGN
C
C     PROGRAM EVALUATES COEFFICIENTS OF FITTED MODEL, THEIR VARIANCE,
C     AND PERFORMS AN ANALYSIS OF VARIANCE ON THE RESPONSE SURFACE
C
C     VARIANCE OF COEFFICIENTS IS ESTIMATED FROM REPLICATES AT CENTRE PT
C
C     DEFINITIONS
C        NSETS=NO OF SETS OF MEASURED RESPONSE
C        NTEST=NO OF EXPERIMENTS PERFORMED
C        NCOEF=NO OF COEFFICIENTS TO BE ESTIMATED
C        MCOEF=NO OF COEFFICIENTS IN 2ND ORDER MODEL
C        NREPS=NO OF REPLICATES AT CENTRE POINT
C        X=MATRIX OF CODED VARIATE LEVELS OF SIZE NCOEF*NTEST
C        Y=MEASURED RESPONSE
C        XTRANS=TRANSPOSE OF X MATRIX
C        C=MATRIX DEFINING COEFFICIENTS OF ESTIMATED PARAMETERS
C        CY=C*Y
C        C1=INVERSE OF C MATRIX
C        B=MATRIX OF ESTIMATED COEFFICIENTS
C        YMEAN=MEAN OF REPLICATE RESPONSES
C        YVAR=ESTIMATED VARIANCE OF MEASUREMENT
C        BVAR=MATRIX OF VARIANCE OF FITTED COEFFICIENTS
C        YEST=EXPECTED RESPONSE FROM FITTED SURFACE
C        SSYDIF=SUM (Y-YEST)**2
C        SS=ESTIMATED SUM OF SQUARES
C        DF=DEGREES OF FREEDOM
C        YTOT=SS DUE TO ERROR
C        SSYDIF=SS DUE TO RESIDUAL
C        ZMS=MEAN SQUARE
C        F=F RATIO RELATIVE TO ERROR MEAN SQUARE
C        FF=F RATIO RELATIVE TO RESIDUAL MEAN SQUARE
C
C     INPUT AND OUTPUT ORDER OF COEFFICIENTS (B) IS
C        B1=(X1*X2*X3)**0
C        B2=X1
C        B3=X2
C        B4=X3
C        B5=X1*X2
C        B6=X1*X3
C        B7=X2*X3
C        B8=X1*X2*X3
C        B9=X1*X1
C        B10=X2*X2
C        B11=X3*X3
C     SUBSCRIPTS 1,2,3 REFER TO INDEPENDENT VARIABLES
C
      DIMENSION X(25,25),Y(25),XTRANS(25,25),C(30,30),CY(25),C1(30,30),
     1N1(25),B(25),BVAR(25,25),TERM(25),YEST(25),YDIFF(25),YDIFFS(25),
     1SS(25),DF(25),YY(25),ZMS(25),F(25),FF(25)
      NSETS=2
      MCOEF=11
      DO 1000 KKK=1,NSETS
      WRITE(6,21)
      DO 1000 KK=1,3
```

