BATCH AND CONTINUOUS BIOCHEMICAL REACTOR STUDIES USING MIXED MICROBIAL CULTURES

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SCOPE AND CONTENTS:

Using soluble organic carbon in the form of glucose as a growth limiting nutrient, the kinetics of mixed microbial populations (mainly bacterial in content) were studied using completely mixed batch and continuous biochemical reactors, in order to determine if kinetic data obtained from these two processes is identical and reproducible.

Significant differences were found in the metabolic activity of of bacteria growing in batch and continuous culture; also periods of continuous culture were found to alter the kinetics of subsequent batch cultures. Simultaneous batch experiments and consecutive batch experiments were found to be substantially reproducible with respect to kinetic data, but inconsistency was obtained in continuous culture kinetic data. The degree of dispersion of the bacteria was also found to be different in batch and continuous culture; continuous operation gave rise to dispersed growth of bacteria, whereas batch operation gave rise to flocculent bacterial growth.

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NOMENCLATURE

C ₁	-	organic carbon concentration at time t_1
C ₂	-	organic carbon concentration at time t_2
dP dt _{max}		maximum rate of product release
$\frac{dm}{dt}$	-	net rate of change of bacterial concentration
ds dt	-	net rate of change of nutrient concentration
E		enzyme concentration
^E 0	_	initial enzyme concentration
I	-	concentration of enzymatic intermediate
k ₁ , k ₂ , k ₃ , k _m	-	rate constants in Michaelis Menten model
K	-	specific growth rate of bacteria
K max	-	maximum specific growth rate of bacteria
m	-	solids (bacterial) concentration
m ₀		initial solids concentration
ml	-	solids concentration at time t _l
m ₂	-	solids concentration at time t_2
P	-	concentration of product
q	-	volumetric flow rate
R	-	rate of nutrient removal per unit mass of bacteria
V	-	reactor volume
S	-	nutrient concentration
s ₀	~	initial nutrient concentration

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OFAPTER 1

INTRODUCTION

The application of batch data to the design of continuous systems is a procedure which is widespread in the field of chemical engineering. an important example of this is in reactor design, where rate constants determined from batch experiments are used to predict the performance of continuous units.

Many continuous chemical engineering processes can be designed and operated using batch data. The inherent assumption is that for a fixed set of external conditions, changing from batch operation to continuous operation does not alter the physical properties: for example, the rate constant for a particular chemical reaction will be identical in both batch and continuous operation, provided consistent external conditions are maintained.

In the field of waste water treatment however, living microorganisms are used in the process; because of this, the Darwinian principle of natural selection applies, and only the microorganisms which can adapt themselves to a given environment will predominate. For this reason, the microorganisms which grow in a batch unit may be significantly different from those which grow in a continuous unit.

The treatment of waste water by microorganisms can be represented schematically as follows:-Soluble H20



A waste treatment process known as the activated sludge process incorporates the above microbiological reaction.

Reactions (i) and (ii) in the above scheme take place simultaneously. Reaction (i) is the process of oxidative metabolism and results in a supply of energy to the microorganisms. Reaction (ii) is a process of microbiological growth brought about by the conversion of carbon and basic nutrients to new microorganisms.

The inherent difficulties of such a process are:-

(1) the nature of the soluble organic pollutant may vary.

(2) if the nature of the waste varies, the properties of the microorganisms may also vary.

(3) the excess bacteria must be removed (usually by settling).

(4) the bacterial population must be sufficiently active to produce an effluent with the required degree of purification.
(5) the rate of reaction is dependent not only on the concentration of pollutant, but also on the concentration of microorganisms.

The microorganisms which are used in continuous waste treatment are in mixed culture, i.e. the population consists of several different types of bacteria. This is because the variety of pollutants in waste water encourage the growth of many different types of bacteria in addition to the microorganisms which are introduced to the process by virtue of the feed being non sterile. In order to design a continuous waste water treatment process, kinetic data specific to the waste and the microbial population must be obtained in the laboratory. At present it is not clear whether this should be carried out in a bench scale

batch unit, or in a bench scale continuous unit. It is the purpose of this study to investigate the differences in kinetic data obtained from these two types of operation.

CHAPTER 2.

LITERATURE REVIEW

The advantage of taking design data from a batch system, according to Stack (2), is that it affords a speedy and simple technique; its disadvantage is that it cannot be used when the waste to be treated is biostatic or toxic, although these properties in any one wasto would also present considerable problems in a continuous process. Stack (2) uses data taken from a batch process to design a continuous unit, and assumes that the amount of pollutant removed at equilibrium is a constant multiplied by the concentration of pollutant. Eusch (21, 22) points out the dangers of making such an assumption in design; the process should be designed to give a fixed pollutant concentration in the effluent, and not on a percentage removal of pollutant.

The crux of the problem in applying batch biological rate data to continuous systems design, is in finding an expression which relates the growth of bacteria (in terms of cell mass and time) to the rate of removal of limiting nutrient.

The most widely used growth expression is that of Monod (10). This expression is based on a model which assumes the formation of a complex enzymatic intermediate, the net rate of accumulation of which is zero at steady state. This model has been verified for batch and pure cultures by Herbert (11) and Novick (16). Monod's specific growth rate is the product of an inverse function of nutrient concentration, and a maximum growth rate, which is fixed for any biological system.

Another less common growth expression is that of Teissier (32).

Teissier's expression indicates that the growth rate at any time is the product of an exponential function of limiting nutrient concentration and a maximum growth rate, which is again fixed for any one biological system. Contois (12) disagrees with Monod and Teissier and suggests that the important parameter by which growth should be measured is not cell mass, but population density; not only the mass of cells but also the culture volume available to them should be considered. His ideas are verified experimentally, and his results indicate significantly different growth expressions from those of Monod. Laidler (13) and Hinshelwood (8) propose several models of consecutive enzymatic reactions. These are not verified experimentally however.

It has been assumed in all the work on the growth and chemical kinetics of bacterial cells mentioned thus far, that the rate of growth is limited by a single nutrient whether it be organic or inorganic. When the concentration of a particular nutrient becomes limiting, the bacteria can only grow as fast as they can absorb that nutrient. Since bacterial metabolism is a process consisting of many intermediates, it is conceivable that the rate limiting nutrient could be a different chemical compound, depending on the stage of the metabolic reaction. It is unsound, therefore, to trace experimentally the specific growth rates in terms of a unique compound, and in the majority of cases organic or inorganic elements have been used as growth factors eg. carbon or nitrogen.

Monod, Teissier and Contois indicate that the expressions which they derive are equally applicable to batch culture as they are to contin-

uous culture. The expressions have only been verified, however, on pure cultures of bacteria.

The hydraulics and dynamics of continuous pure cultures have been extensively investigated by many workers, notably Herbert (11, 24), Novick (16), Martin and Washington (7) and Schulze (3).

The basis of continuous completely mixed culture investigations is the following mass balance on a continuous culture unit.

Rate of change Amount produced Amount removed of biomass per unit time by - per unit time due concentration growth to hydraulic removal At steady state the left hand side of the above expression is zero and the specific growth rate of the bacteria is equal to the amount of bacteria removed hydraulically per unit time, provided the system is completely mixed.

Thus the rate of bacterial growth can be controlled by the rate at which the fluid is washed out, provided sufficient nutrient is present. Varying the detention time, and hence effluent concentration, enables specific growth rate to be obtained as a function of nutrient concentration.

Spicer (19) provides an interesting mathematical analysis of a pure culture system, operating at equilibrium, which is suddenly subjected to a fluctuation in feed concentration; the direction in which growth must tend to restore equilibrium is predicted theoretically. Other theoretical studies of interest are those of Eackman, Frederickson and Tsuchiya, (4) which predict statistical cell age distribution in growing cultures.

In the mass balance on a continuous completely mixed pure culture

unit, the most widely used expression for the "amount of bacteria produced per unit time" is the Monod expression, and is used extensively by Novick and Herbert. Schulze (3) favors the Teissier expression. He has shown that there is significant difference between the maximum growth rates obtained in a batch culture of Escherichia coli, and the maximum growth obtained in a continuous culture. This difference is said to be due to

"the mass balance is based on the assumption of instantaneous mixing in the growth chamber.... it is reasonable to expect that the experimental apparatus will only approach these conditions."

Washington (5, 7) has pointed out that an important factor in considering the mass balance on a continuous unit, is the decrease of microbial population due to death, although this should be negligible in a continuous culture where nutrient is being introduced at a steady rate.

Mashington's work is carried out extensively on pure cultures, and he justifies this by the following statement:-

"bacteria are the predominant organisms in activated sludge processes, and it is assumed that the overall physiological properties of mixed cultures found in activated sludge systems will, in general, be similar to the properties of pure cultures of bacteria."

Pure cultures of bacteria have been widely used in waste water treatment research, primarily on the assumption that properties which apply to pure cultures can be applied to mixed cultures.

Garrett and Sawyer (9) in an excellent paper published in 1952 point out that:-

"in a mixed culture it can be expected that there will be organisms present that can utilise the end products of other organisms, so that when growth ceases there will be very little soluble organic matter remaining."

Experiments were therefore conducted by Garrett and Sawyer, to determine whether or not the kinetics of removal of a given nutrient by mixed cultures follows the same relationship that has been found to apply to the utilisation of individual substances by pure cultures of bacteria. It is inferred in their work that batch techniques are not sufficiently accurate to predict growth when nutrient becomes limiting. Their results show that the rate of growth of bacteria is directly proportional to the quantity of nutrient remaining, up to a critical concentration, above which it is constant and independent of nutrient concentration; i.e. in a mixed culture of bacteria the specific growth rate is related to nutrient concentration by a linear relationship. Thus the expressions of Teissier and Monod, according to Garrett and Sawyer, do not apply to a mixed culture system. Busch (21), using a rate of soluble organic removal per unit mass of bacteria as growth parameter, also indicates a linear relationship between his "unit oxidation rate" and nutrient concentration, and concludes that in a completely mixed system:-

"oxidation rate is a function of effluent quality and can be determined from batch data, but the organisms utilised must be representative of the prototype population."

This refutes an earlier statement by Busch (25) in which it is stated that:-

"for systems of the type studied, mathematical extrapolation of batch unit data to continuous unit design is not practicable."

Bungay and Krieg (14) also further confuse the issue by stating:-

"Batch studies of mixed cultures will not be reviewed because the medium changes constantly and greatly complicates interpretation of data."

Adams and Hungate (15) have taken data from a batch fermentation system of yeast and fruit juice, and applied it to a continuous system with a considerable degree of success. The organisms were however in log phase of growth and were not limited by any single nutrient. This investigation is not applicable to the activated sludge process, where the culture is mixed, and the nutrient (organic carbon) is invariably in limiting concentrations.

