A CONTINUOUS BIOCHEMICAL REACTOR STUDY USING MIXED MICROBIAL CULTURES

A CONTINUOUS BIOCHEMICAL REACTOR STUDY USING MIXED MICROBIAL CULTURES

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SCOPE AND CONTENTS:

A completely-mixed biochemical reactor was used to study a mixed microbial culture using soluble organic carbon in the form of glucose as a growth limiting nutrient. The effect of various flow rates and feed concentrations was determined by evaluating the corresponding variations in unit organism growth rate, yield and effluent carbon concentration.

The effluent carbon concentration was independent of flow rate and feed concentrations for the range studied. The unit growth rate was similarly independent of the feed concentration but varied directly as the flow rate. No trend indicated that yield varied with either flow rate or feed concentration. Large variations in yield often occurred at any one condition due to changes in the mixed microbial population. However, the reason for this microbial variation was not determined.

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NOMENCLATURE

C	-	limiting nutrient concentration (organic carbon)
C_o	-	initial limiting nutrient concentration
C_1	-	limiting nutrient concentration at time t_1
C_2	-	limiting nutrient concentration at time t_2
$\frac{dC}{dt}$	-	net rate of change of the limiting nutrient concentration
K_C	-	net removal rate of the limiting nutrient by organisms
K_c	-	removal rate of the limiting nutrient per unit mass of organisms
K_m	-	growth rate of organisms per unit mass
K_o	-	maximum growth rate of organisms per unit mass
M	-	concentration of organisms
$\frac{dM}{dt}$	-	net rate of change of organism concentration
Q	-	hydraulic flow rate
V	-	volume of the vessel

CHAPTER 1

INTRODUCTION

In the aerobic biological treatment of a waste, organisms using the waste as food are separated from the liquid, thus purifying the carrier liquid. To operate this biological treatment process, it is necessary to know which variables affect the growth of the organisms. In addition, it is convenient to know the quantity of organisms which will be produced (yield). The purity of the liquid after treatment should be controlled by the variables affecting the organisms.

Previous studies have considered quite extensively the effect of detention time on the concentration of waste in the effluent and on the growth rate of organisms. However, the corresponding effect of different feed concentrations of a waste on these parameters has not been considered. There is a similar lack of information relating feed concentrations to other parameters such as yield and effluent waste concentration. A study, designed to vary both detention time (flow rate) and feed concentration would give further information in this regard. Such a study would also reveal the possibility of optimization with respect to flow rate and feed concentration.

Many studies have used pure cultures of bacteria where only one species is permitted to grow. One justifies working with a pure culture by stating that this species is present in the organisms used in waste treatment and all

organisms are basically similar. Although the selection of one species reduces the possibility of fluctuations, it does not necessarily represent what occurs in a competitive environment with many species, i.e. a mixed culture. It would seem reasonable that mixed cultures may give significantly different results than pure cultures because the organisms which can best adapt themselves to a given environment will predominate. Since a mixed culture predominates in the biological treatment of a waste, factors effecting this culture should be known.

In the operation of a continuous process, the concept of steady state is used to simplify the analysis of the system. In addition, the achievement of steady state indicates that organisms can operate in balance with one another. The parameter usually measured to indicate equilibrium is either the biological or chemical oxygen demand of the effluent waste concentration. The oxygen demand has been traditionally used since it indicates how much oxygen the receiving body of water must supply to the waste for complete stabilization. However, the oxygen demand changes depending on the waste. Modern equipment now permits the measurement of a parameter which is conserved throughout, i.e. organic carbon, rather than an indirect parameter like oxygen demand. This modern method is both simple and accurate and therefore should provide additional information in understanding the biological process.

A study was therefore performed using a mixed culture with the purpose of showing the effect of both flow rate and feed concentration on known parameters using the advantages of rapid organic carbon measurements.

CHAPTER 2

LITERATURE REVIEW

Biological treatment facilities designed for continuous flow had been in operation for many years before the first laboratory study was reported. This study, by Garrett and Sawyer (1952), included the development of a kinematic theory and experimentation using an activated sludge culture. In the biological field, Novick and Szilard (1950) and Monod (1950) used identical kinetic developments to describe the steady state kinetics of continuous culture devices. Even earlier, the mathematical simplicity of the completely mixed continuous flow apparatus for the study of steady state systems was shown by Denbigh (1947) in considering the kinetics of steady state polymerization. Since this time, both discussion and experimentation have been promoted by the question whether the theory of the completely-mixed continuous flow system, as developed for a chemical reaction, will also describe the kinetics of biological culture.

2.1 THEORETICAL DEVELOPMENT

In many chemical reactions, an equation is written to represent how chemicals combine on a molar basis, This is known as a stoichiometric equation and can be expressed as

$$aA \rightarrow rR + sS$$

when a moles of A disappear to form r moles of R and s moles of S. When one studies the chemical kinetics of this reaction one studies the factors that influence the rate of reaction

and the explanations for the rate of reaction, Levenspiel (1965).

A few of the variables which influence the rate of reaction are temperature, concentration, rate of mass transfer and possibly rate of heat transfer. If the reaction involves a number of steps in series, it is the slowest step of the series that exerts the greatest influence and can be said to control. The problem is then to find out which variables affect each of these steps and to what degree.

Referring again to the above single reaction, the rate of change in the number of moles of component A per unit volume is

$$r_A = -\frac{1}{V}\frac{dN_A}{dt}$$

If the rate of disappearance of A is a linear function of the concentration of A, then the rate can be expressed as

$$r_A = kC_A$$

with k the rate constant. When the number of moles of A is uniform throughout the system and the reaction does not change the volume of the system, then

$$r_A = -\frac{1}{V}\frac{dN_A}{dt} = -\frac{dC_A}{dt} = kC_A$$

Another single reaction could have a stoichiometric equation of

$$A + B \rightarrow R$$

If one postulated that the mechanism which controlled its rate of reaction involved the collision of a single molecule of A with a single molecule of B, then the number of collisions

of molecules A and B would be proportional to the rate of reaction. Since the number of collisions is proportional to the concentration, the rate of disappearance of A is given by

$$r_A = kC_AC_B$$

Similar postulates of mechanisms are made for reactions in series. The rate constants are then evaluated and the expression tested by experimental data. If the rate equation derived from the postulated mechanism predicts all experimental data, then the equation is considered satisfactory and is used for design and operational control.

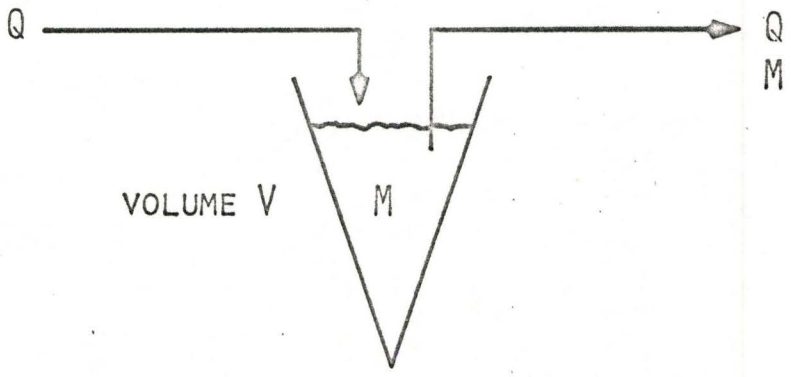
In a biological reaction, a corresponding expression is desirable which will stipulate how the feed rate, feed concentration, temperature and other system variables affect the rate of reaction. When compared to a chemical reaction the problem is much more difficult because of the variability of a living system. Bacteria can change reactant A into a final product along a network of possible reaction paths. The unique path may depend on the species of bacteria, the physical environment (temperature), the chemical environment (concentration, pH) as well as the composition of A. Thus any proposed mechanics will be complex.

In experimentation, the concentration of each of these species of bacteria must be determined to calculate the net rate of reaction. With a pure culture only one type of organism is present and therefore experimental determination and possible variations are greatly reduced as compared to a

mixed culture. In a mixed culture, it is presently impossible to state the exact conditions of experimentation. Therefore, in repeating an experiment, the reaction rate could be either reproduceable or vastly different depending on the culture used. Reproduceable results could be obtained for three reasons: identical conditions, a complex network where more than one reaction path will give the same resultant information, or sufficient restrictions on the system to sitpulate only one result.

The expression for the overall kinetics of any system can be derived by formulating a mass balance. In a mass balance, the mathematical equation for the rate of mass change (growth of organisms) in a completely-mixed continuous flow system with no return flow is expressed as follows:

$$\frac{dM}{dt} = K_m M - \frac{Q}{V} M \qquad (1)$$



where M = mass concentration of material reacting (amount of organisms)

$\frac{dM}{dt}$ = rate of mass change (growth of organisms)

K_m = reaction rate (growth rate) for a unit mass

V = volume of the vessel

Q = hydraulic flow rate into and out of the vessel

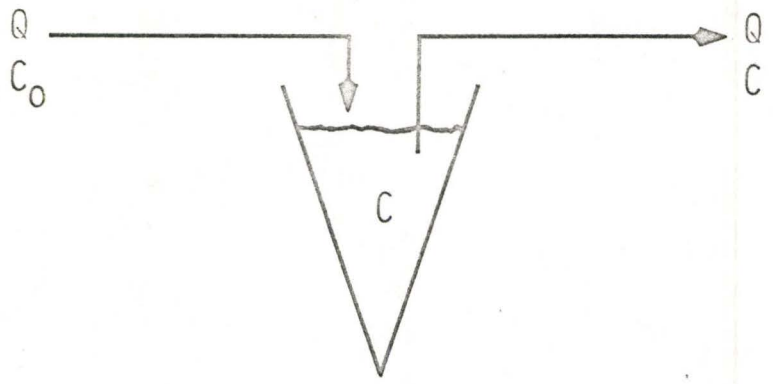
$\frac{V}{Q}$ = mean residence time

This expression states that the rate of mass change is the difference between the amount of growth ($K_m M$) and the amount of wash-out ($\frac{Q}{V} M$) in a unit time. When steady state is reached, the rate of mass change $\frac{dM}{dt}$ is zero. Then the growth rate, K_m, is wholly a function of the hydraulic rate of flow, Q. That is K_m equals $\frac{Q}{V}$ or K_m is equal to the reciprocal of the detention time.

Spicer (1955) showed that the system can only be stable if the growth rate, K_m, decreases as the concentration of organisms increases. This fact applies to an organism dependent on a nutrient factor which is present in such a limiting quantity that small variations in concentration can cause corresponding variations in the growth rate. In this case K_m, the growth rate, is some function of the limiting nutrient concentration.

The net rate of change of nutrient concentration is obtained by another balance. This balance, initially presented by Monod (1950) and also derived by Spicer (1955) and Herbert et al (1956) is represented in the equation

$$\frac{dC}{dt} = \frac{Q}{V} C_o - \frac{Q}{V} C - K_C \qquad (2)$$



where $\frac{dC}{dt}$ = rate of change in concentration of the limiting nutrient

C_o = concentration of nutrient in the influent

C = concentration of nutrient in the vessel and effluent

K_C = function describing the removal rate of the nutrient by the organisms

The term K_C can be expressed as

$$K_C = \frac{K_m M}{Y} = K_c M$$

with Y the yield constant. The yield constant is that fraction of the total nutrient concentration which is synthesized to become the organism mass, M.

When the steady state is attained with respect to the nutrient concentration, then $\frac{dC}{dt}$ will equal zero to

give

$$\frac{Q}{V}(C_o - C) = K_C = \frac{K_m M}{Y} \qquad (3)$$

Theoretically, $\frac{dM}{dt}$ and $\frac{dC}{dt}$ will equal zero at the same time, so that K_m equals $\frac{Q}{V}$. Then $(C_o - C)$ equals $\frac{M}{Y}$ or Y equals $\frac{M}{C_o - C}$. Therefore, three terms can be calculated: the yield constant, Y, by knowing M, C_o and C at steady state; the growth constant K_m, at steady state by knowing the flow rate, Q, and volume of vessel, V; and K_C, the nutrient removal rate for a unit mass of organisms.

As previously mentioned, the growth constant, K_m, should be some function of the limiting nutrient C. The relationship between K_m and C can be experimentally determined by evaluating C at different steady states selected by the Q/V value. A series of yield constants will also result. Thus, it is possible using equations (1) and (2) to calculate the condition at any other steady state by the relationship for K_m and Y.

This development has assumed that the growth rate and unit carbon removal rate are directly related by the yield. This restriction may be incorrect. In addition, the theory has been developed using the premise that steady state can be attained. The limits of variation used to define steady state experimentally is therefore important.

2.2 REPORTED STUDIES

Monod (1949) reported extensive studies of the growth of bacteria (E.coli and B.subtilis) under aerobic conditions

in a simple media containing a single carbohydrate in concentrations of 25 - 300 ppm. These studies were carried out under batch conditions. the rate of growth, studied in relation to the concentration of nutrients, was found under a number of conditions to be correlated by the relationship

$$K_m = k_o \frac{C}{C_1 + C}$$

where k_o = the maximum rate of growth at infinite concentration

C = concentration of nutrient remaining

C_1 = a constant with the dimensions of concentration and equal to the concentration of nutrient when the actual rate is $1/2\ k_o$

It was stated that several mathematically different formulations could be made to fit the data. However, it was both "convenient and logical" to adopt the above hyperbolic equation since it was similar to the Michaelis equation for enzyme reaction.

With pure cultures of bacteria, end-products of organic matter may form which still have a BOD but do not serve as food for further growth of this culture. In a mixed culture, it is possible that there will be organisms present that can utilize the end-products of other organisms so that when growth ceases, there will be very little organic matter remaining other than the organisms. With this idea, Garrett and Sawyer (1952) conducted experiments to determine

> "Whether or not the kinetics of the removal of BOD by mixed cultures follows the same relationship that have been found to apply to the utilization of individual substances by pure cultures of bacteria".

Batch studies were performed which indicated that the rate of growth was a constant for the synthetic waste when the "limiting" organic nutrient was present in an excess concentration. The organic nutrient was one of glucose, peptone, or a combination with equal parts of each. Identical rates were obtained when measuring the soluble BOD remaining or the oxygen utilized.

In the continuous study only peptone at one concentration was used. The apparatus was operated continuously for three days at each of three detention times ranging from 6 to 20 hours. The relation between the rate of growth (calculated by taking the reciprocal of the detention time) and the soluble BOD remaining (ranging from 30 to 80 ppm) was assumed to be linear. The validity of steady state being obtained within three days may be questioned when considering the results of later research, Busch and Myrick (1960).

As continuous flow theory became known, researchers conducted experiments to test its validy and range of applicability. Herbert et al (1956) described in detail the theory of Monod and Novick and Szilard and applied this theory to pure culture work with Aerobacter cloacae using glycerol as the sole carbon source and growth-limiting nutrient. Quantitative data was obtained on steady state bacterial and substrate concentrations at twenty-one different flow rates for one glycerol concentration. They were able to operate a pure culture system for months without any detectable contamination from mutation. Steady state conditions in

bacterial concentrations were within the range of 95 percent confidence limits in tests run for as long as 34 hours. When the flow rate was changed, a 24-hour period was allowed for stabilization before taking samples for bacterial concentration.

Using the values of growth rate and yield constant obtained in batch culture experiments, theoretical and experimental comparisons were made for the continuous flow studies. There was a definite deviation with the bacterial concentration being higher and substrate concentration being lower in the continuous than the values expected based on the batch experiments. Steady state conditions were achieved at flow rates which should have caused "wash-out". The growth rate of batch and continuous cultures may be different or the wash-out rate may be less than predicted. Also, the yield constant (Y) which at low dilution rates had the same value as was found in batch culture experimentation, showed a definite tendency to decrease at higher dilution rates.

A number of experimentors (Monod, Novick and Szilard, Spicer, Herbert) have regarded the growth rate (K_m) of a bacterial culture growing under conditions of nutrient limitations as a function only of the concentration of the limiting nutrient. Contois (1959) from studies of continuous culture of Aerobacter aerogenes indicated that the growth rate was a function of bacterial density as well as the concentration of the limiting nutrient. He also reported the yield constant (Y) to be independent of the growth rate (reciprocal of the detention time at steady state). It should be noted that his detention times

were always less than six hours.

