CARBON BALANCE
IN A BATCH BIO-CHEMICAL REACTOR

by

RODRIGO A. BETANCUR
(Ingeniero Civil)

A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Master of Engineering

McMaster University
September 1970

CARBON BALANCE

IN A BATCH BIO-CHEMICAL REACTOR

MASTER OF ENGINEERING (1970)
(Civil Engineering)

McMaster University
Hamilton, Ontario

TITLE: Carbon Balance in a Batch Biochemical Reactor

AUTHOR: Rodrigo A. Betancur

SUPERVISOR: Dr. J. D. Norman

NUMBER OF PAGES: iv, 57

SCOPE AND CONTENTS:

Measurement of cellular carbon has been accomplished by gravimetric, titrimetric or directly measuring CO_2 produced in the combustion of organic carbon in the Carbon Analyzer. Gravimetric and titrimetric methods are cumbersome, inaccurate and time-consuming and direct measurements in the Carbon Analyzer are inaccurate at concentrations higher than 600 mg/l.

The purpose of this series of experiments is to find an alternative method for measuring cellular carbon. The proposed method is based in a carbon balance in which the soluble and gaseous forms of carbon are monitored. Cellular carbon is calculated by subtracting the sum of the soluble and gaseous forms of carbon from the initial total carbon found in the reactor.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

My thanks go to Dr. J. D. Norman for his encouragement and guidance throughout the period of this study.

The financial assistance provided by McMaster University is also gratefully appreciated.

CHAPTER 1

INTRODUCTORY REMARKS

Proper treatment and disposal of organic wastes is necessary for the protection of receiving waters. For some time the oxygen consuming characteristics of the dissolved wastes have been regarded of paramount importance in the design of waste treatment plants, when assessing the strength of wastes and effluents.

When organic matter is brought into contact with aerobic microorganisms and gaseous oxygen, parallel oxidation and synthesis reactions occur. Part of the organic matter is oxidized to end products as carbon dioxide, water, ammonia and nondegradable by-products with the liberation of energy; concurrent with this degradation, cellular material is synthesized from organic matter and energy.

The measurement of organic pollution in water has been the subject of numerous investigations. Several analytical approaches have been used including nitrogen transformations, biological oxygen depletion and chemical oxygen demand. Traditionally the accepted procedure for the determination of organic pollution is the Bio-chemical Oxygen Demand Test which measures the oxygen consumed by microorganisms during the utilization of organic nutrients. Notwithstanding the inherent problems of sewage dilution effects, elapsed time, interfering nitrification reactions and variation in oxidation rate constants, the BOD test is used as the primary procedure for the determination of readily oxidizable carbonaceous matter in water.

Investigators have long sought a procedure which would provide compatible information in a shorter period of time. Some of them have tried to find a correlation between observed plateaus in the rate of oxygen uptake and BOD while others have attempted to find a suitable oxidizing compound that would react only with degradable organic carbon and in this form replace the time consuming 5 day BOD test, with a more expeditious titrimetric method. Since the advent of the Infra-red Carbon Analyzer it has been possible to measure directly the carbon content of any waste. The carbon analyzer has application in the study of bacterial systems since it makes possible a rapid, accurate determination of the organic content of biological samples in solution.

Traditionally, the strength of organic wastes has been measured in terms of the five day Biochemical Oxygen Demand Test. This is not a consistent parameter, but one that will vary for similar absolute concentrations of different substrates depending on the degree of oxygenation of the compound. Several authors have recommended the use of a conserved parameter such as organic carbon or chemical oxygen demand, to measure biological substrate utilization.

The measurement of cellular carbon has been usually accomplished by gravimetric methods. Biological growth has been represented by empirical formulations derived from quantitative chemistry and few attempts have been made of monitoring cellular carbon in the bioreaction. Determination of organic carbon content of biological samples in suspension has been made at lower concentrations (600 mg/l or less) in the carbon analyzer. At higher concentrations this determination is difficult and inaccurate.

The purpose of this experiment is twofold: a) to determine if CO_2 can be accurately, rapidly and easily measured at the low concentrations observed to evolve from an aerated batch reactor and b) to measure changes in cellular carbon by means of a carbon balance in which easily performed soluble and gaseous carbon determinations are carried out.

The suggested procedure involves a single initial determination of the carbon content of a sample of the whole liquid and successive measurements of soluble and gaseous carbon. The whole liquid and the soluble carbon determinations are carried out in the Carbonaceous Analyzer and the CO_2 carbon is measured with a CO_2 sensitized gas chromatograph.

The results obtained from these series of experiments indicate that the method is suitable to measure changes in cellular carbon with 10% accuracy.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbial Metabolism

Nature is essentially a biological reactor in which numerous and different life cycles are taking place. Degradation of organic wastes and reproduction of microorganisms thriving in the wastes it is one of these cycles. In general, biological activity is used to convert soluble organic matter into gaseous compounds and synthesized cell material; the gases disperse in the atmosphere and the synthesized material is removed by physical methods and disposed of in different ways.

Most of the research done in the field of waste water treatment has been done on soluble organic carbon substrates. Banerji has reported on biological activity on colloidal organic compounds (1).

Busch (3) says about the nature of wastes requiring conventional biological treatment: "Soluble constituents are the only fraction of wastes requiring biological oxidation for their removal, (that, i.e., suspended and colloidal materials can be removed by physical separations, with or without the aid of chemical additives such as coagulants)". Busch later stresses that "In any situation there is literally no excuse for discharge of suspended material to a receiving body of water". In the process of aerobic degradation a portion of the organic matter is converted to new cell material and carbon dioxide as a product of oxidation. This oxidation yields the energy necessary for synthesis and cell maintenance. (8) (13,

The discussions which follow with respect to nutrition and synthesis apply to aerobic or activated sludge process organisms. Bacteria range from autotrophs whose food requirements are mostly inorganic through a wide spectrum of heterotrophs with a large variety of needs. Mixed cultures used as initial seed evolve into cultures of few predominant species which can utilize the available organic matter more efficiently. (20)

Nitrogen, phosphorus, and sulfur are known elements needed in measurable amounts in the metabolism of most activated sludge bacteria. Nitrogen should be present in the form of NH_4^+, the phosphorus as $PO_4^{\equiv}$ and the sulfur as $SO_4^{=}$. Trace amounts of magnesium, potassium, calcium, iron, zinc, copper, cobalt and manganese are also essential for most bacteria. The carriage water is normally relied upon to furnish these (18).

The requirements for synthesis are the presence of living organisms, available food supply and proper environment. A minimum amount of food is required for supplying energy for endogenous respiration. As the food supply is increased above that needed for endogenous respiration, a source of energy and raw materials becomes available for reproduction. The rate of reproduction will increase with the level of the available food supply until the minimum generation time of the organisms becomes the limiting factor.

Sawyer (20) indicates that the N and P requirements in activated sludge should fall within the following limits:

BOD to N --- 17:1 Max.

--- 32:1 Min.

BOD to P --- 90:1 Max.

--- 150:1 Min.

Helmer et al, (8) also point out that defficiencies in nutrients are not likely to be found in domestic sewage but in industrial wastes.

In the presence of oxidizable matter, necessary nutrients and proper environment, microbial cultures will undergo several growth stages, the principal of which are a lag phase, a log growth phase, a maximum stationary growth phase and a death phase (endogeneous). The lag phase is largely eliminated when the culture has been acclimated to the particular substrate. The log phase can be defined as the period during which regular and maximum cell multiplication takes place. The log growth rate is dependent on the mean generation time of the system. The generation time is defined as the interval in which a bacterium develops and completely divides into two cells.

2.2 Bio-Kinetics

In applying physiological concepts to waste-treatment plant design it is necessary to develop mathematical relationships between several process parameters such as rate of growth, rate of substrate removal and rate of oxygen consumption; definite ranges of values for these reaction rate constants and for such parameters as the yield factor have to be established for a variety of process conditions. The relationship between substrate concentration and specific growth rate has been mathematically defined by several authors, i.e., Streeter and Phelps (24), Teissier (25), Garret and Sawyer (6), McCabe and Eckenfelder (15), Hetling and Washington (9), Weston and Eckenfelder (28), and Eckenfelder and O'Connor (5) and Monod (17).

Bacteriologists (17) have preferred a mathematical continuous function specifically known as the Michaelis-Menten equation, (22):

$$k_1 = k_m \left(\frac{S}{S_n + S}\right)$$

where

k_1 = specific growth rate

k_m = maximum growth rate

S = substrate concentration

S_n = constant, equal to substrate concentration at which $k_1 = k_m/2$

This expression is derived from enzyme kinetics and the bacterial cell is considered as an enzyme molecule reacting with the substrate "S" Teissier (25) proposed in 1936 another model:

$$dk_1/dS = c(k_m - k_1)$$

or integrated

$$k_1 = k_m(1-e^{-cS}), \text{ where c is a constant;}$$

This equation is similar to the equation for a first order reaction where the growth rate increases with increasing substrate concentration proportionally to $k_m - k_1$, i.e., to the difference between the existing growth rate and a maximum growth rate. Garret and Sawyer (6) and McCabe and

Eckenfelder (16) have used two separate functions, one for the lower range of substrate concentrations where the growth rate is assumed to increase linearly with substrate concentration, and one for the higher range of substrate concentration where the growth rate is assumed to be constant.

Since most of the kinetic mechanisms reported in the literature express organic substrate as oxygen equivalents and since oxygen is known to participate in several elementary step reactions compressed in the overall metabolic reaction the expression of kinetic rates in terms of oxygen has little meaning. The same can be argued of mechanisms in which organic carbon is the measured parameter and therefore the practice of fitting empirical data to a mathematical model is not justified.

2.3 Measurement of Biodegradability

The wide variety and complexity of organic compounds in most wastes makes it practically impossible to assess, by individual methods of analysis, the various organic constituents of a waste. Furthermore, no chemical method of analysis yields information as to whether or not all or some of the organic material is biologically degradable. Some time ago it was realized that the quantity and nature of each organic compound need not be known in most cases, for the successful treatment of the waste, but that information was required as to the effects produced when the waste was brought into contact with micro-organisms and gaseous oxygen. As a result, a bioassay test was developed which measures the oxygen demand of the biological system when prepared samples of the waste are incubated for a specific period of time at controlled temperature. This test is known as the Biochemical Oxygen Demand (BOD) test (23) and the standard test is carried out for five days

at 20°C. The BOD test is not only used to evaluate the oxygen consuming characteristics of the waste, but to roughly indicate the amound of biologically degradable organic matter present in the sample, i.e., the greater the BOD the greater the organic content.

Busch et al. (4) later shortened the time of the test to 24 to 48 hours. Tneir Total Oxygen Demand (TOD) test is based on the fact that upon attainment of an observed plateau in the BOD curve all substrate has been converted to bacterial cells of known chemical composition ($C_5H_7NO_2$). The resulting cell growth can be determined chemically or gravimetrically in terms of oxygen equivalents.

Hiser and Busch (11) devised a new technique to shorten the BOD test time to 8 hoursusing mass culture aeration and chemical oxygen demand. Oxidation of all oxidizable material in the sample by means of chemical oxidizing compounds, as in the COD test, have been also used to assess the biodegradability of waste waters. Obviously this method will not yield representative results because other than biodegradable matter would be oxidized and as a consequence the amount of oxygen used in the test is not proportional to the amount of bio-degradable matter present in the sample. As a consequence Gilmour et al. (7) suggested the use of potassium persulfate as a means of oxidizing soluble organic matter. Their experimental data show a high correlation with BOD.