```
C
C       DEFINE ARRAYS TO BE ZERO
C
        DO 100 I=1,25
        Y(I)=0.
        CY(I)=0.
        N1(I)=0
        B(I)=0.
        TERM(I)=0.
        YEST(I)=0.
        YDIFF(I)=0.
        YDIFFS(I)=0.
        SS(I)=0.
        DF(I)=0.
        YY(I)=0.
        ZMS(I)=0.
        F(I)=0.
        FF(I)=0.
        DO 100 J=1,25
        X(I,J)=0.
        XTRANS(I,J)=0.
        C(I,J)=0.
        C1(I,J)=0.
        BVAR(I,J)=0.
  100 CONTINUE
        WRITE(6,22)
        WRITE(6,23)
        WRITE(6,24)
        WRITE(6,25)
        READ(5,1) NTEST,NCOEF,NREPS
C
C       DEFINE CODED EXPERIMENTIAL LEVELS
C
        READ(5,2)((X(I,J),J=1,MCOEF),I=1,NTEST)
        DO 101 I=1,NTEST
        READ(5,3) Y(I)
        WRITE(6,20) I,(X(I,J),J=1,MCOEF),Y(I)
  101 CONTINUE
C
C       TRANSPOSE X MATRIX
C
        DO 102 I=1,NTEST
        DO 102 J=1,NCOEF
        XTRANS(J,I)=X(I,J)
  102 CONTINUE
C
C       DEFINE MATRIX OF COEFFICIENTS FOR ESTIMATES OF PARAMETERS OF
C       FITTED RESPONSE
C
        WRITE(6,26)
        DO 103 I=1,NCOEF
        DO 103 J=1,NCOEF
        DO 103 L=1,NTEST
        C(I,J)=C(I,J)+XTRANS(I,L)*X(L,J)
  103 CONTINUE
        WRITE(6,27)((C(I,J),J=1,NCOEF),I=1,NCOEF)
        DO 104 I=1,NCOEF
        DO 104 J=1,NTEST
```

```
      CY(I)=CY(I)+XTRANS(I,J)*Y(J)
  104 CONTINUE
      WRITE(6,28)
C
C     INVERT C MATRIX
C
      DO 105 I=1,NCOEF
      DO 105 J=1,NCOEF
      C1(I,J)=C(I,J)
  105 CONTINUE
      N=30
      NN=NCOEF
      ZERO=1.E-10
      CALL INVMAT(C1,N,NN,ZERO,IERR,N1)
C
C     C1 IS NOW THE INVERSE OF C
C
      WRITE(6,27)((C1(I,J),J=1,NCOEF),I=1,NCOEF)
      WRITE(6,29)
C
C     EVALUATION OF COEFFICIENTS OF FITTED MODEL
C
      DO 106 I=1,NCOEF
      DO 106 J=1,NCOEF
      B(I)=C1(I,J)*CY(J)+B(I)
  106 CONTINUE
C
C     EVALUATION OF VARIANCE OF FITTED COEFFICIENTS
C
      NERROR=NTEST-NREPS+1
      IF(KK.GE.2) GO TO 1001
      SUMY=0.
      SUMYS=0.
      DO 107 I=NERROR,NTEST
      SUMY=SUMY+Y(I)
      SUMYS=SUMYS+Y(I)*Y(I)
  107 CONTINUE
      YMEAN=SUMY/FLOAT(NREPS)
      YVAR=(SUMYS-YMEAN*SUMY)/FLOAT(NREPS-1)
 1001 CONTINUE
      DO 108 I=1,NCOEF
      DO 108 J=1,NCOEF
      BVAR(I,J)=C1(I,J)*YVAR
  108 CONTINUE
      DO 200 I=1,NCOEF
      WRITE(6,30) I,B(I),BVAR(I,I)
  200 CONTINUE
      WRITE(6,38)
      WRITE(6,27)((BVAR(I,J),J=1,NCOEF),I=1,NCOEF)
C
C     ANALYSIS OF VARIANCE
C
      WRITE(6,31)
      WRITE(6,32)
      WRITE(6,40)
      SSYDIF=0.
      DO 109 I=1,NTEST
      DO 201 J=1,NCOEF
```

