CARBON BALANCE

ON

A CONTINUOUS BIOREACTOR

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by

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SCOPE AND CONTENTS:

Using soluble organic carbon in the form of dextrose as a growth limiting substrate, and pure cultures of Escherichia coli, the carbon-containing products of a completely mixed, environmentally controlled, continuous bioreactor were quantitatively analyzed in order to determine if accurate carbon balances are obtainable for a wide spectrum of bacterial growth rates, and if gaseous carbon production exhibits a correlation with bacterial growth rate.

The techniques of experimentation and analysis were developed and refined during the course of the study. Errors were accumulated in the carbon balances, these being considered due to inaccuracies in sampling of the gaseous reactor effluent. Assessment of error significance was made statistically. Soluble and cellular carbon analyses were accurately completed. A limited correlation of gaseous carbon production rate with growth rate was demonstrated.

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CHAPTER 1

1. INTRODUCTION

The removal of unwanted organic material from a water source via biological waste treatment systems is effected by mixed cultures of floc forming bacteria. Subsequent separation of the floc purifies the liquid. This activated sludge biological treatment process generally comprises a well-mixed aeration tank, where microorganisms and organic material react, followed by a clarifier for floc separation. Efficient operation of the process requires maximum organics removal. Thus recycle of solids from the clarifier to the aeration tank increases the microbial concentration and hence the organics removal rate. For optimum operation, the microbial solids content in the clarifier which includes the excess cells of recycle and growth, must be controlled such that: (a) overloading of the clarifier does not occur giving effluent contamination, (b) solids recycle to the aeration tank is sufficient for the required efficiency of waste purification.

Traditionally, the principal variables for determining the strengths of wastes and controlling their treatment by the activated sludge process, have been the oxygen removal characteristics engendered by bacterial utilization of dissolved organics, quantitised in the Biochemical Oxygen Demand test. The inherent inaccuracies in BOD data are well known and include pH and temperature effects, interference from nitrifying bacteria, dilution effects, sampling errors and principally, the

arbitrary period of time which must elapse to give a "5-day BOD" value for a sample. Use of BOD tests for control of treatment processes where optimum operating conditions may vary continually is not meaningful. Use of similar data for plant design does not offer the highest accuracy. The use of a conserved variable, such as organic carbon has been recommended for these operations.

Carbon is the basic food supply of living matter and is continually changed from phase to phase by the biochemical, chemical and physical processes indigenous to bacterial physiology. Organic carbon removal is the basic purpose of waste treatment. It would seem logical that carbon content should be the primary variable for a description of the food supply, (in this case, the biodegradable organic content of a waste water), the microbial concentration in the treatment process, the existence of carbon-containing by-products of metabolism and the effluent quality. Rapid, accurate analysis of organic carbon in all the forms present in a waste treatment system is feasible with currently available analytical equipment. The completion of a carbon balance about such a system can offer accurate information on the effluent quality, the efficiency of operation and drift from optimum conditions, the biodegradability of a waste and aeration efficiency, all within one hour of sampling.

The purpose of this study was to investigate the assessment of a carbon balance about a waste treatment system. A continuous biochemical aerobic reactor without recycle was

chosen. Careful consideration was given to control of the bacterial environment, so that variables affecting cellular growth were minimized and regulated, to maximize an accurate assessment of analytical techniques and resultant experimental errors.

CHAPTER 2

2. LITERATURE REVIEW

2.1. Design Procedures

Engineering design procedures for processes effecting the aerobic biodegradation of industrial wastes containing soluble organics, should by definition determine that a given treatment operation results in a predetermined effluent quality. Since the classical work of Streeter and Phelps (5) on the natural purification of streams, there has been a trend towards increasingly exact design sophistication in the field of biological waste removal, in an attempt to set up a consistent theory for solids growth and pollutant removal (6) (7). Busch (1) (2) reported a rational design approach involving laboratory pilot scale, continuous flow, activated sludge units. The design equations were formulated from steady state balances of organic substrate concentration, measured in oxygen demand units (BOD), across a completely mixed reactor. Busch (2) also stated that the concept of completely mixed systems must be accepted as a requisite for a controlled reactor producing a controlled, predictable effluent, notwithstanding the imperfect mixing often realised in practice.

The kinetic and design data obtained for a given waste (2) were also considered to be valid for that waste alone. The use of an overall rate, applicable to all wastes was not acceptable. Gaudy (4) reported similar studies. McKinney (3) disagreed with Busch in that a fundamental design basis was formulated for all

completely mixed, activated sludge systems, regardless of the waste being treated. Design equations were again formulated from a consideration of mass balances in oxygen demand equivalents.

Keshavan et. al. (8), Schulze (13) and Mancini (14) report laboratory studies on more or less small scale systems, in which oxygen demand units were used to develop design criteria. Eckenfelder (10) offered a procedure to develop a process design standard for aerobic biological processes from limited laboratory data. Considerable assumptions regarding the response of the biological floc in different systems were made. Oxygen equivalents were again used throughout.

Recently (1970), Lawrence (11) et. al. developed a unified basis for design and operation of biological treatment facilities, the unifying parameter being biological solids retention time, a concept originated by Garrett and Sawyer (12). Again, the concept of substrate concentration in terms of indirect oxygen equivalent units was used, and it was considered that relationships developed for pure cultures were applicable to mixed and non-defined cultures.

A review of many of the important contributions in the last decade towards waste treatment plant design shows a diversity of methods and techniques employed, but in nearly all cases, oxygen equivalents in terms of BOD, or COD define the quantity of biodegradable organic pollutant present in the waste.

The measurement of the organic pollution of surface waters, by whatever means, is fundamental to water pollution control and treatment facility design. The use of more fundamental parameters than those of oxygen equivalents would seem to be desirable.

2.2. Development of More Realistic Parameters

The Biochemical Oxygen Demand Test (19), despite its many failings and its insecure basis, is still extensively used as the principle parameter in design for treatment of wastes. Stumm (9) et. al. query the use of complex operational parameters of the BOD type in design studies. They allude that it is not possible to obtain conclusive and conceptual relationships on the kinetics of substrate utilization from collective metabolic parameters of the BOD type, particularly in multi-substrate media, where oxidation is mediated by mixed cultures, each organism possessing different metabolic activities and different nutritional requirements. Even in single substrate systems, an exact correlation between BOD or COD and substrate concentration does not necessarily exist when degradation intermediates or cell waste products can exact a chemical or biological oxygen demand. Obviously, the soluble organic loading in a wastewater will also be a complex mixture of organic substances, whose identification and assessment by analytical techniques is of little consequence, when considered against the volume and variability of the wastewater, especially as no chemical analysis gives any indication of the biodegradability of a given substance.

These facts do not preclude successful full-scale treatment of a waste, but a technique of quantitative measurement of pollution is necessary, and hence the use of the BOD test (19). Numerous reports (28) (17) (18) (22) (23) in the literature have tried to improve the test and rectify some of the disadvantages and inaccuracies, i.e., variable temperature, pH, dilution effects, nitrifying bacteria and length of test, etc. Busch (16) states that the most desirable approach for assessing the biodegradable content of a waste, would be to consider a complete material balance across the system, each reactant and each product being quantitatively measured, thus overcoming the inaccuracies and the indirect approach of quantitative oxygen demand techniques.

Correlations between BOD, chemical oxygen demand (COD) and total organic carbon (TOC) have often been considered, although neither the quantity of chemically oxidizable material (COD), nor the TOC present, are necessarily equivalent to the quantity of biodegradable material in the waste. Determinations of the organic carbon concentration of an aqueous sample have been carried out chemically (20), although these methods have been superseded with the advent of the infra-red carbonaceous analyzer (24) (25). Ford (21) discussed the implications in correlating oxygen and carbon-based parameters, and concluded that TOC is a fundamental pollution parameter, and can be correlated to COD in industrial wastewaters. BOD/TOC correlations are possible in domestic effluents.

Thus the inherent inaccuracies of BOD data, and the volume of literature dealing with methods for its improvement, emphasize the need for more definitive parameters for following organic waste utilization in a waste treatment process. With the advent of the infra-red carbonaceous analyzer (24) (25), it is feasible to monitor the carbon content of waste treatment or bacteriological systems in a minimal time. The method is based on the rapid combustion of a micro sample in oxygen, and measurement of the carbon dioxide evolved with a non-dispersive infra-red analyzer. This instrumental method has three basic advantages, (i) a direct measure of organic, oxidisable carbon is made - the primary parameter in bacterial synthesis of pollutants, (ii) complete oxidation of any form of carbon introduced is effected (25) and no serious sources of interference have been found, (iii) economy of samples and materials is maximised, and time of analysis is greatly reduced, it being possible to run 30 or 40 carbon samples per hour. The only limitation of the method is that inorganic carbon (e.g., dissolved CO_2, carbonates) has to be removed by acidification before the organic carbon determination is carried out. Van Hall and Stenger (25) attempted to correlate COD and BOD data with organic carbon values obtained with a carbonaceous analyzer, but the results were not promising. Rickard and Riley (26) surveyed the utility of infra-red carbon analysis in following the metabolism of organic compounds during the batch growth of a bacterial culture in a defined medium. The relationships

that were obtained between cellular carbon, exogenous soluble carbon and viable cell count during bacterial growth were detailed, together with synthesis rates of cellular material. A carbon balance across the system was achieved, with a maximum error of 5%. Gaseous carbon produced by the organisms as carbon dioxide was measured by difference. A distinction was emphasised between the soluble carbon contained in residual, unused substrate, and the soluble carbon present as by-products of cellular metabolism. The by-product carbon concentration was obtained by subtracting a value for soluble substrate carbon from total soluble carbon, the total soluble carbon value, measured on the infra-red analyzer, being inclusive of both substrate and by-product carbon. A value for cellular carbon was simply obtained by injecting a washed sample of cells from a known volume of reactor fluid into the carbon analyzer. This report appears to be the first to attempt a carbon balance via use of the infra-red carbon analyzer, across a bacterial growth system. Schaffer (27) et. al. extensively evaluated the application of the infra-red analyzer to the waste treatment field, and verified the suitability and precision of the method on a wide variety of materials. Particle size reduction by grinding methods were used for insoluble carbon containing materials, with success. An indication of the utility of the technique for process stream analysis was obtained. The relationship between the COD, BOD and organic carbon values was investigated at various points throughout a representative wastewater

treatment plant. A successful attempt was also made to run a material balance on carbon in a full scale plant. A good balance was obtained across the primary settler. A fairly consistent error in the material balance across the secondary portion of the plant was obtained, which was assumed to be that removed by the aeration tank off-gases as carbon dioxide. The carbon held in solution as free CO_2 or bicarbonate was considered to be minimal in comparison with the overall balance. A total balance across the entire plant gave a similar error - again assumed due to gaseous carbon production. An attempt was made to collect, chemically absorb, and analyze the gaseous carbon produced in the aeration tanks. A better closure of the material balance was demonstrated using the results of this analysis.

Busch (16) considered that the use of the infra-red carbonaceous analyzer would be most effective, in obtaining a measure of the carbon distribution between cell synthesis and carbon dioxide production, thus facilitating an assessment of the total bacterially available carbon in the influent, and the completion of a material balance about the system. The availability of a definitive test for the bacterially available carbon content of a waste, an effluent, or a receiving water which could be simply completed in 1-2 hours was considered to be of great potential in design or treatability studies. In design studies, minimum oxygen requirements could be estimated from the oxygen equivalent of the net change in total carbon,

i.e., the total change in soluble carbon less the carbon content of synthesized cells. This amounts to the carbon removed as gaseous products. The oxygen produced by the system as CO_2 (i.e., the oxygen equivalent of the total carbon change of the system), is related to the minimum oxygen requirement of the system by a ratio given from the equation of reaction between oxygen and the degradable substrate.

As well as calculations of gaseous carbon evolution from a waste treatment system, quantitative laboratory analyses have been made. Betancur (29) estimated the gaseous carbon evolved from a batch biochemical reactor, and completed material balances about the system with the use of a gas chromatograph to measure the volume/time ratio of carbon dioxide produced. Watson (30) used an infra-red carbon dioxide analyzer to measure CO_2 concentrations produced by bacteria in continuous culture, and control the specific growth rate of the organisms by linking the gas concentration signal to a pump supplying substrate to the reactor. Schaffer (27) absorbed effluent carbon dioxide in alkaline solutions and determined the resultant concentration using the infra-red carbonaceous analyzer. Ludzack (31) et. al. absorbed CO_2 in barium hydroxide solution and titrimetrically estimated the gas concentration present.

Hiser (32) in a recent report, goes a step further in considering the measurement of carbon as a primary parameter in waste treatment systems. It is suggested that carbon parameters be used to describe the food and culture content of

microbiological systems, and the change in total oxygen demand be used to describe the energy requirements of those same systems. A carbon balance was carried out across a batch biochemical reactor, utilizing an infra-red carbonaceous analyzer, and a chemical absorption method for carbon dioxide. The variability of dissolved inorganic carbon in a bioreactor with varying pH was pointed out, and the analysis problem of solids or suspended organic carbon was highlighted. No solution to these difficulties was proposed.

2.3. Biokinetics

When a soluble organic waste is degraded via bacterial metabolism in the presence of oxygen and nutrients, a fraction of the organic waste is converted into cellular material, the remainder being oxidized to provide energy. It is feasible to apply these physiological concepts to treatment plant design by developing mathematical relationships between the various parameters such as growth rate, substrate removal rate and oxygen consumption rate of the defined system. Many "kinetic mechanisms" have been reported - usually connecting growth rate and substrate concentration (45) (46) (47) (5) (8) (33) (34) (12) (39) (40) (41) (42) (44) (35). The Michaelis-Menten model; one of the most widely accepted equations, is derived by considering the bacteria/substrate reaction as an enzyme catalysed process. Monod (36) verified the Michaelis-Menten model for pure culture batch growth, suggesting the following form:

$$K_1 = K_m \left[\frac{S}{Sn + S} \right]$$

where K_1 = specific growth rate

K_m = maximum growth rate

S = substrate concentration

Sn = constant, equal to the substrate concentration at which $K_1 = K_m/2$.

Monod's expression was verified for batch and continuous pure culture systems by Novick (37) and Herbert et. al. (38). Grieves (39) applied the equation to mixed culture continuous systems. Pollock (43) has compiled a comprehensive review of kinetic models reported in the literature, and states that as the defined kinetic mechanisms are based on either oxygen demand equivalents or organic carbon concentrations, and as the complete set of reactions undergone by either oxygen or carbon in these processes are unknown, the "kinetic" models have little reaction kinetic significance, and represent curve fitting techniques. This point has recently been emphasised by Matales and Chian (48) in stating that the Monod equation implies nothing concerning the mechanisms limiting growth rate. It merely is a convenient correlation of the data.

2.4. Theory of Continuous Culture

The essential feature of the continuous culture of microorganisms, is that the microbial growth takes place under steady state conditions, i.e., at a constant rate and under constant conditions. Such variables as pH, nutrient concentration, metabolic products and oxygen, which inevitably change during batch bacterial growth are all usually maintained at a constant level in continuous culture, or they may be independently varied by the experimenter. All continuous flow systems consist of a reactor into which reactants flow at a steady flow rate, and products are removed. The variable factors controlling this scheme will include the kinetics of the reaction occuring and the residence time distribution of particles passing through the system. Most reactors lie between two extremes - the completely mixed reactor or CSTR, and the plug flow reactor or PFTR. In a PFTR, all particles have the same residence time equivalent to the mean residence time in the reactor. A CSTR exhibits a wide range of residence times about the mean value. The extent of reaction occurring in a PFTR will be the same as in a batch reactor operated at the mean residence time of the PFTR, that in a CSTR will vary due to the range of residence times therein. Danckwerts (49) infers that the PFTR favours reactions whose rate diminishes with time, and the CSTR favours reactions with an increasing rate with time (for example, auto-catalytic reactions). Hence, the CSTR will be the most efficient type of reactor for bacterial metabolism in continuous culture.

It has been a common occurence that reaction conditions in the aeration tanks in the activated sludge process fell short of the PFTR status originally intended. As it is easier to design for completely mixed (CSTR) conditions, and indeed CSTR's allow the system to operate efficiently in a steady rather than a transient state, completely mixed activated sludge systems would seem to be advantageous.

(i) Basic Theory

The theory and operation of continuous cultures has been reviewed by several authors including Herbert (50) et. al., James (51) and Novick (52). Briefly, it may be considered that the growth rate of organisms in the logarithmic phase may be expressed as:

$$\frac{dx}{dt} = K_1 x \qquad (1)$$

where k_1 = specific growth rate (i.e., rate of growth per unit cell weight per unit time.)

x = concentration of bacterial cells

t = time

Now, assume a pure culture of bacteria growing in an aerated and completely mixed constant volume reactor, being fed nutrient at a constant rate. The rate at which the cells are leaving the reactor will be:

$$-\frac{dx}{dt} = \frac{f}{V}.x = Dx \qquad (2)$$

where f = nutrient flow rate

V = reactor volume

$D = \frac{f}{V}$ = dilution rate

The balance equation for the cell concentration in the reactor is then:

$$\frac{dx}{dt} = K_1x - Dx = x(K_1 - D) \qquad (3)$$

This equation infers that when $K_1 > D$, $\frac{dx}{dt}$ is positive, and the cell concentration in the reactor will increase with time.

Conversely, when $K_1 < D$, $\frac{dx}{dt}$ is negative, and the cell concentration in the reactor will decrease and approach zero at $t = \infty$.

Hence, a steady state with a constant cell concentration, is possible only when $\frac{dx}{dt} = 0$. In this condition:

$$K_1 = D = \frac{f}{V} = \frac{1}{t_r} \qquad (4)$$

where t_r = mean retention time.

This implies that under steady state conditions, the specific growth rate of the organisms equals the dilution rate or the reciprocal residence time. If the reactor fluid volume

is constant, the specific growth rate is determined solely by the nutrient feed rate, and can simply be varied by varying the feed rate. This means that a feed rate decrease results in an effluent substrate decrease due to the increased time of contact between cells and substrate. For any value of residence time, as long as the equivalent cellular specific growth rate is below Km, the maximum growth rate, the culture automatically establishes a steady effluent concentration for as long as other interfering conditions are absent. It is this self-regulatory property of the system that makes steady state, substrate limited, continuous operation possible.

Monod (36) showed that in cultures in which the sole growth limiting factor was the concentration of carbon substrate, the quantity of bacteria produced was a constant fraction, Y, of the quantity of substrate utilized.

$$x = Y (S_o - S) \tag{5}$$

where Y = yield constant

S_o = initial substrate concentration

S = substrate concentration at time t.

Differentiating (5) we have

$$\frac{dx}{dt} = -Y \frac{dS}{dt} \tag{6}$$

It should be noted that Y is not necessarily a true constant, either for pure or heterogeneous cultures (53).

Applying the Monod (36) model to equation (4), we have:

$$K_1 = D = \frac{1}{t_r} = Km\left[\frac{S}{Sn + S}\right] \qquad (7)$$

where S refers to the reactor substrate concentration

Sn = constant, equal to the substrate concentration at which $K_1 = Km/2$ (see section 2.3.)

Equation (7) may be solved for S:

$$S = Sn\left[\frac{D}{Km - D}\right] \qquad (8)$$

The rate of change in the substrate concentration in the reactor, can be equated to the rate of feeding the substrate minus the rate at which the substrate is diluted out of the system in the effluent line and the rate at which substrate is consumed by the organisms in the reactor. This balance can be expressed;

$$\frac{dS}{dt} = DS_o - D_S - K_1\frac{\dot{x}}{Y} \qquad (9)$$

From equations (5) and (8), the steady state concentrations of biological solids and substrate can be calculated for any dilution rate if the growth constants Km, Sn and Y are accurately known, and provided these "constants" are true system constants.

(ii) Oxygen Demand of a Growing Culture

In the aerobic respiration of microorganisms, oxygen acts as the ultimate electron acceptor (54). Pirt (55) investigated the relationship between the amount of available oxygen, and the carbon balance in a bacterial culture. Suppose that for each mole of growth-limiting substrate utilized, P moles of oxygen are required. If the concentration of dissolved oxygen is c, the oxygen uptake ($-\Delta c$) in time t, is given by:

$$-\Delta c = P(So - S) \tag{10}$$

Differentiating equation (10)

$$-\frac{dc}{dt} = -P\frac{dS}{dt} \tag{11}$$

where $\frac{-dc}{dt}$ denotes the oxygen uptake rate.

From equations (6) and (11), the oxygen uptake rate is directly proportional to the growth rate:

Therefore:
$$\frac{-dc}{dt} = \frac{P}{Y}\frac{dx}{dt} \tag{12}$$

From equations (1) and (12)

$$\frac{-dc}{dt} = \frac{P}{Y} K_1 x \tag{13}$$

From equations (5) and (13)

$$\frac{-dc}{dt} = P K_1 (So - S) \qquad (14)$$

For a continuous culture in the steady state, the specific growth rate is equal to the dilution rate.

$$\frac{-dc}{dt} = P D (So - S) \qquad (15)$$

Hence, the oxygen demand of the population of organisms in a continuous culture is also directly proportional to the dilution rate and to the amount of substrate removed at steady state.

(iii) Carbon Dioxide Synthesis

The rate of production of CO_2 by a growing population of microorganisms in steady state has been considered by Watson (30). The rate of production of CO_2 by a growing population x, is given by:

$$\frac{d\ CO_2}{dt} = \frac{dx}{dt} \frac{1}{YCO_2} \qquad (16)$$

where YCO_2 is the yield factor with respect to carbon dioxide

$dx\ /d\ CO_2$

From equations (1) and (16)

$$\frac{d\ CO_2}{dt} = \frac{K_1 x}{YCO_2} \qquad (17)$$

From equation (16), the rate of production of CO_2 from bacterial metabolism is directly proportional to the growth rate of organisms, if YCO_2 is constant. Similarly from equation (12) the oxygen uptake rate is directly proportional to the growth rate, providing Y and P are constants. Hence in general, the rate of production of carbon dioxide should provide an equivalent measure of cellular metabolism/growth rate, as does oxygen uptake, if YCO_2, Y and P are true constants.

Pirt (55) demonstrated that in fully aerobic systems in pure culture, practically all the substrate carbon could be accounted for as CO_2 and cells, unless growth rates near the maximum were achieved, where by-products such as acetic acid were produced, at the expense of carbon dioxide. In partially aerobic systems, Pirt (55) concluded that the nature of the end products of carbon metabolism depends on the oxygen deficiency which is defined as the difference between the amount of oxygen available, and the amount necessary for fully aerobic growth. A decrease in carbon dioxide production (in fully aerobic continuous culture) might be considered therefore, as evidence of near-maximum growth rate, or production of alternative end products, or both. Rickard and Riley (26) in a study of non-substrate limited batch systems found considerable by-product formation after several hours incubation, other than carbon dioxide, probably due to an oxygen limited condition occurring in the batch reactor after this lapsed time.