Respirometic techniques have also been used to determine the oxygen demand values of waste waters and aerobic biological systems. (12) Although these methods provide a more precise description of the oxygen demand curve than the BOD tests, their usefulness in assessing biodegradability

is limited to mere indications if a complete mass balance is not carried out wherein each reactant and each product is quantitatively measured.

Since the development of the Infra-red carbonaceous analyzer it has been possible to measure directly the soluble carbon content of a waste before and after bacterial metabolism takes place. The difference between the two measurements has been termed the total bacterially available carbon measurement. (3,21). For treatment process design, minimum oxygen requirements can be estimated from the oxygen equivalent of the change in total carbon, i.e., total change in soluble carbon less the carbon content of the synthesized cells. This amounts to the change in carbon content of the unfiltered mixed liquid or to the amount of carbon dioxide given off during the reaction.

2.4 Soluble and Cellular Carbon Determinations

Soluble carbon determinations in the carbon analyzer are known to be relatively free of interferences. Rickard et al. (19) assert that " Two possible sources of error do exist, however, that must be taken into account. The first of these is caused by the fact that CO_2, carbonates and bicarbonates will give a positive response. These materials can be easily removed from the sample by acidification (pH2) followed by a nitrogen purge (26). The second possible source of error is caused by the loss of highly volatile organics during purging". Rickard et al. also determined the precision that could be expected from the carbon analyzer measurements by making ten measurements of different concentrations of acetic acid and calculating the standard deviation from the mean. The 95 per cent confidence limit was then calculated

and the precision was found to vary from 3 to 7 per cent, the average being 4.4 per cent.

2.5 CO_2 Measurements

Measurements of CO_2 evolved from biochemical reactors are rare in the waste water literature. Some authors have measured CO_2 by assuming a perfect carbon balance in which CO_2 produced is equal to the difference between the initial total carbon measurement and subsequent measurements of the total carbon present in solution and suspension. (19)

CO_2 has been measured by measuring the production of Barium carbonate in a Barium hydroxide solution (14) or directly with the use of a CO_2 continuous analyzer (27).

CHAPTER 3

EXPERIMENTAL PROCEDURES

3.1. Experimental Equipment

3.1.1. The Batch Reactor

The batch reactor, shown in Fig. 1, consisted of a 14 l cylindrical bottle with a 1 1/2" opening at the top and a 1" opening on the side bottom. A water-cooled condenser was placed on the top opening to minimize evaporation. A rubber cork with two 1/4" holes was placed on top of the condenser; a plastic tube was then inserted through one of the holes. This plastic tube conveyed part of the gas that evolved from the reactor to the gas chromatograph. The other hole permitted the excess gas to diffuse in the atmosphere. The pressure inside the reactor was controlled by controlling the size of this hole.

Another rubber cork was placed on the side bottom opening of the reactor. This cork had three openings, one for introducing the oxygen supply, one to draw the liquid samples and another to place an open end manometer. The liquid samples were drawn through a 1/4" O.D. glass tube that reached the center of the bottle and was controlled by a stop-cock tap. The manometric pressure inside the reactor at the time of sampling the gas was equivalent to a 2" high water column.

The volume of the liquid in the reactor was 10.3 l at the beginning of the run and after 8 hours the volume had been reduced by sampling to 9.7 l The average volume of the reactor was then close to the intended

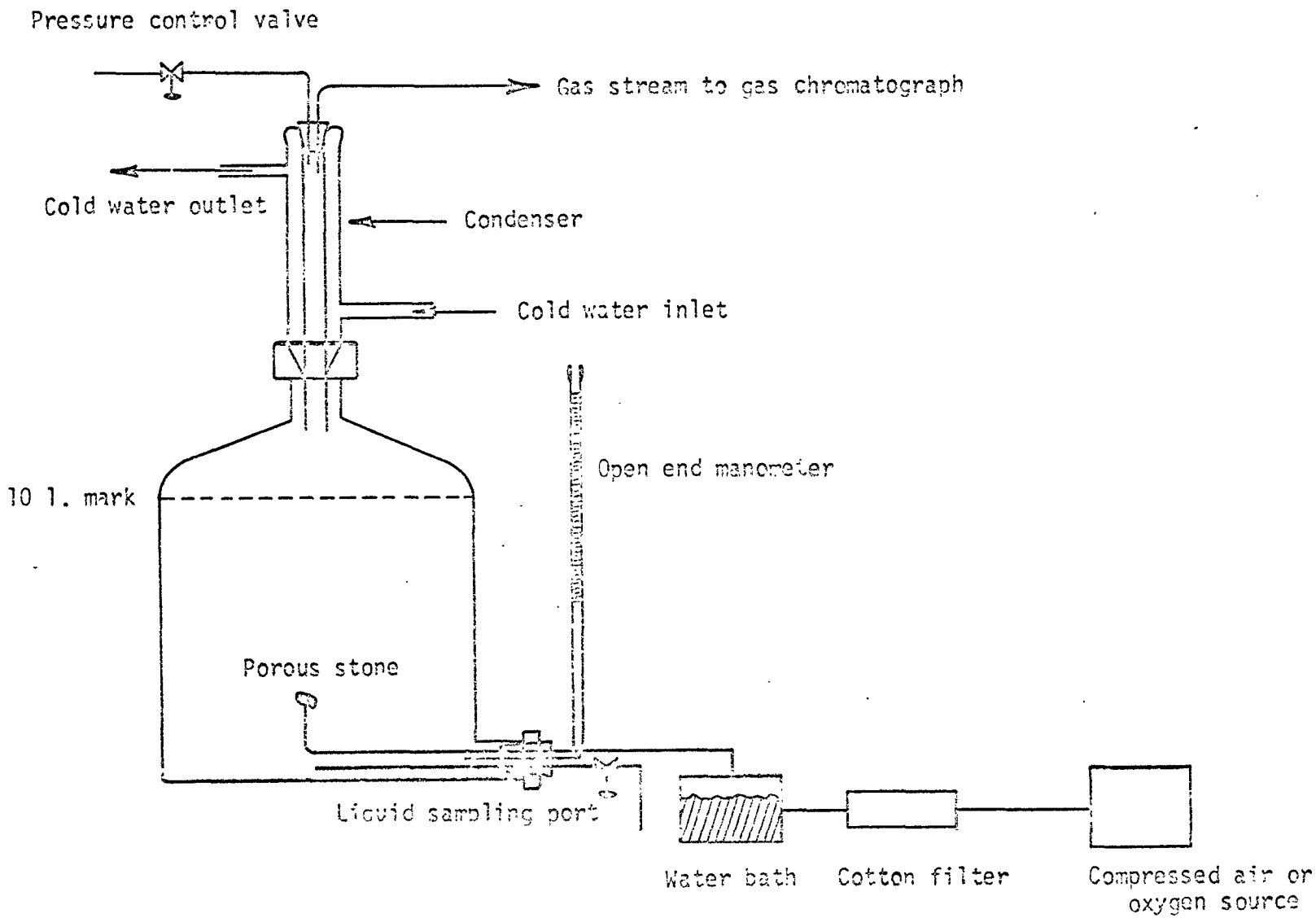

Pressure control valve
Gas stream to gas chromatograph
Cold water outlet
Condenser
Cold water inlet
Open end manometer
10 l. mark
Porous stone
Liquid sampling port
Water bath
Cotton filter
Compressed air or oxygen source

Figure 1.

10.0 l.

The oxygen utilized in these experiments was introduced to the reactor by means of a plastic tube and a porous stone diffuser.

3.1.2. The Gas Chromatograph

The gas chromatograph is a detector cell that measures differences in the thermoconductivity of two inert gas streams, one of which is carrying the sample to be examined: the other sample simply serves as a reference. Differences in thermoconductivity are electronically amplified and plotted on a strip chart recorder.

The stream carrying the sample is forced through a suitably packed column that retards the flow of the component of the sample to be determined.

The gas chromatograph used in these experimental series is a 90-p3 model from Varian Aerograph, the carrier gas was Helium, the column used was a 4 ft. long 1/4" diameter copper tube packed with 80 mesh silica gel which effectively retarded the CO_2 from the rest of the gases 30 to 60 seconds, when the Helium gas was flowing at 60 ml/min.

Signal output was recorded on an Electronik 19 recorder from Honeywell.

Sampling was done by means of a six-way valve with a 0.5 ml loop; the valve guarantees constant volume injections through the chromatograph. The apparatus was calibrated with three different concentrations of CO_2 certified by Matheson of Canada. The CO_2 concentrations of each cylinder were 1.68, 2.65 and 3.47% and are guaranteed to be accurately measured within 5%.

3.1.3. The Infra-red Carbonaceous Analyzer

The apparatus used to measure the carbon content of the liquid samples was a Beckman Carbonaceous Analyzer; made by Beckman under license from the Dow Chemical Company.

The carbon content of the samples is determined by injecting 20 µl of the liquid into a 1800°F combustion chamber by means of a microliter syringe. The sample is instantly vaporized and combusted in the presence of Cobalt Oxide which acts as a catalyst. The sample carbon content is fully converted to carbon dioxide and passed through a non-dispersive type Infra-red analyzer sensitized 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 height of the peak is proportional to the carbon content of the sample. In this way the carbon concentration of several samples may be determined from calibration curves made by injecting samples of known carbon content. (See Appendix 3 and Figures 11 and 12)

3.1.4. The Air and Oxygen Sources

During the acclimation period compressed air from the University compressor was used for mixing and aeration purposes. The dissolved oxygen was kept between 3 and 6 mg/l. In order that any particles of dust in the air did not pass into the porous stone diffuser and clog it, the air was first filtered through glass wool; the air was also pre-saturated in a water bath in order to decrease evaporation.

During the experimental runs pure oxygen was used for oxygenation and mixing purposes. Oxygen was supplied at 30 p.s.i.g. from an oxygen

cylinder controlled with needle valve and gas flow regulator. The oxygen flow was measured with previously calibrated rotameters.

3.2 Experimental Techniques

3.2.1. The Microbial Culture

The microorganisms used in these series of experiments were harvested from a sample taken from the Dundas, Ontario, waste water treatment plant primary clarifier near McMaster University.

A batch biological reactor was started with one liter of Whatman No. 2 filtrate of the raw sample. The seed, substrate and tap water were poured into the aerated vessel to complete approximately 10 liters. The microorganisms were adapted to the substrate for a period of two months prior to the beginning of the first experiment. During this period the batch contents were aerated and mixed with filtered and humidified air taken from the University compresser.

A new batch reactor was started every 24 hours by pouring into a previously washed reactor vessel, 5 liters of the precedent batch supernatant. Substrate, nutrients and tap water were added to complete ten liters.

Microscopic observations were performed once in a while during the adaptation period and at the beginning and end of each run.

3.2.2. The Carbon Source

Most of the work done in the field of biological growth kinetics have assumed growth limitation by a single nutrient so that the rate of disappearance of the nutrient can be related to microbial growth. In

this study the organic carbon has been chosen as the limiting nutrient.

For the purpose of monitoring the changes in the three forms of carbon any source of easily degradable organic carbon could have been used but to simplify the experimental procedures and compare the work of former researchers at McMaster University it was decided to use a simple organic substrate of glucose.

Microorganisms found in raw sewage are known to adapt rapidly to the glucose substrate. Details of the glucose medium and all necessary nutrients can be seen in Appendix No. 1.