A possible reason given for the growth rate expression was an inhibition of the growth process by end-products of that process. Since the yield constant was independent of the growth rate, then the concentration of end-products of the growth process probably was related to the bacterial density. Thus, the appearance of bacterial density in the growth rate expression may be due to what is primarily an effect of concentration of end-products.

Stack and Conway (1959) studied the degradation of a dextrose solution using a mixed culture in a completely-mixed, continuously-fed, oxygen utilometer. They reported that as the detention period was shortened, the amount of work accomplished per unit volume of aeration capacity increased. Also, the amount of nutrient oxidation became less significant with the net result of the production of more solids and the consumption of less oxygen per unit of organic waste removed.

Gaudy et al (1960) reported the results from a completely-mixed unit with recycle. The unit was operated at one detention time (24 hours) and one feed concentration (1000 mg/l glucose) for three months. The time between analyses varied from one to fifteen days. The soluble effluent concentration as COD varied from 125 to 205 mg/l while the biological solids, measured using a membrane filter, ranged from 820 to 1170 mg/l. With a removal efficiency of 80 to 88 per cent they concluded that the unit provided a

"fairly constant biological system based on organic removal efficiency".

The work reported by Busch and Myrick (1960) using a glucose waste found that no food-population equilibrium using a mixed culture could be attained in a continuous system after operation at one organic loading for as long as 103 days. Washington and Symons (1962) reported studies on a completely-mixed system operating on an extended-aeration cycle (24 hours detention). They stated that the "active mass" attained steady-state in two to five weeks while the "endogeneous mass" continued to increase through the fifteen weeks of study without any indication of becoming steady. Based on preliminary studies Hetling, Washington and Rao (1964) felt that steady state could not be maintained using a mixed population similar to activated sludge, however, no data was presented. Similar observations on population dynamics and selection in continuous mixed cultures were reported by Cassell, Sulzer and Lamb (1966). Their experiments revealed that mixed culture systems are very dynamic and that the phenomena of selection and predomination strongly influence the microbial behaviour.

In addition to studying the possibility of steady state a number of researchers have reported work and postulated reasons for variations in solids production both on batch and continuous systems. Rao and Gaudy (1965) presented an extensive summary of studies on carbohydrate wastes concerned with the prediction of sludge yield. Their review of the

literature found that even for the simple carbohydrate glucose, a considerable range of cell yields had been reported (28 to 64 per cent). In order to gain further insight into the constancy of the cell yield they performed a long mixed culture study under "highly controlled operational conditions" using glucose as the only carbon source. This study was carried out using three batch systems which were fed a constant concentration of glucose daily.

Results of this work indicated a statistical range of yields from 48 to 82 per cent from which they concluded that variations in yields were most probably the result of variations in predominance of the microbial populations. These changes in predominance were considered random and were brought about solely by interaction between the organisms (since conditions were controlled). Further it was stated that to expect the cell yield to be solely a function of the structure of the substrate or the free energy of the substrate was a "totally gross simplification". (A discussion which related the free energy content and cell yield was presented by Servizi and Bogan (1963)).

The relationship between solids yield and detention time was traditionally considered a constant. Reports of pure culture studies by Hetling, Washington and Rao (1964) and mixed culture studies by Reynolds and Yang (1966) indicated a straight line relationship for yield with detention time. A non-linear decrease of yield with shorter detention times was noted by Schulze (1964). However, Martin and Washington (1965)

using pure cultures with detention times from 1.67 to 2.5 hours observed a maximum yield at the 2 hour detention time. Genetelli and Heukelekian (1964) experimenting with different substrates noted that sludge yields were influenced by the chemical composition of the substrate but that sludge yields were essentially constant regardless of loading for the same substrate. A fundamental consideration as stated by Hetling, Washington and Rao (1964) is that

> "Yield will vary with different substrates and organisms or even with the same substrate and organism depending on the metabolic pathway by which the substrate is degraded".

CHAPTER 3

EXPERIMENTATION

3.1 DESCRIPTION OF APPARATUS

The main component of the apparatus was the conical reaction vessel. Feed solutions were pumped from storage bottles into the top of the reactor at a constant rate. Aeration was used to completely mix the constant volume reactor and to supply the culture with oxygen. The reactor effluent was wasted continuously. The reaction vessel and associated equipment are shown in Figure 1, and represented schematically in Figure 2.

The reaction vessel consisted of an 8 litre capacity glass percolator which was modified by the addition of a 350 ml fritted-glass disc Buchner funnel to the tapered end. The fritted-glass disc, through which air was supplied, was of medium porosity, giving both a resistance to the downflow of liquid and a good distribution of small bubbles for mixing. A 12 mm diameter glass spout for the effluent flow was attached at the six litre level. A plastic funnel with the interior coated with teflon was mounted in an inverted position to form a cover. A detailed drawing of the reactor is presented in Figure 3.

Air from a compressed air line was filtered through a tube packed with glass wool and was saturated by diffusion through water contained in a plexiglass cylinder. A pressure gauge was attached to the cylinder. The air flow to the reaction vessel was controlled by a Swagelok valve and measured by a RGI flowmeter.

FIGURE 1

PHOTOGRAPH OF EXPERIMENTAL APPARATUS

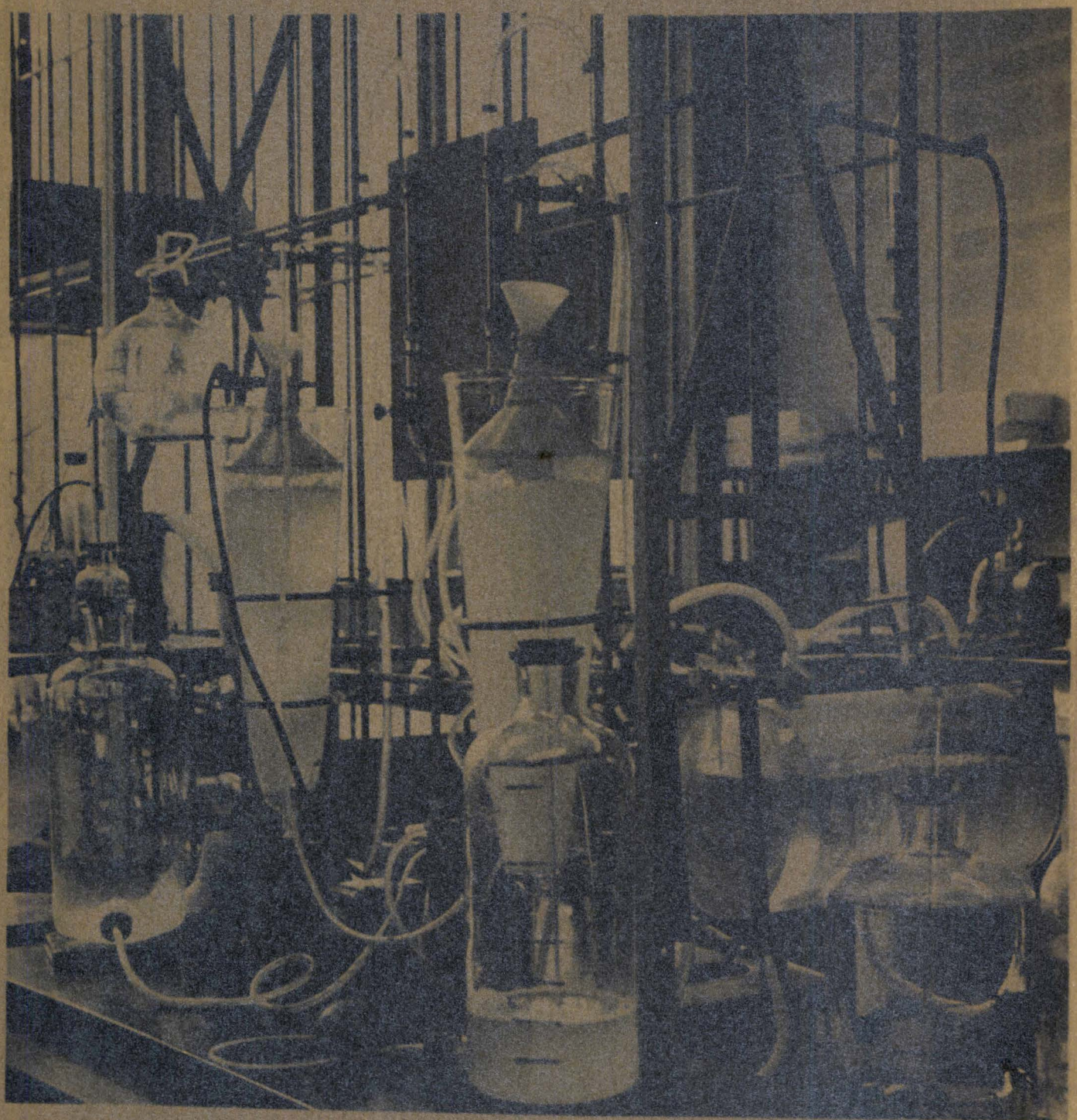


FIGURE 2

SCHEMATIC OF EXPERIMENTAL APPARATUS

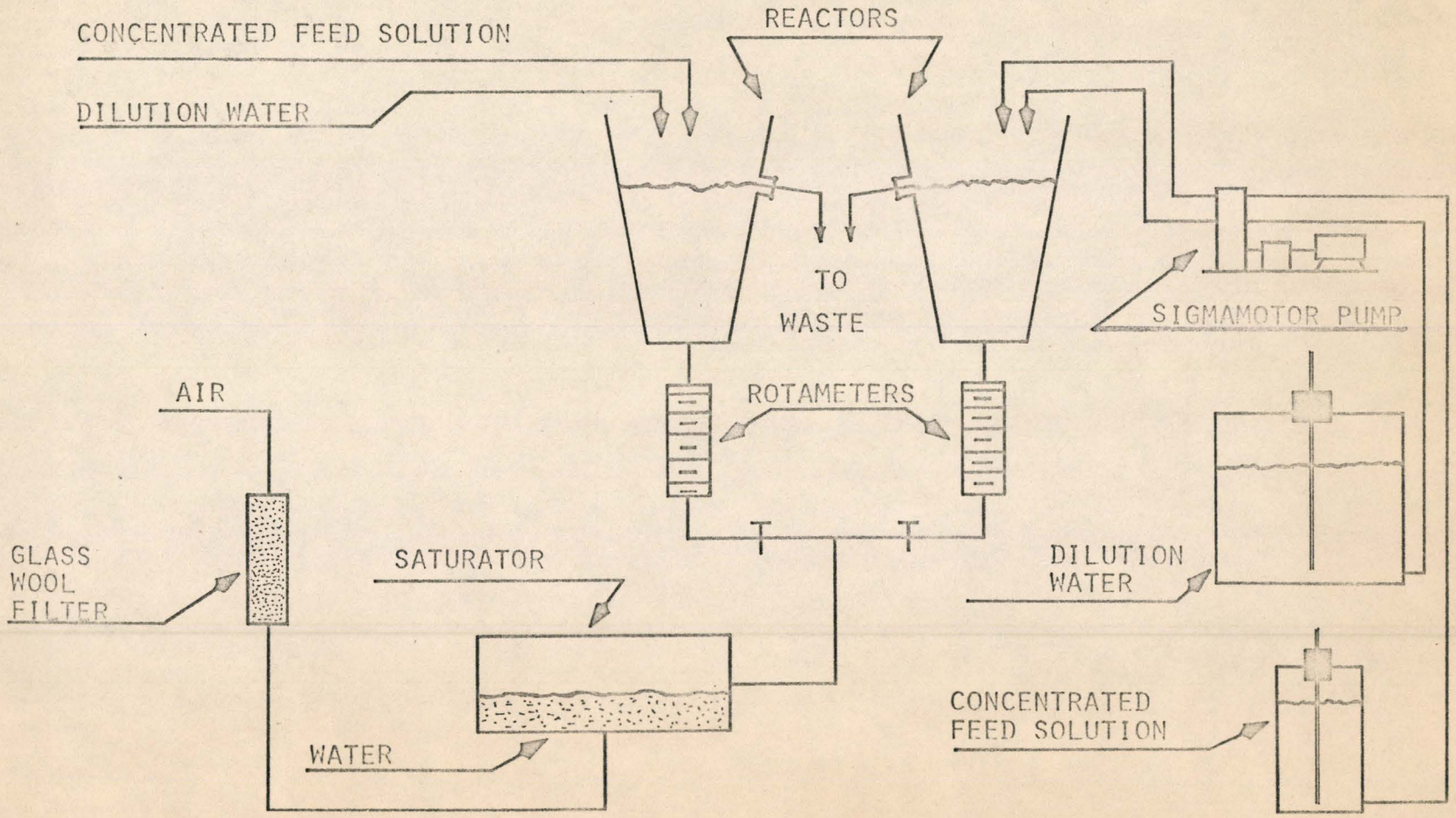
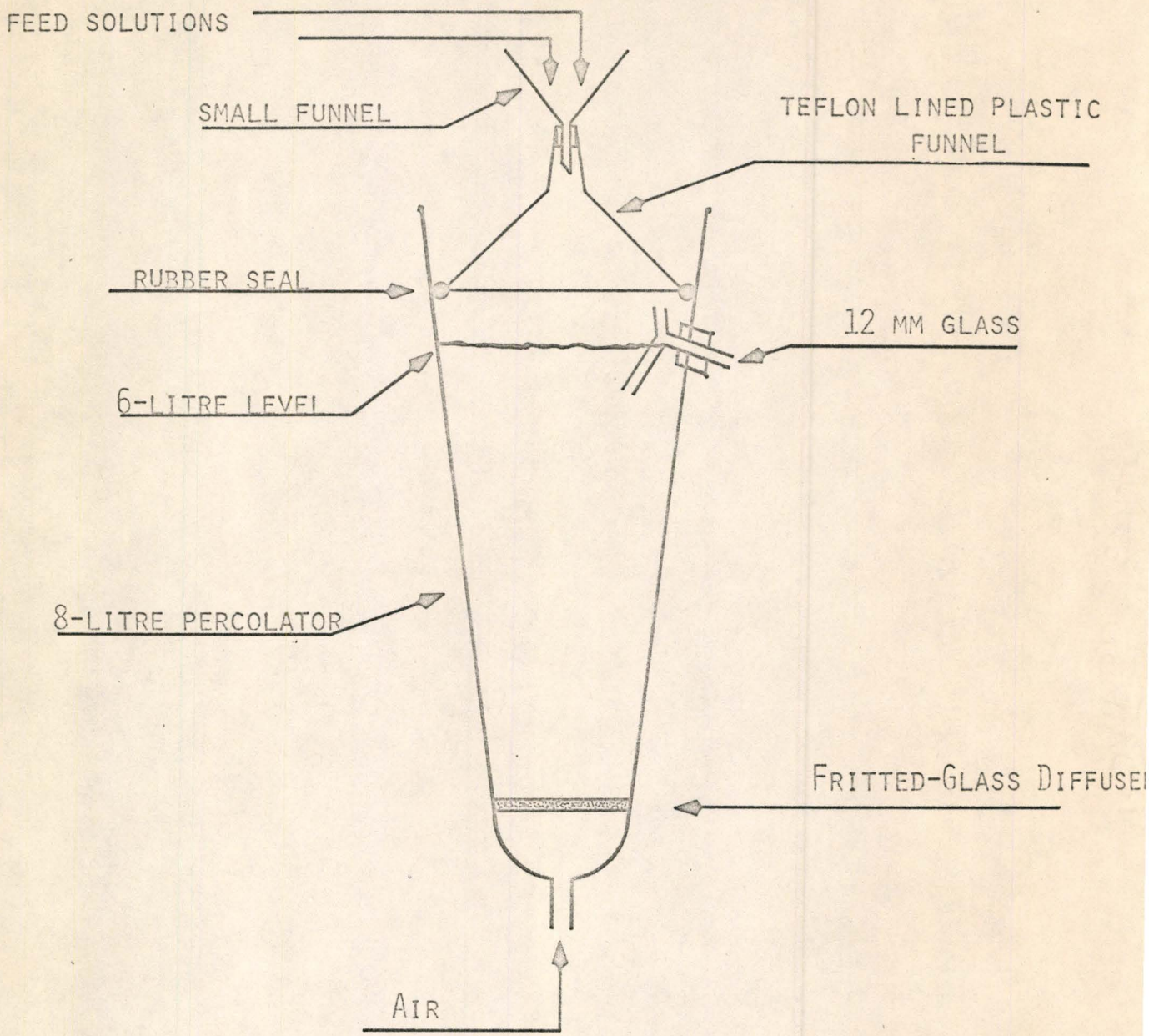


FIGURE 3

CONTINUOUS REACTOR



The liquid feed was pumped to the reactor by a combination of a model T-8 sigmamotor pump, a model DC-F Brailsford effluent sampler, and an electrolytic pump as described by Symons (1963). An air gap existed between the supply lines and the reactor culture to prevent the backgrowth of organisms into the feed lines.