It should be emphasised that YCO_2 and Y are not generally constant for wide ranges of specific growth rates or ranges of temperature, but Watson (30) did obtain good consistency of YCO_2 measurements on two strains of a pure culture at varying temperatures. Also the equilibrium concentration of carbon dioxide over a biochemical reactor fluid surface, will depend on the pH of the fluid. Sawyer (56) presents the CO_2, alkalinity and pH relationships as follows:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

$$M(HCO_3)_2 \rightleftharpoons M^{++} + 2\,HCO_3^-$$

$$HCO_3^- \rightleftharpoons CO_3^= + H^+$$

$$CO_3^= + H_2O \rightleftharpoons HCO_3^- + OH^-$$

Hence for the relationship

$$\frac{dCO_2}{dt} = [CO_2]\ fg$$

to hold, where $[CO_2]$ = concentration of CO_2 in outflowing gas

fg = flow rate of gas phase

the pH must be constant.

It should also be noted that the fixation of evolved carbon dioxide by growing bacteria can occur (60).

2.5. The Practice of Continuous Culture

Busch (1) (2) (16) described equipment which is typical of laboratory "scaling down" of full scale practice. The unit incorporated complete mixing, controlled solids wastage and controlled hydraulic loading, with integral clarification without anaerobic sedimentation. No controls were imposed on pH, temperature or sterility of the unit. Mixed cultures of non-defined bacteria, and a non defined substrate were used. It was claimed that "continuous flow studies in conjunction with batch aeration using organisms from the continuous flow system, can yield all necessary design criteria for aerobic bio-oxidation". It was emphasised that the use an overall reaction rate for all wastes was not acceptable. Keshavan et. al. (8) operated continuous flow equipment incorporating complete mixing via aeration, a synthetic waste of known composition, and continuous inflow of "return activated sludge" of indeterminate composition. No control of pH, temperature or sterility was employed. No claims were made as to any possible general applicability of parameters obtained with this equipment. Schulze (13) reported the use of a completely mixed system without recycle utilizing sterile equipment, a single substrate synthetic "waste", and a pure culture of Escherichia coli. Temperature was accurately controlled throughout and pH was maintained at a reasonably constant value by manual adjustments. Effluent substrate, cellular concentration, dissolved oxygen, oxygen uptake and pH were all monitored for this system. Possible contamination of

the system was considered and the system shut down and sterilised if this occured. Notwithstanding the difference in this "ideal" laboratory system and the activated sludge process, application of the data generated appeared to be possible. Ludzack et. al. (31) operated bench-scale activated sludge units incorporating diffused aeration and sludge settling. Mixed cultures and mixed wastes were employed. No sterility, pH or temperature controls were effected. Foam control was successfully carried out using a silicone compound. A comparison of the performance of this unit with conventional loading, compared favourably with that shown in records of plant units. The early studies of Garrett and Sawyer (12) utilized a well mixed, diffused aeration reactor with temperature control, mixed cultures of organisms and a synthetic waste of known composition. Hatfield and Strong (57) operated continuous units with mixed bacterial flocs and real wastes. Sludge settling and return was incorporated. No control over pH, temperature or foaming was made.

Gaudy et. al. (53) studied the kinetic behaviour of heterogeneous microbial populations in a completely mixed reactor at various dilution rates. The apparatus incorporated constant temperature control, complete mixing and sludge settling for a synthetic waste. Dissolved oxygen and pH were recorded throughout. A later study (58) incorporated sludge recycle and pH control.

The mechanics of continuous bacterial culture and the experimental procedures reported in the waste water literature

are diverse. A further consideration of the microbiological, biochemical and bioengineering literature gives wider applications of the technique.

CHAPTER 3

3. SCOPE OF THE INVESTIGATION

The desirability of improving design techniques, using treatability studies carried out in the laboratory, has been emphasized in the literature. The primary objective of this study is to determine the feasibility of completing a material balance about an environmentally controlled biochemical reactor system utilizing rapid and accurate quantitative techniques, with carbon as the measured variable. Secondary considerations include the assessment of carbon dioxide evolution as a function of growth rate, and a qualitative consideration of the accuracy of the techniques employed. A brief check on the fit of the data to bacterial growth equations is made.

A broad spectrum of experimental techniques is available. This study considers only an aerated continuous biochemical reactor without recycle. No attempt is made to reproduce a small scale treatment system with unknown wastes and non-defined mixed bacterial cultures. The experiments are regarded as an initial assessment of the accuracy and utility of the technique, rather than as producing viable results for use in design or treatment. Hence, attempts are made to control the bacterial growth environment as closely as possible, constraints being placed on the following:

(i) pH

(ii) temperature

(iii) influent substrate concentration

(iv) foaming

(v) reactor fluid volume

Pure cultures of bacteria from a single source are used throughout, and steps taken to avoid contamination. The reactor is considered to be completely mixed, incorporating mixing by mechanical, and diffused aeration systems. A brief study investigates the efficiency of reactor aeration.

The independent variable chosen for the system is the growth rate of microorganisms. Resulting dependent variables include effluent substrate concentration and rate of gas production. The continuous reactor is operated to a substrate limiting condition with constant influent substrate concentration, resulting in constant cellular concentrations at steady state conditions. This system allows examination of gas production and closure of material balances at different cellular growth rates. No studies are made at varying substrate concentrations, at varying suspended solids concentrations, or on biochemical systems with recycle. The variables measured are:

(i) Total influent flow rate.

(ii) Soluble organic carbon concentration in the reactor effluent.

(iii) Total organic carbon concentration in the reactor effluent.

(iv) Volume percentage of carbon dioxide evolved from the reactor.

(v) Suspended solids in the effluent.

(vi) Atmospheric pressure.

(vii) Head pressure in the reactor.

(viii) Oxygen flow rate into the reactor.

CHAPTER 4

4. EXPERIMENTATION

4.1. Description of Experimental Equipment

4.1.1. The Continuous Reactor

The continuous reactor, shown in Figure (1), consisted of a 4 litre capacity glass container with a built-in epoxy spindle, carrying a magnetically driven stainless steel impeller, and a stainless steel fermenter head incorporating twelve ports. Stainless steel baffles were fitted around the inner wall of the reactor. The liquid effluent removal port was located at the two-litre level. Three of the ports in the fermenter head were connected to the substrate, nutrient and alkali sources respectively. A pH probe and needle valve were fitted and the oxygen supply line was connected to the air sparger port. The effluent gases from the reactor were led away for sampling via another port. Other exits in the fermenter head were closed off and an O-ring made up and used as a gas tight gasket between the head and the reactor rim. The reactor was placed in a thermostatically-controlled water bath. The liquid effluent removal port incorporated the two bends as shown, (a) to fit the reactor in the water bath, (b) to prevent oxygen bubbles entering the effluent line during aeration. Complete mixing of the reactants was achieved by a combination of sparger aeration and magnetic mixing. Hence, a representative sample of the reactants was always obtained at the liquid effluent port. Evaporation of the reactor liquid was minimised as the

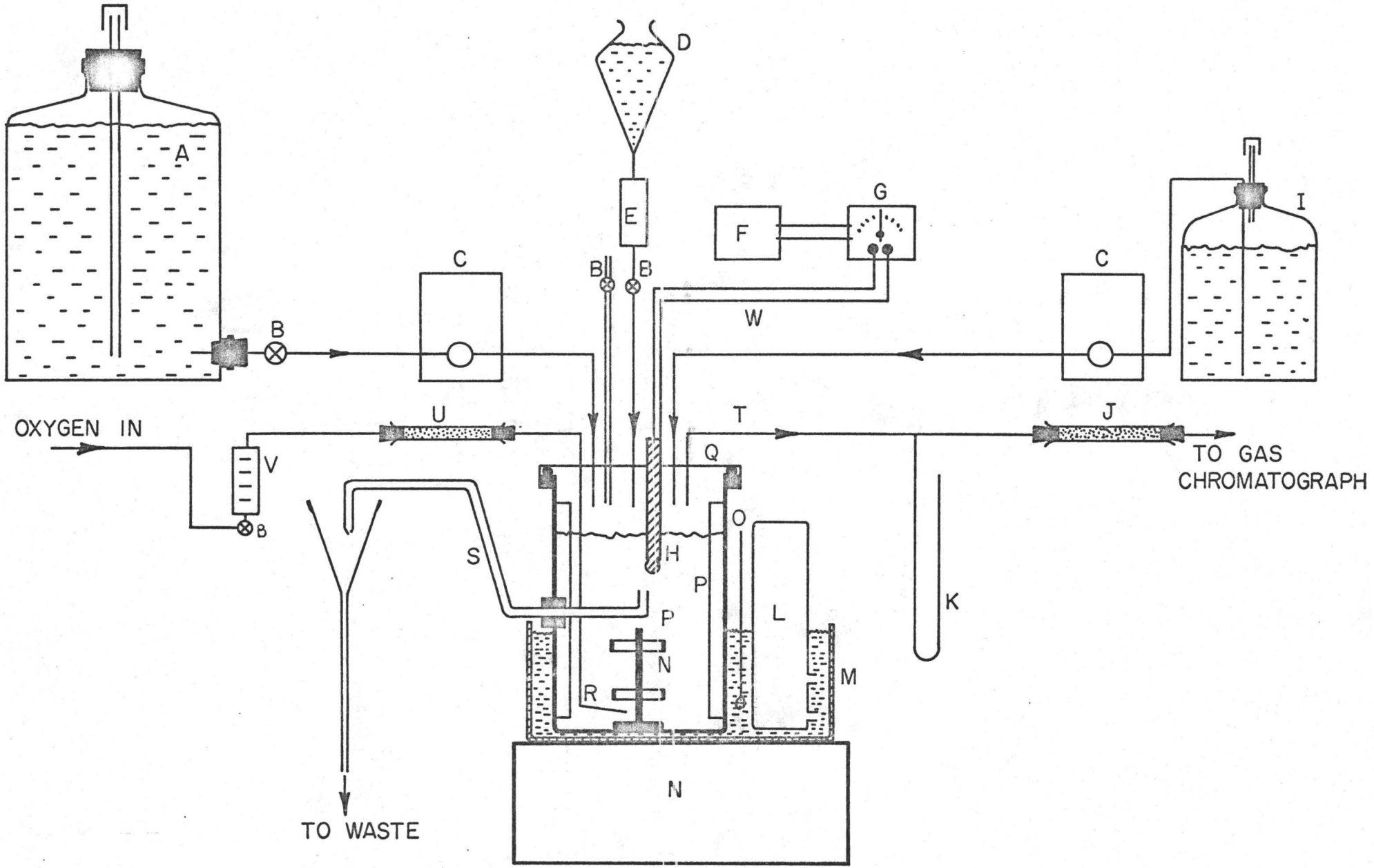
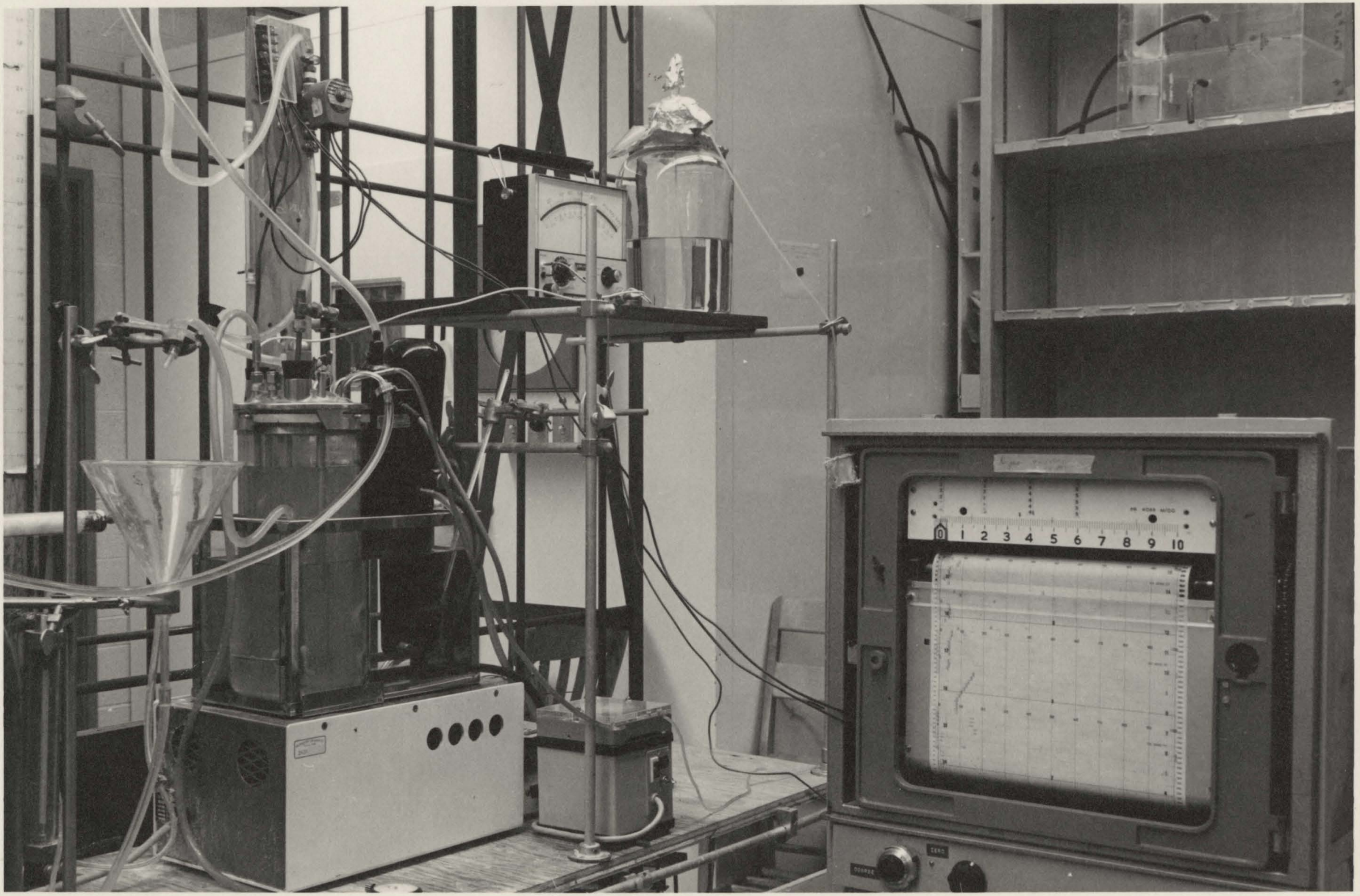


FIGURE. 1 EXPERIMENTAL EQUIPMENT

A DILUTION WATER RESERVOIR
B NEEDLE VALVES
C PERISTALTIC PUMPS
D ALKALI RESERVOIR
E SOLENOID VALVE
F pH RECORDER
G pH METER
H pH PROBE
I SUBSTRATE RESERVOIR
J DRYING TUBE ($CaSO_4$)
K U-TUBE MANOMETER
L THERMOSTAT
M WATER BATH
N MAGNETIC STIRRER AND MOTOR
O THERMOMETER
P CONTINUOUS REACTOR WITH BAFFLES
Q FERMENTER HEAD WITH O-RING SEAL
R AIR SPARGER PORT
S REACTOR LIQUID EFFLUENT PORT
T REACTOR GASEOUS EFFLUENT PORT
U GLASSWOOL FILTER
V GAS FLOWMETER
W ELECTRICAL CONNECTIONS


0 1 2 3 4 5 6 7 8 9 10

gas effluent line acted as an air condenser, and the majority of the water vapour in the exit gases was condensed and returned to the reactor. Foaming of the reactants and solids deposition above the liquid level were very successfully eliminated with the application of a silicone antifoam compound, (Dow Corp. Antifoam A) prior to each experiment, to the upper part of the inner wall of the reactor, and the underside of the fermenter head. The total volume of the reactant liquid throughout the experiments was fixed at 3500 ml. Hence, prior to experimentation, the reactor was calibrated by adding this volume of water and marking the liquid level position.

4.1.2. Feed System for the Continuous Reactor

After several trials, it was considered that the best method of ensuring a steady flow of substrate liquid into the reactor was via mechanical pumping. The dilution water containing trace elements was pumped into the reactor using a Sigma motor peristaltic pump (Model AL 4E40), and the substrate solution with an LKB peristaltic pump (Model 10200). For details of the contents of the feed solutions, see Appendix (B). The sodium hydroxide solution for pH control, was added via gravity.

4.1.3. The Oxygen Supply

A constant oxygen supply to the reactor was required, both to provide a high degree of mixing and an excess of dissolved oxygen. Oxygen was supplied at 20 psi from a cylinder

controlled with pressure reducing and needle valves. An oxygen source was preferred to a compressed air source, due to the presence of carbon dioxide in the latter. The gas was passed through a glass wool plug to remove dust or other unwanted solid material, and then through a carefully calibrated flow-meter, before passing into the reactor. An experimental aeration study, and a theoretical calculation (Appendix C), were carried out to determine if oxygen supply was sufficient.

4.1.4. The pH Controller

The pH control system, shown in Figure (2), utilised the design principles of equipment reported by Callow and Pirt (61). The apparatus included a pH combination electrode (Fisher type No. 13-639-92), a pH meter (Orion Model 401) a recorder (Philips PR 3210 A/00), a solenoid valve (Ascoelectric Limited, No. 8263.A8), and a two-way microswitch (Cherryelectric Prod. Corp. E33). An aluminum baseplate was constructed for the microswitch and bolted to the recorder chassis, such that movement of the recorder pen carriage activated the switch. The switch and solenoid valve were connected in series, and the recorder connected to the pH meter/probe assembly and adjusted such that 0.1 of a pH unit was equivalent to approximately two centimeters of recorder pen travel. A reservoir of 2N sodium hydroxide solution was positioned two feet above the valve, and the alkali feed line to the reactor incorporated the valve. During operation, a continuous decrease in pH of the reactant was evident due to dissolving carbon dioxide and

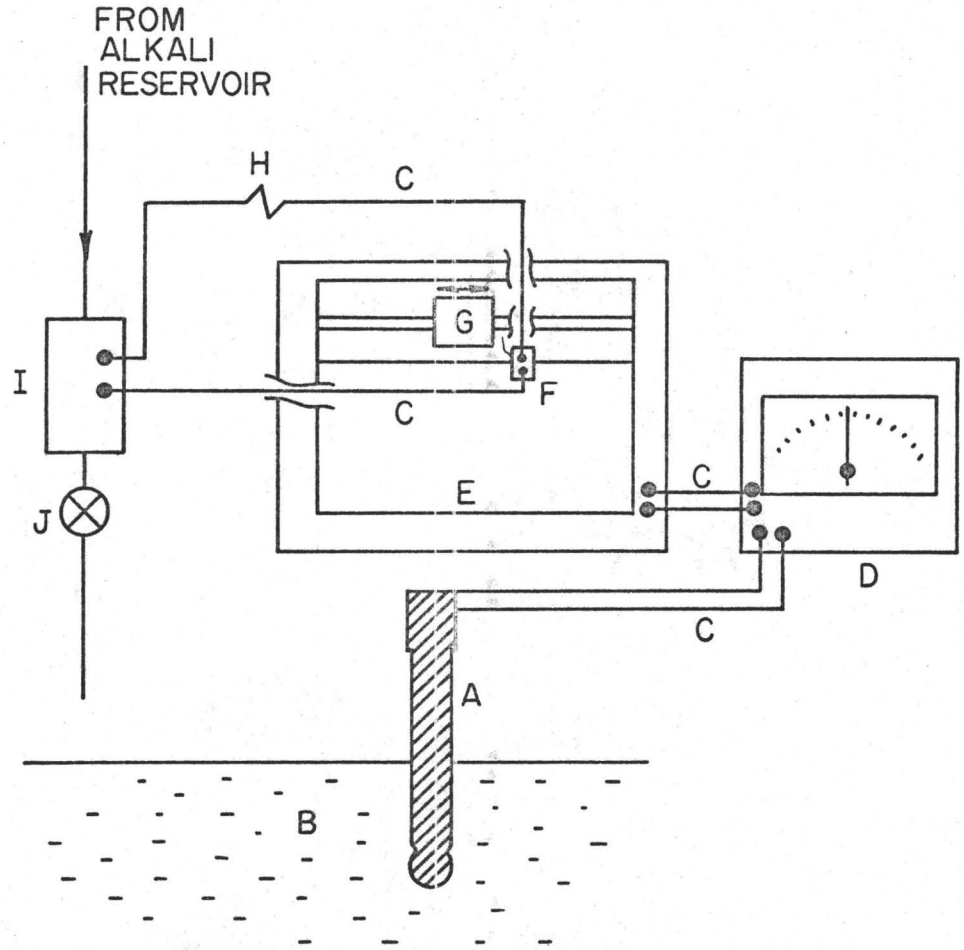


FIGURE.2 pH CONTROL SYSTEM

A	pH PROBE	F	MICROSWITCH
B	REACTOR FLUID	G	RECORDER PEN CARRIAGE
C	ELECTRICAL CONNECTIONS	H	A.C. MAIN SUPPLY
D	pH METER	I	SOLENOID VALVE
E	RECORDER	J	NEEDLE VALVE

addition of acidic dilution water. The resultant drift of the recorder pen carriage activated the switch and opened the valve at a selected pH. Alkali addition quickly reversed the decreasing pH trend, the pen carriage immediately reversed direction and the switch and valve closed, stopping the flow of sodium hydroxide. Due to the very sensitive recorder, pH could easily be controlled to $\pm$ 0.05 of a pH unit in this way. The variables which influenced the degree of control were: (a) the flowrate of alkali to the reactor - adjusted with a needle valve below the solenoid valve, (b) the concentration of sodium hydroxide used, 2N NaOH being found to be the optimum. This control equipment effected a steady pH 6.8 $\pm$ 0.1 for the duration of this study, with no maintenance other than refilling the alkali reservoir, and calibrating and cleaning the combination electrode between runs.