A theoretical analysis of batch reactors versus continuous reactors has been carried out by Slezak and Sikyta (27), in which it is said that the various stages of the batch microbial degradation of organics can be simulated in a series of continuous stirred tank reactors with different residence times. The nutrient variations in a batch reactor with time should be represented by nutrient variations with distance in a plug flow reactor. This may well be applicable to a pure culture process, but in a mixed culture process this would result in the predominance of one group of bacteria in a particular reactor, and the inherent mutualistic relationships of a mixed culture batch reactor would be eliminated.

Gaudy <u>et al</u>. (20) indicate that a continuous culture unit is useful for keeping a mixed culture of bacteria in a phase of constant metabolic and physiological activity, and that bacteria removed from such a culture would be ideal for use in a batch test, although they do not attempt to predict the performance of a continuous unit from batch data. An interesting point arises from their work, however. By using glucose, a nutrient readily absorbed by most forms of bacteria, they were able to simulate the bacterial population of an activated sludge

process by encouraging growth of all types of microorganism. This indicates that an initial inoculum of filtered sewage should keep the same microbial population if glucose is used as nutrient, provided other trace elements necessary for growth are present.

CHAPTER 3

THEORY

3.1 Batch Growth of Pure Cultures of Bacteria

The growth of a batch pure culture, given an excess of nutrient can be represented by the following graph of viable cell numbers versus time.



(1) LAG PHASE

This occurs when the bacteria become acclimatised to the nature and concentration of nutrient. In this phase the bacteria absorb large quantities of nutrient but multiplication of bacteria by cell fission is negligible.

(2) LOG GROWTH PHASE

This immediately follows the lag phase and is characterised by the bacterial population doubling in successive equal generation times. The mathematics of the log growth phase are simple:- the rate of change of bacterial mass is proportional to the mass of bacteria present

 $\frac{dm}{dt} = km$ or $\frac{1}{m} \frac{dm}{dt} = k = SPECIFIC GROWTH RATE$

This states that the rate of change of bacterial mass per unit cell mass is constant, provided that the nutrient is not limiting. As soon as the nutrient becomes limiting the growth curve enters phase (3)

(3) <u>NUTRIENT LIMITING PHASE</u>

This phase is a period of growth where the specific growth rate is gradually decreasing. This can be seen from the growth curve, where the gradient slowly approaches zero.

The nutrient concentration is also being reduced to zero during this phase, so that further growth of bacteria cannot occur.

Michaelis and Menten (1914) have proposed a kinetic model for this period of growth

i.e. Enzyme + nutrient \longrightarrow p + E enzyme + nutrient \longrightarrow intermediate \longrightarrow products enzymatic complex

Assumptions:-

(i) One enzyme active in the reaction

(ii) This one enzyme acts on only one nutrient

(iii) The nutrient is in growth limiting concentrations.

Net rate of production of intermediate =

$$\frac{\mathrm{dI}}{\mathrm{dt}} = k_1 \mathrm{ES} - k_2 \mathrm{I} - k_3 \mathrm{I}$$

the enzyme concentration at any time is equal to the initial enzyme concentration minus the concentration of the intermediate

i.e.:
$$E = E_0 - I$$

At pseudo steady state $\frac{dI}{dt} = 0$
 $I = \frac{k_1 E_0 S}{k_2 + k_3 + k_1 S}$
 $\frac{dP}{dt} = k_3 I = \frac{k_1 k_3 E_0 S}{k_2 + k_3 + k_1 S}$
 $= \frac{k_3 E_0 S}{k_m + S}$
Where $k_m = \frac{(k_2 + k_3)}{k_1}$

Also
$$\frac{dP}{dt}_{max} = k_{3}E_{0}$$

 $\frac{dP}{dt} = \frac{\frac{dP}{dt}_{max}(S)}{(k_{m} + S)}$

i.e. The rate of product release is related to the concentration of nutrient at any time, and also to the maximum rate of product release possible under the given conditions. Monod observed qualitatively that specific growth rate was related to nutrient concentration by a similar relationship:-

$$K = \frac{\max(S)}{k_{m} + (S)}$$

It is felt that the assumptions made in the above derivation would be inapplicable to the growth of mixed cultures of bacteria, and a more important parameter in the case of a mixed culture process would be the rate of removal of organic nutrient per unit mass of bacteria, Busch's "unit oxidation rate". This parameter, in order to be useful in the field of waste water treatment, should still be expressed as a function of limiting nutrient concentration, but it is doubtful whether the mathematical relationship between these two variables is of the same form as Monod's. A linear relation was indicated by Garrett and Sawyer (9), whose evaluations of rate data were made on a continuous unit.

(4) THE STATIONARY PHASE

This immediately follows the nutrient limiting phase, and corresponds to a complete depletion of available nutrient. The net rate of growth of bacteria is zero. This is followed by:-

(5) THE ENDOGENOUS RESPIRATION PHASE

This phase results in a gradual decrease of bacterial numbers due to autolysis, or self digestion of the bacteria. Viable bacteria are able to utilise non viable bacteria as nutrient. It is possible to keep bacteria in the endogenous respiration phase for long periods of time.

3.2 Continuous Growth of Pure Cultures of Bacteria

Consider the completely mixed continuous culture reactor shown below



Solids Balance (Bacterial Balance)

Change = Input - Output + accumulation

 $V \frac{dm}{dt} = q (m_0 - m) + KmV$

K = specific growth rate

At steady state $\frac{dm}{dt} = 0$ and if the feed is sterile, $m_0 = 0$.

$$K = \frac{q}{V}$$

i.e. only the bacteria which have a generation time less than or equal to the detention time will remain in the reactor. The rest will be removed hydraulically. Similarly the rate of removal of nutrient at steady state can be evaluated using a nutrient balance:-

$$V \frac{dS}{dt} = q(S_0 - S) - RmV$$

R is the rate of nutrient removal per unit time per unit mass of bacteria.

At steady state $\frac{dS}{dt} = 0$

$$R = \frac{q}{V} \quad \frac{(S_0 - S)}{m}$$

CHAPTER 4

PURPOSE OF THE INVESTIGATION

Using <u>pure</u> cultures of bacteria, and the theoretical ideas developed earlier, the use of batch data to predict the performance of a continuous completely mixed unit has been proved possible in several instances, (10, 11, 12, 15, 16). When mixed cultures are used the picture is more confused; Busch (21, 22) indicates that batch cultures can be used to predict the performance of continuous units, provided that the cultures used are of similar metabolic characteristics. This assumes that bacteria which predominate in batch mixed culture units can be reproduced in continuous units. Gaudy <u>et al.</u>(20) suggest that mixed cultures taken from a continuous unit would be ideal for study in a batch unit. This again assumes that the nature of the population does not vary when changed from one type of culture to another. In an attempt to elucidate some of these questions, the batch and continuous growth kinetics of a mixed culture of bacteria will be studied on the basis of three variables.

- 1) Suspended solids concentration
- 2) Soluble organic carbon concentration
- 3) Time

in order to determine:-

(1) if there is a difference in bacterial kinetics when cultures are grown in batch and continuous units.

(2) if there is a difference in the types of bacteria which predominate

in batch and continuous culture

(3) if the kinetics are altered by shifting from batch culture to continuous culture and back again.

(4) if batch or continuous kinetic data is reproducible in itself.

CHAPTER 5

EXPERIMENTATION

5.1 EXPERIMENTAL EQUIPMENT

5.1.1 Batch Reactors

The batch reactor, shown in Figure 1, consisted primarily of an 8 - litre glass percolator, the base of which had been cut away, and an inverted sintered glass diffuser attached in its place. The cross sectional area of the diffuser was approximately the same as the cross sectional area of the base of the percolator. When air was forced through the diffuser complete mixing of the aeration chamber was achieved and the possibility of microorganism build up in badly mixed sections of the reactor was eliminated. Air was found to provide adequate mixing of the reactor contents without additional mechanical mixing.

The sampling point in each of the three batch reactors was located at the 6 - litre level, where a small glass neck was fitted to the side of the reactor. The sampling tube itself was a piece of $\frac{1}{4}$ " O.D. glass tubing fitted into a rubber cork in the neck on the side of the reactor. The tube had a right angle bend in it, so that it sampled from a point well inside the reactor. The sample was drawn off by a syphon controlled by a screw clamp.

In order to prevent a solids build up on the sides of the reactor above the liquid level and loss of reactor contents by evaporation, the following modification was made to the reactor. A plastic funnel was cut so that its diameter was just less than the maximum diameter of the



percolator. This funnel was lined with teflon sheeting and the outer rin was encircled by a piece of thick walled rubber tubing, slit lengthways, so that when the funnel was pressed down into the percolator above the liquid level, an airtight seal with the wall was obtained.

This arrangement was to minimize build up of solids around the inside wall of the percolator. Evaporation still had to be eliminated. This was successfully achieved by attaching a water cooled condenser to the neck of the plastic funnel. Due to the airtight seal around the side of the funnel the only path for air passing out of the reactor was up the central tube of the condenser, and the majority of water vapour in the exit gases was condensed, and dripped back into the reactor.

5.1.2 The Continuous Reactor

The continuous reactor was identical in construction to the batch reactor, the difference being in the sample take-off, and the absence of a condenser. The effluent was taken off at approximately the 6 - litre level through a glass "Y" - piece ($\frac{1}{2}$ " I.D.). The "Y" piece was made of $\frac{1}{2}$ " I.D. tubing so that there was less tendency for the solids to clog and block the tube (See Figure 2). This eliminated build up of solids inside the reactor, and a representative sample of the reactor contents was wasted continuously.

5.1.3 Feed System For The Continuous Reactor

It was decided that mechanical pumping of dilution water and concentrated nutrient solution was the best method of ensuring a steady flow of nutrient into the reactor. The dilution water was pumped into the reactor using a Sigmamotor peristaltic pump (MODEL T-6-S). The



LIQUID TAKE-OFF ON CONTINUOUS REACTOR

FIGURE 2.

concentrated nutrient solution was pumped into the reactor by a Brailsford self priming positive displacement pump (Model ES-1) which was powered by a D.C. source. For details of the contents of the nutrient solutions see Appendix (i).

5.1.4 The Air Supply

A constant supply of air to the reactor was required, both to provide an adequate dissolved oxygen level (approximately 6 mg/L), and a high degree of mixing. The air was supplied by the 30 p.s.i.g. air line in the laboratory. In order that any particles of dust in the air did not pass into sintered glass diffuser and clog it, the air was first filtered through glass wool; also as an extra precaution against evaporation of reactor contents, the air was then presaturated with water prior to entering the rotameters, where it was metered continuously using individual calibration curves.

A schematic diagram of the apparatus is shown in Figure 3.



5.2 EXFERIMENTAL TECHNIQUES

5.2.1 Sampling

Samples (approximately 40 ml) were taken from the batch reactor by opening the stop value on the sampling line, and allowing the liquid to siphon into a beaker.

Samples from the continuous reactor were taken both from the actual reactor contents and also the effluent, to ensure that any solids build up in the reactor could be detected.