The effluent from the reactor flowed through a "Y" piece, made of 12 mm diameter glass tubing inserted in the effluent spout, which served to keep the liquid at a constant level and to prevent syphoning while withdrawing from below the liquid surface.

3.2 ANALYTIC TECHNIQUES

During the experimentation the concentration of dissolved organic carbon and non-filterable solids, and the pH level were measured twice daily on the reactor liquid. A microscopic examination was performed approximately once every two days to determine the general types and relative numbers of organisms present.

Microscopic examinations were made using an Olympus microscope with a phase contrast attachment. Sufficient resolution was available to permit the observation of different stages in floc formation and the determination of different types of organisms present.

For the other determinations a 50 ml volume was withdrawn using suction, from approximately 15 cm below the liquid surface of the reactor. Of this, a 10 ml volume, sampled using a

broken tip pipette, was used for each determination. This volume was filtered through a 47 mm diameter Gellman membrane filter of 0.45 micron pore size using a vacuum pump. The filtrate was collected for carbon analyses and the solids retained on the filter was used for the solids determination. The pH level was measured on the liquid remaining in the beaker using a model 76 Beckman pH meter and then the liquid was poured back into the reactor.

Before filtration each membrane filter was washed with a 100 ml of distilled water to remove any soluble carbon and dried in an aluminum weighing dish at 45°C for an hour before storage in a desiccator until weighing and filtration. After filtration, the filter plus retained solids was replaced in the aluminum dish, dried at 45°C for an hour, and cooled in a desiccator until weighing. All weighings were performed on a Mettler balance (Type H15) which could be read to the nearest 0.1 milligram. Solids determinations were done in triplicate.

The standard method for measuring total solids is to dry the material at 103°C for an hour instead of 45°C (Standard Methods 1965). A 45°C temperature was used because of the fact that a 103°C oven was not always accessible. A comparison between total solids measured at 45°C and 103°C is given in Appendix A. A decrease in weight of 0.81 per cent for 103°C conditions, plus or minus 0.63 per cent at a 99 per cent confidence level is indicated for the samples tested. A decrease in the weight of the membrane filter of 16 hundredths

of a milligram was taken into account in the calculations.

The filtrate from filtration was poured into a test tube and titrated with two drops of concentrated hydrochloric acid which reduced the pH to less than 2.0. The decrease in pH converted any inorganic carbon into carbon dioxide which was stripped from the liquid by bubbling through the liquid an inert gas of argon or helium for approximately five minutes. The total organic carbon remaining in the liquid was measured by injecting a 20 µl sample into a model IR315 Beckman infra-red carbonaceous analyzer and recording the magnitude of the resulting output signal. The corresponding carbon concentration in milligrams per litre was determined by injecting samples of sodium oleate of known carbon concentration to give output signals on both sides of the unknown, and linearly interpolating to get the carbon concentration. This standardization of the analyzer was done for each analysis. Three injections were made for each sample. Samples collected in the evening were capped, stored in a 4°C refrigerator, and analyzed with the samples collected the following morning. The glassware used for the carbon analyses was soaked in chromic acid solution (Standard Method 1965), rinsed in tap water and distilled water and oven dried.

3.3 NUTRIENT SOLUTION

The nutrient solution was pumped to the reactor using two tygon feed lines. One feed line supplied tap water supplemented with ferric chloride, magnesium sulphate and potassium phosphate solutions, and the other line supplied

demineralized distilled water supplemented with dextrose and ammonium phosphate. The ratio between tap water and distilled water was 10: 1 or larger. The exact amount of chemicals added is given in Appendix B. Dextrose used as the carbon source was the limiting substance for growth.

A number of methods of supplying the feed solutions were tried before it was decided to pump the two solutions mentioned. The first involved the use of gravity feed with flow rate controlled by a valve but the collection of air in the valve prevented a constant flow rate. When all chemicals were mixed in one container the growth of bacteria caused a blockage in the feed line and a reduction in the carbon concentration being fed. An attempt to cool the feed using a copper coil did not significantly retard the growth of bacteria to make this method suitable. The pumping of two solutions seemed the only method without the sterilization of all equipment and solutions.

In preliminary work, it was assumed that sufficient trace elements were available in the tap water so that only the addition of ammonium phosphate would be necessary. The limited degradation of carbon that resulted indicated a deficiency of at least one element. Chemical analyses of the tap water revealed a limited concentration of iron and potassium, and to a lesser extent, of magnesium. The addition of the chemicals previously mentioned remedied this situation. The results of the chemical analyses of the tap water are given in Appendix C.

3.4 OPERATING CONDITIONS

In the control and analysis of a system, the terms- variables and parameters- are used to describe the system. A variable is a factor which can be externally controlled by adjusting valves, i.e. flow rate. A parameter is a term used to describe a factor which results after a variable has been changed, i.e. detention time, turbulence, effluent carbon concentration.

In this study, the reactor was operated with the feed rate and carbon feed concentration being the only two variables. The feed rate to the reactor was varied to give a range in detention times from 5.6 to 20.0 hours based on the influent flow. The carbon feed concentration ranged from 400 to 1025 milligrams per litre.

The temperature fluctuated between 23°C and 27°C with a maximum change in any given day of two degrees. The air flow through the reactor was approximately 9000 ml/min or 1.5 litres per minute per litre of volume. This flow was maintained throughout and was sufficient to give a saturated dissolved oxygen condition. The pH level of the culture was never adjusted throughout the experiment. The pH range for all conditions was from 4.85 to 8.05 (wash-out) with the pH level normally 6.5 ± 0.5.

3.5 INOCULUM FOR CULTURE

Initially, the reactor was inoculated with raw sewage which had been filtered through a Watman No. 2 filter. Later

the reactor was inoculated using the effluent from a reactor which was operating favourably. No method was used to favour the growth of a specific type of organism. If fungi predominated the reactor was emptied and reinoculated. The growth of all forms of bacteria and protozoa was considered permissible.

CHAPTER 4

RESULTS

4.1 INITIAL EXPERIMENTATION

Initial laboratory work involved refining the control of flows to the system and modifying the feed media until a suitable solution was developed. The schedule of experimentation which followed this initial work, is outlined in Figure 4.

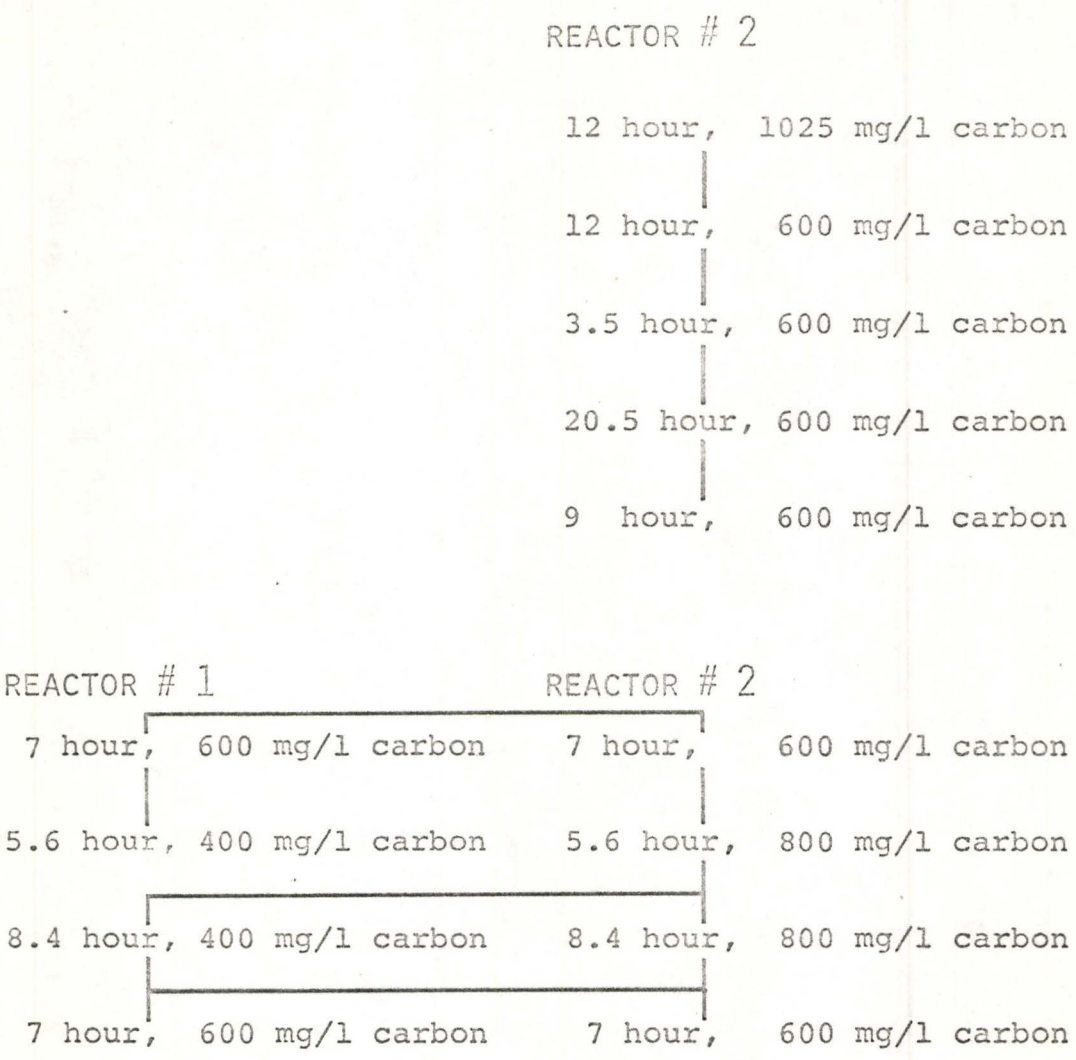
The first series of experiments determined the range of flows and feed concentrations, and the effect these variables had on measured parameters. The data from all the experimental conditions is presented in a tabulated and graphical form in Appendix D.

Feed concentrations of 1025 and 600 mg/l carbon were run at a 12 hour detention time. The difference in effluent carbon or carbon removal rate for the change in feed concentration was slight. With the change in feed concentration the organism mass decreased to adjust to the decrease in carbon feed. This resulted in the same unit carbon removal rate existing for both feed concentrations. This would indicate that a given unit carbon removal rate is defined by a 12 hour detention period.

Experimentation at a 3.5 hour detention period was only run for approximately 8 detention times. It was then concluded that washout was occurring, so the detention time was adjusted to 20.5 hours. Results of analyses revealed that the effluent carbon concentration was no lower at 20.5 hours than at 12 hours. Similar results were obtained at a 9 hour detention time until organisms producing a

FIGURE 4

EXPERIMENTAL SCHEDULE



water soluble, coloured pigment predominated. The results of these organisms will be discussed later, under "Effect of Pigment Production".

It should be noted that the effluent carbon at the 9 hour detention was even less than the 12 or 20.5 hour periods. Thus, detention times as short as 9 hours had no limiting effect on the effluent carbon.

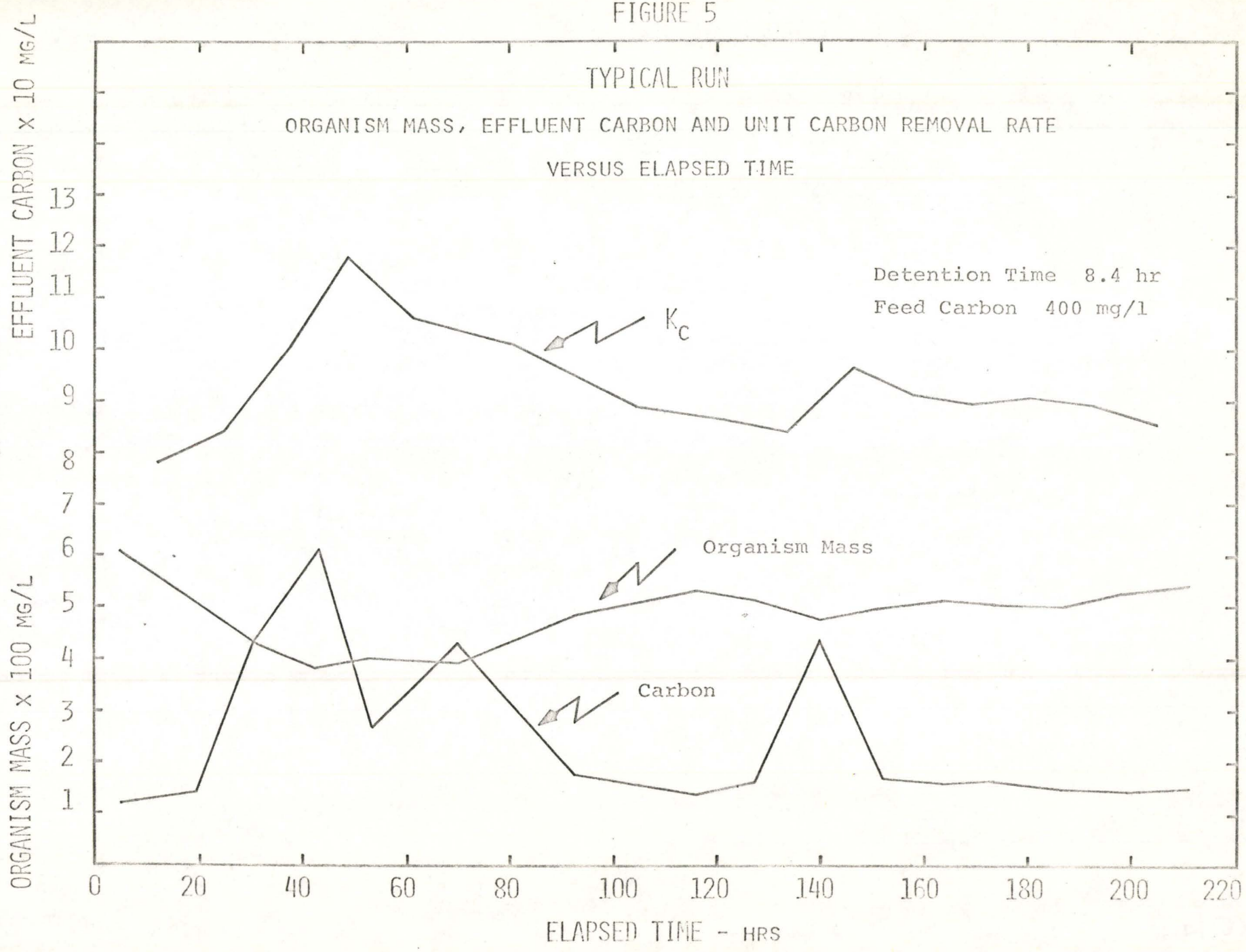
4.2 PARALLEL RUNS

With the knowledge that detentions of 3.5 hours would cause washout and 9 hours had no adverse effect, an experimental design known as a "central composite rotatable design" was set-up using 7 hours, 600 mg/l feed as the centre. Detention times of 5.6 and 8.4 hours were the quarter points at feed concentrations of 400 and 800 mg/l. Experiments were not performed for all conditions in the design because the variation in repeating a condition was at least as great as the variation between conditions. This made the results unsuitable for statistical analysis.

A typical plot, similar to those in Appendix D, of variations of three parameters - effluent carbon, organism mass and carbon removal rate - is presented in Figure 5 for the conditions of 8.4 hours, 400 mg/l feed. The method used to calculate the carbon removal rate is contained in Appendix E.