4.1.5. The Gas Chromatograph

Gas-solid chromatography was used to measure the concentration of carbon dioxide evolved from the continuous reactor. A Varian Aerograph 90 - P3 chromatograph was used for all experiments. Quantitative detection depends on differential gas adsorption across an environmentally controlled column of adsorbent. For the particular application required in this work, several modifications were made to the gas analysis system (Figure (3)). Between the reactor vessel and the chromatograph, two items were included in the gas effluent

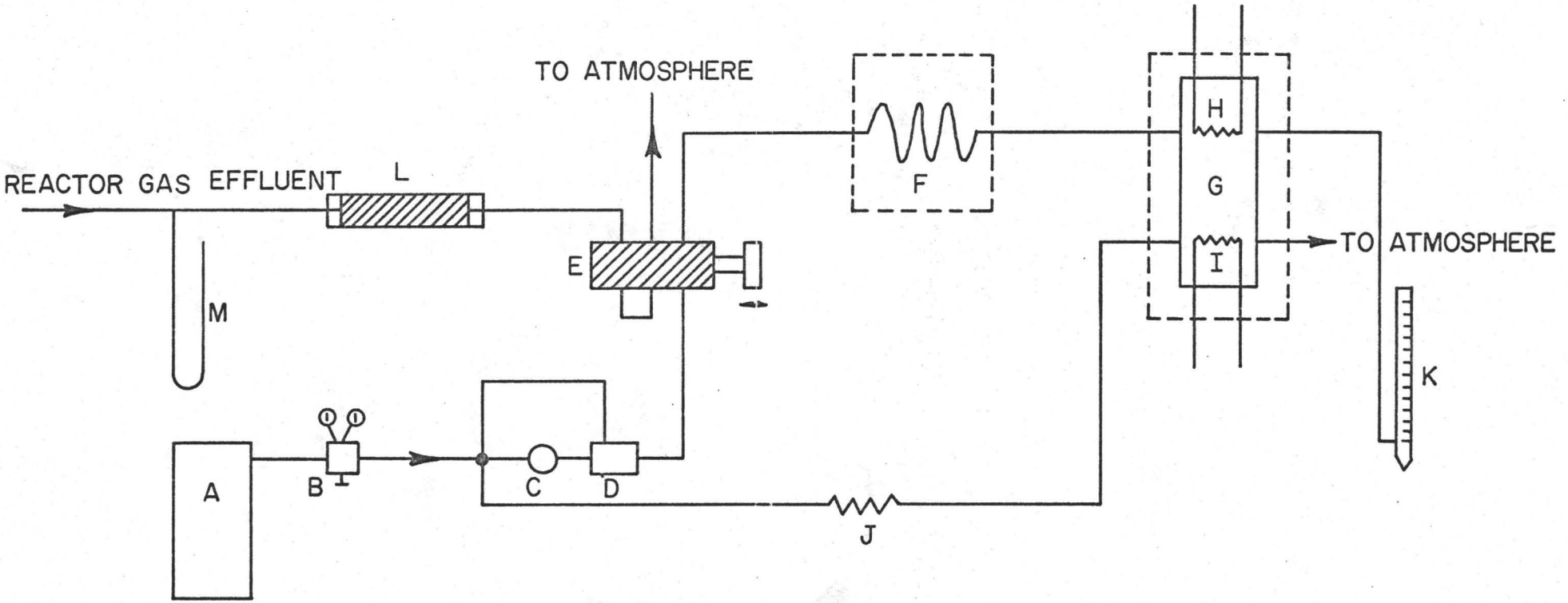


FIGURE.3 GAS ANALYSIS SYSTEM

A HELIUM CARRIER GAS TANK
B PRESSURE REDUCTION VALVE
C CARRIER GAS NEEDLE VALVE
D FLOW CONTROLLER
E GAS SAMPLING VALVE
F CHROMATOGRAPH OVEN AND COLUMN
G DETECTOR BLOCK AND OVEN
H THERMISTOR SENSING FILAMENTS
I THERMISTOR REFERENCE FILAMENTS
J REFERENCE FLOW RESTRICTOR
K BUBBLE FLOW METER
L CALCIUM SULPHATE DRYING TUBE
M U-TUBE MANOMETER

line, (a) a U-tube manometer containing a solution of rhodamine B (density = 1.00) to assess the pressure in the reactor in excess of atmospheric pressure, (b) a drying tube packed with calcium sulphate as adsorbent for any residual water vapour in the effluent gases (CO_2 is not absorbed by $CaSO_4$). A 1/8" x 36" stainless steel column was packed with 80 - 100 mesh silica gel and fitted into the 90 - P3. The column was purged with helium carrier gas at 130°C for 8 hours, at a flow rate of 50 ml/min. Injection of gas samples was effected using a gas sampling valve of 0.25 ml capacity. This valve has two positions: in the closed state, the carrier gas flows uninterrupted through part of the valve into the column, and reactor effluent gases (O_2 + CO_2), pass through a secondary section into the atmosphere. When the valve is opened, the secondary section through which the effluent gas is passing becomes part of the carrier gas stream, and is quantitatively injected therein. The oxygen in the sample is eluted through the column and is detected, showing a single positive peak on the recorder. The carbon dioxide is held up via adsorption on the silica gel, and appears as the next positive peak.

The detection system in the 90 - P3 was also modified. Normally supplied is a thermal conductivity detector incorporating four tungsten filaments, built into a reference cell and a sampling cell. The differential thermal conductivity of the gaseous sample compared with the reference thermal conductivity of the carrier gas defines the sample detection signal. This

detector was replaced by a double 8 kilohm filament thermistor detector (Varian 01-000591-00), due to the ageing of standard tungsten filaments in an oxidizing gas stream. The electronics of the instrument were also necessarily modified. The signal output from the chromatograph was amplified and recorded on a Honeywell Electronik 194 recorder. The chromatograph was calibrated with gas mixtures, each containing a different volume % of carbon dioxide. A second U-tube manometer was used in all calibrations carried out during the experiment in order to exactly equate the calibratory gas pressure with the effluent sample gas pressure at the sample valve.

4.1.6. The Infra-red Carbonaceous Analyzer

Organic carbon analyses were performed using a Beckman Instruments IR 315 infra-red analyzer. 20-microlitre samples were injected with a syringe into a combustion tube at 950°C. Cobalt oxide impregnated asbestos packing in the combustion tube disperses the sample and catalyzes its oxidation by the heated oxygen present, producing carbon dioxide. The carbon dioxide passes through a non-dispersive infra-red analyzer sensitive to CO_2. The signal output of the analyzer is electronically amplified and recorded on a strip chart recorder as a sharp, symmetrical peak. The peak height so produced is directly proportional to the carbon content of the sample. Calibration of the analyzer is effected with known standards (Appendix D).

4.1.7. The Sonication Equipment

An ultrasonic generator (Blackstone Ultrasonic Model SS-2) and probe (Blackstone Model BP-2) was used to disrupt cellular material, prior to determination of the carbon content of unfiltered samples containing bacteria. 5-10 minutes sonication appeared to disrupt the cellular material efficiently. Any prolonged exposure caused a considerable temperature increase.

4.1.8. The Water Bath

Control of the reactor temperature was effected with a thermostat and water bath. 24 $\pm$ 0.5°C was used throughout the experiment so that maximum cellular growth was achieved at an experimentally feasible influent flow rate, within the range of the pumps used. Temperature control also ensured a constant value for maximum growth rate for all experiments (63).

4.2. Experimental Techniques

4.2.1. Equipment Sterility and Assembly

Prior to each run, the fermenter head and reactor vessel were assembled using the O-ring gasket and tested for gas-tightness. Substrate, dilution water, the vinyl tubing (Tygon brand) used in transferring liquid and the assembled reactor were sterilised at 100°C and atmospheric pressure for 30 minutes. After cooling, the liquid substrate and dilution water reservoirs were positioned and connected with the pumps

and fermenter head, the reactor itself being placed in the constant temperature bath. All liquid/gas effluent tubing was sterilized in a similar way and connected. The pH probe was sterilized via immersion in decinormal hydrochloric acid for 30 minutes, and the controller assembly connected up. The aeration line and bleed valve were attached.

4.2.2. Bacterial Storage and Reactor Inoculation

Pure cultures were used throughout this study, the organism selected being Escherichia coli B (ATCC.23226). Sterile agar plates were streaked with bacteria from the original slant, and incubated at 30°C for 48 hours, then stored at 0°C until samples were required.

Inoculation of the reactor presented some problems at first. The final technique involved inoculation of sterile 150 ml samples of nutrient broth (Fisher Scientific, Cat. No. J-1089-C) contained in conical flasks. The broth was incubated at 27°C for 24 hours in a shaker, operating at 1 cycle/sec., at a cycle amplitude of 2 inches. The resultant culture was added to the reactor liquid at pH 6.8 and 24°C. It was generally necessary to allow further growth in the 4 litre reactor in a batch condition with aeration and mechanical mixing prior to the initiation of continuous operation, so that bacterial growth rate was in excess of cellular washout rate. Generally, 150 ml. nutrient broth batches were also stored at 0°C until required.

4.2.3. Calibration of pH Meter

Calibration was carried out between continuous runs using standard pH 6.86 buffer solution. The meter was also temperature compensated for a reactor liquid temperature of 24°C.

4.2.4. Determination of Influent, Effluent Rates; Control of Constant Reactor Volume

The LKB peristaltic pump was used to pump the concentrated substrate (glucose) solution into the reactor. Flow rate determinations were run on a volumetric basis prior to each run, the rate being approximately equal to 85 ml/hour. The feed rate of this pump was kept approximately constant throughout the experiments, and hence the substrate concentration had to be varied for varying total flow rates through the reactor to maintain an overall constant substrate concentration in the influent. These variations are detailed in Appendix (E). The Sigma motor peristaltic pump was used to pump dilution water to the reactor, and also used to vary the overall influent flow rate. Hence for a given flow for any individual experiment, the Sigma motor pump was approximately calibrated prior to the run, at the required dilution water flow rate. Continuous operation involved flow of substrate, dilution water and a small volume of alkali, into the reactor. The overall flow rate was hence the sum of these. Reactor effluent was removed from the vessel via a siphon arrangement (Figure (1)). The reactor volume was controlled by two variables, (i) the vertical

distance between the effluent exit and the reactor liquid level, (ii) the head pressure in the reactor in excess of atmospheric pressure. The head pressure was controlled by adjustments to the oxygen flow rate and to the bleed valve built into the fermenter head. The total flow rate through the reactor system was measured at frequent intervals during each run. Necessary adjustments were made by accelerating or retarding the dilution water flow. After initiating the run, substrate flow, alkali flow, reactor volume and oxygen flow were maintained as constant as possible.

4.2.5. Determination of Oxygen Flow Rate

The flow of oxygen was noted at regular intervals during each run, a carefully calibrated flowmeter being incorporated into the gas influent line. Daily atmospheric pressure readings were also taken.

4.2.6. Microscopic Analysis

Microscopic examinations were made at intervals during the experiments to determine qualitatively if contamination of the reactor contents had occurred. No attempt was made to make a count of the bacteria present.

4.2.7. Sampling

Sampling was carried out at various intervals during each run, depending on the residence time of the reactor contents. For each sample, the total flow rate, the sample time, the oxygen flow rate, the reactor head pressure, the effluent soluble

organic carbon, the effluent soluble + cellular carbon, the suspended solids concentration and the volume % evolution of carbon dioxide were measured. The exact residence time and ppm of carbon entering the reactor at the time of sampling were also calculated. Reactor temperature was noted. Liquid samples were taken from the effluent. Removal of 100 ml of liquid briefly halted continuous operation, but depending on the total flow rate, the volume was soon made up, and continuous operation proceeded. Experiments were continued until no further change in effluent concentration and suspended solids concentration was evident, i.e., a "steady state" had occurred. This might involve 100 hours of operation.

4.2.8. Determination of Suspended Solids Concentration

A Gellman millipore filter (47 mm diameter, 0.45 micron pore size) was washed with distilled water under vacuum filtration. The filter was then placed on an aluminum weighing dish, dried for one hour at 100°C, weighed (in the dish) and replaced in the vacuum filtration apparatus. A known volume of the sample culture was then filtered under vacuum, the solids being retained on the filter, and the filtrate passing into a clean test tube. The filter + solid residue was replaced in the dish, dried and reweighed. Four determinations were carried out per sample.

4.2.9. Determination of Soluble Organic Carbon Concentration

The carbon concentration of the filtrate was determined using the Beckman infra-red analyzer (Model IR 315). Standard

solutions of sodium oxalate were made up (Appendix D), so as to give 500, 250, 100, 50, 25 ppm carbon concentration. The analyzer was calibrated using these solutions, prior to each sample, a calibration curve being plotted, as the analyzer response to carbon content was non-linear. The sample to be analyzed was acidified with 3 drops of concentrated hydrochloric acid and purged with nitrogen for 3 minutes to remove any carbon present as inorganic carbonate. Successive 20 microlitre injections of the sample were made into the analyzer until consistent values were obtained. The carbon content of the sample was then interpolated from the calibration curve. For low carbon concentration samples, the analyzer signal was amplified and a low (50 - 100 ppm) range of sodium oxalate standards made up, and the relevant calibration curve plotted.

4.2.10. Determination of Soluble + Cellular Organic Carbon Concentration

An unfiltered portion of the continuous reactor sample was sonicated for approximately 5 minutes, acidified and purged with nitrogen as above, and analyzed in the infra-red analyzer in the same way as before.

4.2.11. Determination of Gaseous Carbon Evolved from the Continuous Reactor

The reactor effluent gas and reactor liquid were sampled simultaneously. The chromatograph was adjusted and used throughout the study under the following conditions:

Carrier gas flow rate	=	50 ml/min
Bridge voltage	=	5.6 volts
Column temperature	=	130°C
Detector temperature	=	30°C
Injector temperature	=	25°C
Gas inlet pressure	=	30 psi
A.C. furnace power setting	=	37
Injector power	=	0
Detector power	=	0
Attenuator	=	32
Chart speed	=	0.5 ins/min
Recorder span	=	0.1 mV
Carrier gas	=	helium
Sample size	=	0.25 ml

The effluent gas was sampled with the gas sampling valve, after noting the oxygen flow rate and reactor head pressure. Two or three determinations were made to obtain consistent results. Immediately after each sample was taken, the chromatograph was calibrated with a gas mixture containing a known volume percentage of carbon dioxide, the calibratory gas being passed through a manometer to obtain the same inlet pressure as the sample.

Prior to the use of the chromatographic method a chemical adsorption technique for estimation of carbon dioxide was tried. This was inaccurate however, for the low concentrations found in this study.

4.2.12. Dissolved Inorganic Carbon

Several of the filtered liquid samples taken were infra-red analyzed before and after acidification. Little difference in peak height was recorded, and not enough for the difference to be meaningful. Measurement of dissolved inorganic carbon was attempted chromatographically by purging a filtered acidified liquid sample with nitrogen, and sampling the off gases with the gas sampling valve on the 90 - P3. The four results obtained were not consistent - improvements in technique being required to give meaningful results.

4.2.13. pH Environment of Bacteria

Rose (63) states that, "all microorganisms require the presence of hydrogen ions in the environment, in order that they may grow. The optimum concentrations required are usually quite low, and in higher concentrations, the ion can have a toxic or lethal effect on microorganisms. The limits of hydrogen ion concentration for growth of most microorganisms are from around pH 4.0 to 9.0. For most organisms there is a fairly narrow range within these limits that is most favourable for growth. Bacteria, in general, prefer media of pH values near neutrality, and cannot usually tolerate pH values much below 4-5". Rose also states that the pH value of the environment can materially affect metabolic processes other than growth, for instance morphology.

Hence pH control in this study was effected close to neutrality at pH 6.8, to give a standard environment for bacterial metabolism and gas production.

4.3. Experimental Procedure

4.3.1. Gas Chromatograph Response Linearity

The gas chromatograph was tested for linearity of response to volume percent of carbon dioxide in the sample, with gas mixtures containing varying concentrations of CO_2. Results are shown in Appendix F.

4.3.2. Accuracy of Pump System

The reactor was assembled as described in 4.2. As detailed in Appendix E, the reservoir substrate concentration was varied for different residence times in the reactor, so as to maintain an overall carbon concentration entering the reactor equal to 500 ppm. The effluent from the reactor operating at a 5-hour residence time, with the corresponding reservoir substrate concentration, was monitored over a period of time with all equipment in a sterile condition. Infra-red carbon analysis showed 500 $\pm$ 10 ppm carbon in the reactor after ten and 15 hours operation. The pump system was thus considered sufficiently accurate for the main experimental study.

4.3.3. pH Control

A standard inoculation procedure was carried out, and continuous operation at 5 hours residence time initiated to determine if (a) pH control was required; (b) if required, was manual control sufficient. It was found necessary to control the pH of the system if any kind of steady condition was to be reached. Manual control was not accurate nor practicable for any prolonged experimental study. A buffered dilution water

reservoir was used in a second preliminary 5 hour residence run. Again, poor control of pH was effected. Thus, the automatic control system previously detailed was incorporated.

4.3.4. Continuous Reactor Experiments

A series of experiments was initiated with varying influent flow rates, equivalent to reactor residence times between two and ten hours. Automatic pH and temperature control and a full sampling procedure were carried out on these runs which were continued to a "steady state" condition. In certain cases, repeat runs were made. Table (1) gives an outline of the experimental schedule.

4.3.5. Dissolved Oxygen Measurement

Attempts were made to monitor the dissolved oxygen of the reactor contents, to determine if the bacteria were always in the fully aerobic state. The results were not accurate, possibly due to the presence of electrolytes in the reactor liquid. Thus a theoretical estimation of bacterial oxygen requirements, and a brief oxygen transfer study of the continuous reactor was carried out. It was shown that the solution rate of oxygen could be conservatively maximized at a rate greater than the depletion rate effected by the bacteria at their maximum growth rate and concentration. Details are given in Appendix C. The oxygen supply to the reactor was increased after run (4) to minimize errors in the gaseous carbon measurement.

4.3.6. Table (1)

Experiments were run at the following residence times. The repeat runs at 5 hours residence were for technique and

equipment modification as described.

TABLE (I)

Run No.	Residence Time Hours	Overall Influent flowrate ml/hr	Influent Carbon ppm
1	5	700	500
2	5	700	500
3	5	700	500
4	5	700	500
5	6	583	500
6	3	1166	500
7	2	1750	500
8	2.5	1400	500
9	2.25	1555	500
10	10	350	500

CHAPTER 5

5. EXPERIMENTAL RESULTS

5.1. Continuous Reactor Study

Figures (4) to (11), inclusive, show graphically the results of Runs 1,3,4, 5, 6, 8, 9, and 10. The accumulated data for these experiments are tabulated in Appendix (A).

All graphs show ppm carbon as ordinates against time (hours) as abscissae. Each plot shows the variation with time of cellular carbon, remaining soluble carbon, gaseous carbon and cellular plus soluble carbon (after sonication). Ideally, all reactions would be followed from an initial condition where substrate carbon is a maximum and cellular carbon a minimum. This is only possible however, at high residence times in the continuous reactor. At low residence times, the microorganisms have to be brought to a high growth rate in a batch condition, prior to the initiation of continuous operation, otherwise the rate of washout of organisms exceeds their growth rate, and reactor failure occurs. In several cases, the organisms are virtually at the steady condition dictated by the given residence time, when sampling is initiated.

The following experimental conditions applied:

Run (1): Manual pH control was used. This was unsatisfactory and gave solids variability. Reactor oxygenation rate was low and probably insufficient to maintain fully aerobic conditions. No gaseous measurements were undertaken and no material balance

KEY TO SYMBOLS USED

Figures (4) to (11):

△ = sonicated carbon

⊙ = cellular carbon

⊡ = soluble carbon

■ = gaseous carbon

Figures (12) to (18):

⊡ = influent carbon

⊙ = total carbon: soluble + cellular + gaseous

FIGURE (4):

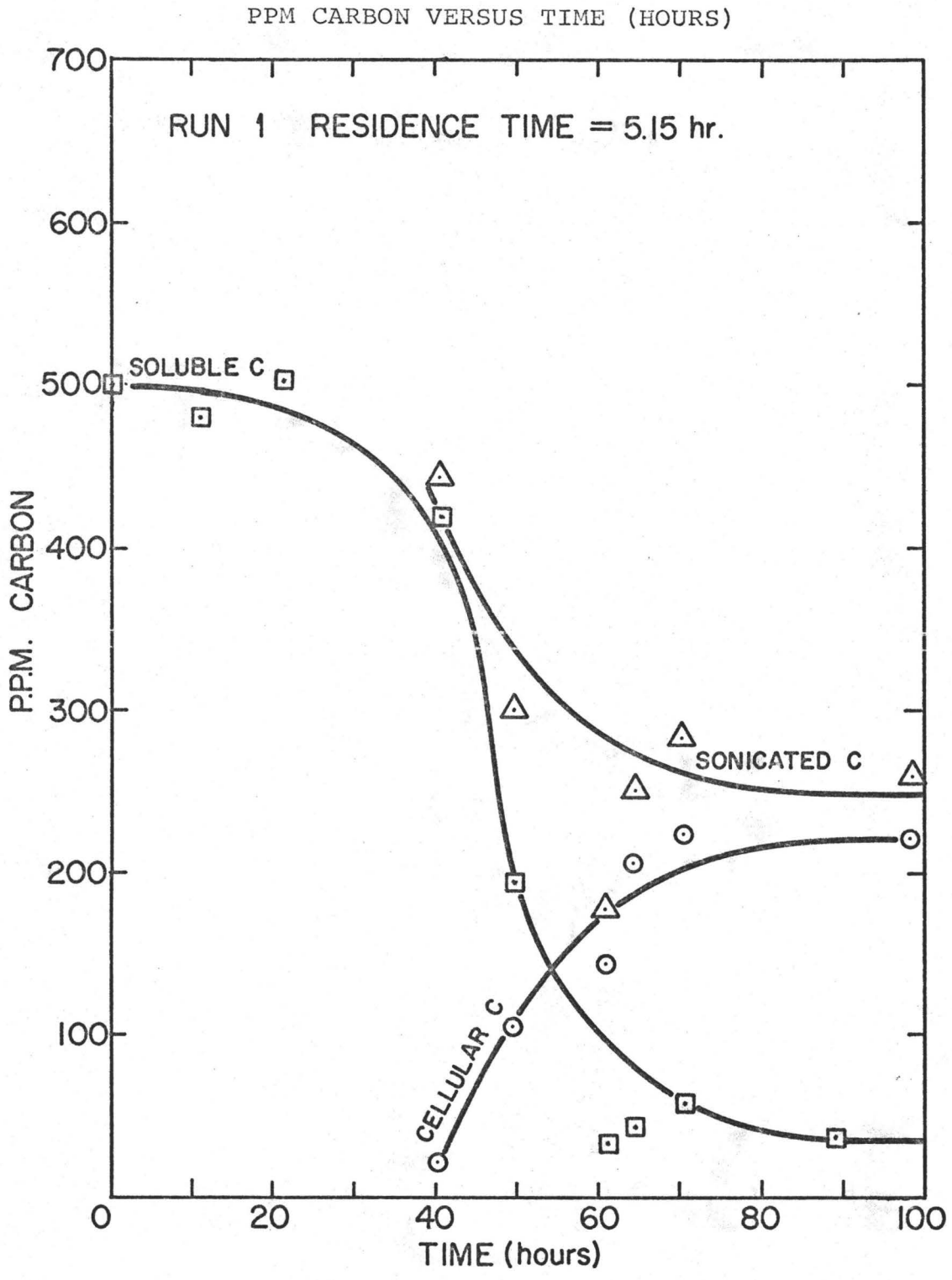

PPM CARBON VERSUS TIME (HOURS)
RUN 1 RESIDENCE TIME = 5.15 hr.
SOLUBLE C
SONICATED C
CELLULAR C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
20
40
60
80
100

FIGURE (5):

PPM CARBON VERSUS TIME (HOURS)