3.2.3. Liquid Sampling

A total of 40 ml was withdrawn every 30 minutes to first 10 ml were wasted to avoid testing unrepresentative samples made up of the liquid trapped in the sample port which had not been properly mixed with the rest of the liquid. Twenty ml were filtered through Millipore filter paper (0.45 μm pore size). In this way 10 ml were left unfiltered.

Filtered samples were further split into two 10 ml samples. One of these samples was CO_2 stripped by acidification and bubbling an inert gas through the liquid for five minutes.

Carbon analysis of the resulting samples were performed by injecting 20 μl several times into the Infra-red Carbon Analyzer. Carbon concentration determinations required several injections of the micro-samples; soluble carbon required generally three to four injections whereas the unfiltered samples required six to ten trials. Readings of soluble carbon were selected after two consecutive injections yielded chart readings

differed in less than 1% of the scale. Carbon determinations on unfiltered samples were averaged after discarding the lowest and highest readings if they were obviously misrepresentative.

Use of the same syringe by the like operator eliminated much of the inaccuracies of the method, especially those due to differences in the injected volume.

A calibration curve was made shortly before each experiment with samples of sodium oxalate carefully diluted in distilled water; the carbon concentration of these samples ranged from 10 to 2500 ppm which is the range in which the totality of the carbon measurements were made (see Appendix 3 for details of the IRCA calibration).

Attempts were made to disrupt the microbial cells in the unfiltered sample by sonication but with the equipment available the sonication period would have been longer than the sampling interval and the temperature rise too large to guarantee representative samples. Samples subject to ultra-sonnic shock during twenty minutes failed to show substantial disruption of the cell material when observed under the microscope, furthermore, the temperature of the samples raised approximately $10^{o}F$ and it was feared that further increase would evaporate significant amounts of water thus increasing concentration readings. The 20 minutes sonicate samples did not show differences in the carbon concentrations when compared to the non-disturbed samples; sonication was discontinued after the first run.

3.2.5. pH Determinations

The pH of the reactor contents was measured at intervals using a

Beckman expanded scale pH meter (Model 76). The instrument was calibrated before use with a phosphate buffer solution of pH 6.86.

3.2.6. Microscopic Observation

Microscopic examinations were made at intervals during the experiments. The observations were purely qualitative. No attempt was made to make a count of the bacteria present.

The apparatus used in the study was an Olympus microscope with a phase contrast attachment.

CHAPTER 4

DISCUSSION OF RESULTS

4.1 The Carbon Balance

The three forms of carbon, i.e., cellular, soluble and gaseous, were measured every half hour. Cellular and soluble carbon concentrations were determined with the aid of the Carbon Analyzer. Samples of the whole liquid and its corresponding filtrate through millipore paper were injected into the Carbon Analyzer; the difference between whole liquid carbon and the filtrate carbon is equivalent to the cellular carbon concentration

The rate of production of CO_2 carbon was measured every two to three minutes with the gas chromatograph. This rate was subsequently integrated with time to obtain the amounts of gaseous carbon evolved from the reactor since the beginning of the run.

Carbon determinations were also performed on the culture shortly before adding the glucose medium to check the level of soluble carbon in the liquid.

The corresponding values of the three carbon forms were converted to mg/l of reactor and added. The figure thus obtained must be constant according to the law of conservation of masses in a batch reaction, i.e.,

$$C_c + C_s + C_g = C_t \qquad (4.1)$$

where C_c is the cellular carbon concentration in mg/l

C_s is the soluble carbon concentration in mg/l

C_g is the gaseous carbon produced since the beginning of the run and is also given in mg/l of reactor

C_t is the total carbon measurement in mg/l

The three forms of carbon were monitored every half hour until the soluble carbon was observed to have attained its lowest value for at least one hour.

The observed or calculated results are plotted in Figures 3 to 10 and listed in Tables 3 to 10.

The calculated values of C_t are represented by the upper line of points in the same figures. A least squares line was fitted through each set of points.

The values of cellular carbon were calculated from equation (4.1) using values of C_t equal to the first total carbon measurement or when this value was believed to be incorrect by the value of the least squares line intercept with the carbon concentration axis. The resulting cellular carbon values can be compared with the values obtained with the aid of the Carbon Analyzer, in Figures 3 to 10 or in Tables 3 to 10.

Maximum cellular carbon concentrations were observed near the time in which soluble carbon reached its lowest value and when CO_2 rate of production was sharply reduced.

Table 1 lists the amounts of glucose added per liter of reactor and the theoretical and observed increases in soluble and suspended carbon concentrations.

TABLE 1

Run No.	Glucose Added in g/lt of reactor	Theoretical Increase in Soluble C	Observed Increase in Carbon Measurement			
			Soluble Carbon	% diff.	Total Initial C	% diff.
1	2.50	1000 mg/l	727 mg/l	- 27.3	878 mg/l	- 12.2
2	2.50	1000 mg/l	926 mg/l	- 7.4	1078 mg/l	7.8
3	2.50	1000 mg/l	937 mg/l	- 6.3	954 mg/l	- 4.6
4	2.50	1000 mg/l	1023 mg/l	2.3	1042 mg/l	4.2
5	2.25	900 mg/l	637 mg/l	- 29.2	814 mg/l	- 9.6
6	2.00	800 mg/l	822 mg/l	2.8	868 mg/l	8.5
7	2.25	900 mg/l	876 mg/l	- 2.6	854 mg/l	- 5.1
8	2.375	950 mg/l	934 mg/l	- 1.6	929 mg/l	- 2.2

In runs 1 and 5 the first total carbon measurements along with the soluble carbon values are believed to be in error as indicated by the large differences in theoretical and observed concentrations. The cause of the error is believed to be the fact that the Carbon Analyzer had not been in use for some time previous to the starting of the runs and these determinations were done shortly after turning it on. This assumption is confirmed by the fact that the second and third measurements of the increases in soluble and total carbon are closer to the theoretical value as can be seen in Table 2.

TABLE 2

Observed Increases in Soluble and Total Carbon (in mg/l)

Run No.	First Reading		Second Reading		Third Reading		Theoretical
	Sol. C	Total C	Sol. C	Total C	Sol. C	Total C	C
1	727	878	916	1082	914	991	1000
5	637	814	861	998	853	826	900

The observed production of CO_2 up until the rate of production was sharply reduced or at the moment of maximum solids concentration was listed in Table 3. In general, the amount of CO_2 produced up to this moment varied between 35 and 40 percent of the soluble carbon added to the reactor as glucose.

TABLE 3

Run No.	Observed Values of Gaseous Carbon as % of the Amount of Glucose Carbon Added	
	at the time of calculated maximum solids carbon	at the time of observed lowest soluble carbon
1	37.0 (6 hrs.)	43.1 (7 hrs.)
2	38.4 (4.5 hrs.)	43.2 (5 hrs.)
3	35.5 (5 hrs.)	35.5 (5 hrs.)
4	40.8 (6 hrs.)	40.8 (6 hrs.)
5	37.0 (6 hrs.)	43.1 (7 hrs.)
6	38.5 (4.5 hrs.)	43.2 (5 hrs.)
7	35.6 (5 hrs.)	35.6 (5 hrs.)
8	41.8 (6 hrs.)	41.8 (6 hrs.)

In general, soluble and gaseous carbon plots are smooth curves while the measured cellular carbon determinations are somewhat jumpy. In general, only 3 to 4 injections of the reactor liquor filtered sample to the Carbon Analyzer were necessary for each soluble carbon determination. Although the unfiltered samples seemed to be optically well dispersed, it is possible that microscopic floc formation and differential microbial concentrations throughout the container affected the carbon readings to such an extent that sometimes up to ten sample injections to the Carbon Analyser were necessary to obtain two consecutive readings differing less than 1% of the scale. After the experiments were concluded it was decided to average the readings that were not obviously unrepresentative, to obtain the cellular carbon value instead of using two consecutive equal readings as is usually done.

In the first run it was attempted to disrupt the cellular mass with ultrasonic waves. Sonication of part of the unfiltered sample yielded equally uneven results. The ultrasonic equipment available did not noticeably disrupt the cells after 20 minutes and raised the temperature of the sample more than $10^{o}C$. Sonication procedures were discontinued after the first run.

The presence of inorganic carbonates or dissolved CO_2 was checked. No appreciable difference was noticed between the acidified and nitrogen purged sample and the non-purged sample.

4.2 Carbon Dioxide Determinations in the Gas Chromatograph

The oxygen flow was regulated and controlled with a gas regulator, a needle valve and a high-precision previously-calibrated flow meter or

rotameter graduated in percent of maximum gas flow. The flow of oxygen was adjusted in such a way as to fulfill metabolic and mixing requirements and at the same time avoiding excessive dilution of the carbon dioxide produced in the reactor. Previous observations indicated that an oxygen flow of 1.4 litres per minute per litre of reactor liquid would suffice these conditons.

As chromatographic measurements require constant pressure sampling the pressure inside the reactor was kept constant by means of a manometer placed in the side bottom hole and adjusting the flow through the top opening.

A continuous stream of the gas given off by the reactor was forced into the gas sampling device of the gas chromatograph. To avoid condensation of water vapor at the sampling port it was necessary to install a condenser between the reactor and the sampling device.

The reproducibility of the gas chromatograph when injecting samples of known concentration is good; variations were noticed when pressure was varied at the sampling port; although variations in pressure were observed of half an inch in the manometer water column, all sampling was done under the same pressure (two inches of water).

Carbon dioxide determinations in the form of the integrated rate of production do not have the same degree of reliability as soluble carbon determinations because of the possibility of introducing a constant sign error that accumulates with integration.

Among the sources that would produce a constant error in the CO_2

rate of production determination are differences in the oxygen flow measurements, accumulation of condensates in the sampling device and the imprecision in the determination of CO_2 concentration in the gas cylinders used to calibrate the gas chromatograph.

4.3 Carbon Determinations in the Infrared Carbon Analyzer

Although the unfiltered samples injected in the IRCA seemed to be optically well dispersed, it is possible that microscopic floc formations and differences in concentration throughout the container affected the carbon readings, because the results obtained did not produce smooth curves as did the filtered samples. These differences are amplified when the filtered sample readings are subtracted from the unfiltered samples to obtain the cellular carbon.

Measurements of unfiltered samples are difficult and time consuming; due to the differences found in successive injections to the IRCA it was necessary to repeat the procedure up to ten times to get reliable results. Soluble carbon determinations needed only three to four injections from each sample and numerous experiments have proven the reliability of the IRCA for the purpose. (2)

4.4. Individual Analysis of Each of the Eight Runs

In Run No. 1 the slope of the least squares line indicates that less than 1% per hour of the total carbon was not being recovered. The loss has been attributed to an increased concentration of cellular matter in the scum or slime on the walls of the reactor.

The first measurement of total carbon is believed to be in error, possibly because the Carbon Analyzer had not been in use for some time before the starting of the experiment, and therefore the cellular carbon was calculated from the least squares intercept.

The maximum difference between observed and calculated cellular carbon was 10.5% of the initial total carbon.

In Run No. 2 the loss of total carbon amounts to less than 1% per hour of the initial total carbon. The first total carbon measurement, though, coincided with the least squares intercept.

In Run No. 3 the loss of carbon was larger than in most runs and this is believed to be due to a small increase in the oxygen flow which tended to decrease the concentration of CO_2 in the gas given off by the reactor. This fact is indicated by the lower CO_2 glucose added ratio observed at maximum cellular carbon (see Table 3). The least squares slope indicates a loss of little more than 2% per hour of the total carbon. The maximum difference between calculated and observed cellular carbon was 16%.