From the experimental schedule, it is noted that 5.6 hour, 800 mg/l was the conditions previous to this run. Thus,

FIGURE 5


TYPICAL RUN
ORGANISM MASS, EFFLUENT CARBON AND UNIT CARBON REMOVAL RATE
VERSUS ELAPSED TIME
Detention Time 8.4 hr
Feed Carbon 400 mg/l
K_c
Organism Mass
Carbon
ORGANISM MASS x 100 MG/L
EFFLUENT CARBON x 10 MG/L
K_c x 0.01 mg/l carbon / mg/l solids-hr
ELAPSED TIME - HRS
0
20
40
60
80
100
120
140
160
180
200
220
1
2
3
4
5
6
7
8
9
10
11
12
13

with an increase in detention time and decrease in feed concentration one would expect a decrease in effluent carbon. However, there was an increase in effluent carbon before equilibrium resulted. This may possibly indicate that a change in influent conditions can stimulate a change in metabolic activity. This change may give adverse results as indicated by the graph.

Since influent conditions are maintained constant for a given run, the terms from equation (3), as previously presented in the theory, can be rearranged to give a linear equation for organism mass, Appendix F. This equation

$$M = \left(-\frac{1}{K_c}\ \frac{Q}{V}\right) C + \frac{1}{K_c}\ \frac{Q}{V}\ C_o$$

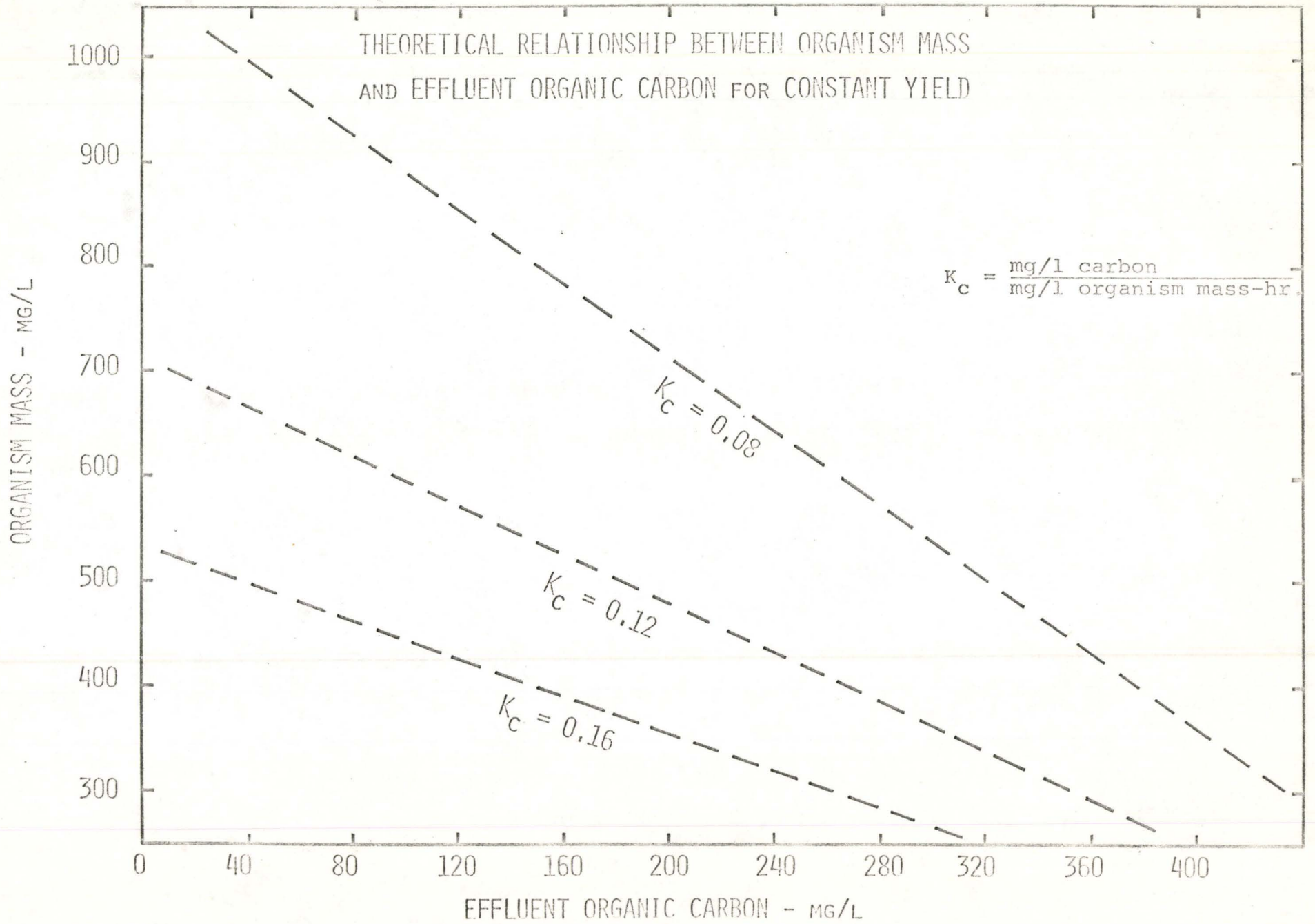
expresses mass (M) as a function of effluent carbon concentration (C) for given experimental conditions (Q and C_o) and rates of unit carbon removal (K_c). The relationship for 7 hours, 600 mg/l feed is graphically presented in Figure 6.

When experimental data is plotted on this graph, a number of qualitative effects can be determined by observation.

(1) When the organism mass increases at the same effluent carbon concentration, then the <u>yield</u> has increased to decrease the unit carbon removal rate.

(2) When the path of change between data points is parallel to the lines of constant carbon removal rate, then some "internal" factor is effecting the rate since the unit rate is not dependent on the "limiting" carbon concentration.

FIGURE 6


THEORETICAL RELATIONSHIP BETWEEN ORGANISM MASS
AND EFFLUENT ORGANIC CARBON FOR CONSTANT YIELD
$K_c = \frac{\text{mg/l carbon}}{\text{mg/l organism mass-hr}}$
$K_c = 0.08$
$K_c = 0.12$
$K_c = 0.16$
ORGANISM MASS - MG/L
1000
900
800
700
600
500
400
300
0
40
80
120
160
200
240
280
320
360
400
EFFLUENT ORGANIC CARBON - MG/L

(3) "Steady state" is when all the data is at one point.

Using the above comments, the results of various runs are considered by observing the data as plotted according to the method described. The first parallel run at 7.0 hours, 600 mg/l feed is presented in Figure 7. The variation in effluent carbon is small compared to the feed carbon concentration. Most of the variation in rate is due to a change in yield with the yield approximately doubling between the extremes.

The results of a second parallel run at these same conditions of detention time and feed concentration are plotted in Figure 8. The variations in effluent carbon and organism mass are a result of "internal" effects since the locus of change is along the lines of constant unit carbon removal rate. In this parallel run, the path of variations for the two reactors was very similar even though the range in the magnitude of the parameters was large. The final effluent carbon concentration and unit carbon removal rate for this run are comparable to the previous run at 7.0 hours, 600 mg/l feed condition.

It was noted that there was considerable foaming of the reactor contents at the higher effluent carbon concentration. This foaming condition is thought to be a result of the organisms present rather than the carbon concentration.

FIGURE 7

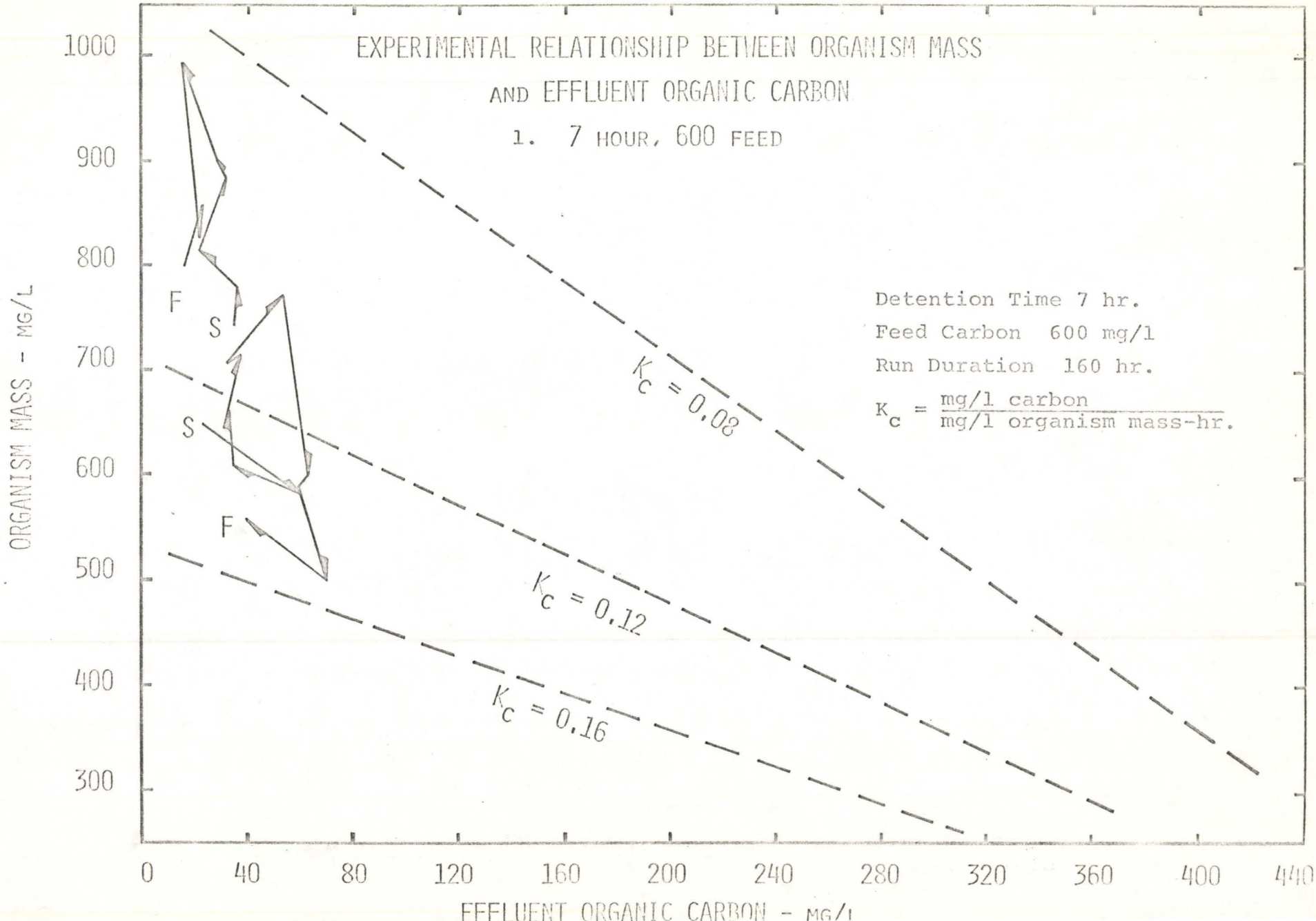
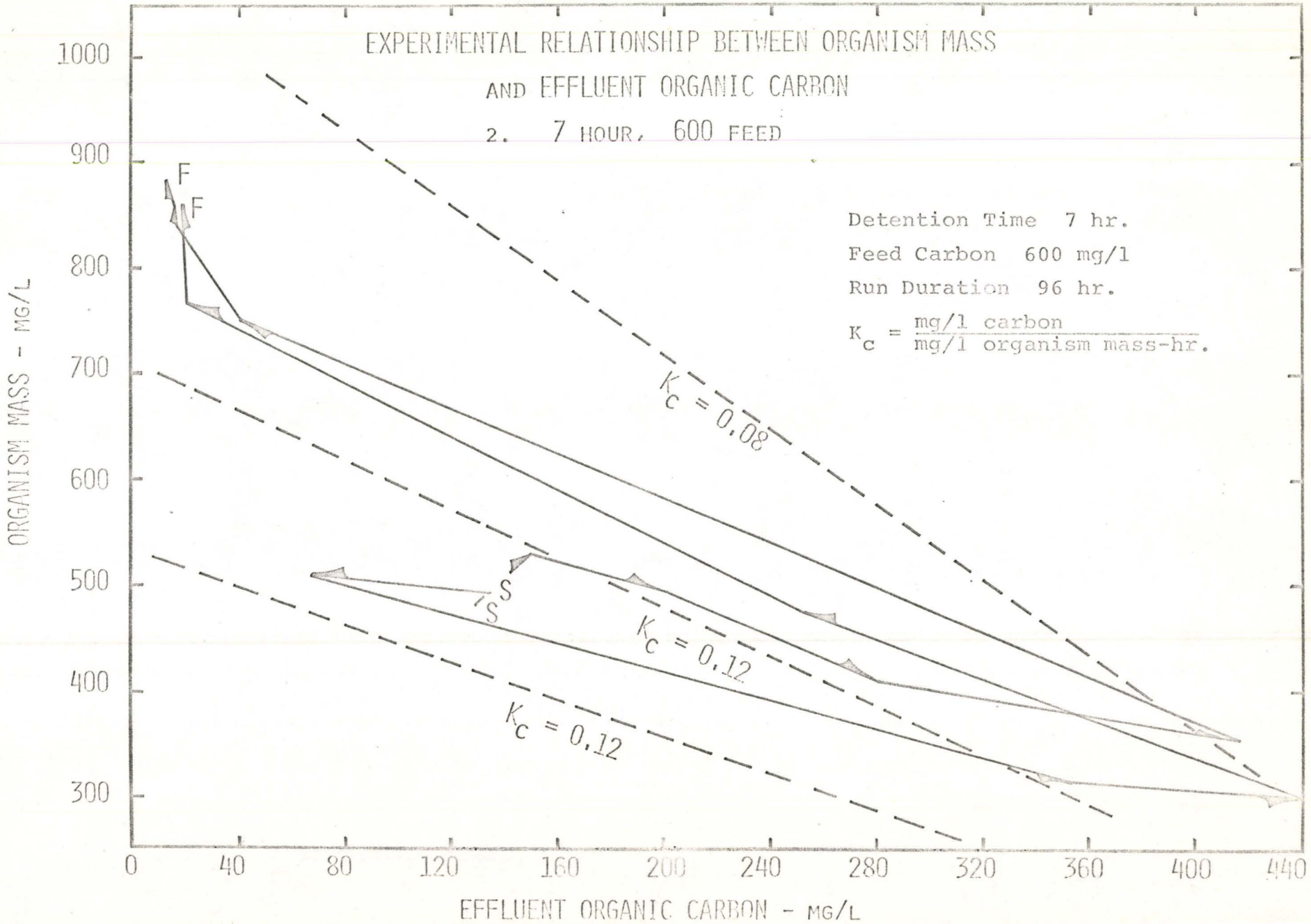

EXPERIMENTAL RELATIONSHIP BETWEEN ORGANISM MASS
AND EFFLUENT ORGANIC CARBON
1. 7 HOUR, 600 FEED
Detention Time 7 hr.
Feed Carbon 600 mg/l
Run Duration 160 hr.
$K_c = \frac{\text{mg/l carbon}}{\text{mg/l organism mass-hr.}}$
$K_c = 0.08$
$K_c = 0.12$
$K_c = 0.16$
F
S
S
F
ORGANISM MASS - MG/L
1000
900
800
700
600
500
400
300
0
40
80
120
160
200
240
280
320
360
400
440
EFFLUENT ORGANIC CARBON - MG/L

FIGURE 8


EXPERIMENTAL RELATIONSHIP BETWEEN ORGANISM MASS
AND EFFLUENT ORGANIC CARBON
2. 7 HOUR, 600 FEED
Detention Time 7 hr.
Feed Carbon 600 mg/l
Run Duration 96 hr.
K_c = mg/l carbon / mg/l organism mass-hr.
$K_c = 0.08$
$K_c = 0.12$
$K_c = 0.12$
F
F
S
S
ORGANISM MASS - MG/L
1000
900
800
700
600
500
400
300
0
40
80
120
160
200
240
280
320
360
400
440
EFFLUENT ORGANIC CARBON - MG/L

Effluent carbon concentrations at 7, 9, 12, and 20.5 hours were very similar. Equally low carbon concentrations were obtained at the 5.6 hour detention time. Thus, detention time seems to have little effect on the effluent carbon concentration over the range studied.