```
      TERM(J)=X(I,J)*B(J)
      YEST(I)=YEST(I)+TERM(J)
  201 CONTINUE
      YDIFF(I)=Y(I)-YEST(I)
      YDIFFS(I)=YDIFF(I)**2
      SSYDIF=SSYDIF+YDIFFS(I)
  109 CONTINUE
      DO 110 I=1,NCOEF
      SS(I)=B(I)*CY(I)
      DF(I)=1.
  110 CONTINUE
      YTOT=0.
      DO 111 I=NERROR,NTEST
      YY(I)=Y(I)-YMEAN
      YTOT=YTOT+YY(I)**2
  111 CONTINUE
      NT=NCOEF+3
      SS(NT-2)=YTOT
      DF(NT-2)=FLOAT(NREPS-1)
      SS(NT)=SSYDIF
      DF(NT)=FLOAT(NTEST-NCOEF)
      SS(NT-1)=SS(NT)-SS(NT-2)
      DF(NT-1)=DF(NT)-DF(NT-2)
      DO 112 I=1,NT
      ZMS(I)=SS(I)/DF(I)
  112 CONTINUE
      DO 113 I=1,NT
      F(I)=ZMS(I)/ZMS(NT-2)
      FF(I)=ZMS(I)/ZMS(NT)
  113 CONTINUE
      DO 114 I=1,NT
      WRITE(6,33) I,SS(I),DF(I),ZMS(I),F(I),FF(I)
  114 CONTINUE
      Z=0.
      DO 115 I=1,NTEST
      Z=Z+Y(I)**2
  115 CONTINUE
      ZZ=0.
      DO 116 I=1,NCOEF
      ZZ=ZZ+SS(I)
  116 CONTINUE
      ZZ=ZZ+SS(NT)
      WRITE(6,34)
      WRITE(6,35)
      WRITE(6,37) Z
      WRITE(6,39) ZZ
      WRITE(6,36)
      IF(KK.GE.2) GO TO 1000
      WRITE(6,41)
 1000 CONTINUE
      STOP
C
C     FORMAT STATEMENTS
C
    1 FORMAT(3I4)
    2 FORMAT(11F5.1)
    3 FORMAT(F20.7)
   20 FORMAT(1H ,I3,2X,F5.1,1X,F5.1,1X,F5.1,1X,F5.1,1X,F5.1,1X,F5.1,1X,F
```

```
  15.1,1X,F5.1,1X,F5.1,1X,F5.1,1X,F5.1,2X,F10.5)
21 FORMAT(1H1,19H SECOND ORDER MODEL )
22 FORMAT(1H ,11H ECHO CHECK  )
23 FORMAT(1H0,36H CODED LEVELS OF EXPERIMENTAL DESIGN  )
24 FORMAT(1H0,6H TRIAL ,15X,25H FACTORS AND COEFFICIENTS ,30X,9H RESP
  1ONSE  )
25 FORMAT(1H ,6X,3H X1,3X,3H X2,3X,3H X3,3X,3H X4,2X,4H X12,2X,4H X13
  1,2X,4H X23,1X,5H X123,2X,4H X11,2X,4H X22,2X,4H X33 )
26 FORMAT(1H1,9H C MATRIX )
27 FORMAT(11F11.5)
28 FORMAT(1H0,18H INVERTED C MATRIX )
29 FORMAT(1H1,20H FITTED COEFFICIENTS /)
30 FORMAT(5X,2H B,I2,2H =,F20.9,5X,10H VARIANCE=,F20.14)
31 FORMAT(1H1,21H ANALYSIS OF VARIANCE )
32 FORMAT(1H0,7H SOURCE, 5X,15H SUM OF SQUARES, 5X,5H D.F., 5X,12H ME
  1AN SQUARE,15X,7H F TEST  )
33 FORMAT(1H0,2H B,I3,3X,F15.5,5X,F10.5,5X,F10.5,5X,F10.5,5X,F10.5)
34 FORMAT(1H0,15H ERROR FOR I=12  )
35 FORMAT(1H0,18H RESIDUAL FOR I=14  )
36 FORMAT(1H1,18H FIRST ORDER MODEL  )
37 FORMAT(1H0,22H TOTAL SUM OF SQUARES=  ,F18.5  )
38 FORMAT(1H0,16H VARIANCE MATRIX  )
39 FORMAT(1H0,26H ESTIMATED SUM OF SQUARES=  ,F15.5  )
40 FORMAT(1H ,61X,6H ERROR ,7X,9H RESIDUAL  )
41 FORMAT(1H0,29H INTERACTION EFFECTS INCLUDED  )
   END
```

```
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```

APPENDIX VI

PRELIMINARY TESTING, TEMPERATURE AND PRESSURE EFFECTS

TEMPERATURE EFFECTS

Thermal Death

Extended recycling of the test slurry through the apparatus in an effort to obtain steady-state operation preparatory to sample collection resulted in an increase in slurry temperature. Preliminary testing with a hydrocyclone of D_c = 0.5 inches accepting a volumetric flowrate of 1.8 l/min increased the temperature of the test slurry from an initial value of 23°C to approximately 45°C for an operating period of 0.5 hours. Such a change in the physical environment was expected to have a significant effect on the number of viable micro-organisms in the mixed culture and the rates of their metabolic processes.