In Run No. 4 the carbon losses are considerably less than in previous runs. This improvement is believed to be caused by the lower initial cellular carbon concentrations. Cellular carbon determinations are usually more difficult and less accurate than determination of the forms of carbon. The

TABLE 4

RUN NO. 1

UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLIDS CARBON		TOTAL CARBON
			Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept
1760	790	0	970	970	1084	1760
1960	975	4	781	985	895	1964
1860	960	13	787	900	901	1873
1860	940	29	791	920	905	1889
1860	880	51	829	980	943	1911
1760	790	80	890	970	1004	1840
1730	700	112	948	1030	1062	1842
1700	610	150	1000	1090	1114	1850
1550	490	191	1079	1060	1193	1741
--	-	-	--	--	--	--
1440	230	280	1250	1210	1364	1720
1420	195	325	1240	1225	1354	1745
1310	110	370	1280	1200	1394	1680
1310	90	409	1261	1220	1375	1719
1400	80	431	1249	1320	1363	1831
1400	80	446	1234	1320	1348	1846
1330	80	458	1222	1250	1336	1788
1250	80	467	1213	1170	1327	1717

Figure 3.

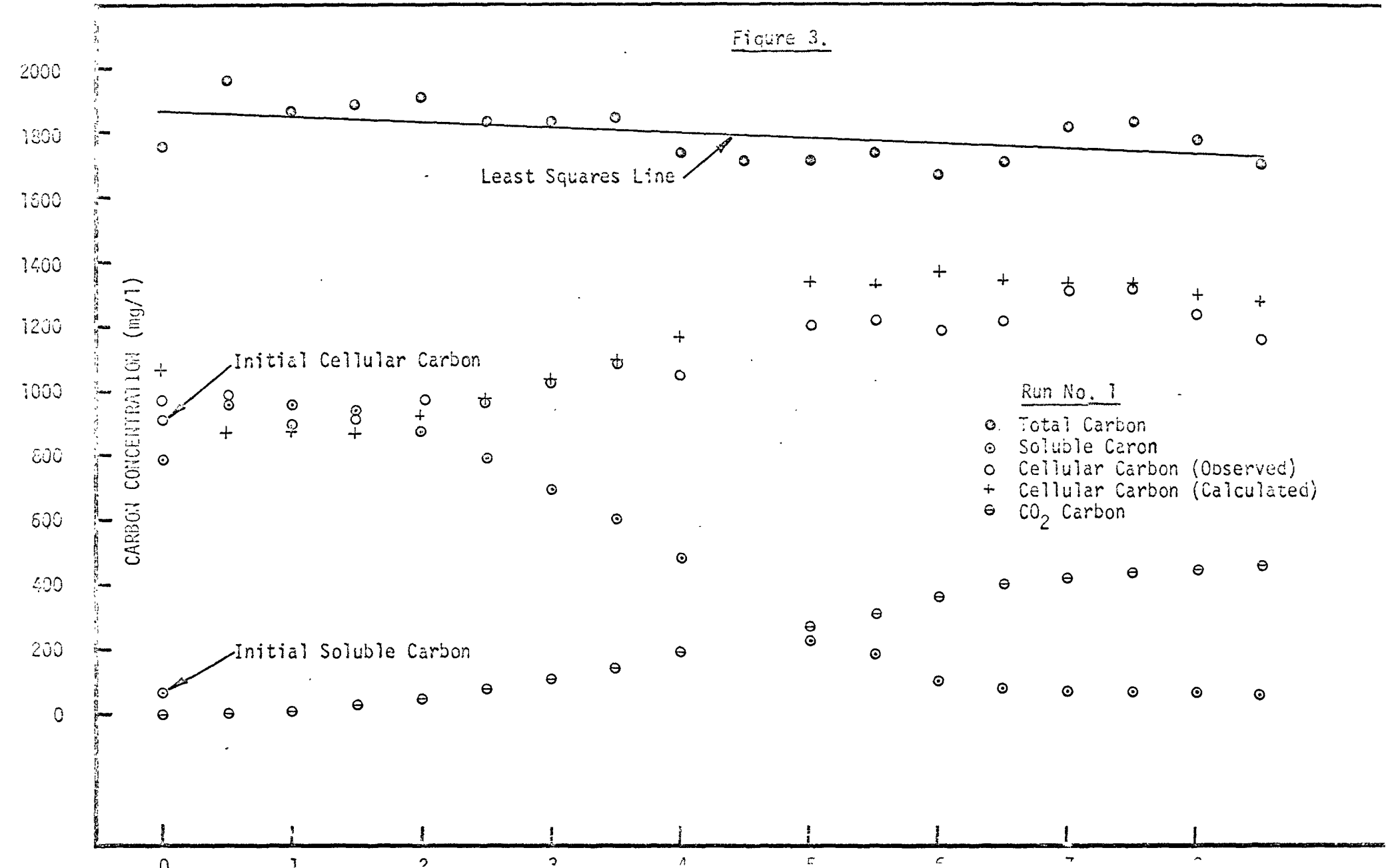

2000
1800
1600
1400
1200
1000
800
600
400
200
0
CARBON CONCENTRATION (mg/l)
Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 1
Total Carbon
Soluble Caron
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
0
1
2
3
4
5
6
7
8

TABLE 5

RUN NO. 2

:ME IRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	2500	980	0	1520	1520	1547	2500
.5	2580	980	13	1507	1600	1534	2593
.0	2400	900	40	1560	1500	1587	2440
.5	2460	840	77	1583	1620	1610	2537
.0	2400	740	119	1641	1660	1668	2519
.5	2260	620	165	1715	1640	1742	2425
.0	2260	510	215	1775	1750	1802	2475
.5	2300	370	269	1861	1930	1888	2569
.0	2060	230	327	1943	1830	1970	2387
.5	1920	80	384	2036	1840	2063	2304
.0	1960	70	432	1998	1890	2025	2392
.5	2060	70	458	1972	1990	1999	2518
.0	1960	70	474	1956	1890	1983	2434

Figure 4.

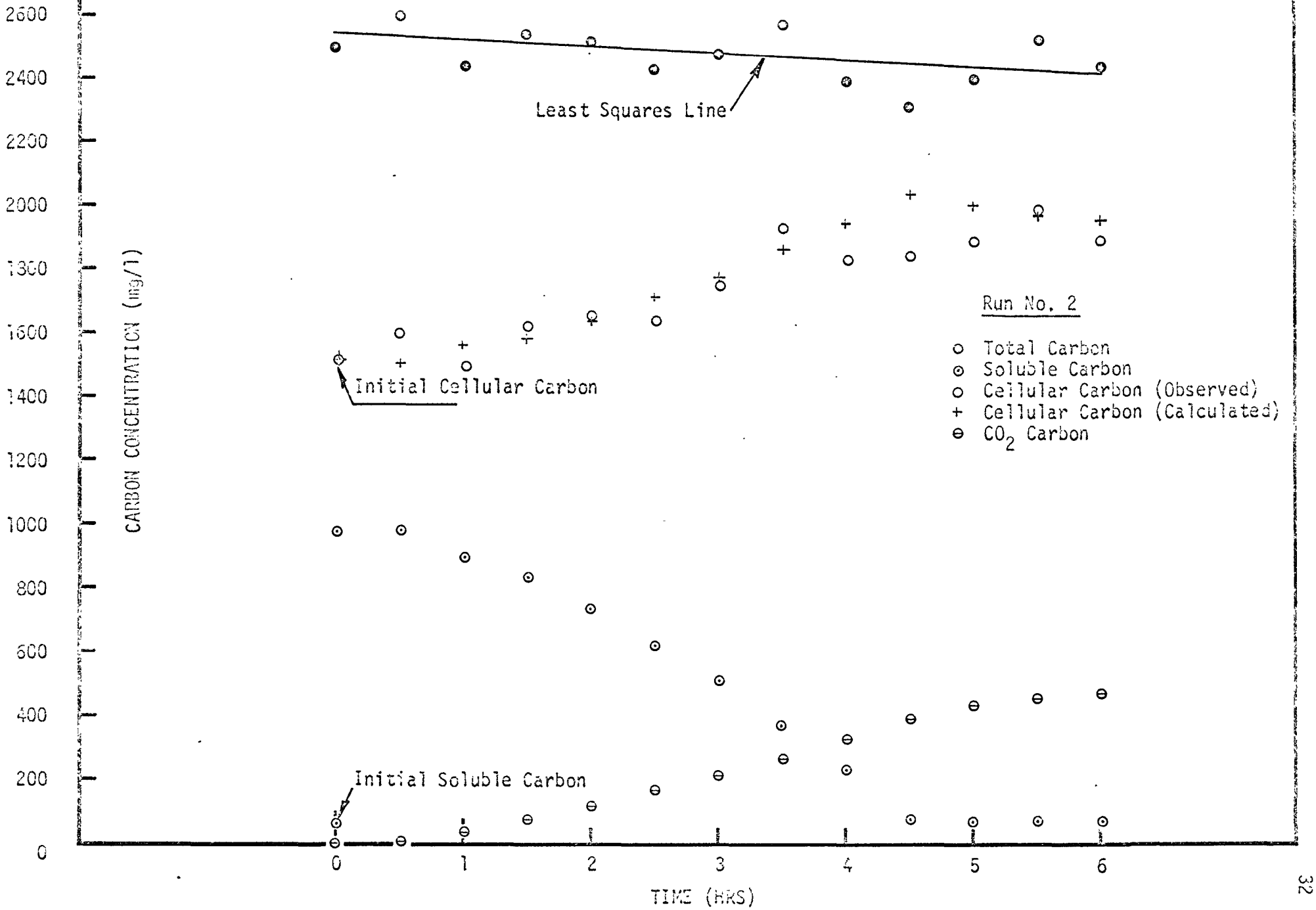

2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
CARBON CONCENTRATION (mg/l)
Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 2
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
0
1
2
3
4
5
6
TIME (HRS)

TABLE 6

RUN NO. 3

IME HRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	2250	1000	0	1250	1250	1207	2250
.5	2160	1030	9	1211	1130	1168	2169
.0	2080	920	28	1302	1160	1259	2108
.5	2030	820	56	1374	1210	1331	2086
2.0	2030	700	90	1460	1330	1417	2120
2.5	1990	650	129	1471	1340	1428	2119
3.0	1840	530	174	1546	1310	1503	2014
3.5	1890	400	224	1626	1490	1573	2114
4.0	1740	300	277	1673	1440	1630	2017
4.5	1670	120	327	1803	1550	1760	1997
5.0	1630	80	355	1815	1550	1772	1985
5.5	1560	120	370	1760	1440	1717	1930
6.0	1480	100	381	1769	1380	1726	1861

Figure 5.