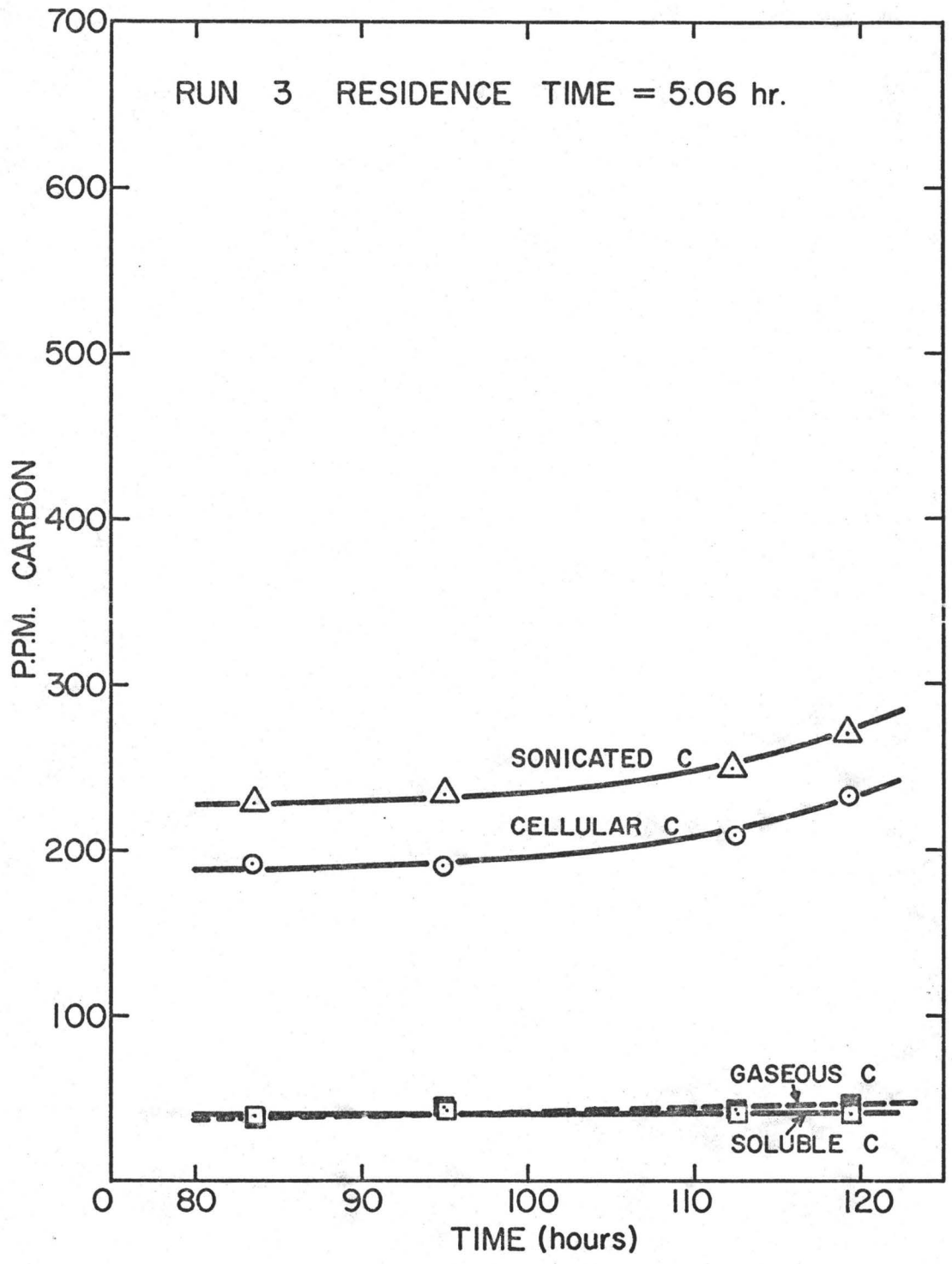


FIGURE (6):

PPM CARBON VERSUS TIME (HOURS)

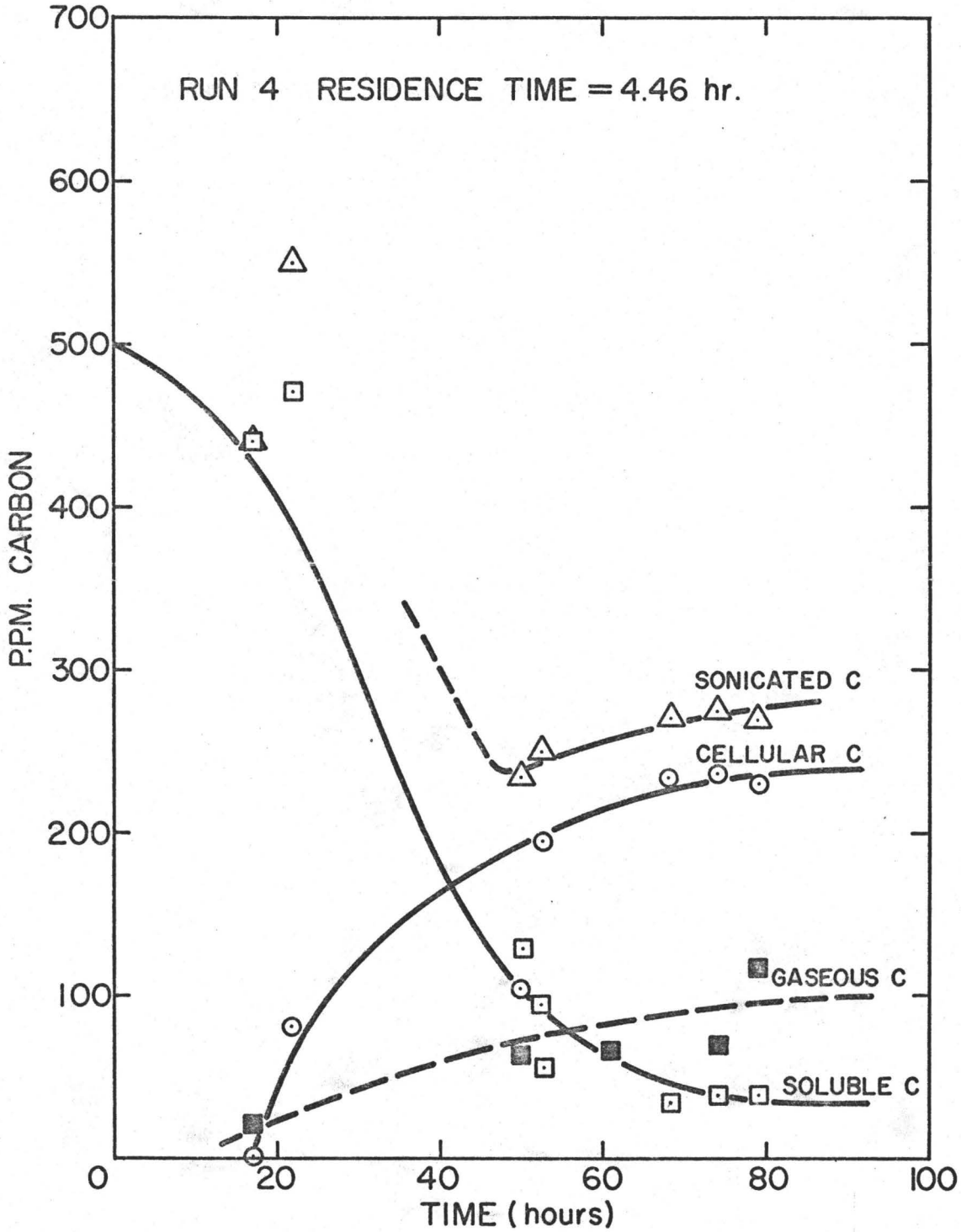


FIGURE (7):

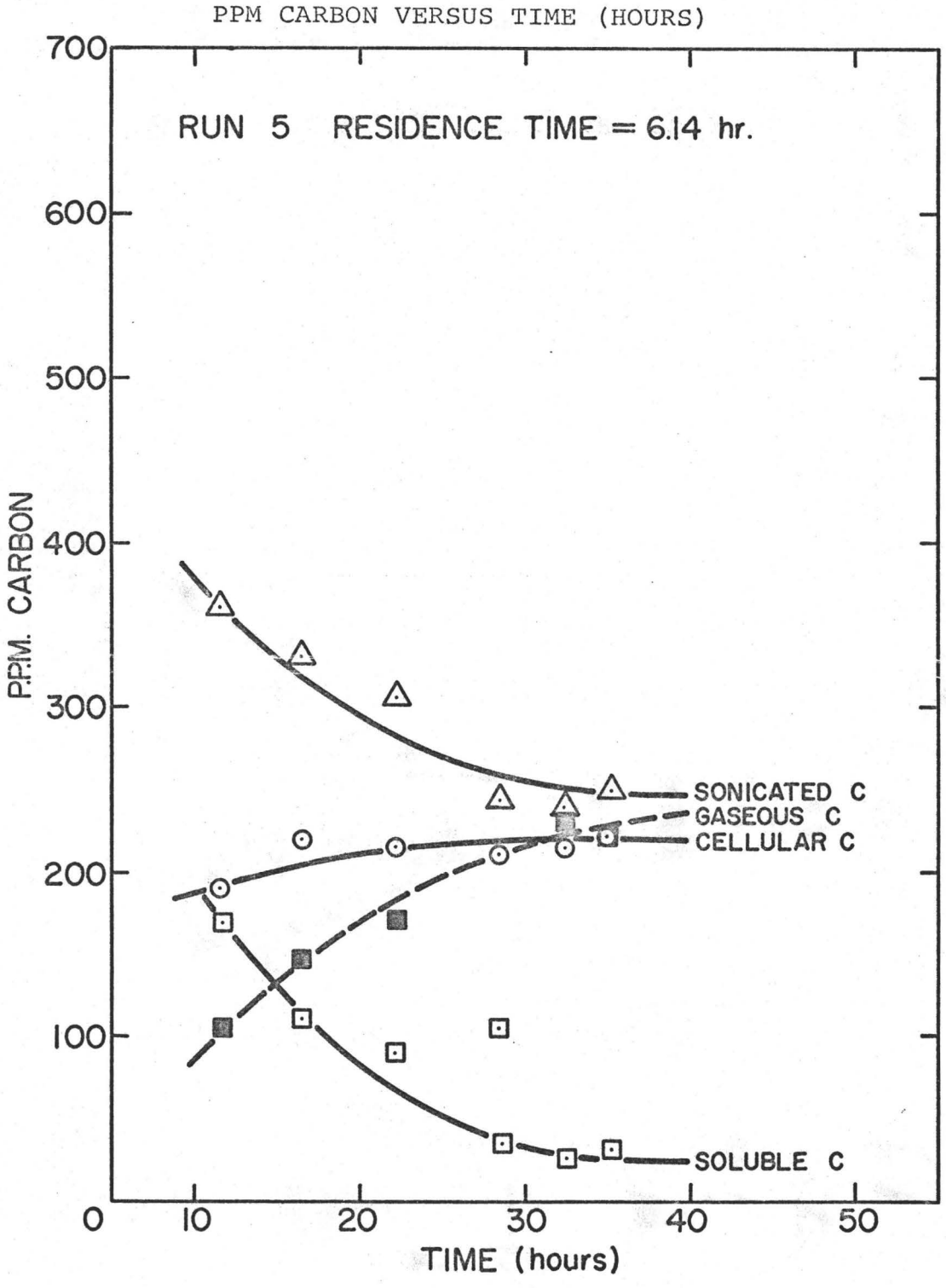

PPM CARBON VERSUS TIME (HOURS)
RUN 5 RESIDENCE TIME = 6.14 hr.
P.P.M. CARBON
TIME (hours)
SONICATED C
GASEOUS C
CELLULAR C
SOLUBLE C
700
600
500
400
300
200
100
0
10
20
30
40
50

FIGURE (8):

PPM CARBON VERSUS TIME (HOURS)

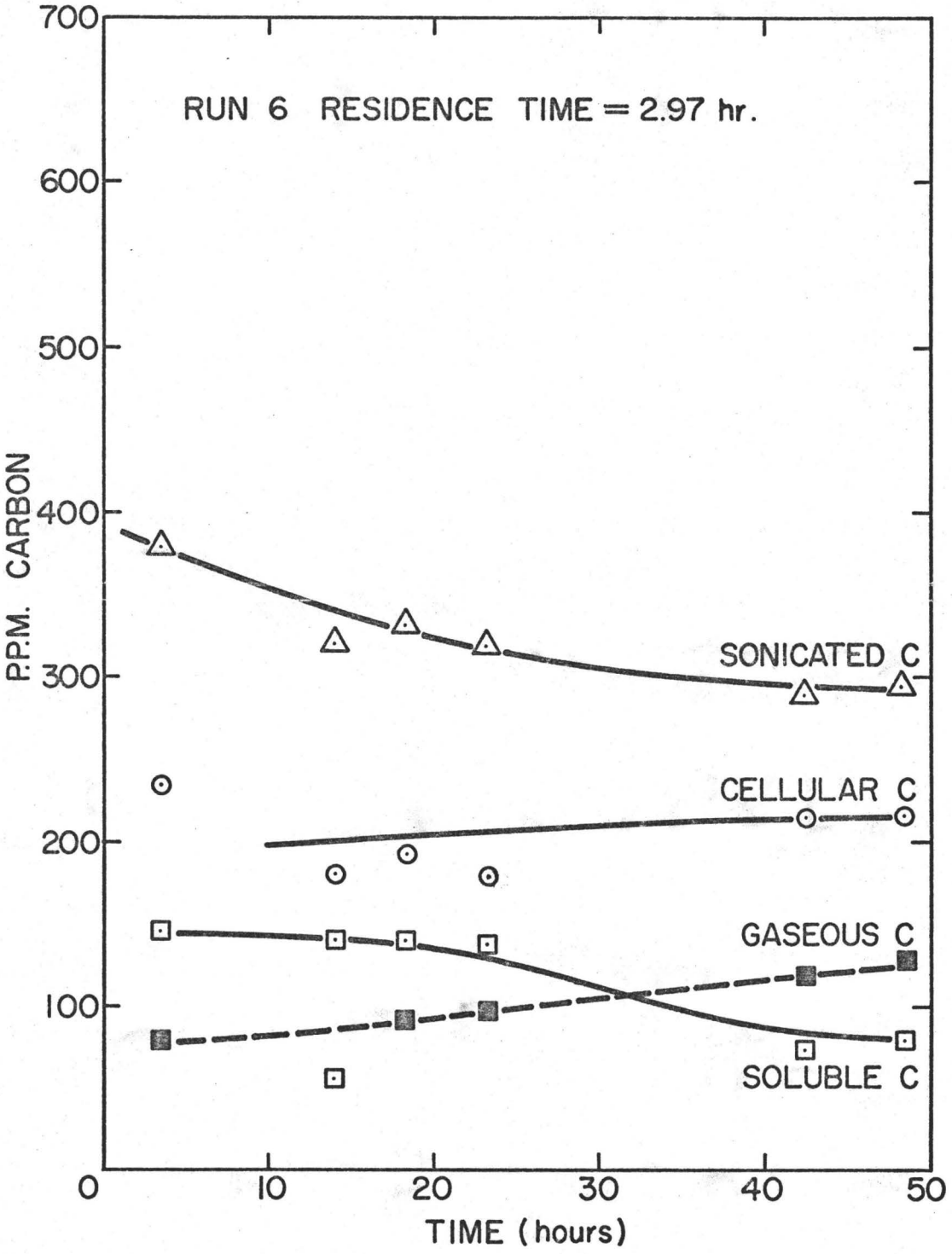


FIGURE (9):

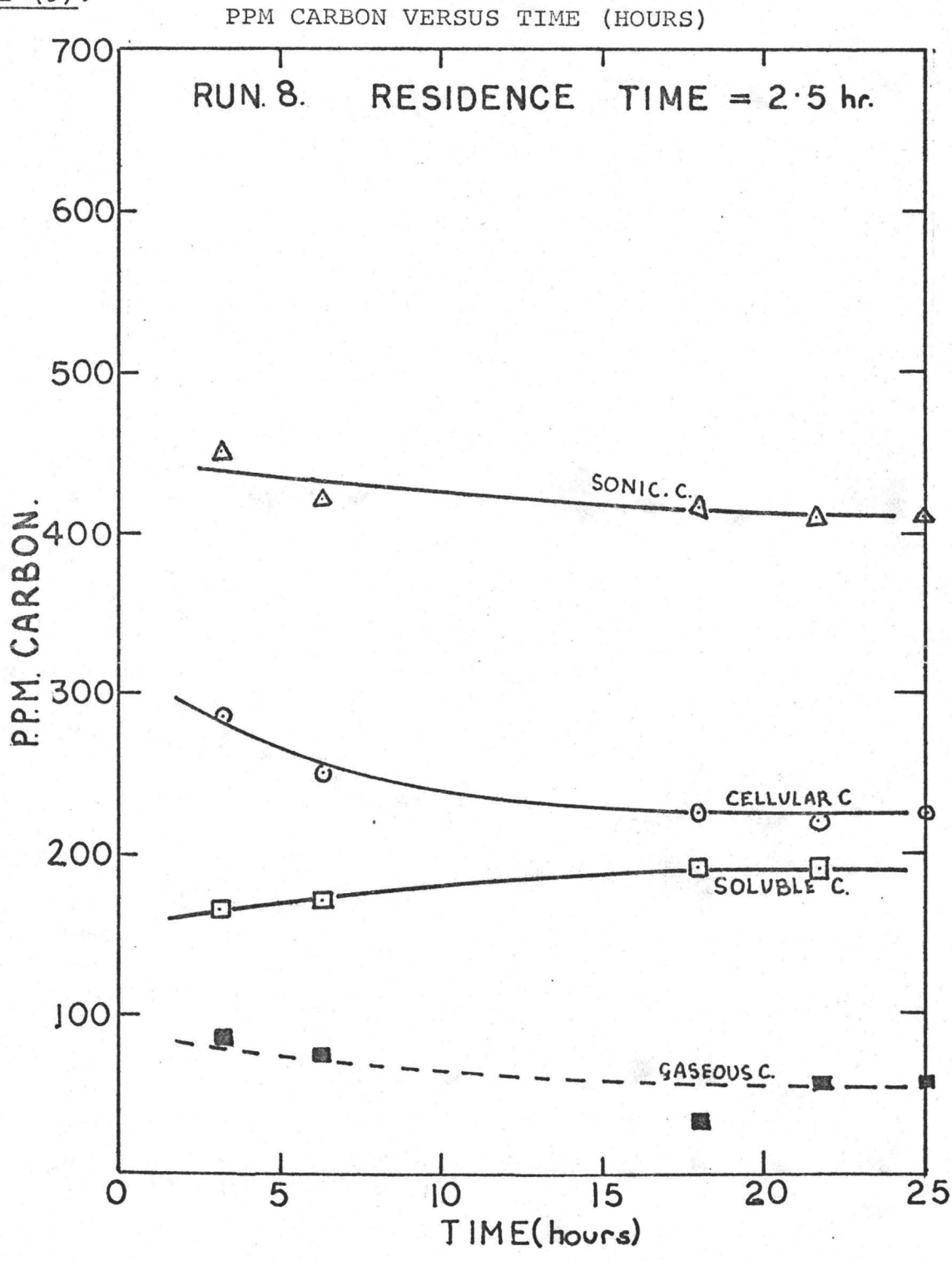

PPM CARBON VERSUS TIME (HOURS)
RUN. 8. RESIDENCE TIME = 2·5 hr.
700
600
500
400
300
200
100
P.P.M. CARBON.
SONIC. C.
CELLULAR C
SOLUBLE C.
GASEOUS C.
0
5
10
15
20
25
TIME(hours)

FIGURE (10):

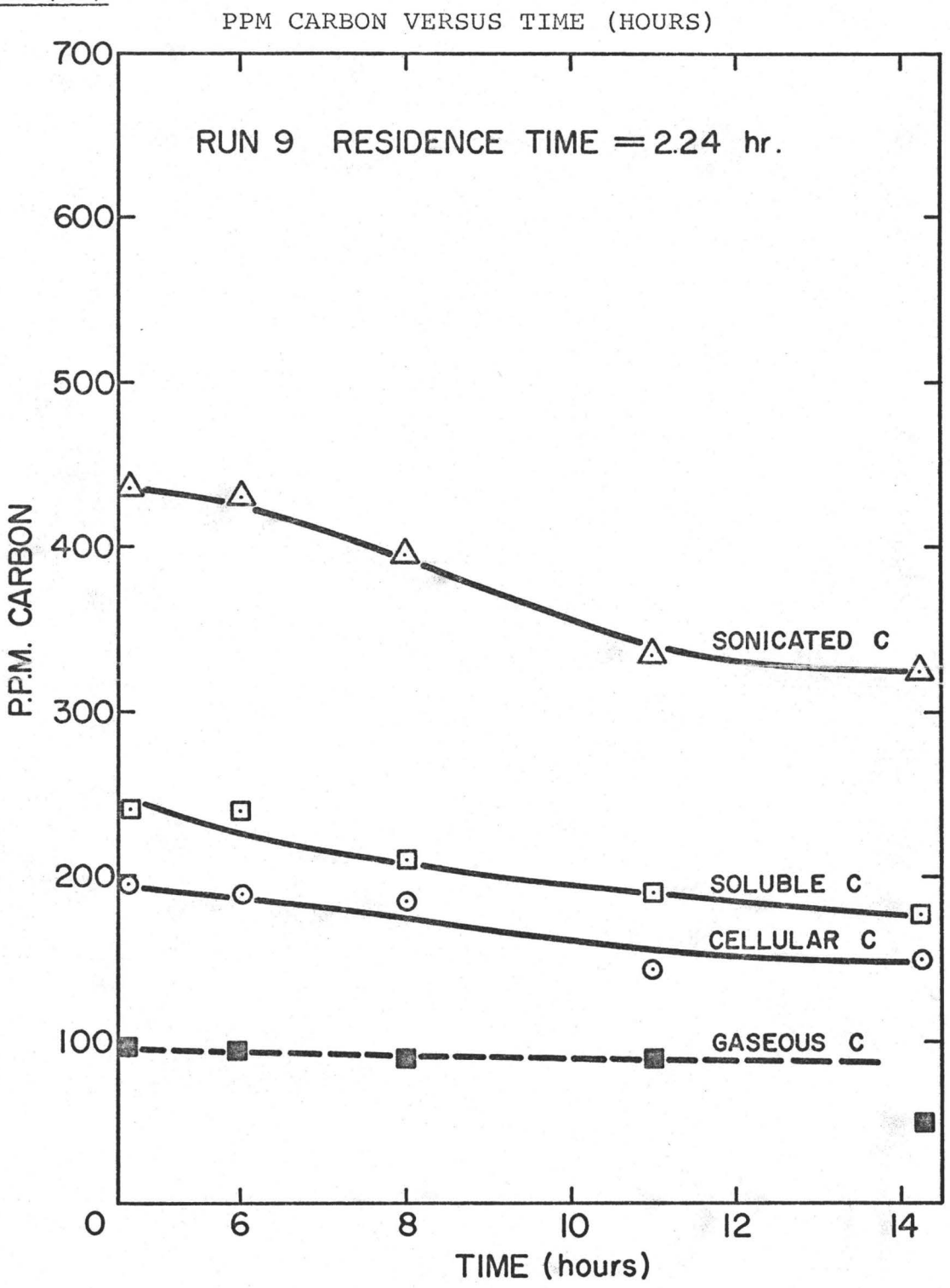
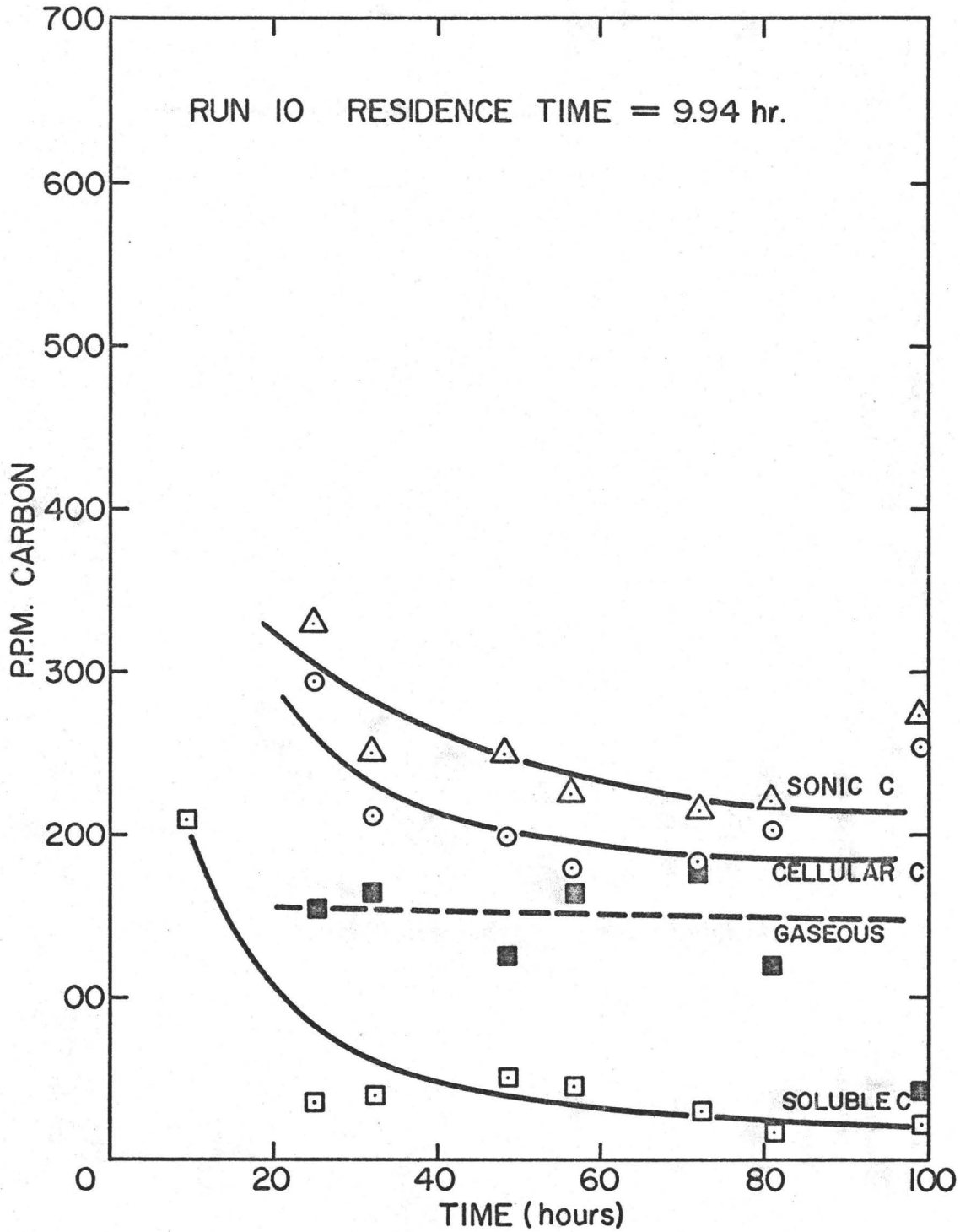