The resistance of protoplasm to temperature increases is dependent on the strength of the weakest chemical bonds. Lamanna and Mallette (1965) report values of from 1.3 to 8.3 K_{cal}/mole, depending on atomic configuration, for the lowest bonding energies in bacterial protoplasm. Since proteins are thought to be formed by hydrogen bonding, their rupture is probably responsible for thermal death. Lamanna and Mallette (1965) conclude that hydrogen bonds are broken during the heat denaturation of proteins and that losses in biological activity are associated with this process. Thermal death has also been ascribed to the melting of deoxyribonucleic acid, a nucleic acid essential for the processes of reproduction. The minimum temperature reported for this phenomenon is 70°C and hence "thermal death" effects in this study are related to an inability to metabolize substrate rather than an inability to reproduce.

Each micro-organic species has a characteristic temperature for which metabolic activity is possible. Since the test slurry consisted of a mixed microbial population incubated at approximately 23°C, it was expected that some fraction of the culture would experience a significant decrease in the rate of metabolic activity for a temperature increase of about 20°C. Studies by Lamanna and Mallette (1965) indicate that psychrophilic bacteria metabolize substrate well at temperatures not much higher than 20°C but exhibit essentially no growth above 30°C. Further, the desaturation of protenaceous material is an irreversible process for some vegetative cells. (Dean and Hinchelwood (1966)). Therefore, exposure to an environment with a temperature far removed (say 15°C) from the incubation temperature could result in the permanent sterilization of some species. To minimize the possibility of thermal death in this study, the test slurry was cycled through the apparatus for a period sufficient to obtain a steady-state particle size distribution (i.e. eliminate the presence of microbial flocs) but short enough to result in temperature increases in the test slurry of less than 5°C. Thus, the possibility of thermal death was minimized.

EFFECT OF TEMPERATURE ON SPECIFIC GROWTH RATE

Each species of micro-organisms is characterized by an optimum temperature defined relative to the velocity of substrate disappearance (Pelczar and Reid (1965)). This temperature is a function of the chemical and physical properties of the environment as well as the relative rates of protoplasm synthesis and protein or enzyme denaturation.

Dean and Hinchelwood (1956) report that below the optimum temperature the multiplication rate of bacteria follows approximately the Arrhenius-

Van't Hoff relationship.

$$\ln K = \frac{\Delta H_a}{RT} + C$$

where ΔH_a = heat of activation

R = gas constant

T = temperature (^oK)

C = constant

k = reaction rate constant

Since, however, growth depends upon a complex system of reactions, each of which have their individual temperature coefficients, the dependence of reaction rate constant on temperature must be determined empirically. Above the optimum temperature the rate falls very rapidly as a result of the inactivation of enzymes which increase in entropy due to a breakdown of low energy hydrogen bonds. This phenomon is also complex and must be evaluated empirically. Lamanna and Mallette (1965) define a temperature coefficient

$$Q_{\Delta T} = \frac{K_{T_2}}{K_{T_1}}$$

which expresses the effect of temperature on the velocity of biological reactions by a ratio of the overall reaction rate constants for substrate disappearance at the extremes of a temperature range. By differentiating the Arrhenius-Van't Hoff relationship with respect to temperature:

$$H_a = \frac{R\, T_1 T_2 \ln Q_{\Delta T}}{T}$$

The heat of activation is characteristic of a given reaction and for most biological reactions, is independent of temperature (Lamanna and Mallette (1965)). Therefore, the change in reaction rate constant for small changes in temperature would be expected to be small. Data