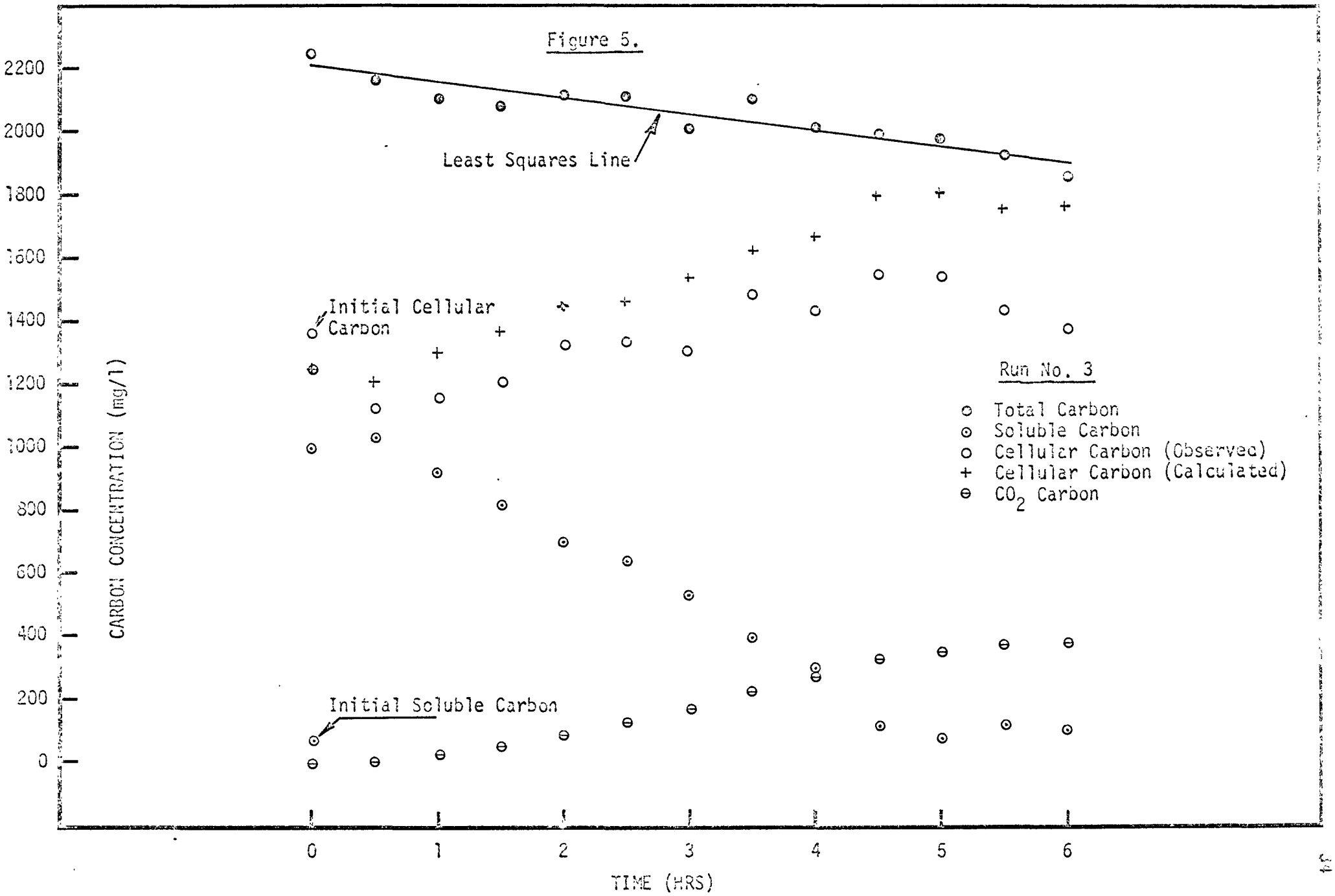

Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 3
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
CARBON CONCENTRATION (mg/l)
TIME (HRS)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6

TABLE 7

RUN NO. 4

ME IRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	1600	1050	0	550	550	541	1600
.5	1580	920	6	674	660	665	1586
.0	1550	860	19	721	690	712	1569
.5	1520	840	38	722	680	713	1558
.0	1520	820	60	720	700	711	1580
.5	1500	790	85	725	710	716	1585
.0	1470	740	114	746	730	737	1584
.5	1400	650	150	800	750	791	1550
.0	1360	560	191	849	800	840	1551
.5	1360	440	236	924	920	915	1596
.0	1290	320	289	991	970	982	1579
.5	1140	170	349	1081	970	1072	1489
.0	1110	70	408	1122	1040	1113	1518
.5	1140	80	441	1079	1060	1070	1581
.0	1070	70	456	1074	1000	1065	1526

Figure 6.

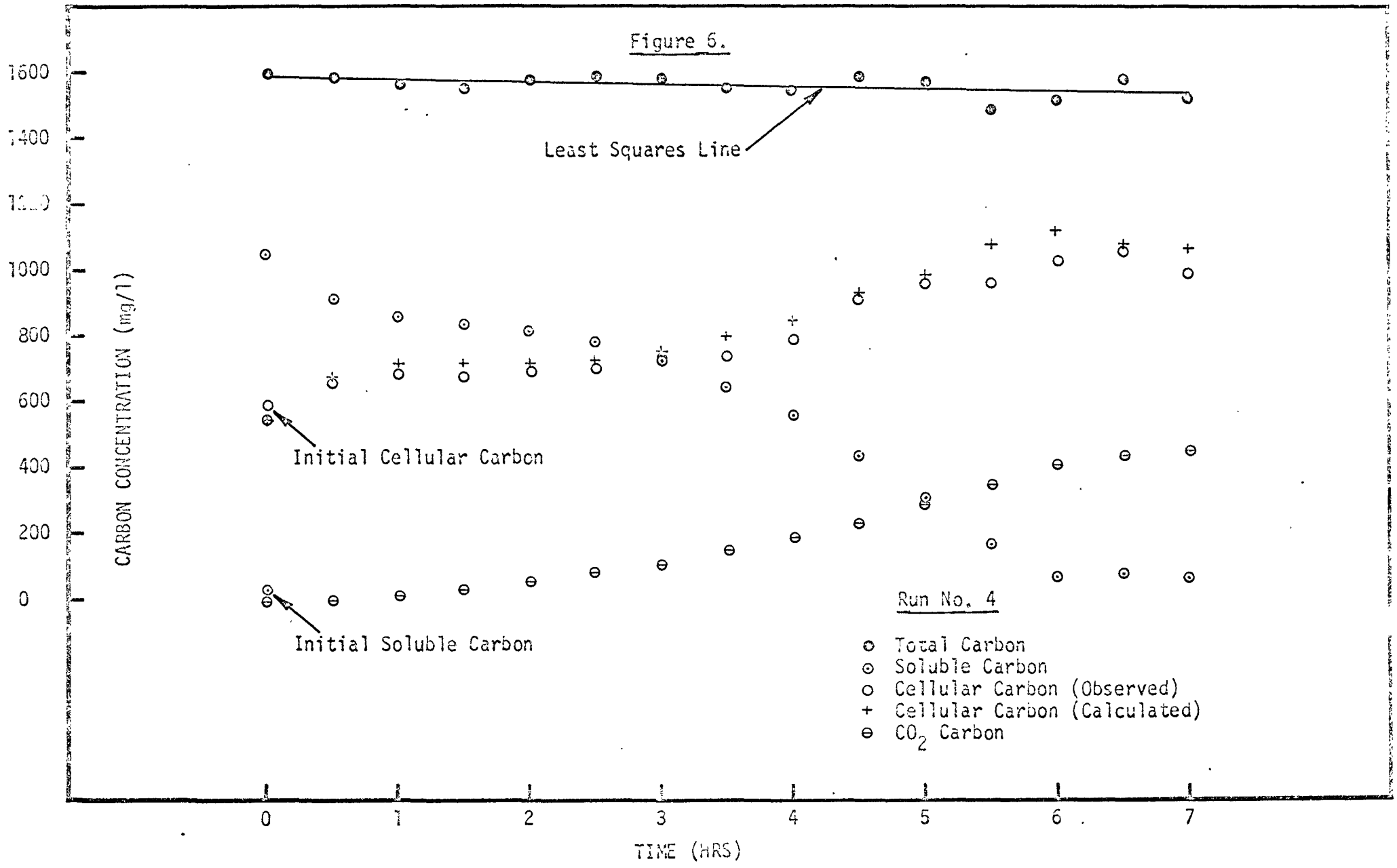

1600
1400
1200
1000
800
600
400
200
0
CARBON CONCENTRATION (mg/l)
Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 4
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
0
1
2
3
4
5
6
7
TIME (HRS)

TABLE 8

RUN NO. 5

IME HRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	1660	700	0	960	960	1057	1660
.5	1840	920	4	736	920	833	1844
.0	1660	900	12	748	760	845	1672
.5	1740	880	26	754	860	851	1766
.0	1660	830	46	784	830	881	1706
.5	1660	740	72	848	920	945	1732
.0	1660	660	101	899	1000	996	1761
.5	1620	575	135	950	1045	1047	1755
.0	1580	460	172	1028	1120	1125	1752
.5	1460	340	211	1109	1120	1206	1671
.0	1340	225	252	1183	1115	1280	1592
.5	1320	190	293	1177	1130	1274	1613
.0	1240	100	333	1227	1140	1324	1573
.5	1240	80	369	1211	1160	1308	1609
.0	1280	70	388	1202	1210	1299	1668
.5	1280	70	401	1189	1210	1286	1681
.0	1240	70	413	1177	1170	1274	1653
.5	1160	70	420	1170	1090	1267	1580

Figure 7.

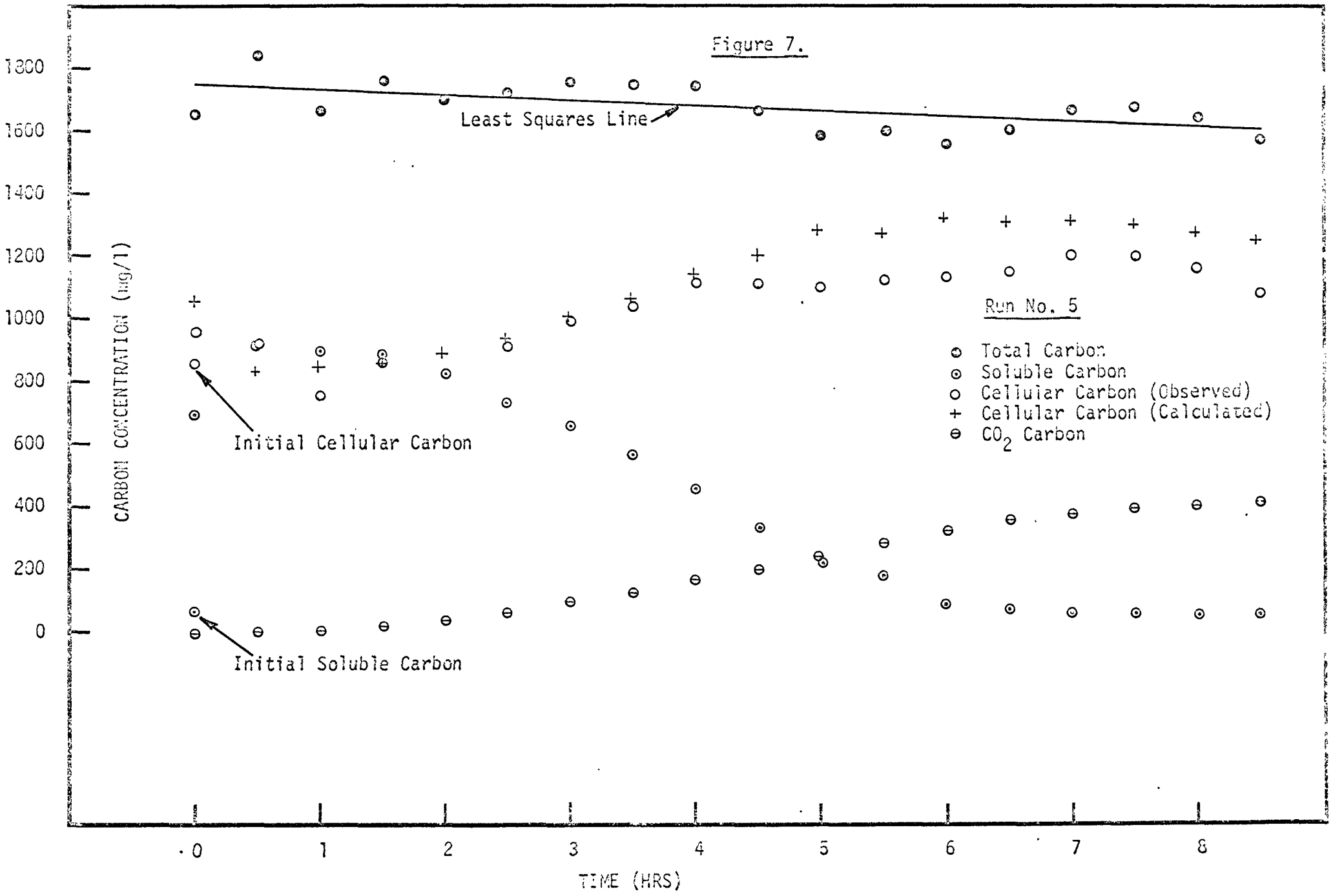

1800
1600
1400
1200
1000
800
600
400
200
0
CARBON CONCENTRATION (mg/l)
Least Squares Line
Run No. 5
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
Initial Cellular Carbon
Initial Soluble Carbon
0
1
2
3
4
5
6
7
8
TIME (HRS)