PPM CARBON VERSUS TIME (HOURS)
RUN 9 RESIDENCE TIME = 2.24 hr.
P.P.M. CARBON
TIME (hours)
SONICATED C
SOLUBLE C
CELLULAR C
GASEOUS C
700
600
500
400
300
200
100
0
6
8
10
12
14

FIGURE (11):

PPM CARBON VERSUS TIME (HOURS)



completed. This run was used to improve experimental techniques.

Run (2): pH control via phosphate buffering of the dilution water found to be unsatisfactory. Considerable bacterial flocculation occurred.

Run (3): A low flowrate of oxygen to the reactor gave inaccurate flowmeter readings. Large errors in the gaseous carbon measurements apparently resulted. This was the first run using the automatic pH controller.

Run (4): This was initiated as a repeat of Run (3). Data are plotted from when "steady-state" conditions were approached. The initial results for 0 → 50 hours were invalidated due to failure of continuous operation.

Run (5): The first run using an increased oxygen flowrate. Again, sampling of the reactor was initiated only when "steady-state" conditions were approached.

Run (6): The short residence time appeared to promote bacterial flocculation: some difficulty in obtaining reproducible solids data resulted.

Run (7): An attempt at 2 hours residence time in the reactor. It was not possible to initiate rapid growth of bacteria, sufficient to allow continuous operation. Contamination of the reactor also occurred, and the run was abandoned.

Run (8): A continual reduction in residence time was used to increase the bacterial growth rate to the required level. At this point, the flow rate to the reactor was stabilized, and sampling initiated.

Run (9): The continuous reactor was worked up to 2 1/4 hours residence time from a batch condition, over 8 hours. A "steady-state" existed at the initiation of sampling. A continual decrease in effluent carbon still occurred with time. It was noticed that at this very high growth rate, close to the maximum for the conditions of this study, very considerable bacterial flocculation and filamentation took place. Retention of excess bacterial flocs occurred within the reactor via adherence to the reactor walls, baffles, etc. A breakdown of the CSTR configuration possibly resulted, the increasing solids decreasing the effluent carbon below the theoretical value for a perfectly mixed system.
Run (10): The reactor was successfully operated for 100 hours. Considerable variation in measured gaseous carbon was evident towards the end of the run.

Curves drawn through the experimental points in Figures (4) to (11) show the trend of the data, but are not the result of curve fitting techniques.

5.2. Carbon Balance

Cellular, soluble and gaseous carbon were measured at intervals for the continuous reactor system. Accuracy of the infra-red determination of the soluble and cellular + soluble (sonicated) carbon samples was acceptable. The first four or five injections into the analyzer showed good repeatability.

The concentration of cellular carbon in ppm, was calculated by simply subtracting that value obtained for soluble carbon from that for an unfiltered, sonicated sample. This implies that the volume of cells in the unfiltered, sonicated sample is not enough to reduce the volume of soluble carbon reactor liquid in that sample significantly below 20 microlitres, the infra-red analyzer injection volume. That this implication is substantiated is shown in Appendix (G). The values obtained for cellular and dissolved carbon are listed in columns 4, 5, and 6 in the data tables (Appendix A). Chromatographic analysis gave the values for percentage volume of gaseous carbon dioxide (column 8, data tables), these values being interpolated from peak height measurements of the sample and of calibratory gas mixtures. A value for corrected gaseous carbon evolved per litre of reactor influent was calculated in milligram units. This calculation, including gas volume corrections for reactor head pressure, atmospheric pressure and reactor temperature, is given in Appendix (H). For each sample taken from the continuous reactor, the current exact influent carbon concentration, and the current residence time were both calculated knowing individual pump speeds and the reservoir substrate concentration.

Figures (12) to (18), using the same axes as Figures (4) to (11), show this theoretical carbon intake into the continuous reactor for the duration of each run, with each value calculated at the time of sample removal. The measured carbon mass balances resulting from the analysis of these samples, are

also plotted, these balances being the sums of values obtained for soluble effluent carbon, cellular carbon and corrected gaseous carbon evolved per litre of feed, (with the carbon feed concentration as close to 500 ppm as possible). For each run, the x coordinates are identical for the two sets of data which appear.

5.3. Error Assessment

If exactly 500 ppm carbon were continually introduced into the reactor, and an exact carbon balance completed for the duration of each run, the two data sets would coincide, in Figures (12) to (18), on a horizontal line (Y = 500 ppm: X = $0 \rightarrow n$), where n = number of hours for which the reactor is operated. A perfect system of this type would thus show no correlation of ppm carbon with time, at any stage of a continuous biochemical reaction. That imperfect balances occur, gives an indication of the possible errors exhibited by the system.

For the purposes of the following qualitative analysis, the data was initially assumed to be best fitted by linear least squares straight lines, as shown in Figures (12) to (18).

Run (3): Run (4).

Considerable discrepancy between the influent carbon ppm and total effluent carbon balance was apparent, with greater data scatter in run (4). Neither the increasing nor decreasing errors demonstrated, were apparently due to faulty technique. Influent carbon values remained at 500 $\pm$ 15 ppm. Error was considered due to an inaccurate measurement of oxygen

FIGURE (12):

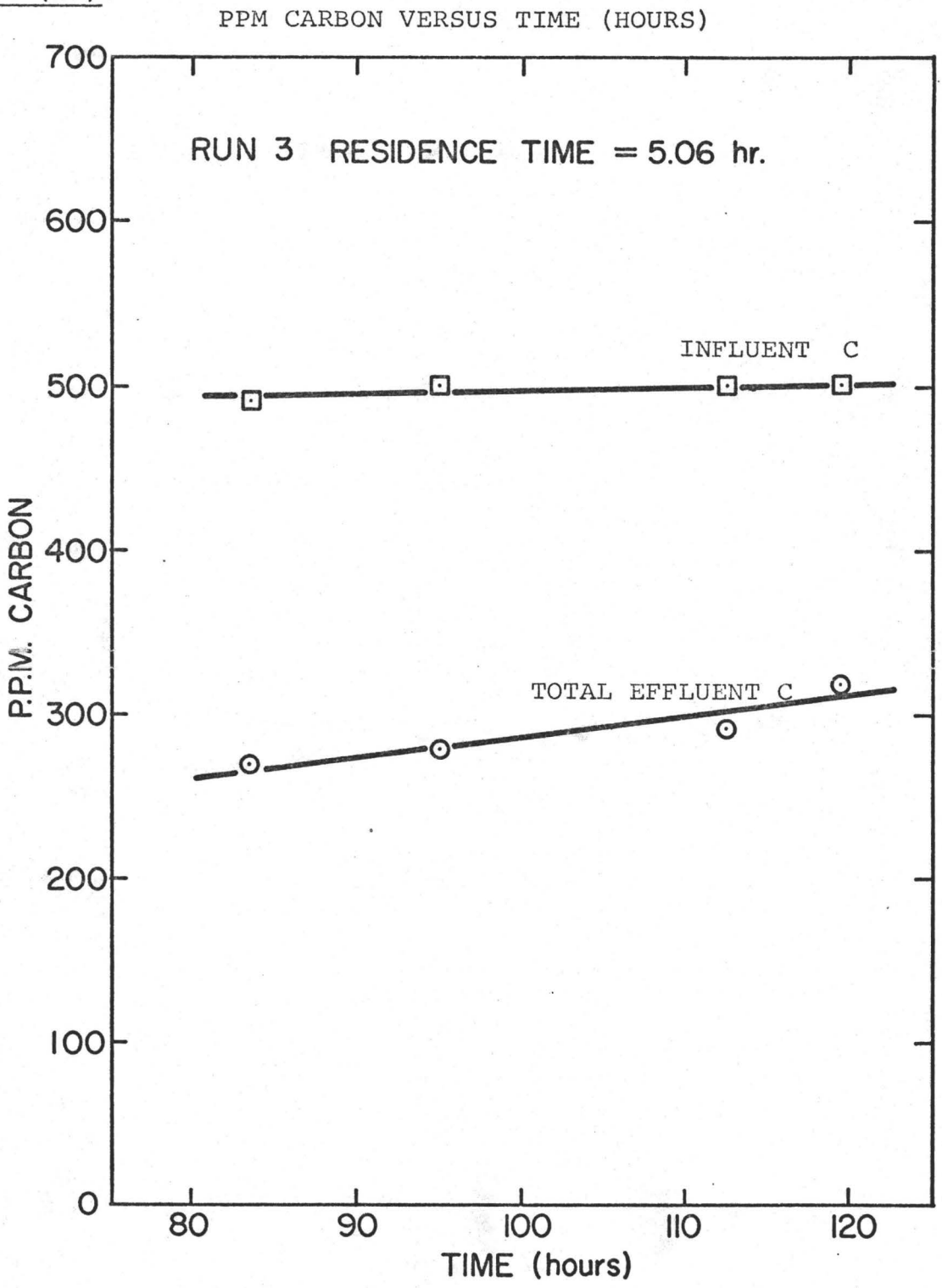

PPM CARBON VERSUS TIME (HOURS)
RUN 3 RESIDENCE TIME = 5.06 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
80
90
100
110
120

FIGURE (13):

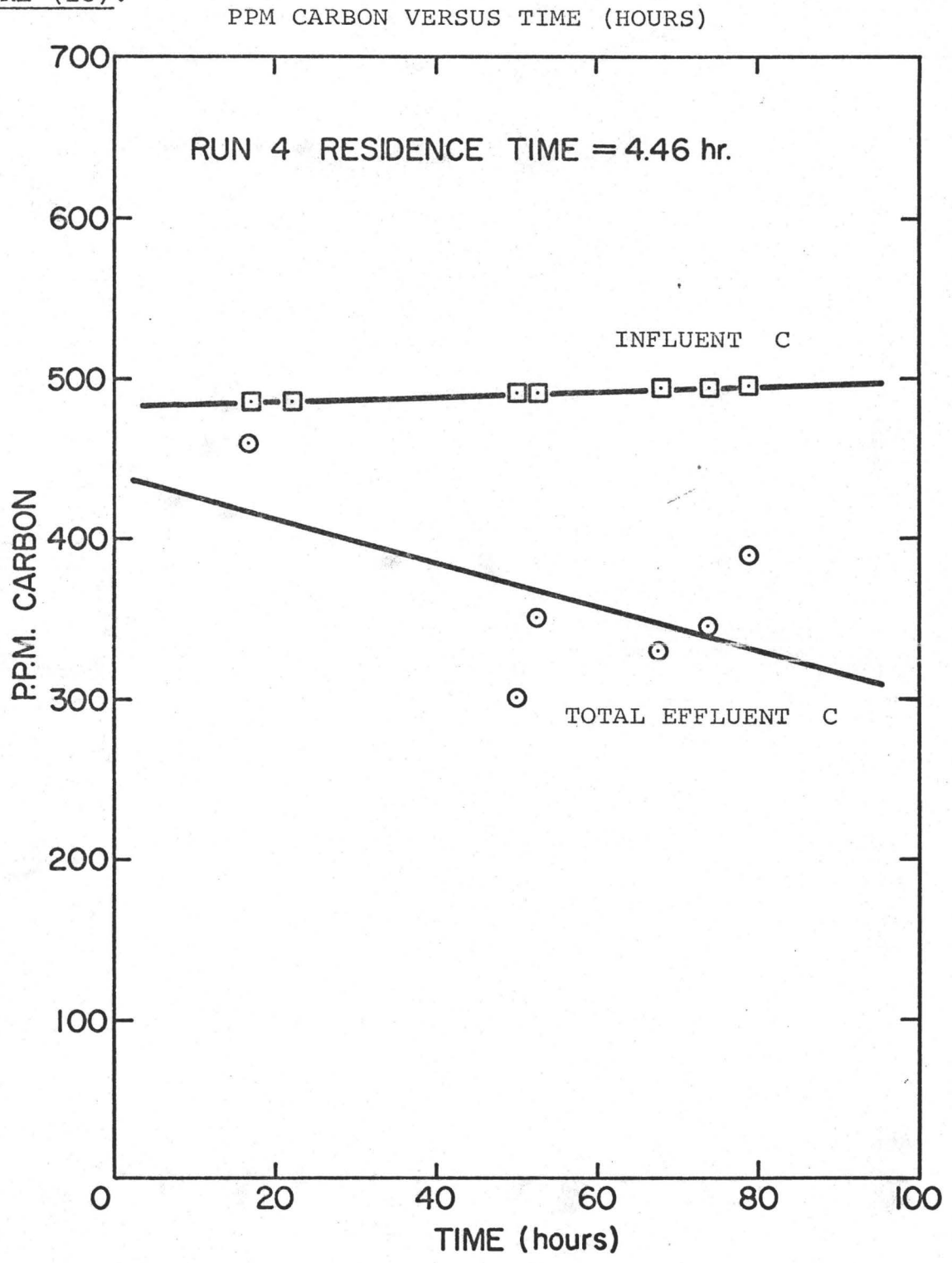

PPM CARBON VERSUS TIME (HOURS)
RUN 4 RESIDENCE TIME = 4.46 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
20
40
60
80
100

FIGURE (14):

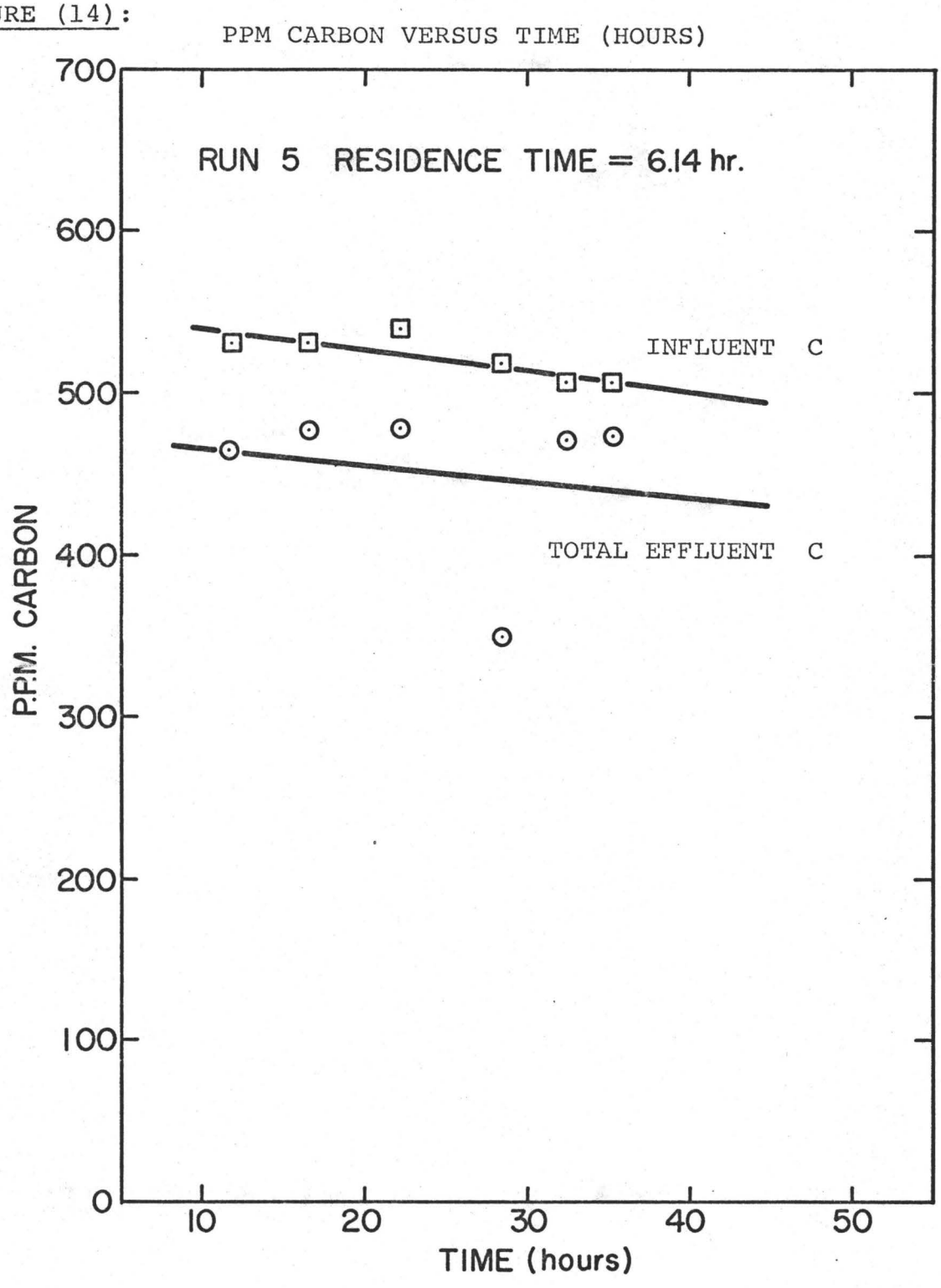

PPM CARBON VERSUS TIME (HOURS)
RUN 5 RESIDENCE TIME = 6.14 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
10
20
30
40
50

FIGURE (15):

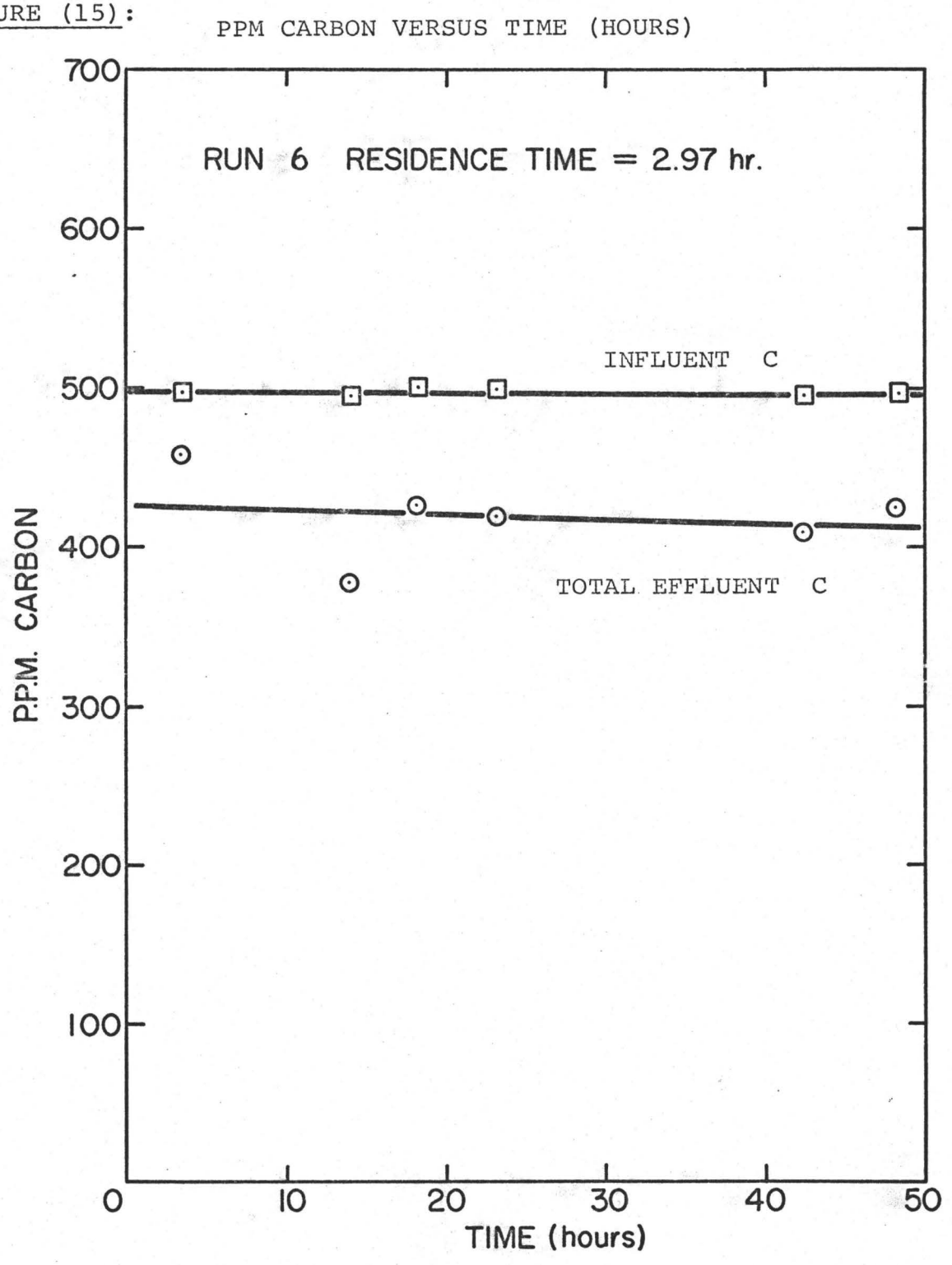

PPM CARBON VERSUS TIME (HOURS)
RUN 6 RESIDENCE TIME = 2.97 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
10
20
30
40
50