Parallel runs at 8.4 hours but with different feed concentrations, 400 and 800 mg/l, are presented in Figures 9 and 10 respectively. These runs had a duration in excess of 200 hours. In contrast to the parallel runs at 7.0 hours, these runs did not follow a similar locus. This may be a result of differences in feed concentration but is probably due to a change in predominance of a species of organisms. This predominance however, may have occurred because of the difference in feed concentration.

4.3 EFFECT OF PIGMENT PRODUCTION

During early experimentation with the reactors, the culture changed from the tradional yellow-white colour to tan, orange, and red. The intensity of colour and predominance of any one colour varied with time. This culture was discarded and the reactors were reinoculated with a new culture for the study described under Initial Experimentation. During the latter part of the 9 hour, 600 mg/l feed run, "pigment" producing organisms again predominated. A plot of organism mass and effluent organic carbon with elapsed time is presented in Figure 11. It is noted that the effect of the pigment, or else the surviving organisms result in a lesser

FIGURE 9

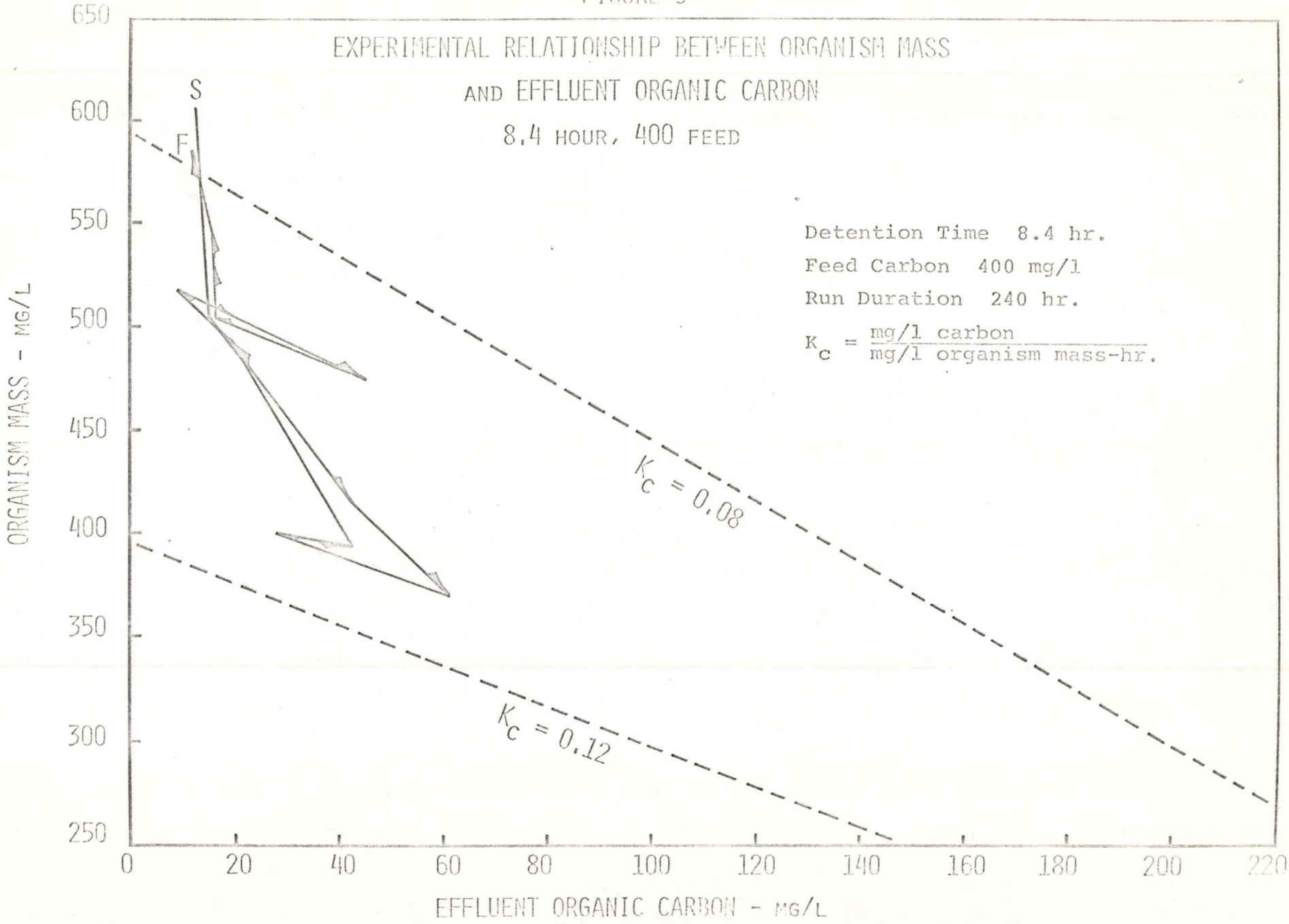

EXPERIMENTAL RELATIONSHIP BETWEEN ORGANISM MASS
AND EFFLUENT ORGANIC CARBON
8.4 HOUR, 400 FEED
S
F
Detention Time 8.4 hr.
Feed Carbon 400 mg/l
Run Duration 240 hr.
$K_c = \frac{\text{mg/l carbon}}{\text{mg/l organism mass-hr.}}$
$K_c = 0.08$
$K_c = 0.12$
ORGANISM MASS - MG/L
650
600
550
500
450
400
350
300
250
0
20
40
60
80
100
120
140
160
180
200
220
EFFLUENT ORGANIC CARBON - MG/L

FIGURE 10

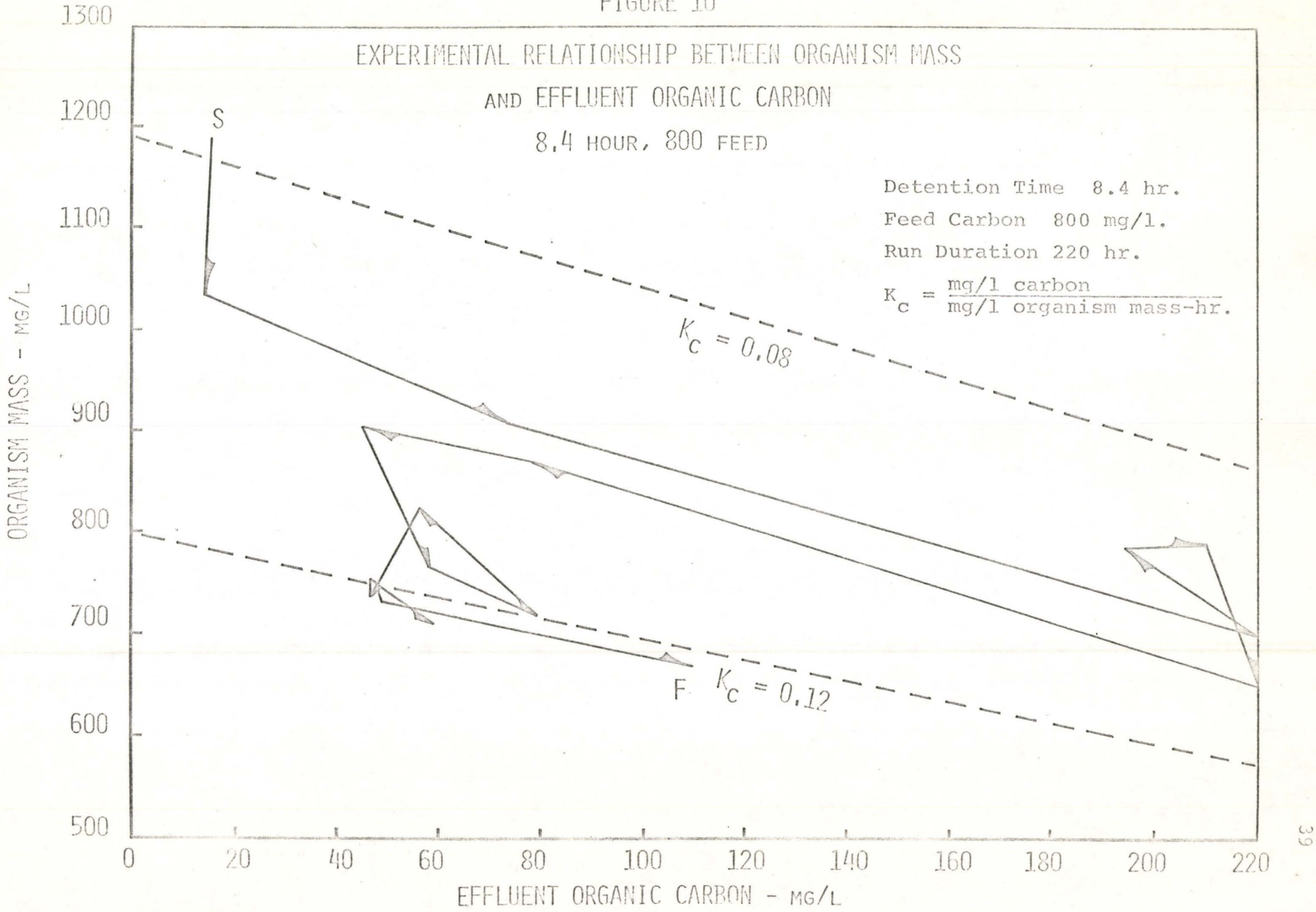
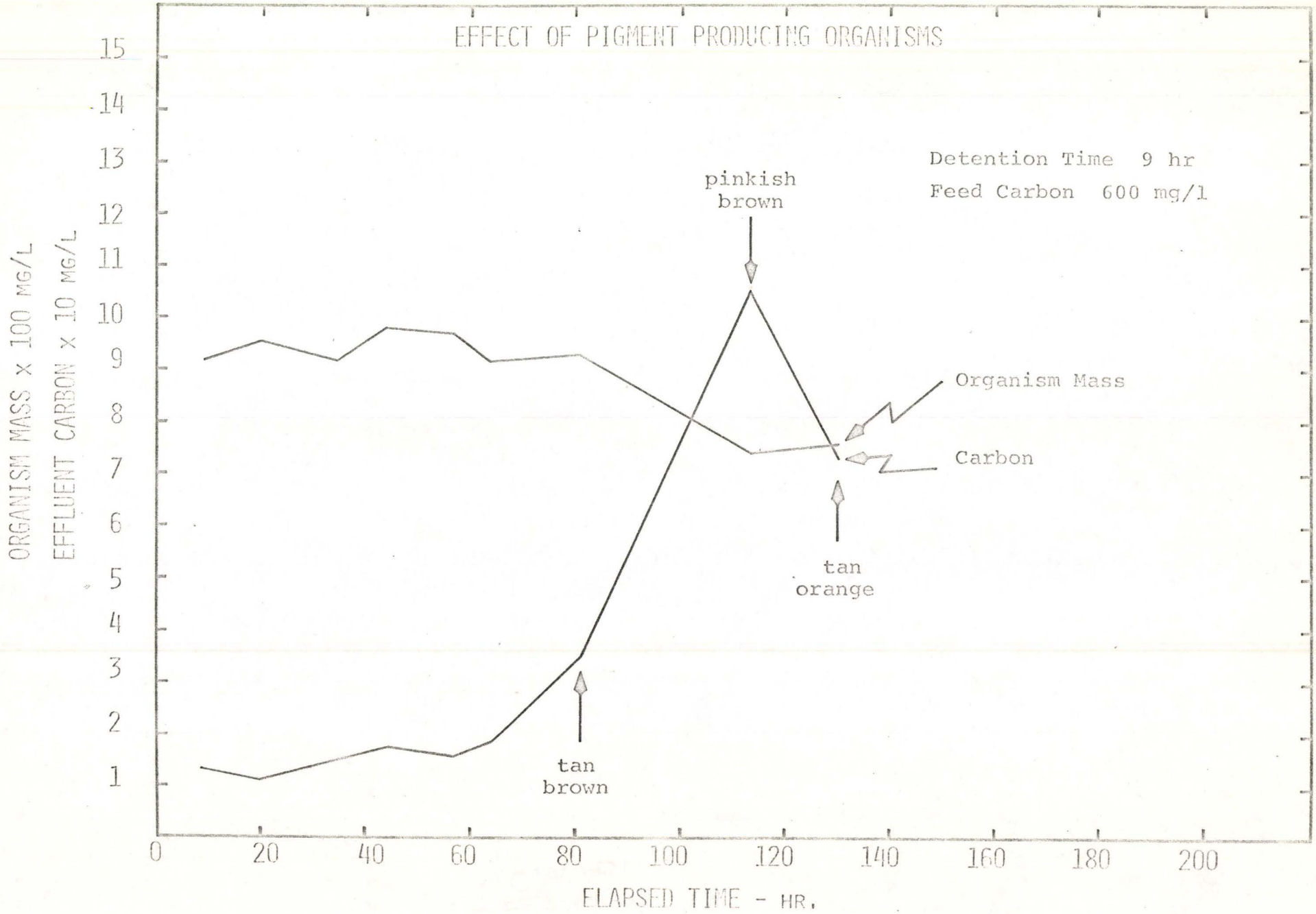

EXPERIMENTAL RELATIONSHIP BETWEEN ORGANISM MASS
AND EFFLUENT ORGANIC CARBON
8.4 HOUR, 800 FEED
Detention Time 8.4 hr.
Feed Carbon 800 mg/l.
Run Duration 220 hr.
$K_c = \frac{\text{mg/l carbon}}{\text{mg/l organism mass-hr.}}$
S
$K_c = 0.08$
F
$K_c = 0.12$
ORGANISM MASS - MG/L
EFFLUENT ORGANIC CARBON - MG/L
1300
1200
1100
1000
900
800
700
600
500
0
20
40
60
80
100
120
140
160
180
200
220

FIGURE 11


EFFECT OF PIGMENT PRODUCING ORGANISMS
Detention Time 9 hr
Feed Carbon 600 mg/l
pinkish brown
Organism Mass
Carbon
tan orange
tan brown
ORGANISM MASS x 100 MG/L
EFFLUENT CARBON x 10 MG/L
ELAPSED TIME - HR.
0
20
40
60
80
100
120
140
160
180
200
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

carbon reduction.

A review of the literature (Kluyver 1956) indicated that these pigment producing organisms are a rare Pseudomonad species known as Pseudomonas aureofaciens. It is noted that pigment produced from these organisms was water-soluble. The work of Cassell, Sulzer and Lamb (1966) also indicated the production of various colours; however, these pigments were only alcohol-soluble.

4.4 MICROSCOPIC OBSERVATIONS

Sufficient information was not obtained from microscopic studies to determine any trend in predominance of organisms. It is thought that changes in predominance of the species may cause the variations in yield and effluent organic carbon.

The organims were mainly dispersed rather than in clumps of floc. Variation in results of organism mass and effluent organic carbon did not seem related to the amount of dispersion of the organisms.

CHAPTER 5

DISCUSSION

5.1 UNIT GROWTH RATE CONCEPT

The work reported by Monod (1949), based on batch studies, gave a functional relationship between the unit growth rate and the limiting nutrient concentration (carbon in this study). The relationship in the form of a hyperbolic equation stated that there was a maximum growth rate when the limiting nutrient was no longer limiting. When the nutrient was limiting, the unit growth rate was controlled by this limiting concentration. The hyperbolic form was based on a correlation rather than some postulated mechanisms as used in chemical reactions.

When equations were developed to express the overall reactions in a completely-mixed reactor, a unit growth rate term was used. These equations when solved for the steady state condition indicated that the unit growth rate was equal to the reciprocal of the detention time.

Experimental work, aimed to determine the relationship for the growth rate as a function of the limiting nutrient, was then performed by setting the detention, which gave the growth rate, and evaluating the limiting nutrient which resulted. This was the method used by Garrett and Sawyer (1952), for mixed culture, and by Herbert et al (1956) and Schulze (1964) for pure cultures.