5.2.2 Determination of Suspended Solids Concentration

The suspended solids content of the sample taken was determined in the following way. A Gellman millipore filter (47 mm diameter, 0.45 micron pore size) was washed by drawing distilled water through it, using Millipore vacuum filtration apparatus: 10 to 20 ML. of distilled water was usually sufficient for this purpose. The filter paper was then placed on an aluminium weighing dish, and allowed to dry for at least one hour at 40°C in an oven. The filter paper was then weighed with the aluminium dish, and once more placed in the vacuum filtration apparatus. A known volume of culture was then filtered through under vacuum. The solids collected on the filter paper, and the filtrate passed into a clean Erlenmeyer flask. The filter paper was then placed in the same aluminium weighing dish, dried again, and weighed. The difference in the two readings gave the weight of suspended solids.

The weighings were carried out on a Mettler balance (Type H15) which could be read to the nearest 0.1 mg.

5.2.3 Determination of Soluble Organic Carbon Concentration

The filtrate from the solids determination was placed in a clean dry test tube and stored in the refrigerator until required. The carbon concentration of the filtrate was then determined using a Beckmann infrared analyser (MODEL IR315) in the following way.

The performance of the carbon analyser was found to vary from day to day. A standard solution of sodium oxalate was found to give readings which differed on successive days by as much as two divisions on a 100 division scale, depending on the gain being used. The following procedure was therefore adopted.

The sample to be analysed was treated with a few drops of concentrated hydrochloric acid to remove any carbon present as inorganic carbonate. It was then stripped with argon for five minutes to remove carbon dioxide, and successive 20 microliter injections of the sample were made into the analyser until four readings differed by less than two divisions. Two standard solutions were then taken, stripped of carbon dioxide, and 20 microliter injections of these were put into the analyser. The standard solutions were chosen (see Appendix (iii)) so that their scale readings straddled the reading of the unknown sample. It was arranged that the standards were as close together as possible in carbon content (differing by 25 mg/liter usually), so that a linear interpolation between the readings given by the two standards could be made. The carbon concentration in the unknown sample was then calculated assuming a linear fit between the readings given by the standards.

The strength of the standards used varied depending on the concentration of the unknown sample, and also on the gain used on the

carbon analyser. It was ensured that the same hypodermic syringe be used throughout the testing period, so that inaccuracies due to calibration of the syringe could be eliminated.

5.2.4 pH Determination

The pH of the reactor contents was measured at intervals using a Beckmann expanded scale pH meter (model 76). The instrument was calibrated before use, using a phosphate buffer solution of pH = 6.86 5.2.5 <u>Microbial Analysis</u>

Microscopic examinations were made at intervals during the experiments. The observations were of a purely qualitative nature. No attempt was made to make a count of the bacteria present. Photographs were taken on runs B22 and C6 (Figure 16).

5.2.6 Settling Tests

On several occasions during the course of the experimental work the air to one or all of the reactors was shut off and the culture allowed to stand for 30 minutes. The settling properties of the bacterial suspension were estimated from the volume of settled floc at the bottom of the reactor. Once again the observations were of a purely qualitative nature.

5.2.7 Determination of Continuous Reactor Feed Rate

The Brailsford positive displacement pump was used to pass the concentrated nutrient solution into the reactor. Under a P.D. of 12 volts, and a current of 1 amp, this pump was found to have a cycle time of 2.66 minutes; the total flow rate of nutrients into the reactor were timed over one complete cycle of this pump. A carbon analysis was made on the liquid collected, in order to determine the inlet carbon concentration.

5.2.8 Air Flow Rates

Throughout the period of experimentation readings on the rotameters were maintained which corresponded to an air flow rate of 6000 ml/min at 1 atm. and 70°F. Occasionally the sintered glass diffuser became clogged with bacteria and dust, and the pressure of the air line was insufficient to give the required flow. To remedy this, concentrated sulphuric acid was poured into the empty reactor, and this was found to clean the sintered glass efficiently.
5.3 EXPERIMENTAL PROCEDURE

5.3.1 Batch Runs

Batch runs were carried out simultaneously on the three batch reactors.

A batch run was commenced by equalizing the carbon and solids concentrations in the three reactors by mixing the contents of each together. The mixture was then inoculated with a suitable amount of nutrient and the mixture redistributed equally into the three reactors. Readings of carbon and solids concentration were made at half hourly intervals until the carbon content showed no appreciable change on successive readings (usually after about 2 hours). Readings of pH were normally taken at the beginning and end of the run. Microbial examinations were made during the run.

It was usual to reinoculate the reactors with fresh nutrient at the termination of a run, so that the next run could continue directly from the end of the previous one. The reinoculation was done without equalizing the carbon concentrations.

At the beginning of a batch run a test was made on the filtering properties of the bacterial floc. If it filtered easily 20 ml samples were used in the subsequent run; if it did not filter easily 10 ml samples were used. This was a necessary precaution because the filtration had to be complete by the time that the next sample was taken, and filtering times greater than $\frac{1}{2}$ hour could not be tolerated.

5.3.2 Continuous Runs

These were carried out in one reactor. A nutrient solution of fixed carbon concentration was passed into the reactor until steady state was obtained i.e. effluent carbon and solids concentrations did not vary with time.

The procedure consisted of allowing the continuous reactor to be operated for two days, and then readings of effluent solids and carbon concentration were taken at daily intervals until two successive daily readings were substantially constant. These values were taken as being the steady state values.

The method of analysis of batch and continuous data is described in Appendix (iv).

CHAPTER 6

EXPERIMENTAL RESULTS

6.1 The Experimental Schedule

The experimental schedule (Figure 4) indicates the paths followed by two samples of the initial inoculum culture A and culture B, during the period of experimentation. The numbers shown represent the batch and continuous runs made at each stage. Where three batch run numbers are bracketed together, this indicates that three simultaneous runs were made. Two or three sets of bracketed run numbers, at any given point, indicate that two or three runs were made consecutively in each of the three reactors.

For details of the nature of the initial inoculum see Appendix (iii).



FIGURE 4. EXPERIMENTAL SCHEDULE

6.2 Simultaneous Batch Runs

Figures 5 and 6 indicate typical values of raw and smoothed data (See Appendix (iv)) taken from three batch reactors run simultaneously. The solids concentrations show a tendency to go through a maximum, indicating the transition from the nutrient limiting phase to the endogenous respiration phase. The carbon concentrations all vary in a similar manner until the endogenous respiration phase occurs; the change of carbon concentration with time is then effectively zero. The unit carbon removal rates, calculated from the above data, are plotted as functions of soluble carbon concentration, and are shown in Figure 7. The rate curve is substantially the same for each of the three runs. The rate curves indicate that there is a positive value of carbon concentration, approximately 20 mg/L, at which the unit rate of removal of carbon is zero.







6.3 Two Consecutive Batch Runs

Figure 8 indicates raw and smoothed carbon and solids data taken from two consecutive batch runs made on one batch reactor. The initial carbon concentration at the beginning of each run is approximately the same. The graphs of solids concentration versus time seem to indicate that the culture passes from the nutrient limiting phase to the endogenous respiration phase in both cases. The carbon concentration on the second run shows a more rapid decrease than in the first run. This is due to the increased bacterial concentration at the beginning of the second run.

The graphs of unit removal rate versus carbon appear to be substantially the same for both runs. There is again a positive carbon concentration at which the unit carbon degradation rate is zero. This value is approximately 20 mg/L which is the same as the value obtained from the three simultaneous batch runs. (See Figure 9)





6.4 Three Consecutive Batch Runs

Figures 10 and 11 indicate raw and smoothed carbon and solids data taken on three consecutive batch runs. The carbon concentrations at the beginning of each run are different. The first two batch runs are reinoculated with carbon before they pass into the endogenous respiration phase. This manifests itself on the first two rate curves (Figure 12) where the unit carbon removal rate does not reach zero. In the third batch run, the curve of solids versus time appears to go through a maximum. This may be due to the bacteria passing into the endogenous respiration phase, which results in a zero value of carbon removal rate.





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6.5 Unit Rate Variations for Culture A

Figure 13 shows the variations in the unit rate of removal of carbon exhibited by the initial culture at different stages of the experimental schedule. The points labelled C2, C4 and C6 are the steady state unit rates of carbon removal at which the continuous cultures were operated. The arrowed dotted lines indicate the direction of transferrence of the culture.

Runs C2, C4 and C6 were operated at detention times of approximately 10 hours, 7 hours and 7.5 hours respectively. The inlet carbon concentrations were approximately 550 mg/L on runs C2 and C4, and approximately 475 mg/L on run C6.

From the values of detention time, it can be seen that the specific growth rates in runs C4 and C6 should be almost the same, both being greater than the specific growth rate in run C2. Theory indicates, therefore, that there should be a greater chance of a particular bacterial species remaining after run C2 than after runs C4 and C6. The subsequent batch run, B7, made directly after run C2, indicates that the unit rate of carbon removal is considerably <u>higher</u> than either of the other two batch runs shown.

It could be inferred from this that the greater the chance the bacteria have of remaining after continuous culture, the greater is their ⁻ metabolic activity during subsequent batch culture. However, it can also be observed from Figure 13 that:-

(a) the unit rate of removal of carbon exhibited by the initial

inoculum shows a tendency to be increased by subsequent continuous culture.

(b) in two out of three cases, the continuous culture operates at higher unit rates of carbon removal than does the preceding batch run operating at the same carbon concentration.

Thus, from figure 13, continuous cultures appear to be more efficient than batch cultures with respect to carbon removal, but they may eliminate bacteria which are important to the mutualistic processes of the batch operation.



6.6 Unit Rate Variations for Culture B

Figure 14 represents the unit rates of carbon removal exhibited by culture B at various stages of the experimental schedule. The initial culture was first operated in a continuous unit, as opposed to culture A, which was first operated on a batch unit.

Continuous run numbers Cl, C3, C5 and C7 were operated at detention times of approximately 10 hours, 10.5 hours, 7 hours and 8.5 hours respectively. The inlet carbon concentrations were approximately 575 mg/L, 450 mg/L, 530 mg/L and 375 mg/L respectively.

The values of detention time indicate that the bacteria in run C5 have less chance of remaining in the continuous reactor than the bacteria in runs C3 and C1. From the discussion of figure 13, it was inferred that the higher the detention time in continuous culture, the higher is the unit removal rate in subsequent batch culture. This would suggest that batch run B28, following continuous run C5, would exhibit a <u>lower</u> unit rate of carbon removal than batch runs B4 and B19.

This is not the case, however. Batch run B28 (following continuous run C5 on a <u>7 hour</u> detention time) exhibits a <u>higher</u> unit rate of carbon removal than does either batch run B4, (following continuous run C4 on a <u>10 hour</u> detention time), or batch run B19, (following continuous run C3 on a 10.5 hour detention time).