presented by Lamanna and Mallette (1965) indicate values of $Q_{\Delta T}$ for $\Delta T = 10^{o}C$ to be less than 2 for a spectrum of temperatures ranging from 0 to 120°C and values of heats of activation less than those representing normal enzyme inactivation. Sometimes, biological processes exhibit a considerable change in $Q_{\Delta T = 10^{o}C}$ with temperature. (Dean and Hinchelwood (1966)). This has been ascribed by Lamanna and Mallette (1965) to a strong dependence of temperature coefficient on the sensitivity of the internal and external environments to temperature changes. Since protoplasm is a heterogeneous system the state of particular phases, the viscosity and the pH or oxidation-reduction potential may be affected differently by a temperature change. Therefore, in a biological system temperature variation may lead to uncontrollable and unknown changes in numerous variables which are reflected in the temperature coefficient.

Lamanna and Mallette (1965) question the validity of applying traditional chemical theories to define the effects of temperature on rates of biological processes. These theories are based on the assumption that a chemical event can be halted only at absolute zero. Since vital biological activities cease at temperatures far removed from absolute zero, the Arrhenius Van't Hoff relationship may not be valid. Lamanna and Mallette (1965) propose a theory of molecular resistance to define biochemical reactions based on the fact that the free diffusion of reactants in protoplasm determines the rate of a biological event. Based on the hypothesis that the hydration of ions and molecules in the aqueous environment provide the resistance to free diffusion they found that the relationship

$$\frac{V_2}{V_1} = \left(\frac{T_2 - x}{T_1 - x}\right) \quad b$$

described the dependence of reaction rate on temperature, where

V_2 and V_1 = the rate of biological processes at temperature T_2 and T_1 respectively

x = the minimum temperature at which the event takes place

b = the temperature coefficient

Wuhrmann (1954) studied the effect of temperature on the unit rate of oxygen uptake for mixed cultures of bacteria growing on a sugar. He found the following expression to fit his data:

$$\log \frac{r_1}{r_2} = 0.0315 \ (T_1 - T_2)$$

when r_1 = unit oxygen uptake rate at temperature T_1

r_2 = unit oxygen uptake rate at temperature T_2

For a 5°C temperature increase from an initial value of 23°C, the increase in the velocity substrate disappearance would appear to be significant. To reduce the influence of temperature on the evaluation of the relative specific growth rates of the effected slurry separation, samples were allowed to cool slowly to approximately 23°C before inoculation into the nutrient environment.

Therefore, the possibility of thermal death was minimized by permitting a maximum slurry temperature increase of 5°C and the possibility of measuring non-representative specific growth rates was minimized by evaluating the velocity of substrate disappearance at the initial temperature of the test slurry.

PRESSURE EFFECTS

Since the test slurry was subjected to static pressures of up to 200 psig in the inlet port of the hydrocyclone separators, preliminary

testing was initiated to determine the effects of pressure on the metabolic activities of the micro-organism population. Pressure could modify the viscosity and elasticity of protoplasm and result in changes in the rates of metabolic processes. Lamanna and Mallette (1965) studied the effects of hydrostatic pressure on E.COLI and found that enzymes were not damaged at pressures below 2000 atmospheres. Further studies made on the intensity of light energy emitted as a function of temperature and pressure for a luminescent bacterial culture indicated that for temperatures close to that of incubation the rate of metabolic activity, measured as luminescent enzyme production, was relatively independent of pressure to approximately 100 atmospheres. Stanier et al (1963) have noted that bacterial cells are comprised of approximately 75 per cent water and since water is relatively incompressible pressures of a few atmospheres would not have a significant effect on the metabolism of the microbes.

Since the levels of pressure exerted in this investigation were much lower than those reported to be harmful to bacteria, the influence of pressure on metabolic rates was considered negligible.

Lamanna and Mallette (1965) suggest that a sudden release of pressure may be harmful to bacteria, which phenomena may be prevalent in the rapid discharge of the partitioned slurries from the hydrocyclone.