The results of these experimentors gave basically a linear relationship for unit growth at low nutrient levels

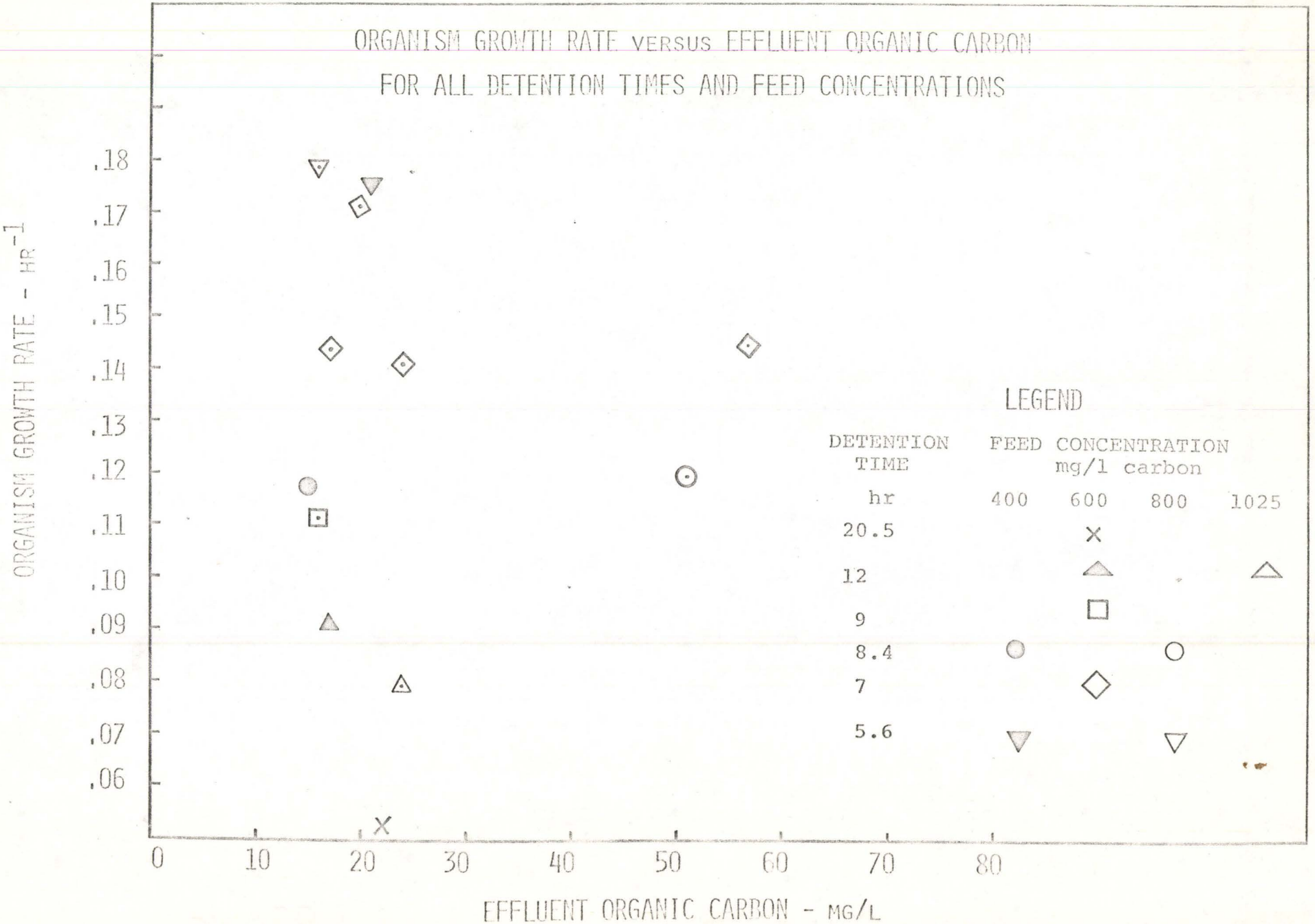
evaluated at steady state. For studies conducted to short detention times (one hour) the nutrient concentration increased in a hyperbolic manner.

The results of this study, as presented in Figure 12, suggest that there is no specific relationship for the conditions tested. Although a range of 5.6 to 20.5 hours was studied, which gave a respective range in growth rates from 0.179 to 0.053 hours^{-1}, the effluent carbon remained essentially constant. High effluent carbon concentrations were more a result of adverse conditions for organism growth than the feed carbon concentration.

If the bacterial growth was controlled as a result of a limiting nutrient concentration, then mass transfer theory should explain the results. With an increased concentration of limiting nutrient, the transfer of nutrient would increase permitting a higher growth rate. This statement describes the results from most studies. The results of this study indicated the growth rate to be independent of the effluent carbon (limiting nutrient). Therefore, it was not the mass transfer rate of carbon which controlled the reaction, but some other factors.

Since the effluent carbon is basically constant with growth rate (except for two conditions) this suggests that there will always be a residual carbon concentration (approximately) 15 - 20 mg/l carbon for this study). Until the detention time is less than 5.6 hours, there is sufficient time to reduce all available carbon (except for the two "adverse" conditions). The fact that growth rate increases is a result of it being equal to

FIGURE 12


ORGANISM GROWTH RATE VERSUS EFFLUENT ORGANIC CARBON
FOR ALL DETENTION TIMES AND FEED CONCENTRATIONS
ORGANISM GROWTH RATE - HR-1
.18
.17
.16
.15
.14
.13
.12
.11
.10
.09
.08
.07
.06
0
10
20
30
40
50
60
70
80
EFFLUENT ORGANIC CARBON - MG/L
LEGEND
DETENTION TIME
hr
FEED CONCENTRATION mg/l carbon
400
600
800
1025
20.5
12
9
8.4
7
5.6

the reciprocal of the detention time at steady state. As the detention time is shortened to less than 5.6 hours, a point will be reached where time does not permit organisms to be reproduced in sufficient quantities to reduce the carbon to the residual level. This condition may be controlled by the generation time of the organisms, by the limitations of transfer of nutrients, or possibly both.

5.2 DEPENDENCE OF EFFLUENT NUTRIENT (CARBON) CONCENTRATION.

The effluent nutrient, as discussed under "Unit Growth Rate", is basically controlled by the flow rate (detention time) provided conditions are satisfactory for gorwth. The concentration of nutrient being fed, however, only affects the effluent nutrient until conditions come to equilibrium. At equilibrium the effluent nutrient will be dictated by the detention time, provided the nutrient concentration is above some residual level. The effect caused by various feed concentrations is to change the mass of organisms to a number sufficient to reduce the nutrient to a concentration specified by the detention time. These statements are stipulated by the equations presented in the Theoretical Development, Section 2.1.

This dependence of effluent nutrient and organism mass on flow rate and feed concentration is in agreement with the unit rate concept. A unit rate is determined by the flow rate. The organism mass then adjusts until the mass acting at some unit rate can decrease the nutrient feed to the concentration

determined by the detention time.

Although there are many studies which report the relationship of effluent nutrient concentration with detention time, the effects of various feed concentrations is very limited. The results of this study, using the data as plotted in Figure 12, indicate that the effluent concentration is not dependent on the feed concentration. This is in agreement with the theory, The effect of detention time, as discussed previously, was not significant over the range studied.

5.3 CONCEPT OF YIELD

The yield factor Y is given as the ratio of the weight of organism mass produced to the weight of nutrient removed. The importance of knowing and optimizing the yield has been emphasized with industrial fermentation processes. In waste treatment a minimum yield is desirable since this results in lower costs for solids disposal. Experimentation using pure cultures has resulted in smooth curves while those with mixed cultures have shown considerable variation in results.

Herbert et al (1956) found the yield to decrease with a decrease in detention time as did the work reported by Schulze (1964). Both studies were with pure cultures. Other pure cultures studies such as Contois (1959) which observed the yield to be independent of detention time or Martin and Washington (1965) which reported a maximum

yield, indicate a non-uniformity of effects.

Mixed culture studies have been fewer in number. The "highly controlled" study by Rao and Gaudy (1965) found a range of yields from 48 to 82 per cent. The results of Reynolds and Yang (1966) indicated a straight line relationship for yield with detention time. Genetelli and Heukelekian (1964) felt that sludge yields for one substrate were constant regardless of loading. All their studies were done at a detention of six hours.

Reported studies indicate a number of relationships to seemingly exist. In considering what may effect the yield, different approaches can be followed. If the reaction was strictly a chemical reaction a stoichiometric relationship would exist to give a constant yield independent of detention time.

With a biological system, it was stated by Hetling, Washington and Rao (1964) that the yield will vary "depending on the metabolic pathway by which the substrate is degraded". This statement appears fundamentally correct for more or less energy is produced depending on the series of reactions from reactant to product. The species of organism and enzymes it uses may further effect the yield.

Rao and Gaudy (1965) considered it a gross simplification to expect the cell yield to be solely a function of the structure of the substrate or the free energy of the substrate. This "thermodynamic" approach by correlating free energy to yield pre-concludes that only one metabolic pathway will be

followed; that is, the work done in reducing the substrate is only related to the free energy (a state function) by following one path of reactions.

Relating yield with detention time may give a unique correlation because of a "sorting" process due to detention time. As the detention time is decreased, organisms of short generation time will be in an advantageous situation. These organisms may have a unique reaction path for growth, giving a different yield. There may also be an influence due to an increase in concentration of limiting nutrient or decrease of organism mass permitting a less restricted environment for growth.

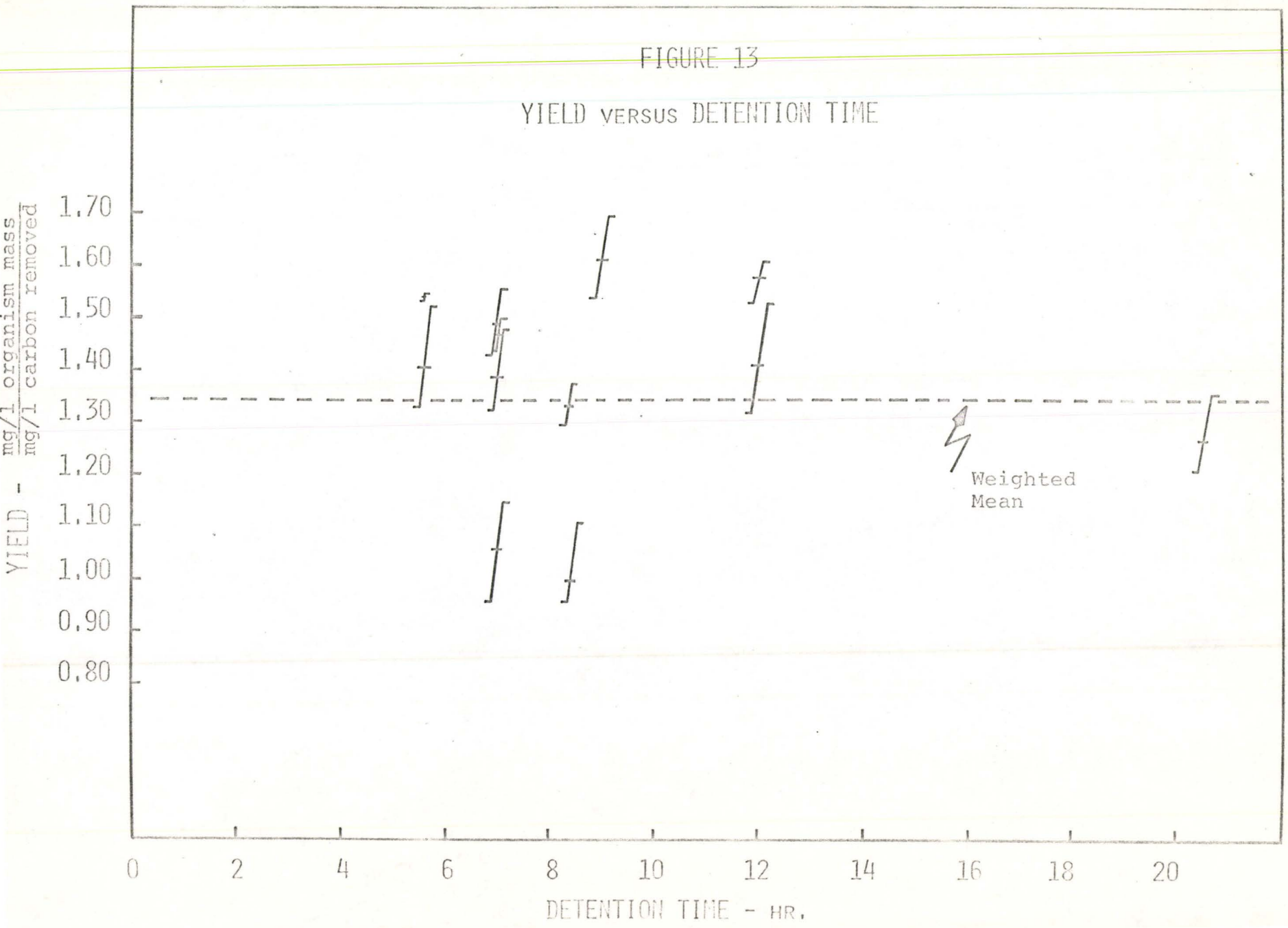
A graphical representation of the yields with detention time for this study is presented in Figure 13. There is considerable variation of yield both at one detention time and with detention time. These variations suggest that conditions within the reactor, i.e. organisms, have more influence in determining the yield of a mixed culture than a controlled variable such as detention time. Experimentation at two different feed concentrations at three detention times gave no trend to suggest that yield was dependent on feed concentration.

5.4 MASS OF NUTRIENT REMOVAL PER UNIT VOLUME OF REACTOR

In formulating equations to describe the overall process in the reactor, mass balances were used for organism mass and nutrients. The mass of nutrients removed involved both the flow rate and the feed concentration. Stack and

FIGURE 13

YIELD VERSUS DETENTION TIME



Conway (1959) in studying the degradation of a synthetic dextrose waste stated that as the detention period was shortened the amount of work accomplished per unit volume of aeration capacity increased. In this study, it has been noted that the same low effluent carbon concentration could be obtained for short as well as for longer detention times even at the same feed concentration. This means that the mass of nutrient (carbon) removal has increased per unit volume of reactor since the detention time has decreased.

This effect may be partially explained by repeating a previous statement, that only when the detention period is sufficiently short to affect the effluent nutrient will there be a limiting condition possible such as to make the mass of nutrient removal constant as the detention time is decreased. In this work as well as that of Stack and Conway, a condition of this limiting nature was not experienced.

5.5 STEADY STATE CONDITION

Steady state for a continuous flow system has been reported to be both obtainable and unobtainable with the majority of studies using pure cultures of organism achieving the steady state condition. The longest run by Busch and Myrick (1960) was for 103 days; during this period no equilibrium could be attained. Cassell, Sulzer and Lamb (1966), with runs from 40 to 101 days in length, observed that all parameters which reflected biological activity fluctuated continuously. All of these studies were with mixed cultures. The

results of this study have also exhibited fluctuations in the parameters measured. Not all runs had the same extremes in fluctuations and most runs had some period (30 hours or more) during which the variation was slight. However, if the run was performed for a sufficient period, it is felt that pronounced fluctuation would occur.

The population dynamics in the work by Cassell, Sulzer and Lamb (1966) was indicated by the colour of alcohol-soluble pigments. Four different pigments were observed during the study and each exhibited a characteristic absorption peak on a spectrophotometer. The absorbance peaks of a culture were compared to each different pigment absorption to estimate the concentration of each pigment. From quantitative analyses of pigment composition, it was indicated that at all detention times (4.5 to 76.5 hours) the pigment concentrations was subject to daily fluctuation. Two different types of fluctuations occurred: one or two pigments predominated continuously, but fluctuated in concentrations from day to day; and several pigments appeared and disappeared in irregular fashion, in addition to showing fluctuations in concentration. They also noticed that detention time was a selective factor in determining the dominant pigment. At shorter detention times certain pigments were not observed.

Fluctuations in biological parameters (absorption and microscopic analysis) were largely non-random in time.

It was assumed, therefore, that the fluctuations were the result of various microbial interactions occurring in mixed cultures. The fluctuations in performance parameters (organism mass and soluble COD) were less pronounced; however, the performance was closely associated with the behaviour of the microbial populations.

In the work of Cassell, Sulzer and Lamb, the dynamics which were evident, were considered to occur due to microbial species competing with each other for the available nutrients. Various species had advantageous conditions during the run and thus predominated. The certain species that predominated may have been determined by predatory bacteria which holds the populations of organisms in check.

Shilo (1966) provided a summary of the discovery of the Bdellovibrio bacteria (1962) and its various characteristics. Bdellovibrio bacteria are totally dependent for their existence on other bacterial species, of which they are parasites. The bacteria can attack and lyse (dissolve) other species of bacteria and have a world-wide distribution. They are present in soil, sea water and especially prevalent in sewage. One very unique property is that they are active against certain groups of bacteria and completely inert against others. Also, Bdellovibrio bacteria can exist for several months in a host-free media.