From Figures 13 and 14, therefore, there would appear to be no consistent quantitative relationship between specific growth rate in continuous culture and the variations in unit carbon removal rate during



subsequent batch culture. However, as can be seen from Figures 13 and 14, periods of continuous operation do tend to increase the unit rate of removal above that exhibited by the initial culture. The rate of carbon removal in continuous culture is generally higher than that exhibited by a batch culture operating at the same carbon concentration.

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6.7 Batch and Continuous Unit Rates

Figure 15 gives an overall picture of unit rates of carbon removal obtained from batch and continuous experiment during this study.

The batch rate data shown are those which correspond to the steady state carbon concentrations obtained from a continuous unit. The unit rates of carbon removal exhibited by a continuous culture are compared only to the batch unit rates which immediately precede and follow that particular continuous culture. The curve shown is the best approximate fit through the batch data.

The unit rates of carbon removal exhibited by continuous cultures are erratic but mostly significantly higher than unit rates obtained from batch cultures operating at the same carbon concentration. The reason for this could be twofold.

Firstly, the bacteria which predominated in continuous culture were highly dispersed, whereas bacteria which predominated in batch culture were flocculent in nature; this would suggest that the interfacial area per unit mass of bacteria available for nutrient absorption is lower in batch culture than in continuous culture, and the efficiency of nutrient removal would therefore be expected to be lower in the batch unit.

Secondly, only viable bacterial cells can predominate in continuous culture units. Non viable cells are removed hydraulically. In batch units, however, non viable cells are allowed to remain in the reactor, and the efficiency of carbon removal per unit mass of bacteria is effectively lowered by the presence of these non active cells. This could also account for the lower efficiency of carbon removal in the batch units.



6.8 Effect of Temperature on Reaction Rate

During the period of experimentation no attempt was made to regulate the temperature of the reactor contents. This is because the laboratory used was air conditioned and variations in the ambient temperature were infrequent. However, it is possible that even small fluctuations in temperature may effect the rate constant of the biochemical reaction studied.

Wuhrmann (31) has related the unit oxygen uptake rate to temperature by the following relationship:

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

Where r_1 = unit oxygen uptake rate at temperature T_1

 r_2 = unit oxygen uptake rate at temperature T_2

This relationship has been found to hold for a mixed culture of bacteria using a sugar as carbon source.

Assuming this expression to hold under the conditions of this study, and considering the most extreme case where the difference in in temperature of reactor contents in two separate runs could be as great as 3°C it can be shown that

 $r_1 = 1.09 r_2$ The differences in unit rate of carbon removal due to variations in temperature would therefore appear to be negligible compared to the differences which were obtained due to intermittent periods of batch and continuous operation, and a temperature dependence of reaction rate was justifiably neglected.

6.9 Qualitative Observations

The culture selected at the beginning of the period of experimentation showed a predominance of bacterial growth, and had excellent settling properties. The culture was divided equally between each of the four reactors (three batch and one continuous) and during the ensuing experiments the following observations were made.

1) The bacteria, which were initially flocculent, rapidly lost their flocculent nature when grown in a continuous culture unit. The bacteria which grew under continuous conditions were of a dispersed or free swimming nature, and their settling properties were non existent. This was observed each time a batch culture was put on continuous operation. Figure 16 (a) shows a photograph of a typical continuous culture. Bacteria are predominant, and these are shown in both flocculent and dispersed growth. The bacterial flocs are much smaller, however, than those shown in figure 16 (b) which is a photograph of a sample of a typical batch culture. Yeast type fungi are shown both inside and outside the bacterial floc, and a scarcity of dispersed bacteria is apparent.

2) The predominance of bacterial flocs could only be restored by placing the continuous culture in a batch unit and allowing it to be aerated, without further addition of nutrients, for a period of two or three days. This caused the bacteria to flocculate. It did, however, also encourage the growth of other types of microorganism, notably fungi (mycelial and yeast types) and protozoa. See figure 16.

FIGURE 16.



FIGURE 16 (a) TYPICAL CONTINUOUS CULTURE



FIGURE 16 (b) TYPICAL BATCH CULTURE (X 400)

3) The continuous cultures were always difficult to filter (approximately $\frac{1}{2}$ hour for a 10 ml sample), whereas batch cultures filtered easily.

4) The pH of the continuous unit was found to vary much more than the pH of the batch unit. As steady state was approached on the continuous unit the pH showed a tendency to increase. The pH of a batch culture, however, did not vary significantly during the course of a run.

pH	batch	6 . 5 → 7.5
pН	continuous	5.5 → 7.5

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

a) The unit removal rate of organic carbon (as dextrose) by a mixed culture of bacteria was significantly lower on a batch unit than on a continuous unit operated at the same carbon concentration.

b) Batch experiments gave rise to flocculent bacterial growths but during continuous culture the bacteria were evenly dispersed throughout the culture medium.

c) The unit removal rates of carbon during batch experiments were changed considerably by intermediate periods of continuous operation. This would suggest that certain species of bacteria may be removed hydraulically during the continuous period; also that these species do not return to predominance during subsequent batch culture.

d) The unit rate of carbon removal does not vary substantially in <u>consecutive</u> batch experiments when the initial carbon concentration is of the same order of magnitude in each.

e) The unit rate of carbon removal does not vary substantially in <u>simultaneous</u> batch experiments when the initial carbon concentration is of the same order of magnitude in each.

f) Batch experimentation in this study has shown that there is a positive organic carbon concentration, $(15 \rightarrow 25 \text{ mg/L})$, at which the unit rate of carbon removal by a mixed culture is zero. This is significantly different from the model of Michaelis and Menten, which

indicates that the unit rate of removal of nutrient should only be zero when the limiting nutrient concentration is zero.

g) The reactors used in this study gave significantly different design data when used in batch operation than when used in continuous operation, both with respect to kinetic data, and the nature of microorganisms which predominated.

7.2 Recommendations

a) A more accurate method of measuring bacterial concentration would be valuable. A possibility for this would be to lyse the bacteria by sonication, and then measure the concentration of bacteria in the original suspension in terms of bacterial carbon content, using the carbon analyser.

b) This study has indicated that the rate of reaction may be influenced by the degree of dispersion of the bacterial population; to investigate this, the growth of bacteria could be studied using population density, instead of suspended solids concentration, as a variable for measuring growth rates.

c) In order to investigate thoroughly the effect of hydraulic loading on bacterial species, a cascade of continuous completely mixed reactors with different residence times could be operated. This would encourage growth of different types of bacteria in each unit, and the metabolic activities of different types of microorganism could be evaluated.

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APPENDICES

APPENDIX (i)

Nutrient Media

In order to investigate the metabolic activity of mixed cultures of bacteria, it is necessary to supply one nutrient in growth limiting concentrations, so that bacteria can only grow as fast as they can absorb that nutrient. This limiting nutrient is known as a growth factor.

Since organic carbon, nitrogen and phosphorus are required in some form by all types of bacteria, it is usual to select one of these as a growth factor. Carbon was selected for use in this study.

For adequate nutrition of bacteria a C:N:P ratio of 40:5:1 is sufficient (28). By ensuring that nitrogen and phosphorus in any nutrient medium are far in excess of this requirement, carbon is thus made the limiting nutrient.

Dibasic ammonium phosphate (NH4)2 HPO4 was used to provide a source of nitrogen and phosphorus, and dextrose (C6 H12O6) was used as the organic carbon source. Mixing dextrose and ammonium phosphate in a 3:1 ratio by weight ensured that the carbon was in limiting concentrations. The nutrient solution was made up in tap water.

It was found, however, that the growth of bacteria was not satisfactory using the above medium. This suggested that the bacteria were limited by some other nutrient. For this reason three other nutrient solutions were made up.

1) Ferric chloride (FeCl3.6H20) solution - concentration

1.0 mg/ml.

2) Potassium phosphate (K2HPO4) solution - concentration 50 mg/ml.

3) Magnesium sulphate (MgSO4 7H2O) solution - concentration 50 mg/ml.

These solutions were added to provide basic nutrients (Fe, K, Mg) which may be lacking in tap water.

These solutions were added to the dextrose / ammonium phosphate medium using the following arbitrary formulae.

1) ½ ml FeCl3 solution/200 mg carbon/litre of feed

2) 1 ml K₂HPO₄ solution/200 mg carbon/litre of feed

3) 1 ml MgSO4 solution/200 mg carbon/litre of feed

N.B. nutrient solutions for the continuous reactor had carbon concentrations of approximately 500 mg/L organic carbon, and for the batch reactors approximately 100 mg/L

This modified nutrient medium gave satisfactory growth in the batch reactors. It was necessary, however, for operation of the continuous reactor to mix large quantities of nutrient solution. When the nutrients were mixed together in the same container, it was found that rapid growth of bacteria occurred in the feed line, thereby continually reducing the inlet carbon concentration. It was observed, however, that if the ammonium phosphate / dextrose solution was made up in distilled water, as opposed to tap water, the growth of bacteria was considerably reduced.

It was therefore decided that the feed to the continuous reactor be divided into two streams
1) A feed stream containing the required amounts of dextrose and ammonium phosphate, made up in distilled water.

2) A dilution water stream made up in tap water to provide the trace elements required for growth. This contained ferric chloride, potassium phosphate and magnesium sulphate, added in the same amounts as previously mentioned.

The required concentration of carbon in the feed stream was calculated based on a total flow of liquid; also when measuring flow rates, and carbon concentration of the inlet stream, it was necessary to make determinations on the liquid mixture rather than the individual solutions.

APPENDIX (ii)

Standard Solutions For Carbon Analysis

Previous experience had indicated that sodium oxalate solution, made up in distilled water, was an ideal standard for the carbon analyser. The standards were stored in a refrigerator at 4°C, and it was found that their carbon content did not fluctuate, irrespective of their period of storage. Slight fluctuations which did occur in the performance of the carbon analyser were though to be due to experimental error rather than variation in content of the standard solution.

APPENDIX (iii)

The Initial Inoculum

The microbial inoculum selected at the start of the period of experimentation was taken from two batch reactors in the laboratory which had been operated for a period of approximately 1 year. They were chosen because:-

i) the settling properties of the culture were good.

ii) samples of the culture were easily filtered.

iii) the culture was well acclimated to the nature of feed being used in this study.

APPENDIX (iv)

1. Analysis of Batch Data

Raw data were taken during batch runs at equal intervals of time. In most cases the interval was half an hour. The batch runs were usually of $2 \rightarrow 2.5$ hours duration, and, with samples taken every half hour, only five or six points were obtained from which rate data could be evaluated. To generate more data linear interpolations were made at equal time intervals between raw data points, and the raw and interpolated data were then smoothed according to a 5 - point smoothing formula (30). The unit removal rate of carbon was then calculated as follows:

Typical curves of carbon and solids concentration versus time are shown below.