FIGURE (16):

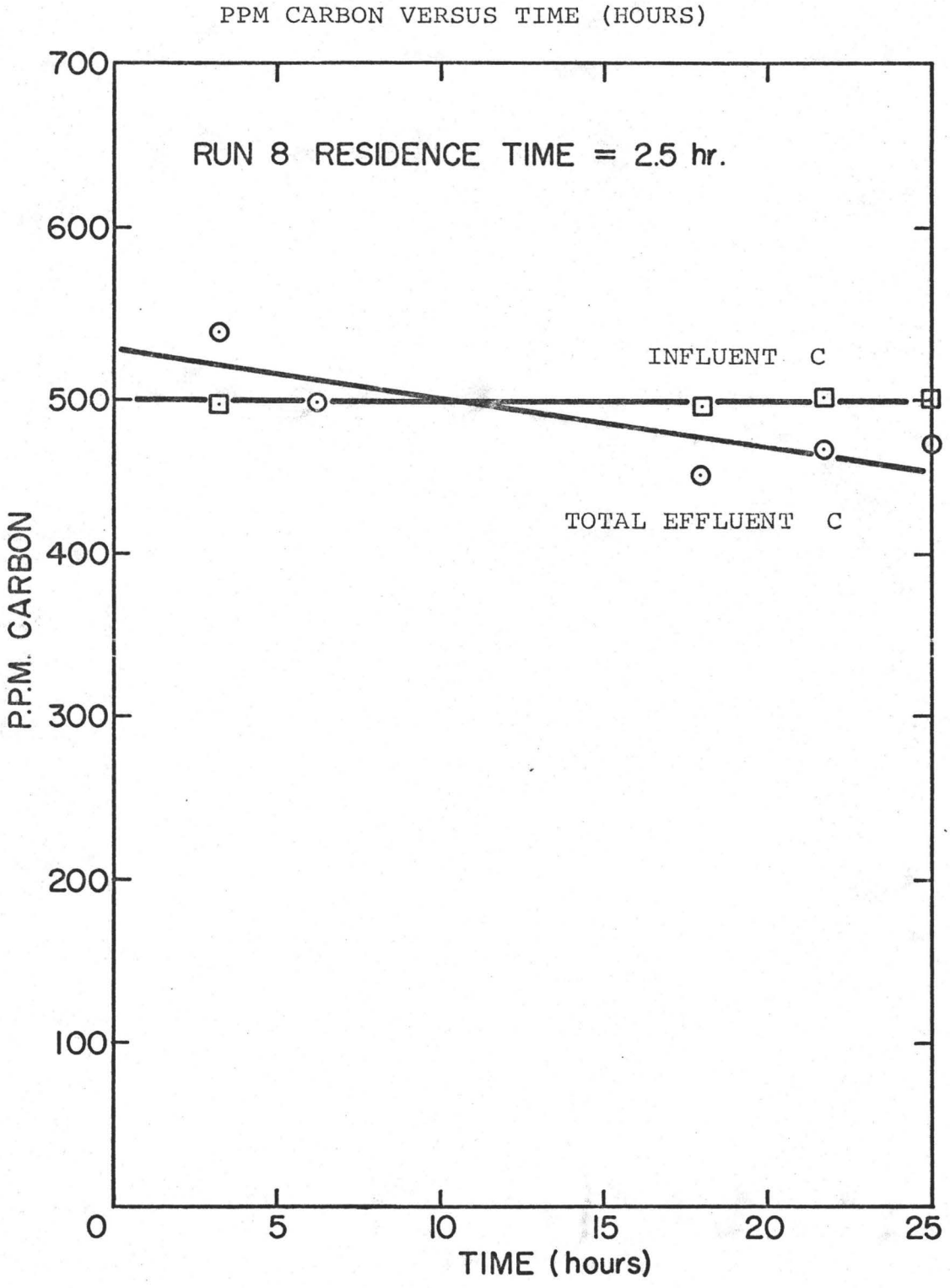

PPM CARBON VERSUS TIME (HOURS)
RUN 8 RESIDENCE TIME = 2.5 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
5
10
15
20
25

FIGURE (17):

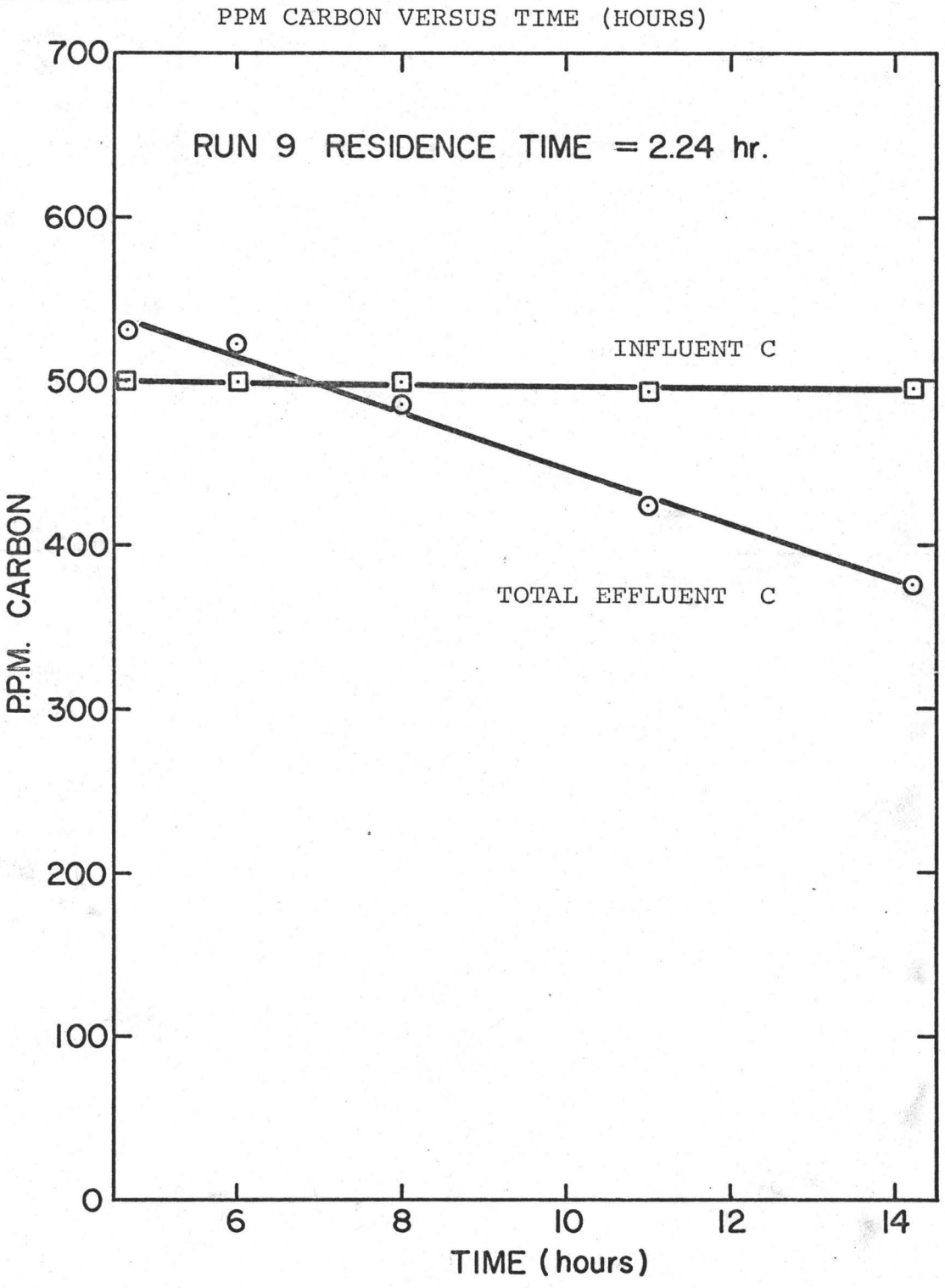
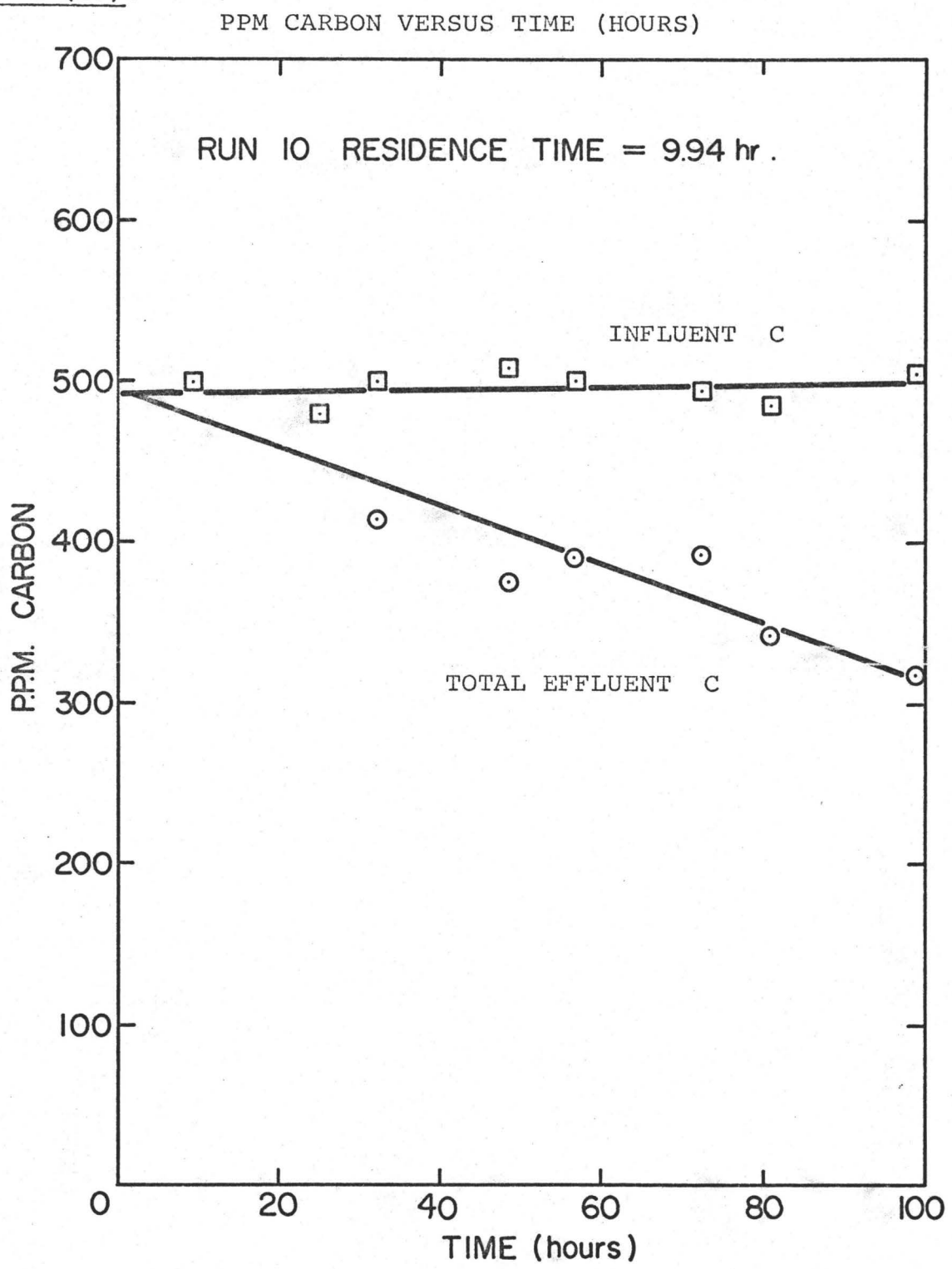

PPM CARBON VERSUS TIME (HOURS)
RUN 9 RESIDENCE TIME = 2.24 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
6
8
10
12
14

FIGURE (18):


PPM CARBON VERSUS TIME (HOURS)
RUN 10 RESIDENCE TIME = 9.94 hr.
INFLUENT C
TOTAL EFFLUENT C
P.P.M. CARBON
TIME (hours)
700
600
500
400
300
200
100
0
20
40
60
80
100

flow rate, with the flowmeter operating outside its optimum range.

Run (5):

An improvement in the carbon balance is evident. The data appears linear apart from a single low result. Influent carbon was somewhat higher than required, at 531-506 ppm. A mean value of the sum of % balance errors (data table, column 13), for each sample gives an approximate 90% material balance.

Run (6):

This is similar to Run (5), with 85% materials balance.

Run (8):

This run shows a positive balance error, and intersection of linear least squares fitted lines: the balance was considered close to 100%.

Run (9): Run (10):

Both these runs showed initial positive balance errors, which decreased to zero and then show increasing negative errors. Run (9) exhibited decreasing effluent soluble carbon with time, possibly due to loss of CSTR configuration, detailed in section 5.1. Evidence of bacterial growth in the substrate feed line in Run (10) suggests a decreasing influent carbon concentration, not shown in the calculated influent carbon data. Balances from 100% decreasing to 70% were obtained.

From this initial data assessment, carbon balances about the system appear to be feasible, at the 75% to 100% level of closure after the improvement of initial experimental techniques.

5.4. Error Analysis

A series of statistical tests was performed on the accumulated data to further refine the estimate of the carbon balance error. The algorithm showing the test sequence, and the details of the statistical evaluations are given in Appendix I. Significance of testing was considered at the 95% confidence level. At this level:

(a) All the data was found to be best fitted by linear least squares lines,

(b) All the data except for run (8) had high significance for fitting separate lines through the data points. Run (8) had high significance for a common line through all points.

(c) The slopes of the fitted least squares lines for the influent carbon data for runs (3) (6) (8) (9) and (10), are not significantly different from zero, whereas for runs (4) and (5), these lines are different from zero. Only runs (4) and (5) can be said to exhibit a small, but significant error with time in the influent carbon concentration to the reactor, run (4) increasing, and run (5) decreasing with time.

(d) The slopes of the fitted least squares lines representing influent carbon data and total effluent carbon data have no significant difference between them for runs (3) (4) (5) (6) (8) and (10). Significance of slope difference is evident in run (9). An estimate of the percentage

experimental error in the carbon balance across the entire run, may be made in all cases, except run (9), by evaluating the difference in areas under the fitted lines, considering the two lines to be parallel. These error estimates appear in Table (2). Run (8) has insignificance of slope difference and high confidence for the use of one line through all the data, as would be expected, as the two least squares lines intersect. A 100% balance is considered to have been completed in this case. Run (9) in showing retention of different slopes, and hence separate least squares lines, albeit with intersection, is the only example of a statistically significant error increase with time, with respect to the material balance data, for the duration of continuous reaction. Run (10), from experimental observation was expected to show a similar effect although statistically this is not apparently the case at the 95% confidence level.

TABLE (2)

Run	% error in carbon balance for complete run
3	-42%
4	-24%
5	-14%
6	-16%
8	0%
9	+8% → -25%
10	-18%

A consideration of Figures (12) to (18), and Table (2) emphasizes the varying degrees of success in achieving a material balance about the continuous reactor system. From experimental experience, it was found that errors in determining soluble and cellular carbon were small. The techniques used are adequate. Major errors in runs (3) and (4) were due to a low oxygen flow rate through the reactor, outside of the range at which the flowmeter was accurate. Use of incorrect flow rates in the calculation of gaseous carbon produced/litre feed, leads to a large accumulative error; this measurement has to be made accurately. Conversely, at higher oxygen flows more amenable to accurate measurement, and also in excess of the requirement for fully aerobic operation, the carbon dioxide in the gas effluent is considerably diluted, requiring greater sensitivity in detection by the chromatograph. It was considered that a major source of error in all the remaining runs was due to inaccuracy in measurement of carbon dioxide. The statistically significant error increase in run (9) was possibly attributable to microorganism growth in the substrate feed line (which occurred in runs (9) and (10)). This would lead to a reduction in ppm carbon reaching the reactor, a reduction possibly increasing with time. The "error" increase could, in fact, mirror an actual decrease in incoming substrate concentration, even if this was calculated to be constant from pump speed and dextrose concentration.

A breakdown of the ideal CSTR configuration tended to invalidate several attempts at continuous reaction. This became

particularly noticeable at high bacterial growth rates where accumulation of bacteria within the reactor via flocculation was often initiated. This led to a lower unused substrate concentration in the effluent than would occur if bacterial flocculation was absent. This effect was possibly another cause of error in run (9). Minor experimental problems also included variation in reactor fluid volume leading to residence time variation and consequent growth rate and substrate removal variation. Small variations in pump speed also gave inaccuracies in the calculation of influent carbon.

However, the major error in completing the material balance was considered to originate from the measurement of gaseous carbon. The gas chromatograph was extremely accurate in measuring CO_2 down to .01% by volume, as evidenced by the repeatability of calibration values with known standards. The problem was considered due to the method of sampling utilized, where the gaseous carbon concentration of an 0.25 ml. volume of gaseous effluent was integrated over the time taken to introduce one litre of feed to the reactor, to give a value of gaseous carbon produced/500 ppm carbon substrate influent. Continuous analysis of the gaseous effluent would reduce this sampling error.

5.5. Carbon Dioxide Evolution as a Function of Growth Rate

The relationship between the rate of production of carbon dioxide and the growth rate of microorganisms was investigated. It has been shown (30) (55) that the production rate of CO_2 is

directly proportional to the growth rate, if the yield factor is a constant, except at growth rates near the maximum, where by-products inclusive of possible residual organic material begin to form. This formation occurs at the expense of CO_2 production. By-product formation is also induced at low to medium growth rates, if the system deviates from the fully aerobic state.

The experimental gaseous carbon and growth rate data were analyzed to see if this type of trend could be found. Values for the rate of evolution of gaseous carbon per hour at steady state were calculated, knowing the mean steady state rate of evolution of gaseous carbon per litre of feed and the residence time. These values were plotted against the steady state growth rates (reciprocal residence times) as in Figure (19). Visual inspection of this plot reveals little correlation of the two variables, assumed due to the inaccuracy of measurement of CO_2 concentration. A second calculated value of the rate of evolution of gaseous carbon per hour was derived by considering that all the errors in the material balances in Table (2) are due to poor sampling of the CO_2 from the reactor. Thus the balance was completed for each run by subtracting cellular plus soluble carbon ppm from the total carbon in the influent, and assuming this value to comprise gaseous carbon. Figure (20) shows this plot of calculated ppm gaseous carbon per hour against growth rate. Here, the type of situation described by Pirt (55) appears to be substantiated, with an approximately

linear relationship at low to medium growth rates between the two variables, and a sharp reduction of CO_2 production at high growth rates. The intercept on the y-axis for the initial linear relationship where growth rate equals zero, would appear to represent the CO_2 production resulting from endogenous respiration of the strain of E. coli. used. The value for this intercept suggested in Figure (20) is close to endogenous respiration rates reported in the literature for E. coli. (13). The form of Figure (20) indicates again that the major measurement errors occurred in sampling of the gaseous reactor product.

Deviation from a linear correlation between the variables, growth rate and rate of CO_2 production, should not affect the accuracy of a material balance providing carbon is the measured variable. The decrease in CO_2 concentration in the gaseous effluent will be balanced by an increase of carbon-containing organic by-products in the liquid effluent from the reactor, which would be accounted for in a carbon analysis. No attempt was made in these experiments to differentiate between unused dextrose and by-products of cellular metabolism (which would be expected to occur at high growth rates where the concentration of CO_2 appeared to be less than expected), as organic carbon was the primary variable investigated. A simple plot of growth rate against substrate concentration (for all runs) is shown in Figure (21) for growth rates, $0 \rightarrow 0.44$ hr.$^{-1}$ The familiar exponential curve is followed and no great errors appear even when analysis is made of unused substrate carbon plus residual carbon at the higher growth rates. Similar use of the data

in fitting the Monod type (36) model showed no large deviations from linearity.

5.6. Discussion

The aerated, continuous biochemical reactor without recycle, described in this study, was operated successfully for periods of up to 100 hours, at 24°C ± 0.5°C and with automatic pH control to ± 0.1 of a pH unit. An approximate steady state was achieved for all runs, using pure cultures of Escherichia coli and a single substrate. Completion of material balances about this system, with carbon as the measured variable, emphasized a need to perfect the gaseous carbon sampling system. The simplified and carefully controlled system used, does not offer numerical results useful in design or treatment control on a full scale. Completion of a carbon balance about a reactor dealing with an unknown waste and examination of the distribution of carbon in cellular, gaseous and unused forms, assesses the treatability of the waste and the efficiency of the treatment process. As previously emphasized, organic carbon removal is the purpose of conventional waste treatment processes.

A reactor scheme with solids clarification and recycle was not investigated. The system used in this study was run in a substrate limited condition, and not in a microbial mass limited state, as normally occurs in the activated sludge process. The use of substrate limitation and the absence of recycle, simplifed the experimental hydraulic problems, but allowed investigation of carbon balance closure at a wide spectrum of

cellular growth rates, albeit at constant substrate inflow and constant (steady state) cellular concentrations. Materials closure with varying cellular concentration and varying substrate concentrations, with a low rate of cellular growth as in full scale treatment should not present any new problems.