TABLE 9

RUN NO. 6

ME IRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	2200	870	0	1330	1330	1328	2200
5	2250	870	10	1320	1380	1318	2260
0	2100	790	32	1378	1310	1376	2132
5	2140	730	62	1408	1410	1406	2202
0	2100	640	96	1464	1460	1462	2196
5	1990	545	132	1523	1445	1521	2122
0	1990	450	172	1578	1540	1576	2162
5	1930	325	216	1569	1605	1567	2146
.0	1850	200	262	1738	1650	1736	2112
.5	1740	80	308	1812	1660	1810	2048
0	1770	65	346	1789	1705	1787	2116
5	1850	65	368	1767	1785	1765	2218
.0	1770	65	380	1755	1705	1753	2150

Figure 8.

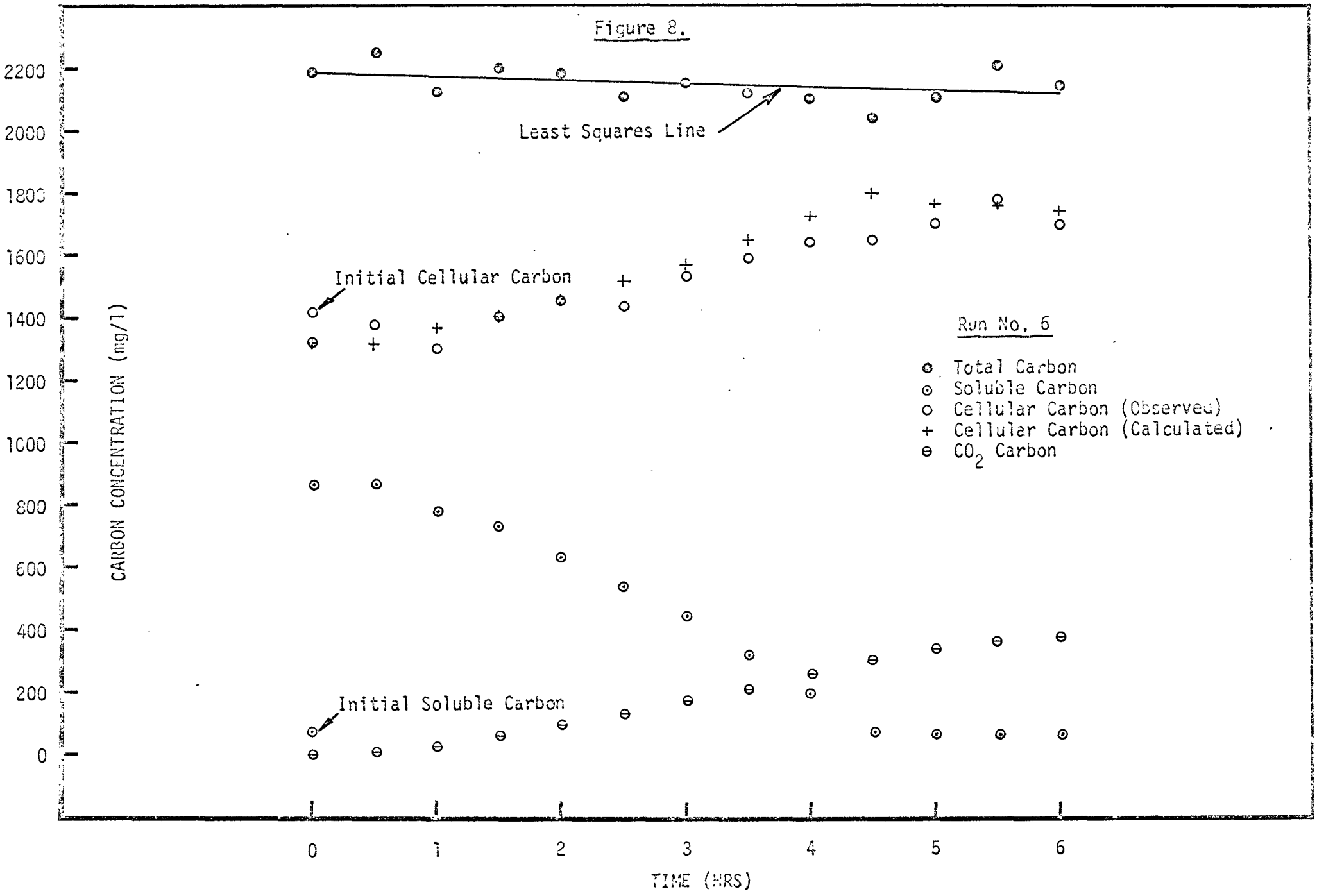

2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
CARBON CONCENTRATION (mg/l)
Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 6
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
0
1
2
3
4
5
6
TIME (HRS)

TABLE 10

RUN NO. 7

ME IRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	2060	930	0	1130	1130	1163	2060
.5	1980	950	8	1102	1030	1135	1988
.0	1900	860	25	1175	1040	1218	1925
.5	1860	770	50	1240	1090	1273	1910
.0	1860	640	81	1339	1220	1372	1941
.5	1820	580	115	1364	1240	1397	1936
.0	1800	500	157	1403	1300	1436	1957
.5	1740	370	202	1488	1370	1521	1942
.0	1620	270	250	1540	1350	1573	1870
.5	1550	100	295	1665	1450	1698	1845
.0	1510	60	320	1680	1450	1713	1830
.5	1440	100	333	1627	1340	1660	1773
.0	1370	70	343	1647	1300	1680	1713

Figure 9.

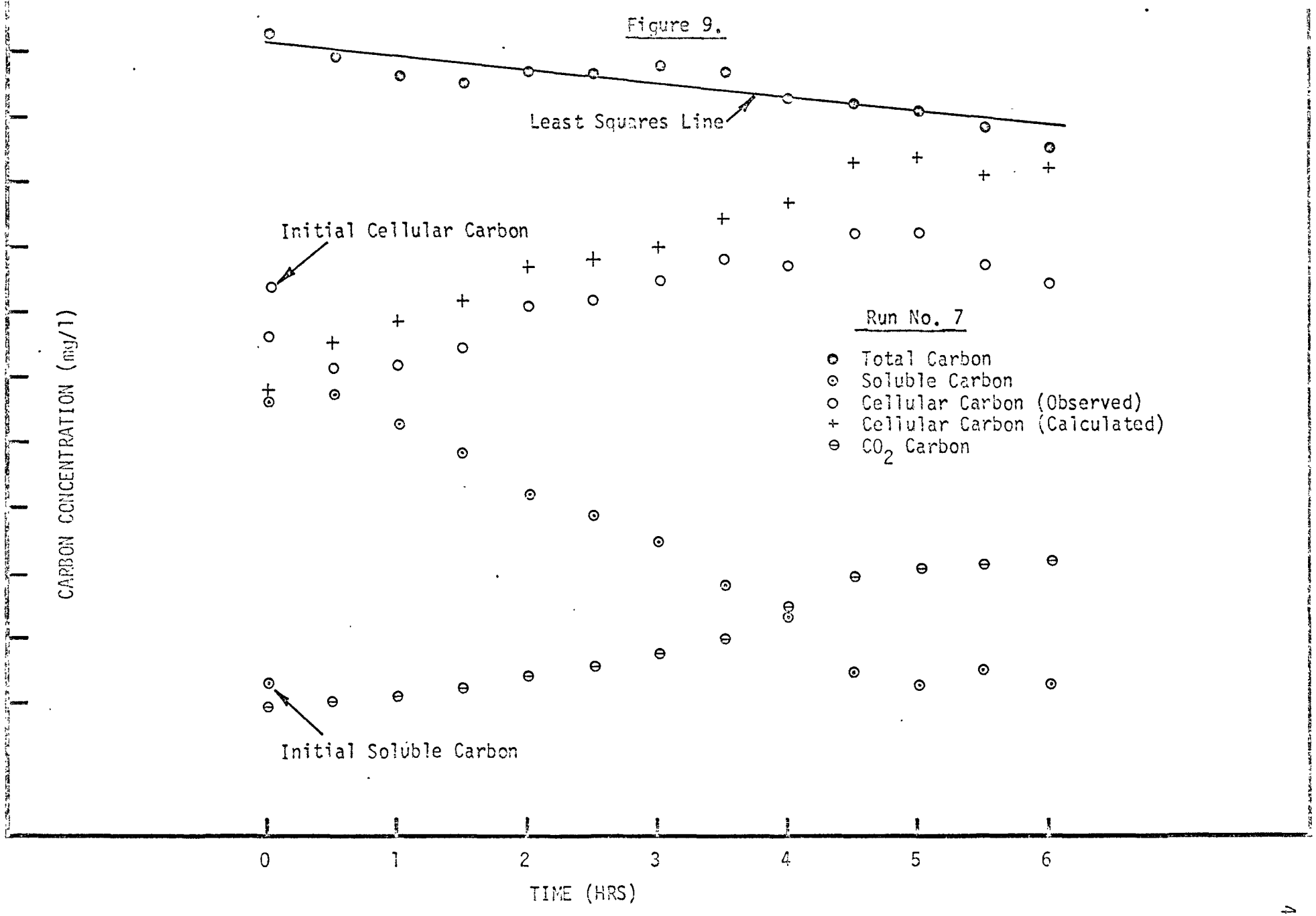
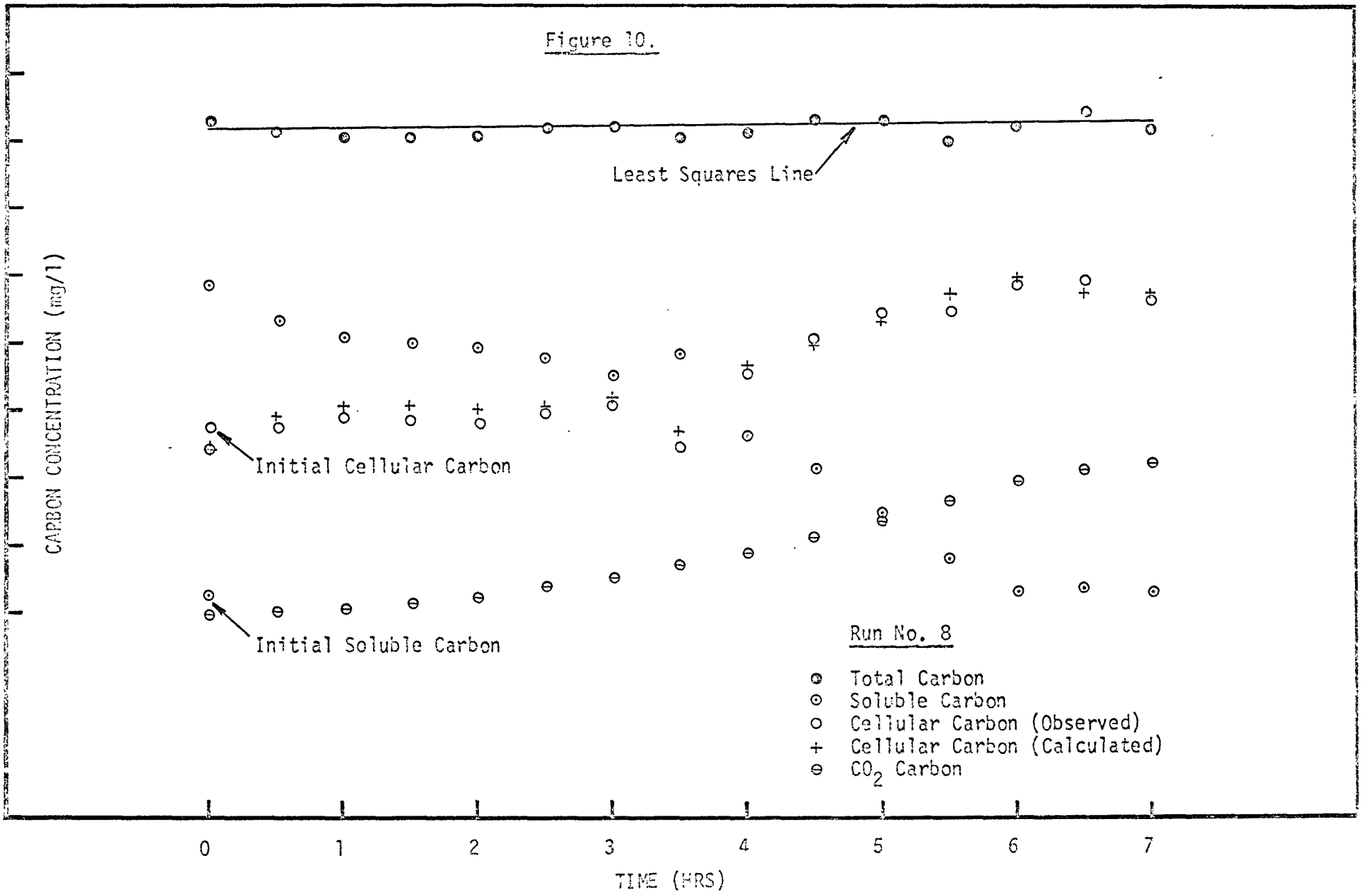

Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 7
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
CARBON CONCENTRATION (mg/l)
0
1
2
3
4
5
6
TIME (HRS)

TABLE 11

RUN NO. 8

IME IRS)	UNFILTERED SAMPLE CARBON	FILTERED SAMPLE CARBON	CO_2 CARBON	SOLID'S CARBON			TOTAL CARBON
				Calc. from 1st Reading	Observed	Calc. from Least Squares Intercept	
	1460	970	0	490	490	455	1460
.5	1420	870	6	584	550	549	1426
.0	1400	820	18	622	580	587	1418
.5	1380	800	36	624	580	589	1416
.0	1360	790	57	613	570	578	1417
.5	1360	760	81	619	600	584	1441
.0	1340	710	109	641	630	606	1449
.5	1270	775	144	541	495	506	1414
.0	1250	530	184	746	720	711	1434
.5	1250	430	228	812	820	777	1478
.0	1190	300	280	880	890	745	1470
.5	1070	165	339	956	905	921	1409
.0	1050	65	397	998	985	963	1447
.5	1070	75	429	956	995	921	1499
.0	1000	65	443	952	935	917	1443

Figure 10.


600
400
200
000
800
600
400
200
0
CARBON CONCENTRATION (mg/l)
Least Squares Line
Initial Cellular Carbon
Initial Soluble Carbon
Run No. 8
Total Carbon
Soluble Carbon
Cellular Carbon (Observed)
Cellular Carbon (Calculated)
CO_2 Carbon
0
1
2
3
4
5
6
7
TIME (HRS)

losses in total carbon were less than 0.5% per hour and the maximum difference between calculated and observed cellular carbon was 7%.

In Run No. 5 the carbon losses were 1% per hour and the maximum difference between calculated and observed cellular carbon was 10%. Here again the first measurement of total carbon is believed to be in error for the same reason as in Run No. 1 and the least squares intercept was used to calculate cellular carbon.

In Run No. 6 the least squares slope indicates a carbon loss of 0.6% and the maximum difference between calculated and observed cellular carbon was 7%.

Run No. 7, like Run No. 3, has higher than usual carbon losses. The reason for this is believed to be an error in the measurement of the oxygen flow. The carbon losses are close to 2% per hour and the maximum differnece between calculated and observed cellular carbon was 17%.

Run No. 8 shows an excess carbon of 0.3% per hour and the maximum difference in calculated and observed cellular carbon was 3.3% which is very good.

Table 12 lists the calculated least squares slope and intercept for each run.

TABLE 12

Run No.	Slope mg/l/hour	Intercept mg/l
1	16.7	1874
2	19.6	2527
3	49.5	2207
4	7.8	1591
5	17.5	1757
6	13.0	2198
7	42.5	2027
8	- 4.7	1425

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Gaseous carbon given off by an aerated, batch bioreactor can be accurately, rapidly and easily measured with a CO_2 sensitized gas chromatograph. The precision of the carbon dioxide measurements depend to a great extent on the availability of gas standards covering an adequate range to accurately calibrate the gas chromatograph.

5.1.2 Determinations of cellular carbon in a batch bioreactor can be carried out by measuring initial total carbon concentration and monitoring soluble and gaseous carbon concentration throughout the reaction.

5.1.3 Carbon recovery in this series of experiments varied from 3 to 16 per cent. This can be used as an indication of the precision that can be obtained with the procedure.

5.1.4 Determination of the initial total carbon concentration using the infrared carbon analyzer, should be based on at least three samples of each condition.

5.2 Recommendations

5.2.1 It is recommended that observed changes in cellular carbon, obtained by the method here suggested, be used to determine microbial growth rates.

5.2.2 It is also recommended that the carbon balance method be used in a continuous reactor.

REFERENCES

1. Banerji, S. K., Ewing, B. B., Engelbrecht, R. S. and Speece, R. E., "Mechanism of Starch Removal in the Activated Sludge Process" WPCF, 40, 1, 16, (1968).

2. Bennet, J., "Batch and Continuous Biochemical Reactor Studies Using Mixed Microbial Cultures", Master's Degree Thesis, McMaster University,(1968).

3. Busch, A. W., "Energy, Total Carbon and Oxygen Demand", 20 Industrial Waste Conference, Purdue University, Lafayette, Ind., 457 (1965).

4. Busch, A. W., Grady, L., Jr., Shivagi, Rao, T., and Swilley, E. L., "BOD Progression in Soluble Substrates - IV - A Short Term Total Oxygen Demand Test", 16th Industrial Wastes Conference, Purdue University, 67 (1961).

5. Eckenfelder, W. W., Jr., and O'Connor, D. J., Biological Waste Treatment, Pergamon Press, New York, (1961).

6. Garret, M. T. and Sawyer, C. N., "Kinetics of Removal of Soluble BOD by Activated Sludge", 7th Industrial Wastes Conference, Purdue University, Lafayette, Ind., 51 (1952).

7. Gilmour, C. H., Merryfield, F., Burgess, F., Punkerson, L. and Carswell, K., "Persulfate Oxidizable carbon and BOD as a Measure of Organic Pollution in Water", 15th Industrial Wastes Conference, Purdue University, Lafayette, Ind., 143 (1960).

8. Helmers, E. N., Frame, J. D., Greenberg, A. E. and Sawyer, C. N., "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, Part II", Sewage and Industrial Wastes, 23, 884, (1951).

9. Hetling, L. J., and Washington, D. R., "Kinetics of the Steady State Bacterial Culture III, Growth Rate", Proc. 20th Industrial Waste Conference, Purdue University, 254, (1964).

10. Heukelekian, H., Orford, H. E. and Mangonelli, R., "Factors Affecting the Quanitity of Sludge Production in the Activated Sludge Process" Sewage and Industrial Wastes, 23, 945, (1951).

11. Hiser, L. L., and Busch, A. W., "An 8-Hour Biological Oxygen Demand Test Using Mass Culture Aeration", WPCF 36, 4, 505 (1964).

12. Hunter, J. V., Heukelekian H., "Determination of Biodegradability Using Warburg Resperometric Techniques", Proc. of the 19th Industrial Waste Conference, Purdue University, 616, (1964).

13. Lamanna and Mallette, "Basic Bacteriology; Its Biological and Chemical Background" 2nd Ed. Baltimore: Williams and Wilkings Co., (1959).

14. Ludzack, F. J., Kroeger, H. L. and Ettinger, M. B., "Interactions of Waste Feed, Activated Sludge and Oxygen as Traced by Radioactive Carbon", WPCF, 40, 6, 782, (1964).

15. McCabe, J., and Eckenfelder, W. W., Jr., "Process Design of Biological Oxidation Systems for Industrial Waste Treatment", Waste Treatment, 156, edited by Pergamon Press Ltd., London (1960).

16. McCable, J. and Eckenfelder, W. W., "BOD Removal and Sludge Growth in the Activated Sludge Process", WPCF, 33, 3, 258, (1961).

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

18. Oginsky, E. L. and Umbreit, W. W., "An Introduction to Bacterial Physiology", W. H. Freeman and Co., San Francisco, California,(1954).

19. Rickard, M. D. and Riley, W. H.,"Carbon as a Parameter in Bacterial Systems, Growth Limitation and Substrate Utilization Studies", 20th Industrial Waste Conference, Purdue University, Lafayette, Indiana, 235, (1965).

20. Sawyer, C. N., "Bacterial Nutrition and Synthesis" in "Biological Treatment of Sewage and Industrial Wastes", Ed. J. McCabe and W. W. Eckenfelder Jr., Reinhold Publishing Corp., p. 3, (1956).

21. Schaffer, R. B. et al, "Application of a Carbon Analyzer in Waste Treatment", WPCF, November, 37, 1545, (1965).

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

23. "Standard Methods for the Examination of Water and Waste Water", 11th Ed., American Public Health Assoc., New York,(1960).

24. Streeter, N. W., and Phelps, E. B., Public Health Bulletin 146, USPHS, (1925).

25. Teissier, G., "Les Lois Quantitatives de la Croissance", Ann. Physiol. Phisicochim. Biol., 12, 527, (1936).

26. Van Hall, Barth and Stenger, "Elimination of Carbonates from Aqueous Solutions Prior to Organic Determination", Anal. Chem., 1965.