The rate of removal of carbon/unit mass of bacteria in the range $t1 \longrightarrow t2$ is given by

$$R = \frac{1}{(m_1 + m_2)} \frac{(C_1 - C_2)}{(t_1 - t_2)}$$

This can be determined for various values of carbon concentration and it is most meaningful to graphically express R as a function of carbon concentration. The values of carbon concentration corresponding to the above value of R is $(C_1 + C_2)/2$.

2. Analysis of Continuous Data

The steady state unit rate of removal of carbon was evaluated by dividing the difference between the influent and effluent carbon concentrations by the steady state solids concentration and the detention time (see section 3.2).

APPENDIX (V)

Experimental Date

Continuous Reactor Data

RUN NO.	DETENTION TIME (HR)	INLET CARBON CONC• (MG/L)	STEADY STATE CARBON (MG/L)	STEADY STATE SOLIDS (MG/L)	CONTINUOUS UNIT RATE (1/HR)	BATCH UNIT RATE (1/HR)	
C1	9.75	577.0	23.60	928.3	0.0637	0.0093 0.0179 0.0202	(84) (85) (86)
C2	9.91	562.1	19.32	919.2	0.0617	0•0088 0•0099	(82) (83)
٢3	10.46	535.0	85•41	612.5	0.1328	0.1482 0.1431 0.1545 0.1084 0.1040 0.0888	(B22) (B23) (B24) (B25) (B26) (B26) (B27)
C5	7.09	530.1	70.52	629.2	0.1188	0.0693 0.0693 0.0718 0.0969 0.1094 0.0940 0.0756 0.1317 0.0804 0.1224	(B13) (B14) (B15) (B16) (B17) (B18) (B19) (B20) (B21) (B32)
6	7.17	470.0	41.30	690.2	0.0949	0.0871 0.0849 0.0874 0.0773 0.0774 0.0795	(B22) (B23) (B24) (B25) (B26) (B27)
С7	8•51	376.2	19.42	469.2	0.0943	0.0431 0.0416 0.0339 0.0325 0.0407	(828) (829) (831) (832) (833)
C8	5.73	420.8	28•76	573.5	0.1366	0.0656 0.0661 0.0504 0.0526 0.0619	(828) (829) (831) (832) (833)

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SOLIDS DATA ON THE CONTINUOUS RUNS WERE TAKEN ON SIX REPLICATE 10 ML. SAMPLES

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Batch Reactor Data

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	RAW DA	TA	SMOOTH	IED DATA	RATE DATA		
TIME	SOLIDS	CARBON	SOLIDS	CARBON CONC	UNIT RATE	CARBON CONC	
(HR)	(MG/L)	(MG/L)	(MG/L).	(MG/L)	(1/HR)	(MG/L)	
0.00	525.0	70.6	524.64	70.77			
0.25			548.85	57.79	0.0968	64.28	
0.50	570.0	46•4	567.94	47.76	0.0740	52.63	
0.75			582.44	39.43	0.0558	43.44	
1.00	595.0	29.7	592.88	33.35	0.0414	36.39	
1.25	· · · · · · · · · · · · · · · · · · ·		599.79	28.86	0.0301	31.10	
1.50	635.0	20.9	603.70	25.61	0.0216	27.23	
1.75			605.14	23.25	0.0156	24.43	
2.00	605.0	21.5	604.63	21.43	0.0120	22.34	
2.25			602.72	19.79	0.0108	20.61	
2.50	600.0	18.0	599.93	17.99	0.0000	18.89	

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RUN NUMBER B2

	RAW DA	TA	SMOOTH	IED DATA	ED DATA RATE DATA		
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON	
	CONC	CONC	CONC	CONC	RATE	CONC	
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)	
0.00	505.0	68.5	504.35	68.57			
0.25			. 537.50	58.51	0.0750	63.69	
0.50	565.0	49•7	561.21	50.14	0.0631	54•47	
0.75	•		577.01	42.54	0.0534	46.34	
1.00	625.0	32.4	586.43	36.01	0.0449	39.27	
1.25			591.02	30.52	0.0373	33.26	
1.50	595.0	20.4	592.31	26.06 ·	0.0301	28.29	
1.75		· · · ·	591.83	22.62	0.0232	24.34	
2.00	590.0	19.8	591.12	20.19	0.0165	21.41	
2.25	•		591.73	18•74 [`]	0.0098	19.46	
2.50	595.0	18.2	595.18	18.26	0.0032	18.50	

RUN NUMBER B3

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	RAW DA	TA	SMOOTH	ED DATA	RATE	E DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT RATE	CARBON
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	565.0	70.3	564.50	70.34	ć.	
0.25			581.88	61.66	0.0606	66.00
0.50	595.0	53.4	592.13	53.68	0.0543	56.67
0.75		•	596.73	46.45	0.0487	50.06
1.00	610.0	33.2	597 . 16	39.97	0.0434	43.21
1.25			594.91	34.28	0.0382	37.12
1.50	630.0	26.2	591.45	29.40	. 0.0329	31.84
1.75			588•28·	25.35	0.0274	27.37
2.00	585.0	21.8	586.88	22.16	0.0217	23.76
2.25	1		588.72	19-86	0-0157	21-01
2.50	595.0	18.4	595.30	18.46	0.0094	19.16

DUPLICATE 10ML. SAMPLES WERE USED FOR SOLIDS DATA.

RUN	NUMBER	84
*****	*****	***

			• • •• •• •• •• •• •• •• •• ••				
RAW DATA			SMOOTH	ED DATA	RATE DATA		
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON	
	CONC	CONC	CONC	CONC	RATE	CONC	
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)	
0.00	205.0	76.1	204.44	76.21			
0.50			239.72	64.86	0.1022 .	70.53	
1.00	270.0	54.5	266.65	55.16	0.0766	60.01	
1.0			286.35	47.00	0.0590 /	51.08	
2.00	315.0	30.4	299.92	40•25	0.0461	43.63	
2.50		2	308.48	34.78	0.0360	37.51	
3.00	320.0	26.0	313.14	30.45	.0.0278	32.61.	
3.50			315.01	27.16	0.0210	28.80	
4.00	315.0	24.5	315.21	24.76	0.0152	25.96	
4.50			314.85	23.13	0.0103	23.94	
5.00	315.00	22.1	315.03	22-14	0.0063	22.64	

RAW DA	ТА	SMOOTH	ED DATA	RATE DATA		
SOLIDS	CARBON CONC	SOLIDS	CARBON	UNIT	CARBON CONC	
(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)	
255.0	71.6	254.90	71.67			
		. 275.34	62•55	0.0688	67.11	
295.0	54.1	294.46	54.55	0.0561	58,55	
	÷	311.46	47.58	0.0460	51.07	
300.0	33.2	325.55	41.54	·0•0379	44.56	
· .		335.94	36.32	0.0315	38.93	
310.0	27.3	341.82	31.83	0.0265	34.08	
		342.42	27.97	0.0226	29.90	
340.0	24.5	336.92	24.63	0.0197	26.30	
· · · ·		324.54	21.71	0.0176	23.17	
305.0	19.1	304.48	19.12	0.0165	20.42	
	RAW DA SOLIDS CONC (MG/L) 255.0 295.0 300.0 310.0 340.0 305.0	RAW DATA SOLIDS CARBON CONC CONC (MG/L) (MG/L) 255.0 71.6 295.0 54.1 300.0 33.2 310.0 27.3 340.0 24.5 305.0 19.1	RAW DATA SMOOTH SOLIDS CARBON SOLIDS CONC CONC CONC (MG/L) (MG/L) (MG/L) 255.0 71.6 254.90 255.0 71.6 254.90 295.0 54.1 294.46 300.0 33.2 325.55 335.94 310.0 27.3 340.0 24.5 336.92 305.0 19.1 304.48	RAW DATASMOOTHED DATASOLIDSCARBONSOLIDSCARBONCONCCONCCONCCONC(MG/L)(MG/L)(MG/L)(MG/L)255.071.6254.9071.67255.071.6294.4654.55295.054.1294.4654.55300.033.2325.5541.54310.027.3341.8231.83340.024.5336.9224.63305.019.1304.4819.12	RAW DATA SMOOTHED DATA RATE SOLIDS CARBON SOLIDS CARBON UNIT CONC CONC CONC CONC RATE (MG/L) (MG/L) (MG/L) (MG/L) (I/HR) 255.0 71.6 254.90 71.67 (I/HR) 255.0 71.6 294.46 54.55 0.0688 295.0 54.1 294.46 54.55 0.0561 311.46 47.58 0.0460 300.0 33.2 325.55 41.54 0.0379 335.94 36.32 0.0315 310.0 27.3 341.82 31.83 0.0265 340.0 24.5 336.92 24.63 0.0197 324.54 21.71 0.0176 305.0 19.1 304.48 19.12 0.0165 0.0165	

RUN NUMBER B6

2.5

	RAW DAT	`A	SMOOTHED DATA	RATE	DATA
TIME	SOLIDS CONC	CARBON	SOLIDS CARBON CONC CONC	UNIT RATE -	CARBON CONC
(HR)	(MG/L)	(MG/L)	(MG/L) (MG/L)	(1/HR)	(MG/L)
0.00	250.0	72.4	249.57 72.39		
0.50	•		274.17 .64.88	0.0574	68.83
1.00	. 295.0	57.3	292.47 57.26	0.0538	61.07
1.50		1. S.	305.37 49.76	0.0502	53.51
2.00	315.0	31.2	313.77 42.61	0.0462	46.18
2.50		а. Т	318.56 36.05	0.0415	39.33
3.00	310.0	27.3	320.66 30.31	0.0359	33.18
3.50			320.95 , 25.61	0.0293	27.96
4.00	320.0	21.5	320.35 22.20	0.0213	23.90
4.50	•	•	319.75 20.29	0.0119	21.24
5.00	320.0	20.0	320.05 20.12	0.0011	20.20

DUPLICATE 10ML. SAMPLES WERE USED FOR SOLIDS DATA.