FIGURE (19): PPM GASEOUS CARBON PER HOUR VERSUS GROWTH RATE

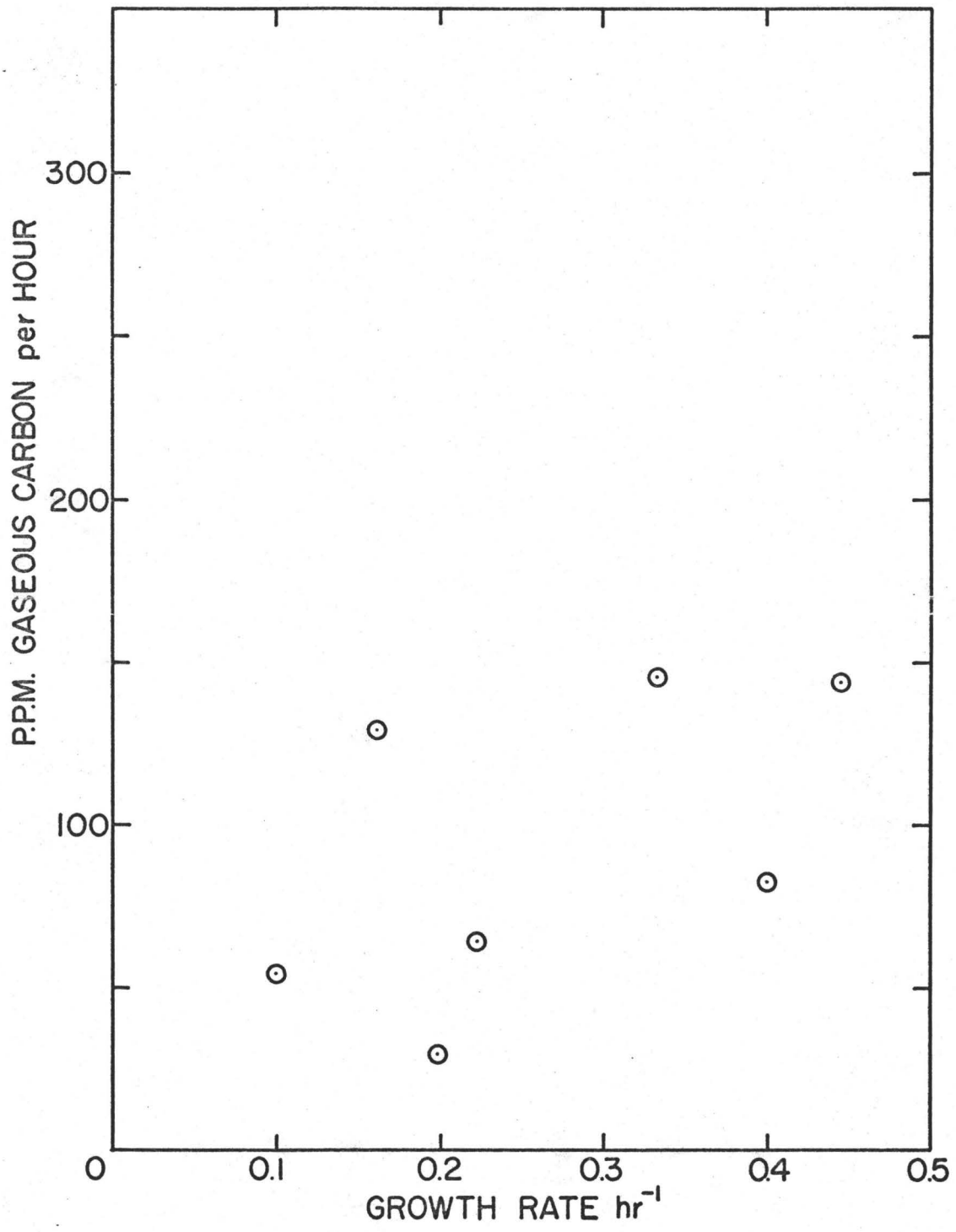


FIGURE (20):

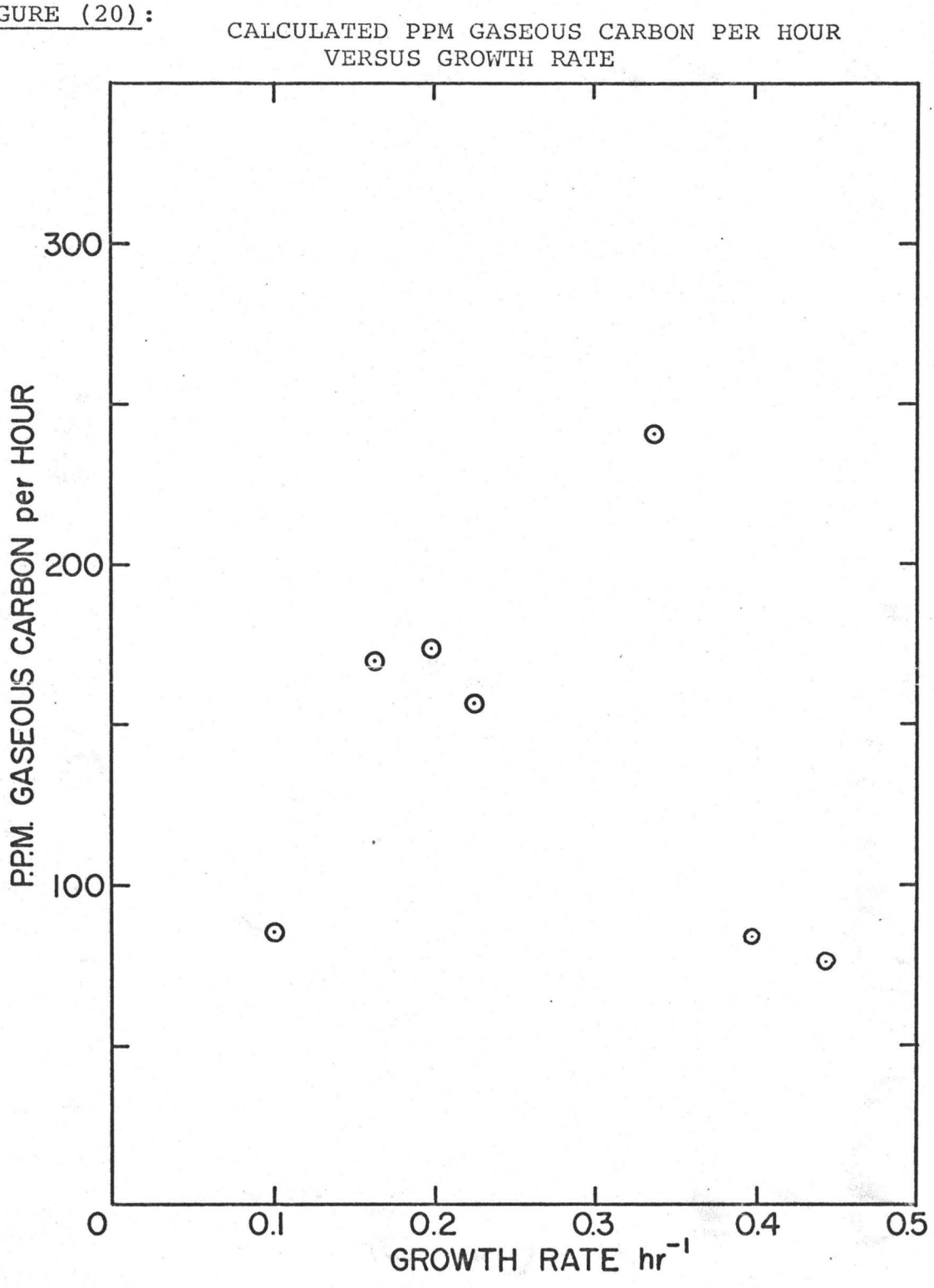
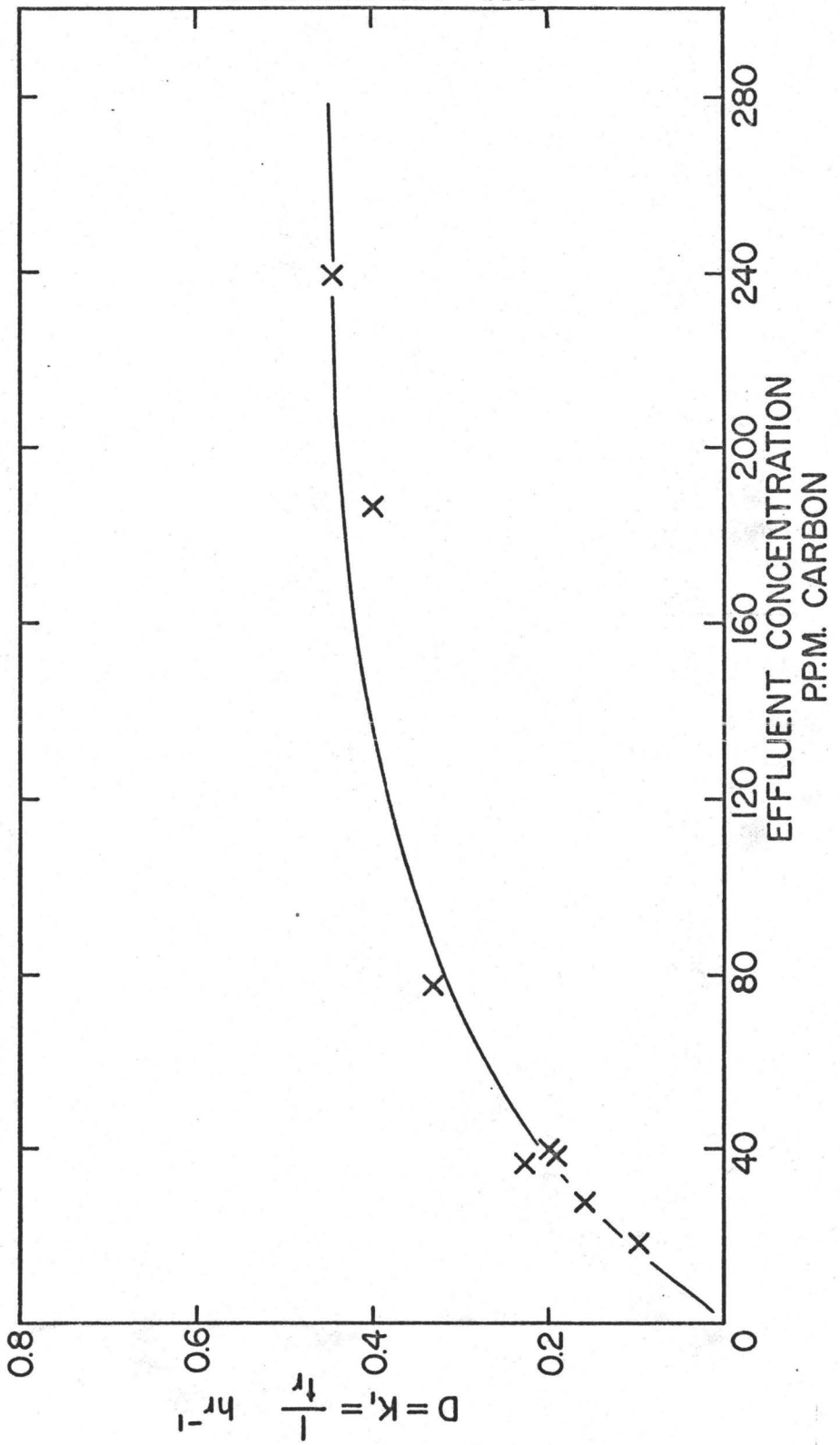

CALCULATED PPM GASEOUS CARBON PER HOUR
VERSUS GROWTH RATE
P.P.M. GASEOUS CARBON per HOUR
300
200
100
0
0.1
0.2
0.3
0.4
0.5
GROWTH RATE hr⁻¹

FIGURE (21): GROWTH RATE VERSUS EFFLUENT CARBON CONCENTRATION: PPM



CHAPTER 6

6.1. CONCLUSIONS

1. Using the equipment developed for this study, successful operation of an environmentally controlled, aerated, continuous biochemical reactor without recycle was achieved for periods of up to 100 hours.
2. A combination of carbon and carbon dioxide analysis, accurately and rapidly gave values for soluble substrate and cellular carbon. Sampling errors occurred in the measurement of gaseous carbon. The chromatographic equipment accurately measures the CO_2 content of a sample to .01% by volume.
3. A carbon mass balance across the continuous biochemical reactor was only partially successful for all runs attempted. Statistical evaluation of the experimental errors confirmed that between 58% and 100% of the influent carbon was accounted for in the various runs.

   Possible sources of discrepancy included:

   (i) The sampling of the reactor effluent gases,

   (ii) Fluctuations in cellular growth rate at "steady-state", with positive and negative reactor fluid volume changes,

   (iii) Bacterial growth in the substrate feed line, reducing the ppm influent carbon.

4. Correlation between the measured rate of CO_2 production and cellular growth rate was not demonstrated. A correlation was suggested using CO_2 production rate values calculated from a theoretical 100% carbon balance up to growth rates at the 0.3 $hr.^{-1}$ level. At very high growth rates by-product formation at the expense of CO_2 was suspected.
5. For the pure culture of Escherichia coli used in this experiment, values of maximum growth rates in accordance with literature (13) values were obtained. A plot of growth rate against substrate concentration followed the typical exponential relationship.

6.2. RECOMMENDATIONS

1. The techniques used in this study, could be applied to obtain defined growth rates with selected cultures and degradation characteristics of specific substrates, if certain improvements are made to the method. These include:
   (a) A more positive method of controlling reactor volume,
   (b) An improved liquid effluent removal system,
   (c) A method of overcoming the sampling and data/time integration error which accumulated in the measurement of CO_2. Continuous infra-red analysis of CO_2 produced in a bioreactor with on-stream analytical equipment might be more satisfactory.

2. Environmental control of biochemical reactors, as described in these experiments is necessary if repeatable results for a given waste or bacterial system are required.

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APPENDIX A

DATA TABLES

RUN (1):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
0	250	-	500	-	-	-
11	250	-	480	-	-	-
21.5	250	-	500	-	-	-
40.25	250	-	420	444	22	58
49.5	250	-	194	300	106	280
61	250	-	35	179	144	380
64.5	250	-	45	251	206	541
70.5	250	-	59	283	224	590
89.0	250	-	38	-	-	-
112.5	250	-	37	257	220	580
119	250	-	38	260	222	584

RUN (1): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell. + Gaseous	Influent C ppm	tr. hr	Error in C Balance
0	-	-	-	500	5	-
11	-	-	-	500	5	-
21.5	-	-	-	500	5	-
40.25	-	-	-	515	5.15	-
49.5	-	-	-	515	5.15	-
61	-	-	-	515	5.15	-
64.5	-	-	-	515	5.15	-
70.5	-	-	-	515	5.15	-
89.0	-	-	-	515	5.15	-
112.5	-	-	-	515	5.15	-
119	-	-	-	515	5.15	-

RUN (3):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
83.5	140	6.50	38	230	192	505
95	140	6.40	45	235	190	500
112.5	140	6.50	41	250	209	550
119.5	140	6.50	40	272	232	610

RUN (3): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
83.5	0.65	38	268	492	4.95	-45.5%
95	0.72	43	278	502	5.06	-44.6%
112.5	0.69	42	292	501	5.05	-41.7%
119.5	0.78	47	319	502	5.06	-36.5%

RUN (4):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
17	220	22.60	440	440	0	0
22	230	25.10	470	550	80	70
50	170	27.10	130	235	105	350
52.5	110	27.00	55	250	195	450
68.25	120	28.70	36	270	234	620
74	110	28.70	38	275	237	600
79	170	28.70	39	270	231	630

RUN (4): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
17	0.23	21	461	485	4.83	- 4.9%
22	0.06	-	--	485	4.83	-
50	1.50	64	299	490	4.40	-38.9%
52.5	2.18	95	350	490	4.40	-28.6%
68.25	1.28	61	331	494	4.46	-33.0%
74	1.55	68	344	494	4.46	-30.4%
79	1.72	117	388	494	4.46	-21.4%

RUN (5):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
11.75	1030	3.55	170	360	190	510
16.5	1030	3.85	110	330	220	550
22.25	1030	3.55	90	305	215	500
28.5	1020	3.50	35	245	210	530
32.5	1020	3.80	26	240	214	530
35.25	1030	3.75	30	250	220	530

RUN (5): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
11.75	0.18	104	464	531	6.43	-12.6%
16.5	0.26	146	476	530	6.41	-10.2%
22.25	0.30	171	476	539	6.52	-11.7%
28.5	0.19	105	350	518	6.27	-32.4%
32.5	0.43	231	471	506	6.14	- 6.9%
35.25	0.41	223	473	506	6.14	- 6.5%

RUN (6):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
3.5	1050	3.75	145	380	235	620
14	1055	4.00	140	320	180	590
18.25	1055	4.00	140	333	193	510
23.25	1050	4.10	140	320	180	500
42.5	1020	3.65	75	290	215	570
48.5	1020	3.65	80	295	215	560

RUN (6): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
3.5	0.29	78	458	497	2.98	- 7.8%
14.0	0.21	57	377	495	2.97	-23.8%
18.25	0.34	93	426	499	2.99	-14.6%
23.25	0.36	98	418	499	2.99	-16.2%
42.5	0.45	119	409	495	2.97	-17.4%
48.5	0.49	129	424	495	2.97	-14.3%

RUN (8):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
3.25	1055	3.85	165	450	285	580
6.33	1050	3.90	170	420	250	490
18.00	1050	3.85	190	415	225	-*
21.75	1050	3.85	190	410	220	-*
25.00	1055	3.90	185	410	225	-*

* Too much flocculation for meaningful solids determination.

RUN (8): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
3.25	0.38	86	536	492	2.50	+ 8.9%
6.33	0.32	73	493	498	2.53	- 1.0%
18.00	0.15	33	448	489	2.48	- 8.4%
21.75	0.24	54	464	496	2.51	- 6.5%
25.00	0.25	57	467	495	2.50	- 5.7%

RUN (9):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
4.66	1060	4.10	240	435	195	580
6.00	1060	4.10	240	430	190	590
8.00	1060	4.20	210	395	185	620
11.00	1055	4.30	190	335	145	480
14.25	1055	4.40	175	325	150	430

RUN (9): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
4.66	0.47	96	531	499	2.24	+ 6.4%
6.00	0.46	93	523	499	2.24	+ 4.8%
8.00	0.44	90	485	499	2.25	- 2.8%
11.00	0.44	89	424	494	2.23	-14.2%
14.25	0.25	51	376	496	2.23	-24.2%

RUN (10):

Hours	O_2 Flow ml/min	Head Pressure ins. H_2O	CARBON PPM Filtered	Sonicated	Solids	Solids Concentration mg/l
9.25	1060	3.80	210	-	-	-
25.00	1140	3.80	36	330	294	740
32.25	1110	3.80	39	250	211	600
48.58	1100	3.75	51	250	199	480
56.75	1080	3.70	46	225	179	460
72.25	1030	3.75	31	215	184	480
81.00	1030	3.75	17	220	203	500
99.25	1000	3.70	21	275	254	680

RUN (10): (Continued)

Hours	% Vol. CO_2	Corrected Gaseous C per litre feed mg.	Total C ppm Solution + Cell + Gaseous	Influent C ppm	tr hr	Error in C Balance
9.25	-	-	-	498	9.96	-
25.00	0.16	155	485	479	9.59	+ 1.3%
32.25	0.17	165	415	501	10.03	-17.2%
48.58	0.13	125	375	508	10.17	-26.2%
56.75	0.18	166	391	500	10.01	-21.8%
72.25	0.20	178	393	495	9.92	-20.6%
81.00	0.14	122	342	486	9.72	-29.6%
99.25	0.05	43	318	506	10.12	-37.2%

APPENDIX B

NUTRIENT MEDIA

An investigation of bacterial metabolism of the type undertaken, requires that one nutrient be supplied in a growth limiting concentration, so that bacteria can only grow as fast as they can absorb that nutrient. As organic carbon, nitrogen and phosphorus are required in some form, in greater then trace quantities by all types of bacteria, one of these is normally chosen as the growth limiting factor. Organic carbon was selected for use in this study. Adequate bacterial nutrition necessitates a C:N:P ratio equivalent to 40:5:1 (7). If nitrogen and phosphorus are present in excess of this requirement, organic carbon will function as the growth limiting nutrient.

$(NH_4)_2\ HPO_4$, dibasic ammonium phosphate was used to provide a nitrogen and phosphorus source, and dextrose (anhydrous d-glucose), $C_6H_{12}O_6$, was used as the organic carbon source. Mixing dextrose and ammonium phosphate in an approximately 2.5:1 ratio by weight ensured that organic carbon was limiting. This substrate solution was made up in distilled water and sterilized at 100°C and atmospheric pressure for 1/2 hour before use.

Trace elements are also required for bacterial metabolism, these being supplied from a reservoir of dilution water. Three solutions were prepared:

(a) Ferric chloride ($FeCl_3 . 6H_2O$) solution, 1.0 gm/litre.

(b) Potassium phosphate (K_2HPO_4) solution, 50 gm/litre.

(c) Magnesium sulphate ($MgSO_4 . 7H_2O$) solution, 50 gm/litre.

These solutions were added to the dilution water reservoir (tap water) in the following way:

(a) 1.25 ml $FeCl_3$ solution/litre of feed.

(b) 2.5 ml K_2HPO_4 solution/litre of feed.

(c) 2.5 ml $MgSO_4$ solution/litre of feed.

Use of tap water as solvent also ensured the presence of calcium as a trace element. The feed streams of substrate ($(NH_4)_2 HPO_4 + C_6H_{12}O_6$ in distilled water), and dilution water + nutrient ($FeCl_3 . 6H_2O + K_2HPO_4 + MgSO_4 . 7H_2O$ in tap water) were supplied from separate reservoirs to the continuous reactor, previous work having showed that this technique minimised growth in the feed lines. The dilution water was also sterilized and then acidified with several drops of concentrated hydrochloric acid, to keep the trace elements in solution.

APPENDIX C

OXYGEN REQUIREMENTS OF E. COLI UNDER THE ENVIRONMENTAL CONDITIONS OF THIS STUDY

Pirt (55) reported experiments considering the changes in end products of bacterial metabolism if oxygen was allowed to become growth limiting. Anaerobic conditions drastically affected the distribution and type of metabolic products formed. A brief study was undertaken to show that fully aerobic conditions existed at all times in this work.

Schulze (13) showed that for E. coli at 30°C and pH 6.7, the respiration rate of the cells varied from 16 mgm. O_2/gm cell weight/hour at zero growth, to approximately 500 mgm. O_2/gm cell weight/hour at the maximum growth rate.

Assume that the mean cellular solids concentration at steady state in the reactor = 600 mgm/litre.
For a constant reactor volume of 3.5 litres, the total cell weight = $\frac{600 \times 3.5}{1000}$ gm. cells.

At the maximum growth rate at 30°C, 500 mgm. O_2/gm cell weight/hour are required. Hence, for the reactor, $\frac{600 \times 3.5}{1000}$ x 500 mgm. O_2/hour will be required at maximum cellular concentration, and maximum growth rate.

Density of oxygen at 24°C and 760 mm. pressure $\simeq$ 1.31 gm/litre $\equiv$ 1310 mgm/litre.

Hence, for the reactor, $\frac{600 \times 3.5 \times 500 \times 1000}{1000 \times 1310 \times 60}$ ml. O_2/min.

= 13.3 ml oxygen per minute are required by the maximum cell concentration at maximum growth rate.*

* This is a conservative estimate, using a maximum respiration rate value at 30°C, whereas reactor temperature equalled 24°C throughout.