Initial studies in which a culture of micro-organisms was partitioned intoacycloned fraction and an "undisturbed" fraction which were inoculated into "identical" physical and chemical environments indicated that

differences in the metabolic activities of the two portions, measured as specific growth rate, could not be ascribed to pressure effects. Therefore, the influence of system pressure on the specific growth rates of the separated microbial slurries was considered negligible.

TEST SLURRY PREPARATION

At the completion of each experimental run, the contents of the overflow and underflow test reactors were mixed to define the seed population for the next run. Preliminary investigation indicated that immediate inoculation of the seed population into a fresh environment resulted in a considerable delay in the initiation of substrate disappearance measured as organic carbon. Since the physical and chemical nature of the new environment was identical to the seed environment, it was not expected that the micro-organisms would require a period of adaptation before being capable of utilizing substrate (Dean and Hinchelwood (1966)). Since the inoculation of the test reactors represented a dilution of an already small concentration of micro-organisms (less than 800 mg/l) it was concluded that the retardation of substrate uptake was an expression of the low initial concentration of viable micro-organisms. Lamanna and Mallette (1965) report similar findings and report that the length of the lag phase decreases with increasing inoculum and quantitatively tends to be a linear function of the logarithm of the number of organisms in the inoculum. Dean and Hinchelwood (1966) report similar results for studies of AEROBACTER AEROGENES. In order to minimize the lag period, the concentration of the seed population was increased by a 24 hour batch growth between adjacent runs. Further,

seed volumes representing 67% of the test reactor volume were used. Dean and Hinchelwood (1966) provide data to indicate that the duration of the lag phase is a function of the age of the microbial cells. Studies in which AEROBACTER AEROGENES were transferred from a growing culture to a fresh supply of the same medium defined by a carbon source of glucose suggested that the lag was a function of the age of the parent cells and the source of nitrogen. Using ammonium phosphate as a nitrogen source (as per this investigation) resulted in a lag duration which varied inversely with cell age to some minimum value after which it varied directly with cell age. This phenomenon is ascribed to a lack of diffusible co-enzyme intermediates required for the utilization of the nitrogen source in younger cells and chemical decay or loss of intermediates by diffusion from the cell for old cultures. These results are substantiated by Stanier et al (1963) who noted a dependence on inoculum volume. For this investigation, 80 per cent of the culture by volume was wasted per day and replaced with tap water in order to minimize the accumulation of toxic metabolic products in the batch seed population. This procedure provided the benefits of reducing the lag period upon inoculation into a fresh medium; permitting a natural selection of microbial species based on characteristic rates of multiplication; and providing a nutrient environment adequately rich in trace elements. Further, the time between feeding the seed population and effecting a slurry separation was standardized to afford some basis for relative evaluation of the test runs.

APPENDIX VII

NUTRIENT MEDIA

Eckenfelder and O'Connor (1961) report that a carbon:nigtogen: phosphorous ratio of at least 40:5:1 is required for bacterial metabolism.

In this investigation, nitrogen and phosphorous were supplied by dibasic ammonium phosphate; $(NH_4)_2HPO_4$ organic carbon was supplied by dextrose $C_6H_{12}O_6$. Organic carbon was defined to be the growth limiting nutrient by maintaining nitrogen and phosphorous in quantities exceeding those of the critical ratio.

The trace elements necessary to sustain bacterial growth were provided at level greater than those defining growth limitation by using tap water, augmented by the addition of iron, potassium and magnesium compounds to complete the nutrient medium.

The nutrient environment employed in this study is defined by the following mass ratios per unit reactor volume:

$$\frac{C_6H_{12}O_6}{(NH_4)_2HPO_4} = 3.0000$$

$$\frac{C_6H_{12}O_6}{FeCl_3 \cdot 6H_2O} = 10^3$$

$$\frac{C_6H_{12}O_6}{K_2HPO_4} = 10^1$$

$$\frac{C_6H_{12}O_6}{MgSO_4 \cdot 7H_2O} = 10^1$$

plus trace elements (tap water).