· . . . `

•	RAW DA	TA	SMOOTH	ED DATA	RATE DATA		
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON	
	CONC	CONC	CONC	CONC	RATE	CONC	
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)	
0.00	285.0	59.3	 284•85	59.54			
0.25			303.03	46.51	0.1773	53.03	
0.50	320.0	35.7	319.18	37.18	0.1201	41.85	
0.75			333.06	30.87	0.0773	34.02	
1.00	365.0	28.0	344.41	26.95	0.0464	28.91	
1.25	•		353.01	24.74	0.0253	25.84	
1.50	360.0	23.7	358.59	23.60	0.0128	24.17	
1.75			360.93	22.87	0.0082	23.23	
2.00	360.0	21.9	359.76	21.88	0.0109	22.37	

		****	*********	****			
	RAW DA	TA	SMOOTH	ED DATA	RATE DATA		
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON	
	CONC	CONC	CONC	CONC	RATE	CONC	
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)	
			. '	•			
0.00	275.00	49.9	274.09	49.82			
0.25			311.09	46.56	0.0447	48.19	
0.50	340.0	42.6	334.59	42.14	0.0547	44.35	
0.75			. 347.76	37.12	0.0588	39.63	
1.00	375.0	26.6	353.81	32.05	0.0578	34.59	
1.25		· .	355.90	27.48	0.0515	29.77	
1.50	355.0	23.1	357.24	23.96	0.0395	25.72	
1.75			361.00	22.03	0.0215	22.99	
2.00	370.0	22.1	370.37	22.24	0.0000	22.14	

	RU	N	Ν	UM	В	Ε	R	B	9	
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			•••••••••••••••••••••••••••••••••••••••	•• ••		
RAW DATA			SMOOTHE	D DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
	•	• · · ·	·			
0.00	310.0	46.6	310.33	46.69		-
0.25	1		313.62	40.64	0.0775	43.67
0.50	320.0	35.4	322.05	35.93	0.0593	38.29
0.75		· .	333.91	32.34	0.0438	·34 • 14
1.00	375.0	26.2	347.49	29.65	0.0316	31.00
1.25	•		361.07	27.65	0.0226	28.65
1.50	375.0	26.1	372.93	26.12	0.0167	26.88
1.75	1. Sec. 1. Sec	. ,	381.36	24.84	0.0135	25.48:
2.00	385.0	23.6	384.65	23.60	0.0129	24.22

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

		RU	IN NUMBER B	10		
		****	*****	***		
	RAW DA	TA	SMOOTH	ED DATA	RATE	E DATA
TIME	SOLIDS CONC	CARBON CONC	SOLIDS CONC	CARBON CONC	UNIT RATE	CARBON CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	380.0	55.6	379.01	55.87		
0.25			413.91	43.02	0.1296	49•44
0.50	440.0	32.6	434.11	34.22	0.0830	38.62
0.75			442.81	28.67	0.0506	31.44
1.00	425.0	24.3	443.20	25.58	0.0279	27.13
1.25	•		438.45	24.16	0.0129	24.87
1.50	430.0	23.9	431.76	23.61	0.0051	23.89
1.75			426.31	23.14	0.0044	23.38
2.00	425.0	22.0	425.39	21.95	0.0011	22.55

RAW DATA		SMOOTH	SMOOTHED DATA		RATE DATA	
SOLIDS	CARBON	SOLIDS CONC	CARBON CONC	UNIT	CARBON CONC	
(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)	
375.0	55.8	374.48	56.06	с. А.		
· · ·		399.52	42.85	0.1365	49.46	
420.0	32.0	416.94	33.57	0.0909	38.21	
*	· ·	427.96	27.53	0.0572	30.55	
460.0	24.8	433.80	24.06	0.0323	25.80	
		435.70	22.45	0.0148	23.25	
435.0	22.1	434.88	22.02	0.0039	22.24	
		432.56	22.10	0.0000	22.06	
430.0	22.0	429.98	21.99			
	RAW DA SOLIDS CONC (MG/L) 375.0 420.0 460.0 435.0 430.0	RAW DATA SOLIDS CARBON CONC CONC (MG/L) (MG/L) 375.0 55.8 420.0 32.0 460.0 24.8 435.0 22.1 430.0 22.0	RAW DATA SMOOTH SOLIDS CARBON SOLIDS CONC CONC CONC (MG/L) (MG/L) (MG/L) 375.0 55.8 374.48 399.52 32.0 416.94 427.96 460.0 24.8 433.80 435.0 22.1 434.88 432.56 430.0 22.0 429.98	RAW DATA SMOOTHED DATA SOLIDS CARBON SOLIDS CARBON CONC CONC CONC CONC (MG/L) (MG/L) (MG/L) (MG/L) 375.0 55.8 374.48 56.06 399.52 42.85 420.0 32.0 416.94 33.57 460.0 24.8 433.80 24.06 435.70 22.45 435.0 22.1 430.0 22.0 429.98 21.99	RAW DATA SMOOTHED DATA RATE SOLIDS CARBON SOLIDS CARBON UNIT CONC CONC CONC CONC RATE (MG/L) (MG/L) (MG/L) (MG/L) (1/HR) 375.0 55.8 374.48 56.06 399.52 42.85 0.1365 420.0 32.0 416.94 33.57 0.0909 427.96 27.53 0.0572 460.0 24.8 433.80 24.06 0.0323 435.70 22.45 0.0148 435.0 22.1 434.88 22.02 0.0039 432.56 22.10 0.0000 430.0 22.0 429.98 21.99 1.99 1.99	

RUN NUMBER B12

	RAW DA	TA	SMOOTH	IED DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	370.0	54.9	369.55	55.22	· .	
0.25			399.26	40.93	0.1487	48.07
0.50	425.0	29.5	422.33	31.40	0.0927	36.27
0.75		•	438.98	25.69	0.0531	28.55
1.00	450.0	22.7	449•46	22.84	0.0257	24.27
1.25	·	÷.	454.03	21.90	0.0083	22.37
1.50	455.0	22.3	452.92	21.92	0.0000	21.91
1.75		•	446.37	21.95		
2.00	435.0	21.1	434.65	21.04	•	

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

		RL	IN NUMBER I	B13		. 78.
		***	****	***		
	RAW DA	TA	SMOOTI	HED DATA	RATI	E DATA
TIME	SOLIDS CONC	CARBON CONC	SOLIDS CONC	CARBON CONC	UNIT RATE	CARBON CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	455•0	123•8	454.50	123.75		
0.25			484.43	114.11	0.0822	118.93
0.50	510.0	104.1	507.07	103.85	0.0828	108.98
0.75			523.41	93.33	0.0817	98.59
1.00	525.0	88.2	534.43	82•89	0.0789	88.11
1.25			541.11	72.90	0.0743	77.89
1.50	545.0	. 63.1	544.43	63.70	0.0678	68.30
1.75	· -		545.36	55.65	0.0591	59.67
2.00	. 545-0	49.0	544.90	49.10	0-0481	52.37

RUN NUMBER B14 **************

RAW DATA		SMOOTH	SMOOTHED DATA		RATE DATA	
SOLIDS	CARBON	SOLIDS	CARBON	UNIT .	CARBON	
CONC	CONC	CONC	CONC	RATE	CONC	
(MG/L)	(MG/L)	(MG/L),	(MG/L)	(1/HR)	(MG/L)	
460.0	111.3	459.83	111.14		•	
· ·		478.10	107.39	0.0320	109.26	
495.0	102.2	494.06	101.23	0.0560	104.31	
· .		. 507.95	93.33	0.0631	97.28	
515.0	87.2	520.01	84.32	0.0701	88.82	
		530.50	74.86	0.0720	79.59	
540.0	65.0	539.65	65.60	0.0692	70.23	
	•	547.71	57.20	0.0618	61.40	
555.0	50.2	554.93	50.30	0.0500	. 53.75	
	RAW DA SOLIDS CONC (MG/L) 460.0 495.0 515.0 540.0 555.0	RAW DATA SOLIDS CARBON CONC CONC (MG/L) (MG/L) 460.0 111.3 495.0 102.2 515.0 87.2 540.0 65.0 555.0 50.2	RAW DATA SMOOTH SOLIDS CARBON SOLIDS CONC CONC CONC (MG/L) (MG/L) (MG/L) 460.0 111.3 459.83 478.10 495.0 102.2 515.0 87.2 520.01 530.50 540.0 65.0 539.65 540.0 50.2 554.93	RAW DATASMOOTHED DATASOLIDSCARBONSOLIDSCARBONCONCCONCCONCCONC(MG/L)(MG/L)(MG/L)(MG/L)460.0111.3459.83111.14478.10107.39495.0102.2494.06101.23507.9593.33515.087.2520.0184.32540.065.0539.6565.60540.050.2554.9350.30	RAW DATA SMOOTHED DATA RATI SOLIDS CARBON SOLIDS CARBON UNIT CONC CONC CONC CONC RATE (MG/L) (MG/L) (MG/L) (MG/L) (1/HR) 460.0 111.3 459.83 11.14 460.0 111.3 459.83 11.14 460.0 102.2 494.06 101.23 0.0320 495.0 102.2 494.06 101.23 0.0560 507.95 93.33 0.0631 515.0 87.2 520.01 84.32 0.0701 530.50 74.86 0.0720 540.0 65.0 539.65 65.60 0.0692 547.71 57.20 0.0618 555.0 50.2 554.93 50.30 0.0500	

RUN NUMBER B15 *****

	RAW DA	TA	SMOOTH	ED DATA	RATE	E DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
٥	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	460.0	124.1	459.38	124.06		
0.25	· ·		487.40	114.84	0.0779	119.45
0.50	510.0	105.3	506.37	105.09	0.0785	109.96
0.75	•	•	518.26	95.05	0.0784	100.07
1.00	520.0	85.5	525.04	84.96	0.0774	90.00
1.25	,	· •	528.65	75.05	0.0753	80.00
1.50	530.0	65.2	531.08	65.55	0.0717	70.30
1.75		•	534.26	56.71	0.0664	61-13
2.00	540.0	48.7	547.17	48.76	0.0592	52.73

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

		****	***	****				
	RAW DA	TA	SMOOTI	SMOOTHED DATA		RATE DATA		
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON		
	CONC	CONC	CONC	CONC	RATE	CONC		
(HR)	(MG/L)	(MG/L)	(MG/L.)	(MG/L)	(1/HR)	(MG/L)		
0.00	545.0	148.2	545.26	148.00				
0.25			548.87	142.59	0.0395	145.29		
0.50	555.0	135.4	556.65	134.20	.0.0607	138.40		
0.75			567.85	123.37	0.0771	128.79		
1.00	580.0	114.2	581.74	110.63	0.0887	117.00		
1.25			597,57	96.52	0.0957	103.57.		
1.50	620.0	81.4	614.59	81.57	0.0986	89.04		
1.75		• •	632.07	66.33	0.0978	73.95		
2.00	650.0	50.8	649.28	51.23	0.0937	58.83		
2.25			665.46	37.10	0.0866	44.21		
2.50	680.0	24.1	679.87	24.19	0.0768	30.64		

RUN NUMBER B17

		,				
	RAW DAT	A	. SMOOTH	ED DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR) [`]	(MG/L)
0.00	585.0	151.6	586.26	151.25		•.
0.25			. 567.35	146.31	0.0343	148.78
0.50	560.0	138.3	567.68	136.25	0.0709	141.28
0.75		•	582.59	122.29	0.0971	129.27
1.00	600.0	110.0	607.43	105.61	0.1122	113.95
1.25		·	637.52	87.40	0.1170	96.50
1.50	650.0	66.1	668.25	68.86	0.01130	78.13
1.75	•		694.85	51.19	0.1037	60.03
2.00	720.0	33.8	712.76	35.58	0.0887	43.38
2.25	· · ·		717.30	23.21	0.0692	29.40
2.50	705.0	15.0	703.79	15.30	0.0446	19.25