The time required to complete a parasitic cycle from attacking a host to lysing of the host, depends on the previous

activity of the parasite. The parasitic cycle has been reported by Starr and Baigent (1966) to be as short as 5 hours. Therefore, in the population dynamics of this study, Bdellovibrio bacteria may have been responsible for the fluctuations. The parasite could attack a predominant species causing a cessation of growth, which would result in an increase in effluent carbon until other bacterial species predominated. During other periods of a run, no specific parasite would be available to attack the predominant bacteria and thus a period of relatively "steady state" would result.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

(a) The unit growth rate of organism was independent of (i) feed carbon concentration

(ii) effluent carbon concentration

over the range of detention and feed carbon concentrations. This unit growth rate was during a "steady-state" condition.

(b) The yield of organisms did not change in any specific manner to indicate a dependence of yield on

(i) flow rate

(ii) feed carbon concentration

(iii) effluent carbon concentration

The variation in values at one experimental condition could be greater than the variation between two different conditions.

(c) The effluent carbon concentration was independent of (i) flow

(ii) feed carbon concentration

for the conditions studied. This effluent carbon concentration was during a "steady-state" condition. Effluent concentration, when greater than normal, were a result of conditions within the biological system rather than experimentally controlled conditions.

(d) The mass of carbon reduced per unit volume of reactor increased as the detention time decreased for the same feed carbon concentration. This was a result of the effluent carbon concentration not being limited for the detention times studied.

(e) The condition of "steady-state" was approached during some experimental conditions. Fluctuations which occurred could result from

(i) variations in the predominance of species competing for existence

(ii) predominant species being attacked by parasitic bacteria, i.e. Bdellovibrio, causing changes in the predominant species.

6.2 RECOMMENDATIONS

(a) This study has indicated that in order to comprehensively study a mixed biological system, it is necessary to know as early as possible, when fluctuations are occurring. It is, therefore, recommended that some technique be developed whereby a rapid measurement will determine a change in composition of the biological population. A spectophometric technique as used by Cassell, Sulzer and Lamb may be suitable. The continuous monitoring of pH should also be considered.

(b) During periods of fluctuation, the carbon reduction was a function of biological mass and was independent of external variables (flow rate, feed concentration). To study this biological mass it is recommended that techniques be developed to determine

(i) types of organisms present during fluctuations

(ii) changes in predominance of organisms

(iii) possible effect of parasitic bacteria

(c) Since glucose is rapidly assimilated by organisms, further studies to determine the effect of flow rate on effluent carbon concentration should be confined to short detention times (less than 5.6 hours).

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APPENDIX A

DIFFERENCES IN WEIGHT FOR DRYING AT 45°C AND 103°C

The membrane filter paper was dried and weighed at both 45°C and 103°C. These are the results:

filter paper after 45°C (grams)	filter paper after 103°C for 70 min. (grams)	difference (grams)
1.4506	1.4503	0.0003
1.4596	1.4596	0.0000
1.43105	1.4310	0.00005
1.4829	1.4828	0.0001
1.45925	1.4592	0.00005
1.4524	1.45235	0.00005
1.47315	1.4730	0.00015
1.4714	1.47125	0.00015
	Average difference	0.00016 gm.

After filtrations, 12 filters were dried and weighed at both 45°C and 103°C. These are the results:

Sample	(1) Filter paper + solids after 45°C (grams)	(2) Filter paper + solids after 103°C (grams)	(1) - (2) y'	100000 ((1) - (2) -.00016) y
1	1.47105	1.4709	0.00015	-1
2	1.4619	1.4617	0.00020	4
3	1.4469	1.4467	0.00020	4
4	1.4559	1.4556	0.00030	14
5	1.45865	1.45835	0.00030	14
6	1.4526	1.4524	0.00020	4
7	1.46455	1.4643	0.00025	9
8	1.47695	1.47675	0.00020	4
9	1.46635	1.4662	0.00015	-1
10	1.4547	1.4544	0.00030	14
11	1.4699	1.4697	0.00020	4
12	1.4686	1.46845	0.00015	-1

$n = 12$ $\quad \Sigma y^2 = 752$ $\quad s^2(y) = 24.240$

$\Sigma y = 68$ $\quad \Sigma' y^2 = 752 - 5.667 \times 68$ $\quad s^2(\bar{y}) = 2.020$

$\bar{y} = 5.667$ $\quad = 266.644$ $\quad s(\bar{y}) = 1.422$

$\bar{y}' = 5.667 \times 10^{-5}$ $\quad s(y') = 1.422 \times 10^{-5}$

The variation at the 99 per cent confidence level is $\pm 3.16 \times 1.422 \times 10^{-5} = \pm 4.38 \times 10^{-5}$. The average weight of the 12 samples used was 0.00697 grams. Therefore, a decrease in weight 0.81 per cent for 103°C conditions, plus or minus 0.63 per cent at a 99 per cent confidence level, Volk (1958).

APPENDIX B

NUTRIENT MEDIA

For adequate nutrition of bacteria a C:N:P ratio of 40:5:1 is sufficient (Eckenfelder and O'Connor, 1961). By ensuring that nitrogen and phosphorus in any nutrient medium are far in excess of this requirement, carbon is made the limiting nutrient.

Dibasic ammonium phosphate, $(NH_4)_2HPO_4$, was used to provide a source of nitrogen and phosphorous, and dextrose, $C_6H_{12}O_6$, was used as the organic carbon source. Dextrose has been reported to be used by all bacteria (Kendall, 1928). By mixing dextrose and ammonium phosphate in a 3:1 ratio by weight ensured that the carbon was in a limiting concentration. This solution was made up in distilled water to form the feed stream.

The following stock solutions were used to provide iron, potassium, and magnesium nutrients which were not in sufficient concentrations in the tap water.

(1) Ferric chloride, $FeCl_3 \cdot 6H_2O$

solution concentration 1.0 mg/ml.

(2) Potassium phosphate, K_2HPO_4

solution concentration 50 mg/ml.

(3) Magnesium sulphate, $MgSO_4 \cdot 7H_2O$

solution concentration 50 mg/ml.

These solutions were proportioned to the dextrose-ammonium phosphate feed according to the following arbitrary formulae.

(1) 1/2 ml $FeCl_3$ solution/200 mg carbon/litre of feed

(2) 1 ml K_2HPO_4 solution/200 mg carbon/litre of feed

(3) 1 ml $MgSO_4$ solution/200 mg carbon/litre of feed

These chemicals were added to tap water to form the dilution water stream. The ratio of flow of the dilution water stream to the feed stream was at least 10:1.

The required concentration of carbon in the feed stream was calculated based on a total flow of liquid. When measuring flow rates and carbon concentration of the inlet stream, determinations were made on the liquid mixture rather than the individual solutions.

APPENDIX C

CHEMICAL ANALYSIS OF TAP WATER

The following is a list of analyses results of a sample of tap water used to make up the dilution water.

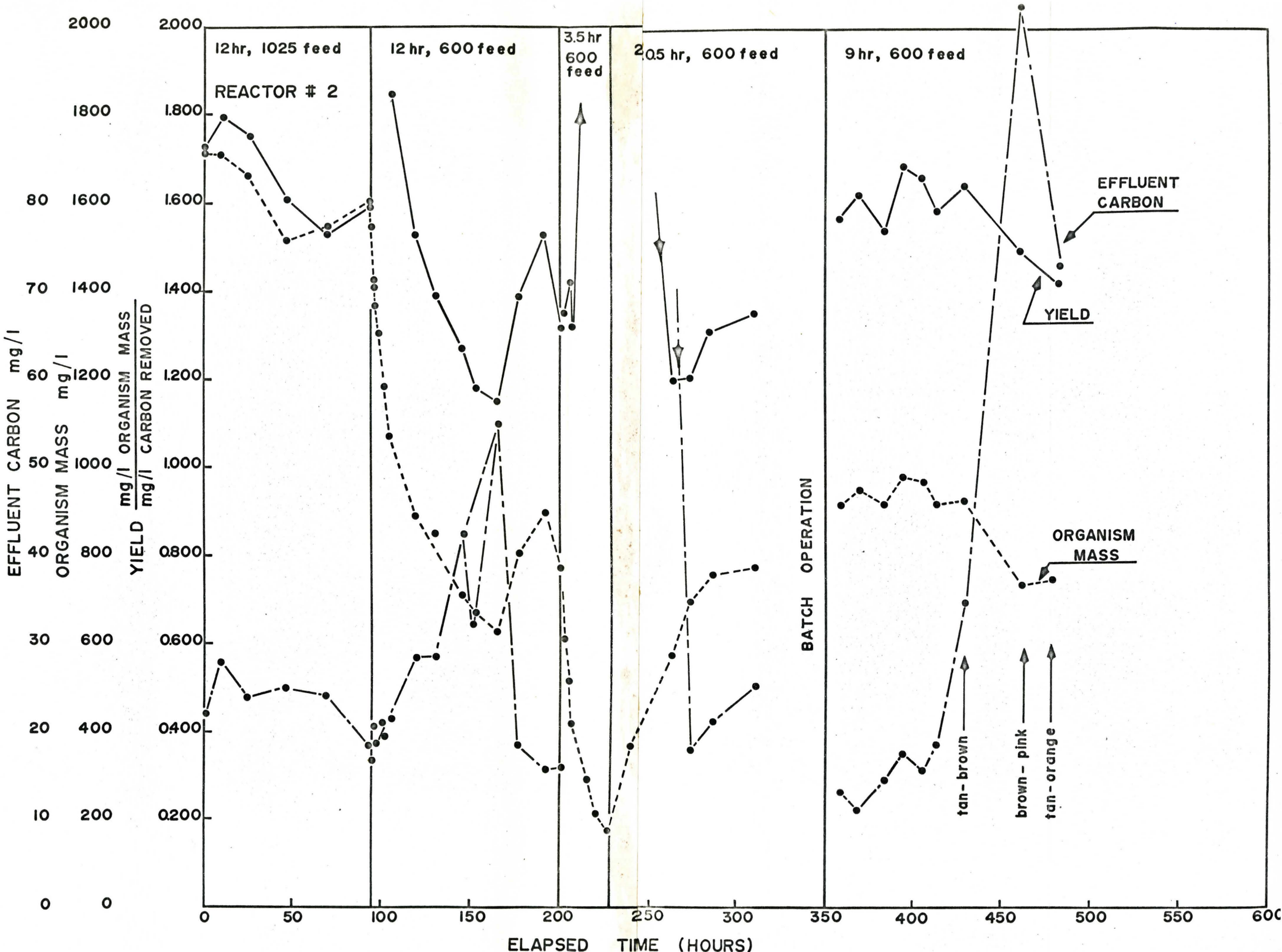
Hardness as $CaCO_3$	142 ppm
Alkalinity as $CaCO_3$	93 ppm
Iron as Fe	0.08 ppm
Potassium as K	1.6 ppm
Magnesium as Mg	16 ppm
Calcium as Ca	30 ppm
Sodium as Na	13 ppm
Sulphate as SO_4	28 ppm
Chloride as Cl	29 ppm
Fluoride as F	0.1 ppm

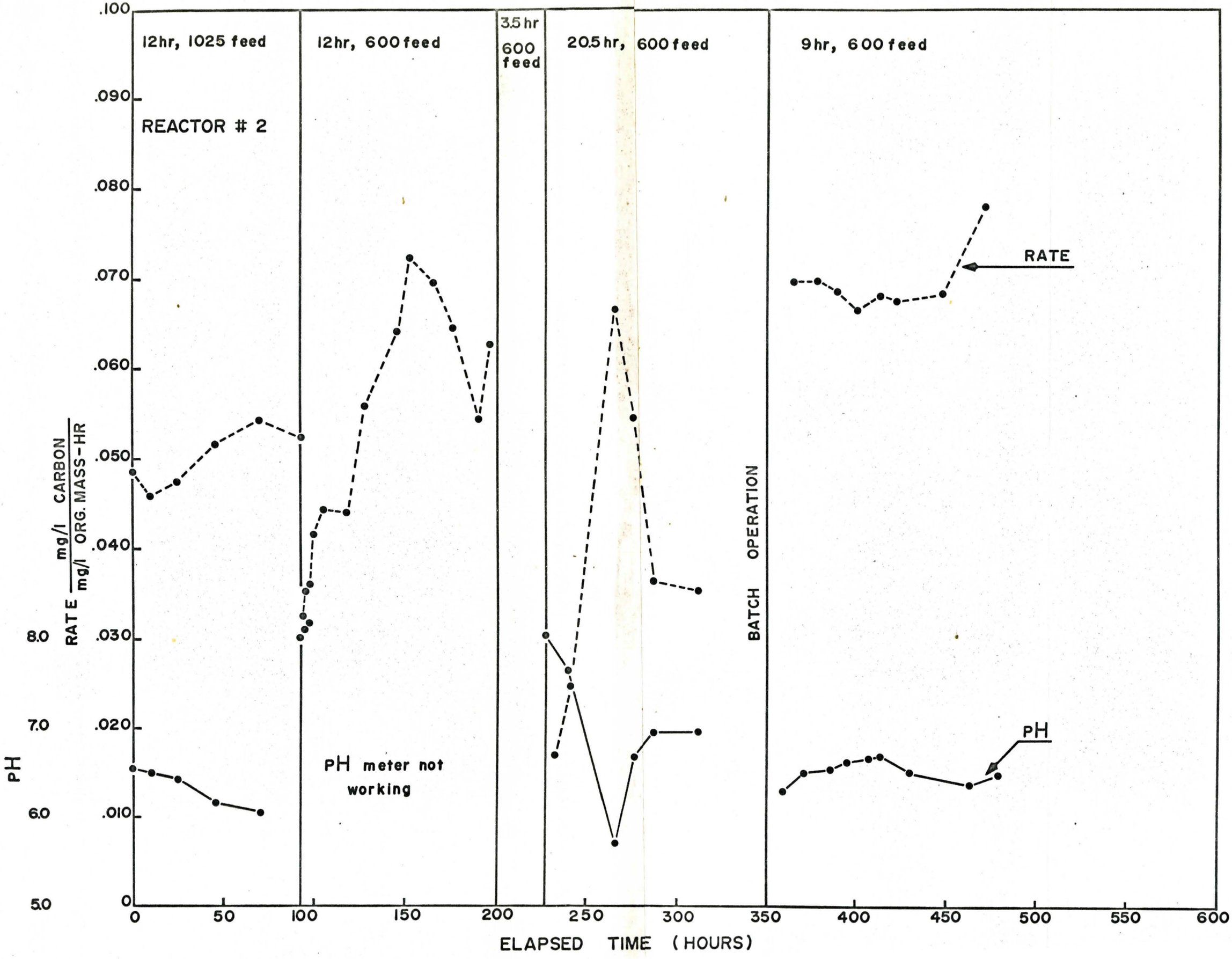
APPENDIX D

RESULTS - TABULATED AND PLOTTED

Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate $\frac{\text{mg/l carbon}}{\text{mg/l mass-hr.}}$	Yield $\frac{\text{mg/l mass}}{\text{mg/l carbon}}$
12 hour detention, 1025 mg/l carbon feed REACTOR # 2						
0	1725	22	6.55		0.048	1.72
9	1710	28	6.52		0.046	1.80
24	1665	24	6.41		0.048	1.75
47	1515	25	6.17		0.052	1.61
70	1545	24	6.04		0.054	1.54
93	1600	19	——		0.052	1.59
12 hour detention, 600 mg/l carbon feed REACTOR #2						
0	1600	19	——		0.030	2.75
1	1545	17	——		0.033	2.65
2	1420	21	——		0.031	2.46
3	1410	19	——		0.036	2.43
4	1365	23	——		0.032	2.37
6	1300	21	——		0.036	2.25
8	1180	20	——		0.042	2.04
13	1070	22	——		0.045	1.85
26	890	29	——		0.044	1.53
37	850	29	——		0.056	1.49
52	710	43	——		0.064	1.27
60	670	32	——		0.072	1.18
73	625	55	——		0.070	1.15
84	805	19	——		0.064	1.39
97	895	16	——		0.054	1.53
104	770	16	——		0.063	1.32