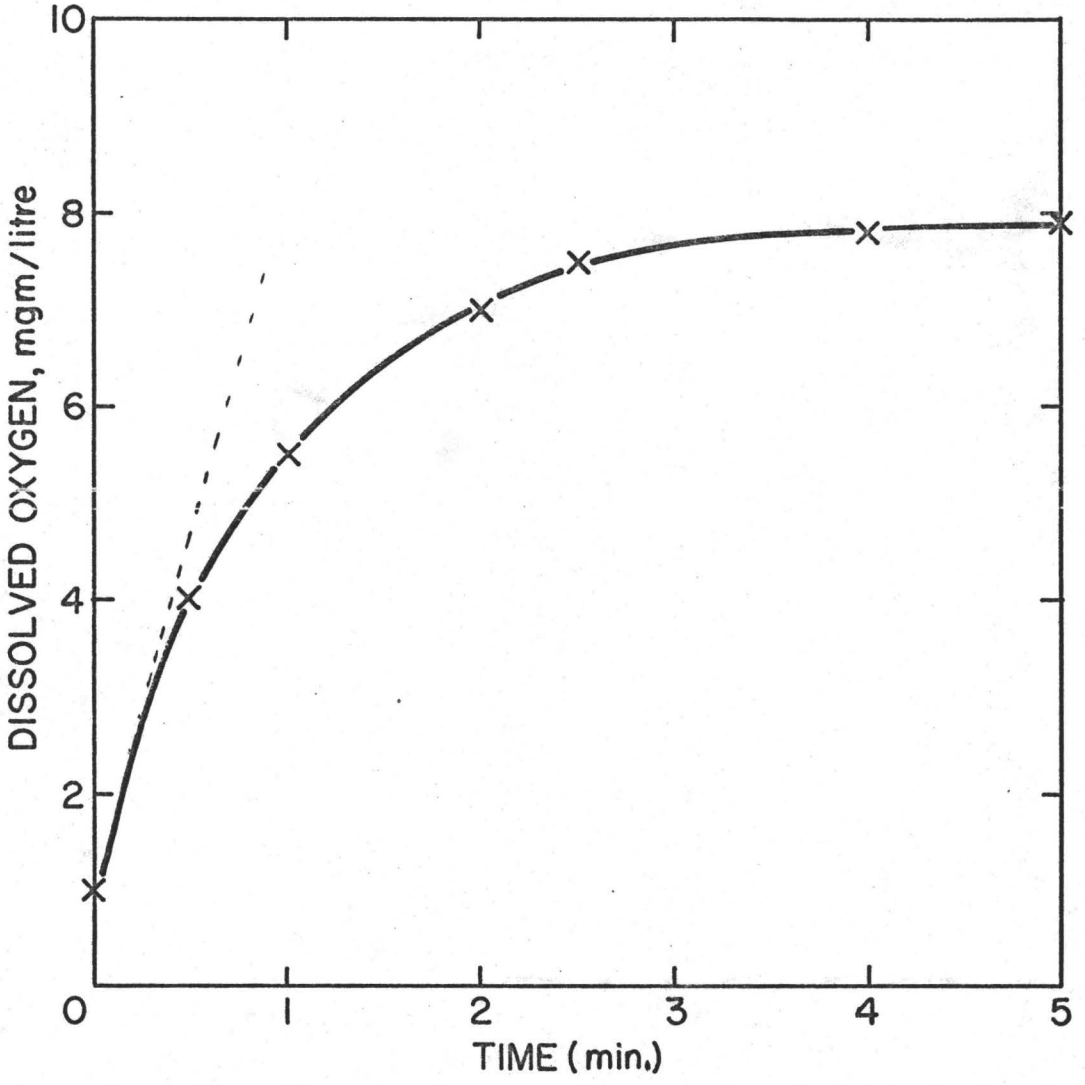
Experimental Proceedure

1. 3.5 litres of tap water were added to the clean reactor vessel and temperature stabilised at 24°C.
2. Nitrogen from a compressed gas cylinder was bubbled through the water with high speed mechanical stirring until the D.O. reading (Y.S.I. D.O. meter) equalled zero.
3. Stirring rate was set equivalent to the rate used throughout all experiments, and aeration initiated at an oxygen flow rate of 1000 ml/min, approximately equivalent to the rate used in most of the experimental study. Aeration was continued until the dissolved oxygen reading showed no further increase.

The entire proceedure was repeated and similar results obtained. The resultant graph of dissolved oxygen (mgm/litre) as ordinates against time in minutes as abscissae is shown in Figure (22).

FIGURE (22):

DISSOLVED OXYGEN (mg/l) VERSUS
TIME (MINUTES)



Results

The plot of D.O. versus time, shows that the solution rate of oxygen can be conservatively maximised at 6.0 mgm/litre/minute at low D.O. values. That is, 6.0 mgm of oxygen/litre/minute are available for cellular uptake.

Oxygen density at 24°C and 760 mm pressure $\simeq$ 1310 mgm/1000 ml.

Therefore, 1 mgm $\equiv \frac{1000}{1310}$ ml

Hence, for a reactor of 3500 ml capacity, solution rate

$= 3.5 \times 6.0$ mgm. O_2/minute

$3.5 \times 6.0 \times \frac{1000}{1310}$ ml O_2/minute

$=$ 16 ml O_2/minute

Hence, reactor oxygenation is sufficient even at maximum growth rates of E. coli.

APPENDIX D

CALIBRATION STANDARDS FOR CARBON ANALYSIS

Earlier work (43) (29) indicated that solutions of sodium oxalate were reliable standards for calibration of the infra-red analyzer. Storage was effected at 4°C, and it was found that carbon content was stable. A standard solution of 500 ppm carbon was prepared by dissolving 2.7891 gm sodium oxalate in 1 litre of distilled water. 50,100,250 ppm carbon solutions were prepared by dilution as required. Calibration curves were drawn up for the infra-red analyzer for each sample taken from the continuous reactor.

APPENDIX E

SUBSTRATE CONCENTRATIONS

Dextrose concentrations of 500 mgm/litre of organic carbon were used as the total influent concentration throughout the study. The dextrose concentration required in the substrate reservoir was calculated as follows:

For the continuous reactor under conditions of steady state with a constant cell concentration

$$K_1 = D = f/V = \frac{1}{tr}$$

where: K_1 = specific growth rate

D = dilution rate

f = total flow rate into reactor

V = reactor fluid volume

tr = mean residence time.

Therefore, $tr = \frac{V}{f} = \frac{3500}{f}$

Hence, for 5 hours residence time,

$$5 = \frac{3500}{f}$$

Therefore, $f = 700$ ml/hour.

If the dextrose is pumped at 78 ml/hr, the dilution water + nutrient pump flow, plus the small alkali flow from the pH controller must be adjusted to total 622 ml/hr.

Now this total inflow of 700 ml/hr must contain a carbon concentration of 500 ppm or 500 mgm/litre. 500 mgm/litre carbon ≡ 1250 mgm/litre dextrose. That is, per hour:

$$\frac{700}{1000} \times 1.25 \text{ gm dextrose}$$

enter the reactor, (i.e., 78 ml of substrate contain

$$\frac{700}{1000} \times 1.25 \text{ gm dextrose)}$$

$$= 11.2179 \text{ g/l. dextrose.}$$

Each dextrose reservoir concentration is calculated thus, and depends on the residence time, and the pump speed. The pump used to transfer the dextrose solution to the reactor, was calibrated constantly throughout the experimental work. The following table gives the dextrose concentrations used.

Residence Time Hours	Reservoir Glucose Concentration gm/l	Run No.
5	11.2179	1
5	11.2179	2
5	11.2179	3
5	11.2179	4
2	25.0659	7
2.25	22.2532	9
2.5	19.6035	8
3	16.7625	6
6	8.2761	5
10	5.0435	10

APPENDIX F

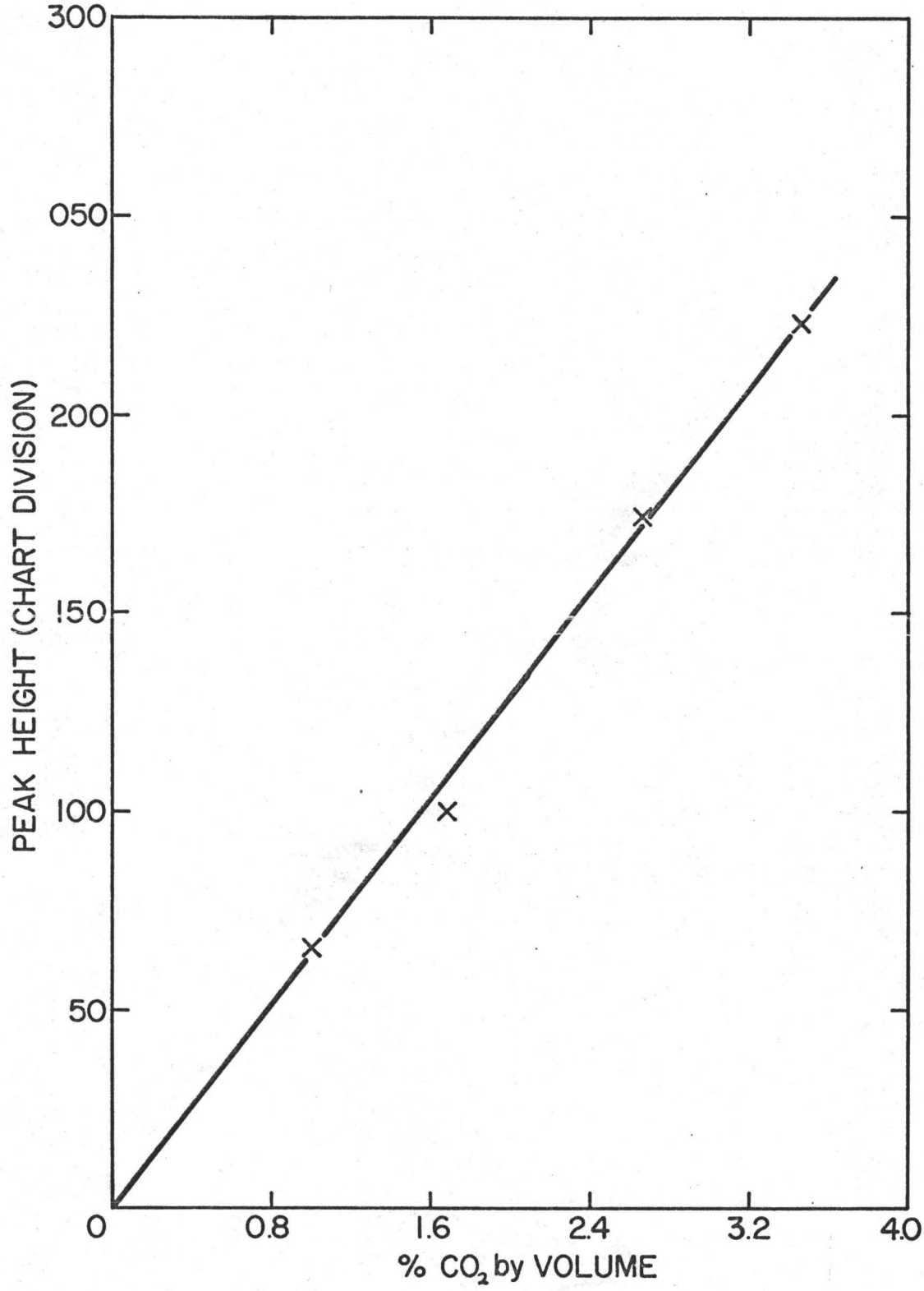
LINEARITY OF 90-P3 DETECTOR RESPONSE

Figure (23) shows a plot of % carbon dioxide by volume against peak height. The sampling was carried out at the same sensitivity as was used in all the subsequent experiments. Each point is the mean peak of ten peaks. Dalnogare and Juvet (64) point out that peak height measurements are usually recommended when small peaks must be measured, or when the band width is narrow. The height and area of chromatographic peaks are affected not only by the sample size, but also by factors influencing the sensitivity of the detector, such as fluctuations in the operating bridge current, the carrier gas flow rate, or the column and detector temperatures. Hence, the chromatograph was calibrated with a known volume % of CO_2 mixture for every sample.

It can be seen that the response of the detection system is essentially linear with respect to carbon dioxide concentrations up to 3.5% by volume.

FIGURE (23):

PEAK HEIGHT VERSUS % CO_2 BY VOLUME



APPENDIX G

ESTIMATION OF BACTERIAL VOLUME IN A 20 MICROLITRE SAMPLE

Let density of bacteria = 1 gm/ml.

= 1000 mgm/ml.

Hence the volume of 500 mgm of bacteria = 0.5 ml.

Hence, 1 litre of an inoculum containing 500 mgm bacteria/litre contains 0.5 cc of bacteria.

Hence, 20 microlitres contains:

$$\frac{20}{1{,}000{,}000} \times 0.5 \text{ ml of bacteria}$$

$$= \frac{20}{1{,}000{,}000} \times 500 \text{ microlitres of bacteria}$$

$$= \frac{1}{100} = .01 \text{ microlitres}$$

Thus, for a 500 mgm/litre bacterial inoculum, a 20 microlitre unfiltered sample contains a negligible volume of cellular material. The equation:

solids carbon = unfiltered (sonicated) carbon - soluble carbon.

(where the carbon values are obtained by infra-red analysis) is correct. Any inaccuracy caused by cellular volume present in the sample is much less than the experimental resolution of the analyzer.

APPENDIX H

CALCULATION OF PPM GASEOUS CARBON EVOLVED FROM REACTOR PER LITRE OF FEED

(a) The gram molecular volume of carbon dioxide is corrected for reactor temperature, reactor head pressure and atmospheric pressure for each sample.

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$

Therefore: $$\frac{406.8 \times 22414}{273} = \frac{412.75 \times V_2}{297}$$

Where: 406.8 ins. water ≡ 1 atmospheric pressure

22414 cc. = G.M.V. of CO_2 at S.T.P.

273°A ≡ 0°C

412.75 ins. water ≡ atmospheric pressure + reactor head pressure at time of sample.

297°A ≡ 24°C

V_2 = corrected G.M.V.

(b) The resulting ambient density of gaseous carbon is calculated.

V_2 = 24032 cc. ≡ 44.0098 gm CO_2 (= molecular weight)

Therefore: 1 cc. CO_2 ≡ $\frac{44.0098}{24032}$ gm CO_2

$$\equiv \frac{44.0098 \times 12.011 \times 1000}{24032 \times 44.0098} \text{ mgm carbon}$$

Therefore: 1 cc. CO_2 ≡ 0.4998 mgm carbon

(c) The quantity of carbon evolving from the reactor per minute is calculated.

Chromatographic analysis gives 0.18375% by volume CO_2 being produced for this sample. Ambient total gas flow through the reactor = 1030 cc/min.

Hence, per minute, $\frac{.18375}{100} \times 1030$ cc. carbon dioxide pass.

$$\equiv \frac{.18375}{100} \times 1030 \times 0.4998 \text{ mgm carbon}$$

i.e., 0.9459 mgm carbon/minute is being evolved.

(d) Calculation of ppm gaseous carbon evolved from reactor per litre of feed.

Carbon entering at the time of sampling = [dextrose pump flow rate ml/hr. x dextrose concentration gm/l x 1000 x 2] divided by:

[Total flow into reactor ml/hr. x 1000 x 5.]

= 531 ppm carbon entering at the time of this sample.

Now 1 litre of liquid enters the reactor every $\frac{6.43}{3.5}$ hr.

= 110.23 min/litre.

Where: 6.43 = residence time

3.5 l. = reactor volume.

Hence, 531 ppm carbon enters every 110.23 min.

Therefore, carbon evolution per litre entry = 0.9459 x 110.23

= 104 ppm carbon.

That is, $\frac{104}{531}$ x 100% of the influent carbon is being converted to a gaseous form at the particular time of sampling.

APPENDIX I

STATISTICAL ANALYSIS OF THE DATA

The two sets of data obtained for each residence time chosen, relate a non-random independent variable free from error (time), with a dependent variable (carbon concentration), subject to various errors. The sequence of statistical tests carried out is defined in the algorithm appended.

(i) Significance of linear versus polynomial regression for the best fit for 14 data sets.

A program was run, which takes each set of data (x = time, hours: y = carbon ppm), and fits polynomials of increasing order for increasing numbers of points. Comparison of second order regression with linear first order regression was done by comparing the difference between the sum of squares removed by the linear regression and the sum of squares of the second order regression with the residual sum of squares of the second order regression at the appropriate degrees of freedom. An F. test was performed for the increase in order. In all sets of data, at the 95% confidence interval, linear regression was found to give the best data fit. Figures (12) to (18) show the linear least squares lines.

(ii) Significance of deviation of slope of influent carbon least squares line from zero.

The t-test to establish whether the slope is significantly different from zero compares the difference between the least squares slope and zero slope with the standard deviation of the slope at the relevant number of degrees of freedom. The 95% confidence limit was used and Table (A) shows the data.

TABLE (A)

Run	T-Statistic	Degrees of Freedom	Significance of Slope Difference From Zero at 95% Confidence.
3	1.6508	2	No significant difference
4	15.1180	5	95% significance of difference
5	2.9970	4	95% significance of difference
6	1.0179	4	No significant difference
8	0.3360	3	No significant difference
9	2.0890	3	No significant difference
10	0.4900	6	No significant difference

(iii) Significance of fitting each pair of data sets with one line or two lines.

For each run, the sum of squares of deviations from the best straight line through all the data and the sums of squares of deviations from the best straight lines with individual slopes (if individual slopes are significant

by test (iv)) or with pooled slopes (if individual slopes are not significant by test (iv)), were compared at their respective degrees of freedom: i.e., the deviations from the best straight line through all the data per set were compared with a minimum deviation obtainable by straight line correlation. This comparison provides a variance ratio which can be tested by the F-test. Resulting F-values are shown in Table (B). Significance or the lack of it at the 95% confidence level, is also shown in Table (B).

(iv) Significance of difference of slopes of the two least squares lines per pair of data sets.

Regression analysis proceedures having been used to fit linear least squares lines to all the sets of data, the t-test to establish whether there is significant difference between two slopes was run. This requires an expression for the pooled estimate of the standard deviation of the slopes. The two variances of estimate from the two correlations are pooled by weighting according to their respective degrees of freedom, to give a pooled variance of estimate $(s(\hat{y})_p$. The t-statistic,

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

with $N_1 + N_2 - 4$ degrees of freedom.

where b_1 = slope of regression line through first data set

b_2 = slope of regression line for second data set.

$\Sigma^1 x_1^2 = \Sigma x_1^2 - \bar{x}_1 \Sigma x_1$

$\Sigma^1 x_2^2 = \Sigma x_2^2 - \bar{x}_2 \Sigma x_2$

$\bar{x}_1$ = mean x values, set one

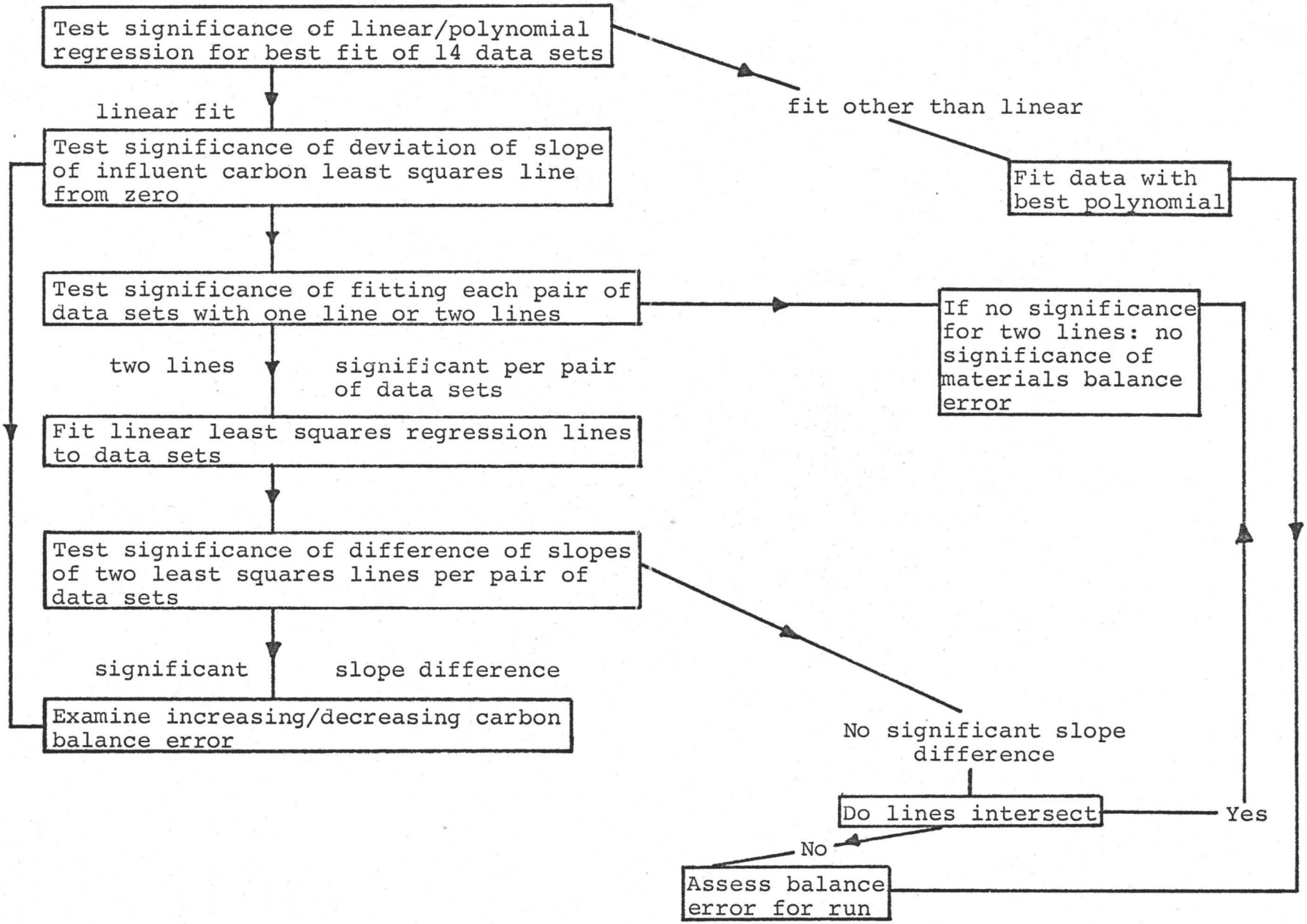
$\bar{x}_2$ = mean x values, set two

The t- statistics resulting from this computation are listed in Table (B). Computer programs run on the CDC 6400 machine are used for test (i) and (iii).

Run	t-Statistic	Degrees of Freedom	*Significance of Different Slopes	F-Value	Degrees of Freedom	* Significance of Separate Least Squares Lines Over One Line For All Data Per Run
3	0.4054	4	No significance	479.16	1 and 4	High significance for separate lines
4	- 0.04902	9	No significance	34.65	1 and 10	High significance for separate lines
5	0.0023	8	No significance	68.76	1 and 8	High significance for separate lines
6	0.1776	8	No significance	106.71	1 and 8	High significance for separate lines
8	0.1645	6	No significance	0.47	1 and 6	High significance for common line
9	3.1799	6	95% confidence of significance	178.47	2 and 5	High significance for separate lines
10	0.2433	11	No significance	36.38	1 and 12	High significance for separate lines

* Significance or lack of it is considered at the 95% confidence level.

TABLE (B)


Test significance of linear/polynomial regression for best fit of 14 data sets
fit other than linear
linear fit
Fit data with best polynomial
Test significance of deviation of slope of influent carbon least squares line from zero
Test significance of fitting each pair of data sets with one line or two lines
If no significance for two lines: no significance of materials balance error
two lines
significant per pair of data sets
Fit linear least squares regression lines to data sets
Test significance of difference of slopes of two least squares lines per pair of data sets
significant
slope difference
Examine increasing/decreasing carbon balance error
No significant slope difference
Do lines intersect
Yes
No
Assess balance error for run