RUN NUMBER B18

	RAW DATA		SMOOTH	SMOOTHED DATA		RATE DATA	
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON	
	CONC	- CONC	CONC	CONC	RATE	CONC	
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	. (MG/L)	
	-						
0.00	555.0	161.7	554.87	161.71			
0.25	· · · ·		565.41	150.54	0.0798	156.12	
0.50	575.0	139.5	574.34	139.58	0.0769	145.06	
0.75			582.14	128.63	0.0758	134 + 11	
1.00	580.0	121.0	589-29	117.45	0.0763	.123.04	
1.25			596.25	105.83	0.0784	111.64	
1.50	580.0	95.0	603.52	93.54	0.0819	99.68	
1.75	,		611.57	80.36	0.0868	86.95	
2.00	620.0	66.7	620.87	66.08	0.0927	73.22	
2.25	· ·•		631.90	50.46	0.0997	58.027	
2.50	645.0	33.4	645.14	33.30	0.1075	41.88	
•							

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

		****	******	****		
	RAW DA	TA	SMOOTH	HED DATA	RATE DATA	
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	675.0	89.0	673.72	88.98		
0.25			722.50	75.37	0.0780	82.17
0.50	760.0	61.6	752.46	61.49	0.0752	68 .43
0.75			766.92	• 48.12	0.0704	54.81
1.00	745.0	28.0	769.19	35.99	0.0632	42.05
1.25			762.58	25.87	0.0529	30.93
1.50	750.0	16.8	750.40	18.51	0.0389	22.19
1.75			735.96	14.66	0.0207	16.58
2.00	725.0	14.8	722.56	15.08	0.0000	14.87

RUN NUMBER B20

	RAW DA	TA 💡	SMOOTH	ED DATA	RATE DATA UNIT CARBON RATE CONC	
TIME	SOLIDS CONC	CARBON	SOLIDS CONC	CARBON CONC	UNIT RATE	CARBON CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	730-0	95.6	729.05	96.10		
0.25		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	768.71	67.57	0.1524	81.84
0.50	800.0	.43.6	794.38	46.64	0.1071	57.11
0.75	•		. 807.04	32.12	0.0726	39.38
1.00	780.0	16.7	807.67	- 22.80	0.0462	27.46
1.25	•	•	797.24	17.48	0.0265	20.14
1.50	780.0	14.8	776.74	14.96	0.0128	16.22
1.75	:		747.15	14.04	0.0048	14.50
2.00	710.0	13.5	709.45	13.53	0.0028	13.78

RUN NUMBER 821

	~ ~ ~ ~			-	
	RAW DATA	SMOOTH	ED DATA	RATE	DATA
TIME	SOLIDS. CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L) (MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
			9. J. J.	1	•
0.00	655.0 102.0	651.50	101.88	- e ¹	
0.25		746.39	88.28	0.0778	95.08
0.50	810.0 73.6	789.11	72.87	0.0803	80.58
0.75		793.62	56.96	0.0804	64.91
1.00	765.0 43.6	773.90	41.85	0.0771	49.40
1.25	· .	743.92	28.87	0.0684	35.36
1.50	705.0 16.9	717.64	19.33	0.0522	24.08
1.75		709.05	14.53	0.0269	16.93
2.00	730.0 15.4	732.10	15.80	0.0000	15.17
	•				

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

81.

RUN NUMBER B25

	RAW DA	TA	SMOOT	HED DATA	RATE	E DATA
TIME	SOLIDS CONC	CARBON CONC	SOLIDS CONC	CARBON CONC	UNIT RATE	CARBON CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	745-0	128-8	745.75	128.77	•	
0.25	14300	12000	754.39	107.71	0.1123	118.24
0.50	770.0	86•4	774.62	86.23	0.1123	96.97
0.75			801.80	·65.51	0.1052	75.87
1.00	895.0	48•2	831.27	46.67	0.0923	56.09
1.25	•		858.37	30.89	. 0.0747	38.78
1.50	885.0	16.7	878.44	19.30	0.0534	25.09
1.75			886.84	13.07	0.0283	16.18
2.00	880.0	12.9	878.90	13.33	0.0000	13.20

RUN NUMBER B26

		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		<b>n</b> <i>n n</i>		
	RAW DA	TA S	SMOOTH	HED DATA	RATE	E DATA
TIME	SOLIDS CONC	CARBON CONC	SOLIDS CONC	CARBON	UNIT RATE	CARBON CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	760.0	126.8	760.23	126.73		
0.25	· · · · ·	•	781.47	106.56	0.1047	116.64
0.50	805.0	85.8	806•50 . 832•88	85•41 64•62	0.1066 0.1014	95•98 - 75•01
1.00	865.0	49.6	858.16	45.54	0.0903	55.08 37.51
1.50	900.0	15.0	895.62	17.82	0.0526	23.66
2.00	900•0	12.5	902•89 899•26	12.97	0.0265	12.42

## RUN NUMBER B27

	RAW DA	ТА	SMOOTH	HED DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RAIE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
	•			-		
0.00	745.0	116.7	745.52	116.35		
0.25	•		770.33	105.47	0.0574	110.91
0.50	800.0	91.5	803.21	89.43	0.0815	97.45
0.75			837.78	70.54	0.0921	79.99
1.00	860.0	49•8	867.66	51.09	0.0912	60.82
1.25	•		886.50	33.40	0.0807	42.25
1.50	900.0	16.3	887.91	19.77	0.0615	26.58
1.75			865.53	12.49	0.0332	16.13
2.00	815.0	13.3	812.98	13.88	00000	13.18
			•			

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

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		RU	IN NUMBER B	322		02.
		****	*****	****		
	RAW DA	TA	SMOOTH	HED DATA	RATE	E DATA
TIME	SOLIDS.	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	550.0	112.7	549.49	112.89		
0.25			589.45	88.92	0.1683	100.91
0.50	625.0	66.7	622.03.	67.86	0.1391	78.39
0.75			647.70	49.89	0.1132	58.87
1.00	665.0	34.7	666.96	35.22	0.0893	42.55
1.25			680.29	24.03	0.0664	29.62
1.50	690.0	14•9	688•20	16.53	0.0438	20.28
1.75			691.17	12.91	0.0210	14.72
2.00	690.0	13.1	6 <u>89</u> 69	13.37	0.0000	13.14

#### RUN NUMBER B23 *****

	RAW DA	TA	SMOOTI	HED DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	540.0	110.8	539.69	110.95		•
0.25			581.15	88.49	0.1603	99.72
0.50	620.0	67.4	618.23	68.30	0.1347	78.40
0.75			. 648.97	50.73	0.1109	59.52
1.00	635.0	33.5	671.40	36.13	0.0885	43.43
1.25			683•58	24.85	0.0666	30.49
1.50	690•0	15.5	683.52	17.25	0.0445	21.05
1.75			669.29	13.68	0.0211	15.47
2.00	640.0	14.2	638.91	14.49	0.0000	14.09

#### RUN NUMBER B24 *****

	RAW DA	TA .	SMOOTH	HED DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR) .	(MG/L)
0.00	560.0	111.5	560.66	111.72		
0.25	•		569.77	87.45	0.1718	99.59
0.50	585.0	65.2	589.06	66.55	0.1443	77.00
0.75			614.61	49.07	0.1162	57.81
1.00	650.0	34.7	642.51	35.08	0.0890	42.07
1.25			668.83	24.63	0.0637	29.85
1.50	695.0	16.3	689.64	17.74	0.0403	21.21
1.75		· · · · ·	701.04	14.61	0.0183	16.20
2.00	700.0	14.9	699.10	15.15	0.0000	14.88

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

82.

		****	*****	****		
	RAW <b>DA</b>	TA	SMOOTH	ED DATA	RATE	E DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	390.0	61.1	389.68	61.13		
0.25			413.74	· 51.06	0.1004	56.09
0.50	435.0	41.3	433.11	41.51	0.0901	46.28.
0.75	•		448.05	32.83	0.0789	37.17
1.00	. 475.0	18.0	458.80	25.31	0.0663	29.07
1.25			465.60	19.29	0.0521	22.30
1.50	470.0	14.1	468•70	15.08	0.0361	17.18
1.75			468.35	13.00	0.0178	14.04
2.00	465.0	13.2	464.78	13.36	0.0000	13.18

#### RUN NUMBER B29 ****

	****	*******	***		
RAW DA	TA AT	SMOOTH	IED DATA	RATE	E DATA
SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
CONC	CONC	CONC	CONC	· RATE	CONC
(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
420.0	60.7	420.09	60.82		
· *		434.58	48.29	0.1173	54.56
450.0	36.9	450.60	37.66	0.0962	42.97
	•	. 466•21	28.89	0.0765	33.27
500.0	13.6	479.43	21.99	0.0584	25.44
		488•32	16.94	0.0418	19.47
495.0	13.0	490.90	13.72	0.0263	15.33
		485.22	12.32	0.0115	13.02
470.0	12.6	469.31	12.72	0.0000	12.52
	RAW DA SOLIDS CONC (MG/L) 420.0 450.0 500.0 495.0 470.0	RAW DATA SOLIDS CARBON CONC CONC (MG/L) (MG/L) 420.0 60.7 450.0 36.9 500.0 13.6 495.0 13.0 470.0 12.6	RAW DATA       SMOOTH         SOLIDS       CARBON       SOLIDS         CONC       CONC       CONC         (MG/L)       (MG/L)       (MG/L)         420.0       60.7       420.09         434.58       450.0       36.9       450.60         466.21       500.0       13.6       479.43         495.0       13.0       490.90       485.22         470.0       12.6       469.31	RAW DATA       SMOOTHED DATA         SOLIDS       CARBON       SOLIDS       CARBON         CONC       CONC       CONC       CONC         (MG/L)       (MG/L)       (MG/L)       (MG/L)         420.0       60.7       420.09       60.82         434.58       48.29         450.0       36.9       450.60       37.66         466.21       28.89         500.0       13.6       479.43       21.99         488.32       16.94         495.0       13.0       490.90       13.72         470.0       12.6       469.31       12.72	RAW DATA       SMOOTHED DATA       RATE         SOLIDS       CARBON       SOLIDS       CARBON       UNIT         CONC       CONC       CONC       CONC       RATE         (MG/L)       (MG/L)       (MG/L)       (MG/L)       (1/HR)         420.0       60.7       420.09       60.82       60.82         434.58       48.29       0.1173       60.0962         450.0       36.9       450.60       37.66       0.0962         466.21       28.89       0.0765       500.0       13.6       479.43       21.99       0.0584         495.0       13.0       490.90       13.72       0.0263       485.22       12.32       0.0115         470.0       12.6       469.31       12.72       0.0000       0.0000

DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

DATA FOR RUN B30 WAS DISCARDED DUE TO LEAKAGE OF CONDENSER CONTENTS INTO THE REACTOR

	RAW DA	TA	SMOOTH	ED DATA	RATE	DATA
TIME	SOLIDS CONC	CARBON CONC	SOLIDS CONC	CARBON CONC	UNIT RATE	CARBON CONC
(HR)	(MG/L)	(MG/L) ·	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	520.0	66•5	518.65	66.56		
0.25			577.80	54.66	0.0868	60.61
0.50	625.0	43.3	617.01	43.65	0.0737	49.16
0.75			638.99	33.84	0.0625	38.75
1.00	640.0	. 16.8	646.43	25.25	0.0518	29.68
1.25			642.03 ·	19.00	0.0405	22.26
1.50	630.0	13.5	628•48	14.58	0.0278	16.79
1.75			608.49	12.58	0.0130	13.58
2.00	585.0	13.1	584.74	13.28	0.0000	12.93

## RUN NUMBER B32

		****	******	*		
	RAW DATA	,	SMOOTHE	D DATA	RATE	DATA
TIME	SOLIDS C	ARBON	SOLIDS CONC	CARBON	UNIT	CARBON CONC
(HR)	(MG/L) (	MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	545.0	73.0	544.32	73.10	•	•
0.25		•	577.63	58.98	0.1007	66•04
0.50	605.0	45.8	601.01	46.43	0.0851	52.70
075			. 615.92	35.65	0.0709	41.04
1.00	635.0	17.6	623.83	26.81	0.0570	31.23
1.25		•	626.21	20.09	0.0430	23.45
1.50	625.0	14.6	624•54	15.66	0.0284	17.87
1.75			620.28	13.69	0.0126	14.68
2.00	615.0	14.2	614.91	14.38	0.0000	14.04

## RUN NUMBER B33

				•		
	RAW DA	ΓA ····································	- SMOOTHE	D DATA	RATE	DATA
TIME	SOLIDS	CARBON	SOLIDS	CARBON	UNIT	CARBON
	CONC	CONC	CONC	CONC	RATE .	CONC
(HR)	(MG/L)	(MG/L)	(MG/L)	(MG/L)	(1/HR)	(MG/L)
0.00	480.0	80.0	478.88	80.11		
0.25			519.40	64.53	0.1249	72.32
0.50	550.0	50.0	543.36	50.70 ····	0.1041	57.62
0.75			554.45	38.79	0.0868	44.74
1.00	555.0	23.1	556.33	28.93	0.0710	33.86
1.25			552.68	21.27	0.0553	25.10
1.50	545.0	14.9	547.19	15.96	0.0386	18.61
1.75		•	543.52	13.14	0.0206	14.55
2.00	545.0	12.8	545.36	12.98	0.0012	13.06

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DUPLICATE 20ML. SAMPLES WERE USED FOR SOLIDS DATA.

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### APPENDIX (VI)

Computer Programs used in this Study

NOMENCLATURE USED IN COMPUTER PROGRAMS

- C SOLUBLE ORGANIC CARBON CONCENTRATION RAW DATA
- CAV AVERAGE SOLUBLE ORGANIC CARBON CONCENTRATION IN A FIXED TIME INTERVAL
- CB1,CB2 BATCH CARBON CONCENTRATIONS WHICH STRADDLE THE STEADY STATE CARBON CONCENTRATION
- CNEW CARBON CONCENTRATIONS GENERATED FROM RAW DATA
- C1 CARBON CONCENTRATIONS WHICH INCLUDE GENERATED DATA
- CS SYMBOL USED IN SMOOTHING ROUTINE TO REPRESENT SMOOTHED DATA
- CR SYMBOL USED IN SMOOTHING ROUTINE TO REPRESENT RAW DATA
- DCDT RATE OF CHANGE OF CARBON CONCENTRATION
- DCDTM UNIT RATE OF ORGANIC CARBON REMOVAL

JRUN, JBRUN NUMBER OF BATCH RUNS

JCRUN NUMBER OF CONTINUOUS RUNS

- NPOINT NUMBER OF RAW DATA POINTS IN A BATCH RUN
- Q VOLUMETRIC FLOW RATE INTO CONTINUOUS REACTOR
- R1,R2 BATCH UNIT CARBON REMOVAL RATES CORRESPONDING TO CARBON CONCENTRATIONS CB1 AND CP2
- R3 BATCH UNIT RATE CORRESPONDING TO THE STEADY STATE CARBON CONCENTRATION ON THE CONTINUOUS REACTOR
- R4 STEADY STATE UNIT RATE OF CARBON REMOVAL IN THE CONTINUOUS REACTOR
- SSC STEADY STATE CARBON CONCENTRATION IN THE CONTINUOUS REACTOR
- SSM STEADY STATE SOLIDS CONCENTRATION IN THE CONTINUOUS REACTOR

T TIME

TAU DETENTION TIME

TNEW GENERATED TIME DATA

- TI TIME VALUES WHICH INCLUDE GENERATED DATA
- V VOLUME OF CONTINUOUS REACTOR

SUSPENDED	SOLIDS	CONCENTRATIONS-RAW	JATA
-----------	--------	--------------------	------

N

- WAV AVERAGE SUSPENDED SOLIDS CONCENTRATION IN A FIXED TIME INTERVAL
- WNEW SUSPENDED SOLIDS CONCENTRATIONS GENERATED FROM RAW DATA

W1 SUSPENDED SOLIDS CONCENTRATIONS WHICH INCLUDE GENERATED DATA

```
С
  c
                  PROGRAM FOR EVALUATING THE UNIT RATE OF CARBON REMOVAL FROM
  c
c
                  BATCH CARBON AND SOLIDS DATA AS FUNCTIONS OF TIME
                DIMENSION C(20) +T(20) +W(20) +TNEW(20) +WNEW(20) +T1(20) +C1(20) +W1(20)
             1.WAV(20).CAV(20).DCDT(20).DCDTM(20).CNEW(20).CS(20).CR(20)
           READ(5:9) JRUN
9 FORMAT(15)
               LL=1
         10 READ(5+1) NPOINT
               READ(5+2) (W(J)+J=1+NPOINT)
                READ(5+2) (T(J)+J=1+NPOINT)
                READ(5+2) (C(J)+J=1+NPOINT)
            1 FORMAT(15)
           2 FORMAT(10F8.2)
 ¢
                 GENERATION OF NEW DATA FROM RAW DATA
 c
               NP=NPOINT-1
               DO 3 J=1+NP
TNEW(J)=(T(J)+T(J+1))/2.0
               CNEW(J)=(C(J)+C(J+1))/2.0
           3 WNEW(J)=(W(J)+W(J+1))/2.0
               DO 4 J=1,NP
               I=2#J
               T1(I)=TNEW(J)
               C1(I)=CNEW(J)
          4 W1(1)=WNEW(J)
               DO 5 J=1.NPOINT
               1=2*J-1
                T1(I)=T(J)
               C1(I)=C(J)
          5 W1(I)=W(J)
 ¢
 c
                 SMOOTHING OF RAW AND GENERATED DATA
 с
               NT=2*NPOINT-1
                KK=1
               DO 21 I=1,NT
         21 CR(I)=C1(I)
         25 JJ≠0
               GO TO 24
        13 DO 20 I=1.NT
20 CR(I)=CS(I)
         24 CS(1)=(1./70.)*(69.*CR(1)+4.*CR(2)-6.*CR(3)+4.*CR(4)-CR(5))
               CS(2)=(1./35.)*(2.*CR(1)+27.*CR(2)+12.*CR(3)-8.*CR(4)+2.*CR(5))
               NTT=NT-2
               DO 11 I=3.NTT
         11 C5(I)=(1./35.)*(-3.*CR(I-2)+12.*CR(I-1)+17.*CR(I)+12.*CR(I+1)-3.*C
             1R(I+2))
               CS(NT-1)=(1+/35+)*(2+*CR(NT-4)+8+*CR(NT-3)+12+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+27+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-2)+*CR(NT-
             1-1)+2.*CR(NT))
               CS(NI)=(1./70.)*(-CR(NT-4)+4.*CR(NT-3)-6.*CR(NT-2)+4.*CR(NT-1)+69.
             1*CR(NT))
               JJ=JJ+1
               IF(JJ.EQ.998.OR.JJ.EQ.999) GO TO 14
IF(JJ.LT.1000) GO TO 13
IF(KK.EQ.2) GO TO 18
        16 DO 17 I=1.NT
       17 Cl(I)=CS(I)
GO TO 14
18 DO 19 I=1.NT
        19 W1(I)=CS(I)
        14 WRITE(6+15) (CS(1)+1=1+NT)
        15 FORMAT(15F8.2/)
               IF(JJ.LT.1000) GO TO 13
              KK=KK+1
              IF (KK .EQ.3) GO TO 22
              DO 23 I=1+NT
       23 CR(I)=W1(I)
              GO TO 25
С
c
c
c
c
                     CALCULATION OF DERIVATIVE AND 1/M(DC/DT) USING A LINEAR
                                                                     APPROXIMATION
       22 NN=2*NP
             WRITE(6,8)
         8 FORMAT(19H UNIT RATE 1/MDC/DT+5X+13HCONCENTRATION)
             DO 6 I=1.NN
             WAV(I)=(W1(I)+W1(I+1))/2.0
             DCDT(1)=(C1(1)-C1(1+1))/(T1(1+1)-T1(1))
             CAV(I)=(C1(I)+C1(I+1))/2.0
             DCDTM(I)=(1.0/WAV(I))+DCDT(I)
         6 #RITE(6,7) DCDTM(1),CAV(1)
7 FORMAT(2X,F10.5,13X,F10.5/)
             LL=LL+1
             IF(LL.LE.JRUN) GO TO 10
             STOP
             END
```

PROGRAM FOR CALCULATING THE UNIT RATE OF CARBON REMOVAL ON A CONTINUOUS RUN FROM STEADY STATE CARBON AND SOLIDS DATA

THE PROGRAM ALSO CALCULATES THE BATCH UNIT RATE OF CARBON REMOVAL CORRESPONDING TO THE STEADY STATE CARBON CONCENTRATION ON THE CONTINUOUS RUN

WRITE(6,3)

С

- 3 FORMAT(11H BATCH RATE, 5X, 15HCONTINUOUS RATE, 5X, 13HCONCENTRATION, 5X 1,14HDETENTION TIME)
- READ(5,1) JCRUN 1 FORMAT(I5)
- J=1 8 READ(5,2) V,Q,CO,SSC,SSM
- 2 FORMAT(5F10.5)
- I=1 READ(5,4) JBRUN
- 4 FORMAT(I5)
- 7 READ(5,5) CB1,CB2,R1,R2

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5 FORMAT(4F10.5)
R3=R1+((SSC-CB1)/(CB2-CB1))*(R2-R1)
TAU=V/Q/60.0
R4=(CO-C)/SSM/TAU
WRITE(6,6) R3,R4,SSC,TAU
6 FORMAT(2X,F8.4,8X,F8.4,8X,F8.2,10X,F8.2)
I=I+1
IF(I.LE.JBRUN) GO TO 7
J=J+1
IF(J.LE.JCRUN) GO TO 8
STOP
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END