27. Watson, T. G., "Steady State Operation of a Continuous Culture at Maximum Growth Rate by Control of Carbondioxide Product", J. Gen. Microbiology, 59, 83 (1969).

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

APPENDIX 1

THE GLUCOSE MEDIUM

The growth of micro-organisms in a liquid medium needs the presence of some inorganic nutrients not found in tap water in sufficient amounts such as Iron, Potassium, Magnesium, Sulphates, Phosphates, Chlorides and Ammoniacal Nitrogen.

The glucose, $C_6H_{12}O_6$, and dibasic ammonium phosphate, $(NH_4)_2\ HPO_4$, in a 3:1 ratio by weight, were diluted in tap water. Stock solutions of the following inorganic nutrients were made:

Ferric Chloride, $FeCl_3.6H_2O$, in a concentration of 1.0 gm/l

Potassium Phosphate, K_2HPO_4, in a concentration of 50.0 gm/l

Magnesium Sulphate, $MgSO_4.7H_2O$, in a concentration of 50.0 gm/l

These solutions were added to the glucose-ammonium phosphate medium in the following manner:

1.0 ml $FeCl_3$ solution / gram of glucose added

2.0 ml K_2HPO_4 solution / gram of glucose added

2.0 ml $MgSO_4$ solution / gram of glucose added

The glucose medium thus prepared has been successfully used by former researchers (2) at McMaster University.

APPENDIX 2

CALIBRATION OF THE GAS CHROMATOGRAPH

The gas chromatograph was calibrated with the aid of compressed gas cylinders containing known concentrations of CO_2. Three cylinders supplied by Matheson of Canada were specifically used: the CO_2 concentrations were 1.68%, 2.65% and 3.45% by volume. Plots of the recorder output signal against the CO_2 concentration of the sample injected yielded a straight line that passed through the origin.

With an attenuation of 32, the recorder output signal was 100% when the 1.68% cylinder was sampled; this means that 1% of the output signal at maximum sensitivity was equal to 1.68% divided by 3200 or 0.000525% CO_2 by volume. The corresponding weight of the carbon in a mole of CO_2 is given by the ratio 12/22.4 gm/l The rate of production of CO_2 was measured every 15 minutes and then integrated to obtain the amount given off by the reactor.

The chart reading can be converted to grams of Carbon/15 min in the following way:

a) multiply the peak height by the attenuation factor (i.e., 1, 2, 4, 8, 16, 32, 64, etc.).

b) multiply the result by the concentration factor, 0.000525%, mentioned above.

c) multiply the result by the oxygen flow every 15 minutes (the oxygen flow was set at 1.4 l /min/l of reactor or 21 l O_2/15 min/l of

reactor).

d) multiply the result by the weight of carbon per mole of CO_2 and divide by the volume occupied by the mole (12 gm/22.4 l). The result is the weight in grams of carbon produced by the reactor in the form of CO_2 every 15 minutes per litre of reactor.

APPENDIX 3

STANDARD SOLUTIONS FOR THE INFRA-RED CARBONACEOUS ANALYZER

Throughout several years of experimentation with the Beckman Carbon Analyzer at McMaster University it has been found that Sodium Oxalate solutions in distilled water is good standards for carbon analysis. Standard samples were stored at $4^{o}C$ and found not to change with relatively long periods of time (2).

Standard solutions ranging from 10 ppm to 2,500 ppm were used; the calibration curves for the two gains used throughout the experiments can be seen in Figures 11 and 12.

Figure 11.

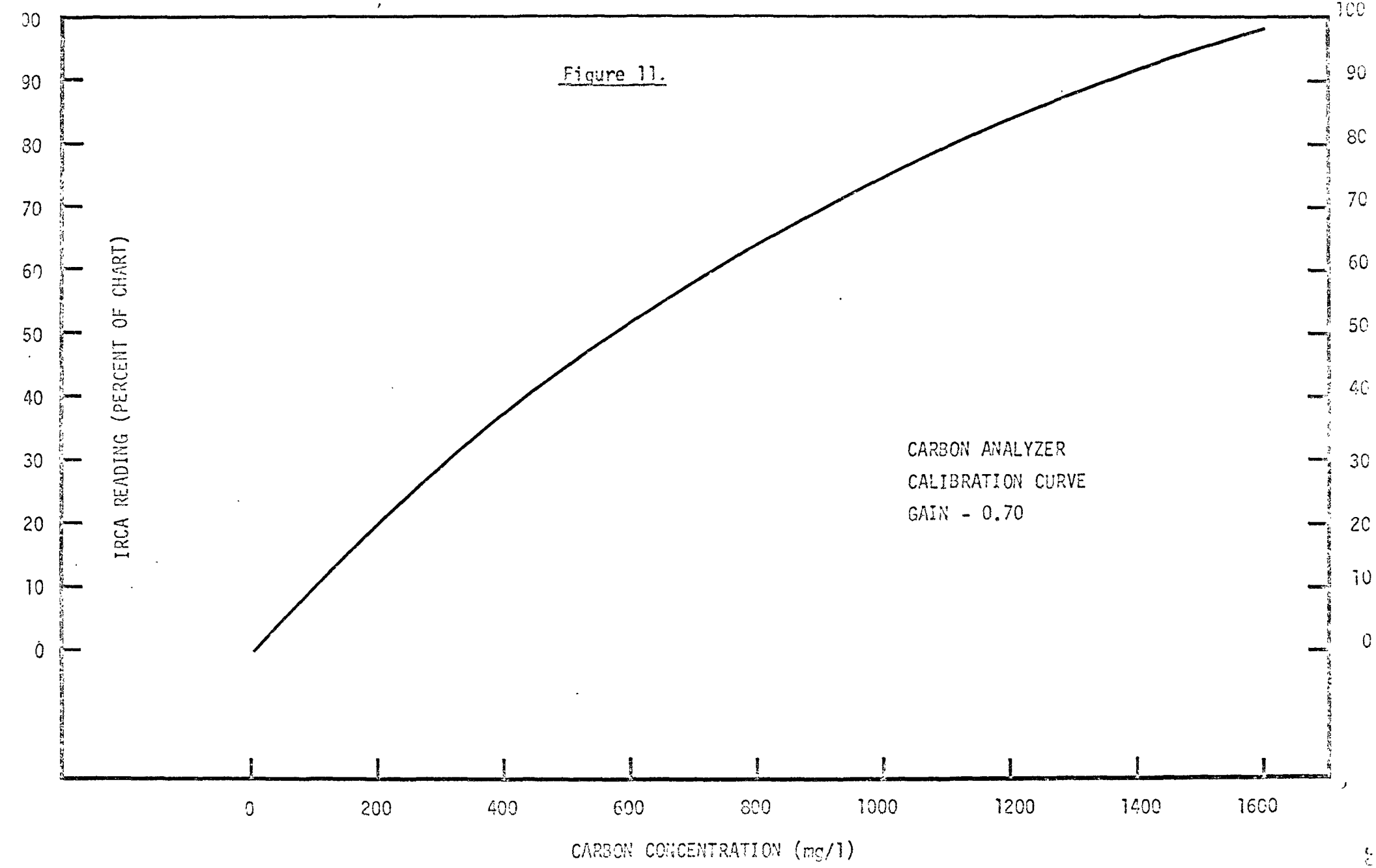
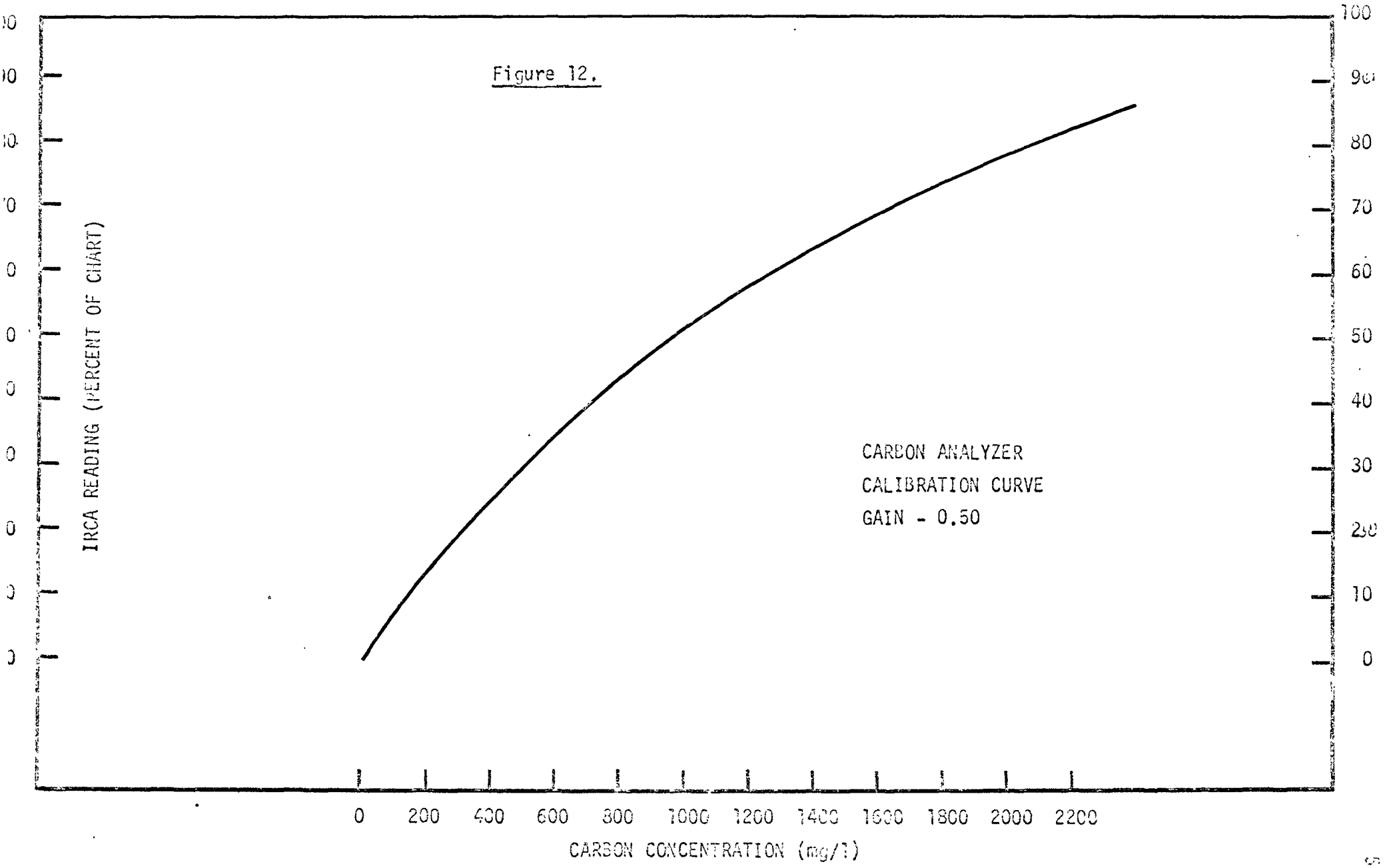

IRCA READING (PERCENT OF CHART)
0
10
20
30
40
50
60
70
80
90
100
CARBON ANALYZER
CALIBRATION CURVE
GAIN - 0.70
0
200
400
600
800
1000
1200
1400
1600
CARBON CONCENTRATION (mg/l)

Figure 12.


IRCA READING (PERCENT OF CHART)
CARBON ANALYZER
CALIBRATION CURVE
GAIN - 0.50
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
CARBON CONCENTRATION (mg/l)