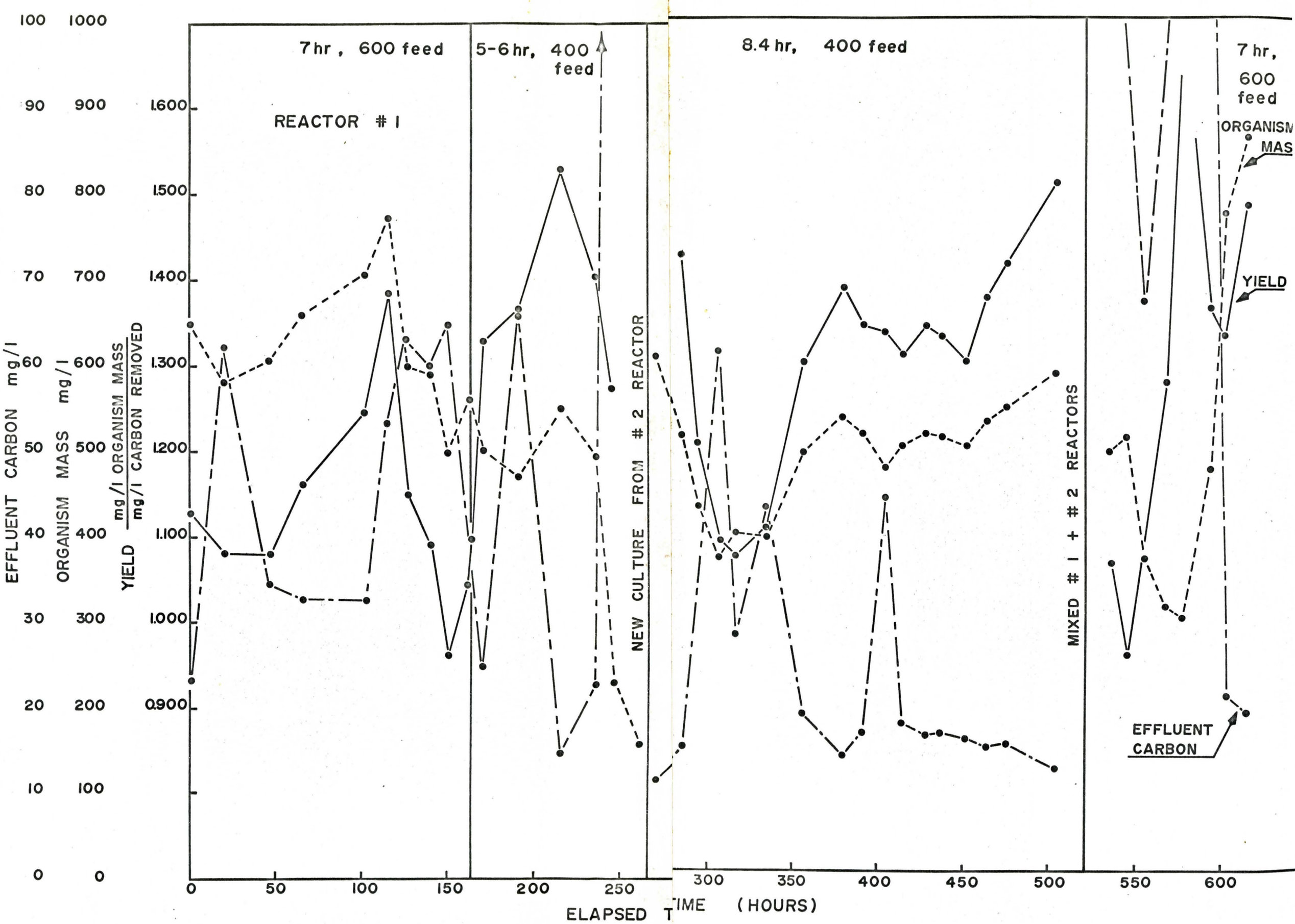
Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate mg/l carbon / mg/l mass-hr.	Yield mg/l mass / mg/l carbon
3.5 hour detention, 600 mg/l carbon feed REACTOR # 2						
2	607	148	6.94		0.115	1.35
4	515	237	7.22		0.116	1.42
6	415	272	7.43		0.174	1.32
16	290	521	7.99		-0.033	5.18
21	210	536	7.95		0.017	9.13
28	172	538	8.05		0.135	2.78
20.5 hour detention, 600 mg/l carbon feed REACTOR # 2						
0	170	538	8.05		———	2.74
13	385	486	7.67		0.025	3.38
37	580	119	5.70		0.067	1.20
47	705	18	6.69		0.055	1.21
60	760	22	6.95		0.037	1.31
85	780	26	6.96		0.036	1.36
9.0 hour detention, 600 mg/l carbon feed REACTOR # 2						
10	920	13	6.30		0.070	1.57
20	957	11	6.50		0.072	1.62
36	925	15	6.56		0.069	1.54
44	985	18	6.65		0.071	1.69
58	970	16	6.69		0.065	1.66
64	920	19	6.70		0.068	1.58
81	930	35	6.52		0.070	1.64
113	740	105	6.38		0.080	1.50
130	750	73	6.48		0.078	1.42


12hr, 1025 feed
REACTOR # 2
12 hr, 600 feed
3.5 hr 600 feed
2 0.5 hr, 600 feed
9 hr, 600 feed
EFFLUENT CARBON
YIELD
ORGANISM MASS
BATCH OPERATION
tan-brown
brown-pink
tan-orange
EFFLUENT CARBON mg/l
ORGANISM MASS mg/l
YIELD mg/l / mg/l ORGANISM MASS / CARBON REMOVED
ELAPSED TIME (HOURS)
0
50
100
150
200
250
300
350
400
450
500
550
600
2000
1800
1600
1400
1200
1000
800
600
400
200
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
80
70
60
50
40
30
20
10


12hr, 1025 feed
12hr, 600 feed
3.5 hr
600
feed
20.5 hr, 600 feed
9hr, 600 feed
REACTOR # 2
RATE
pH
pH meter not
working
BATCH OPERATION
RATE mg/l CARBON / mg/l ORG. MASS-HR
pH
ELAPSED TIME (HOURS)
.100
.090
.080
.070
.060
.050
.040
.030
.020
.010
0
8.0
7.0
6.0
5.0
0
50
100
150
200
250
300
350
400
450
500
550
600

Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate $\frac{\text{mg/l carbon}}{\text{mg/l mass-hr.}}$	Yield $\frac{\text{mg/l mass}}{\text{mg/l carbon}}$
1. 7.0 hour detention, 600 mg/l carbon feed REACTOR # 1						
0	650	24	6.00	—	—	1.13
21	582	62	5.60	—	0.129	1.08
47	608	35	6.32	—	0.134	1.08
68	660	33	6.35	—	0.123	1.16
92	715	38	6.61	26	0.092	1.25
102	708	32	6.38	25.5	0.115	1.25
116	772	54	6.13	27	0.101	1.39
128	600	63	6.13	26.5	0.122	1.15
140	590	60	6.11	—	0.131	1.09
150	500	65	5.90	26.5	0.148	0.96
162	560	40	6.29	—	0.140	1.05
5.6 hour detention, 400 mg/l carbon feed REACTOR # 1						
6	500	25	6.54	27	0.130	1.33
27	470	66	—	—	0.122	1.37
52	550	15	6.74	25	0.121	1.53
72	495	23	6.73	23	0.126	1.41
82	230	220	7.48	25	0.054	1.29
96	160	—	7.73	23.5	—	—

Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate mg/l carbon / mg/l mass-hr.	Yield mg/l mass / mg/l carbon
	8.4 hour detention, 400 mg/l carbon feed REACTOR # 1					
5	612	12	6.71	25.5	0.079	1.58
20	513	15	6.71	23.5	0.083	1.42
30	430	43	6.73	25.5	0.092	1.20
43	370	61	6.60	24	0.103	1.09
54	400	28	6.54	26	0.103	1.08
70	395	43	6.62	24.5	0.105	1.11
92	495	19	6.72	———	0.089	1.30
116	535	14	6.77	24.5	0.087	1.38
127	515	16	6.89	26.5	0.088	1.34
140	475	44	6.54	25.5	0.084	1.34
151	500	18	6.91	———	0.086	1.31
164	515	16	6.88	———	0.088	1.34
174	510	16	6.88	27.5	0.089	1.33
188	500	16	6.86	———	0.091	1.30
199	530	15	6.85	26.5	0.086	1.38
212	545	15	6.75	24.5	0.084	1.41
240	585	12	6.56	24	0.079	1.51
2.	7.0 hour detention, 600 mg/l carbon feed REACTOR # 1					
14	495	135	6.00	23	———	1.06
24	510	67	6.14	23.5	0.162	0.96
34	370	———	6.92	———	———	———
48	315	353	7.19	24	0.074	1.28
58	300	452	7.34	23	0.038	2.01
74	475	251	5.30	22	0.132	1.36
82	770	21	6.32	23	0.142	1.33
96	860	19	6.37	23.5	0.096	1.48


7 hr, 600 feed
5-6 hr, 400 feed
8.4 hr, 400 feed
7 hr, 600 feed
REACTOR #1
NEW CULTURE FROM #2 REACTOR
MIXED #1 + #2 REACTORS
EFFLUENT CARBON mg/l
ORGANISM MASS mg/l
YIELD mg/l ORGANISM MASS / mg/l CARBON REMOVED
ORGANISM MASS
YIELD
EFFLUENT CARBON
ELAPSED TIME (HOURS)
0
50
100
150
200
250
300
350
400
450
500
550
600
0
100
200
300
400
500
600
700
800
900
1000
0
10
20
30
40
50
60
70
80
90
100
0.900
1.000
1.100
1.200
1.300
1.400
1.500
1.600

7hr, 600 feed

REACTOR # 1

5.6hr, 400feed

NEW CULTURE FROM # 2 REACTOR

8.4 hr, 400 feed

MIXED # 1 + # 2 REACTORS

7 hr, 600feed

.1625

RATE

PH

0.377

RATE $\frac{\text{mg/l CARBON}}{\text{mg/l ORG.MASS-HR}}$

PH

ELAPSED TIME (HOURS)

.150 .140 .130 .120 .110 .100 .090 .080 .070 .060 .050

8.0 7.0 6.0 5.0

0 50 100 150 200 250 300 350 400 450 500 550 600

Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate mg/l carbon / mg/l mass-hr.	Yield mg/l mass / mg/l carbon
1. 7 hour detention, 600 mg/l carbon feed REACTOR # 2						
0	745	35	3.90	—	—	1.32
21	780	37	4.85	—	0.103	1.38
28	—	—	5.95	—	—	—
47	815	22	5.55	—	0.104	1.38
56	885	32	6.21	—	0.090	1.56
80	902	28	6.39	23	0.091	1.58
92	994	16	6.31	26	0.085	1.70
105	890	20	6.38	25.5	0.093	1.53
117	818	22	6.34	27	0.099	1.44
129	856	22	6.42	26.5	0.095	1.51
140	860	23	6.28	—	0.092	1.56
153	800	18	6.37	26.5	0.100	1.44
5.6 hour detention, 800 mg/l carbon feed REACTOR # 2						
6	890	36	6.18	27	0.150	1.17
27	887	100	—	—	0.138	1.27
52	1015	75	5.35	25	0.128	1.40
72	1210	16	6.11	23	0.118	1.54
82	1210	16	6.18	25	0.116	1.54
96	1210	17	6.20	23.5	0.116	1.54

Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate $\frac{\text{mg/l carbon}}{\text{mg/l mass-hr.}}$	Yield $\frac{\text{mg/l mass}}{\text{mg/l carbon}}$
8.4 hour detention, 800 mg/l carbon feed REACTOR # 2						
6	1190	16	6.19	25.5	0.079	1.52
20	1035	14	5.75	23.5	0.090	1.32
31	910	73	5.95	25.5	0.089	1.25
45	700	220	5.40	24	0.083	1.20
54	785	195	5.60	26	0.095	1.30
70	790	210	5.70	24.5	0.088	1.34
94	650	221	5.80	——	0.114	1.03
95	added 15 ml of both $FeCl_3$ and K_2HPO_4 solutions					
99	873	77	5.95	——	0.130	1.21
117	905	46	6.10	24.5	0.101	1.20
128	765	59	5.75	26.5	0.114	1.03
140	715	80	6.24	25.5	0.117	0.99
152	825	56	5.90	——	0.110	1.11
165	740	47	6.06	——	0.122	0.98
174	755	47	5.75	27.5	0.119	1.00
189	710	60	6.16	——	0.123	0.96
200	750	47	5.80	26.5	0.122	1.00
213	735	49	6.01	24.5	0.122	0.98
216	670	110	6.32	24	0.097	0.97

Elapsed Time (hr)	Organism Mass (mg/l)	Effluent Carbon (mg/l)	pH	Temperature °C	Carbon Removal Rate mg/l carbon / mg/l mass-hr.	Yield mg/l mass / mg/l carbon
2. 7 hour detention, 600 mg/l carbon feed REACTOR # 2						
0	480	129	6.11	----	------	1.02
10	530	150	6.43	23	0.106	1.25
24	495	200	6.43	23.5	0.112	1.24
34	410	279	6.89	----	0.094	1.27
47	355	415	7.24	24	0.045	1.92
60	755	40	6.00	23	0.145	1.35
72	840	18	5.75	22	0.101	1.44
82	860	17	6.13	23	0.097	1.48
96	880	15	6.21	23.5	0.095	1.50

7 hr, 600 feed

REACTOR # 2

5.6 hr, 800 feed

8.4 hr 800 feed

7 hr, 600 feed

MIXED # 1 + # 2 REACTOR

YIELD

ORGANISM MASS

EFFLUENT CARBON

EFFLUENT CARBON mg/l

ORGANISM MASS mg/l

YIELD $\frac{\text{mg/l ORGANISM MASS}}{\text{mg/l CARBON REMOVED}}$

ELAPSED TIME (HOURS)

7 hr, 600 feed

5.6 hr, 800 feed

8.4 hr, 800 feed

7 hr, 600 feed

REACTOR # 2

MIXED # 1 + # 2 REACTOR

RATE $\frac{\text{mg/l CARBON}}{\text{mg/l ORG. MASS-HR}}$

RATE

PH

PH

ELAPSED TIME (HOURS)

.16 .15 .14 .13 .12 .11 .10 .09 .08 .07

8.0 7.0 6.0 5.0

0 50 100 150 200 250 300 350 400 450 500 550 600 650

APPENDIX E

CALCULATION OF CARBON REMOVAL RATE

Using equation (2)

$$\frac{dC}{dt} = \frac{Q}{V} C_o - \frac{Q}{V} C - K_C$$

and changing it from a differential form to time increments, then

$$\frac{C_2 - C_1}{t_2 - t_1} = \frac{Q}{V} (C_o - C) - K_C$$

or $$K_C = K_c M = \frac{Q}{V} (C_o - C) - \frac{C_2 - C_1}{t_2 - t_1}$$

Therefore, the carbon (nutrient) removal rate for a unit mass can be calculated by substituting the effluent carbon concentration C_1 at time t_1 and C_2 at time t_2 into the following equation:

$$K_C = \frac{1}{M}\left(\frac{Q}{V} (C_o - C) - \frac{C_2 - C_1}{t_2 - t_1}\right)$$

The carbon concentration C, and organism mass M, can be the values determined at time t_2. For small changes in carbon concentration, $\frac{C_2 - C_1}{t_2 - t_1}$ becomes negligible and the expression for K_C is that of APPENDIX F.

APPENDIX F

DERIVATION OF EQUATION FOR ORGANISM MASS AS A FUNCTION OF EFFLUENT CARBON

From equation (3)

$$K_C = K_c M = \frac{Q}{V} (C_o - C)$$

or $$K_c = \frac{Q}{V} \left(\frac{C_o - C}{M} \right)$$

By rearranging

$$M = \frac{1}{K_c} \frac{Q}{V} (C_o - C)$$

or $$M = - \frac{1}{K_c} \frac{Q}{V} C + \frac{1}{K_c} \frac{Q}{V} C_o$$

This equation is of the same form as the standard equation for a straight line $y = mx + b$ if K_c and Q are constant. Thus, it is possible to plot the organism mass, M, as a function of effluent carbon, C, for various unit carbon removal rates, K_c.