EFFECT OF INITIAL BIOLOGICAL SOLIDS CONCENTRATION ON BIOCHEMICAL RESPONSE IN DISCONTINUOUS ACTIVATED SLUDGE SYSTEMS

By

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CHAPTER I

INTRODUCTION

The importance of the activated sludge process in treating both industrial and domestic wastes is well known in the field of Sanitary Engineering, and various aspects of this process have been studied extensively by several investigators. Waste purification in any biological process involves synthesis and respiration. The synthesis mechanism manufactures the new cells and respiration provides energy for synthesis. This somewhat oversimplified outlook has been accepted, and several investigators represent it schematically as follows:

$$\begin{array}{l} \text{Organic matter} \\ + \\ \text{Oxygen} \\ + \\ \text{Organisms} \end{array} \longrightarrow \begin{array}{l} \text{Synthesis (protoplasm and/or new cells)} \\ \\ \text{Energy + waste products } (CO_2, H_2O, NH_3) \end{array}$$

The BOD loading affects the purification of wastes in any treatment process, and extensive work has been accomplished on this aspect. Using the pure organic compound glucose as a typical carbohydrate, several investigators have studied the effect of feeding increasing amounts of this compound on the efficiency of purification.

Literature Review

Both domestic and industrial wastes can be treated successfully in activated sludge processes. It is not unusual that domestic wastes often contribute carbohydrates. The presence of large amounts of carbohydrates has been cited to be the cause for various operational difficulties at treatment plants. For example, large amounts of carbohydrate wastes are thought to permit growth of the filamentous organism Sphaerotilus and cause trouble in settling tanks which follow the activated sludge aeration tank, i.e., the filaments contribute to sludge bulking. Smith (1)(2) has studied metabolic changes which occur with heterogeneous seed when excessive amounts of glucose are present. He pointed out that large amounts of glucose permit the growth of the filamentous organism Sphaerotilus and make the sludge bulk. Ruchhoft et al (3) isolated the organisms from a bulking sludge and concluded that the major cause of bulking was the presence of Sphaerotilus. Ingols and Heukelekian (4)(5) have made the same observations and concluded that higher amounts of glucose permit the growth of the filamentous organism Sphaerotilus. However, they pointed out when large amounts of mixed liquor suspended solids were maintained in high concentrations of glucose and an abundant nitrogen source was supplied, underaeration might be the probable cause for the predominance of this organism. The predominance of Sphaerotilus has also been attributed to the lack of ability of the Zooglea organisms to compete with Sphaerotilus at higher glucose concentrations. Okun (6) has confirmed the ideas of Smith and Ingols. He observed that extensive growths of Sphaerotilus occurred at low oxygen tensions, although this organism was entirely absent at higher oxygen concentrations under the same conditions of loading. However, he felt that abnormal amounts of glucose concentrations encourage the growth of Sphaerotilus even under high oxygen tensions.

Kraus (7) concluded that the phenomenon of bulking may be caused either by a change in the predominance of the species in the system or a rapid increase in synthesis. Kraus' conclusions are in agreement with McKinney's statements regarding requirements for flocculation (8)(9).

Various investigators have suggested permissible BOD loadings for domestic wastes. Greeley (10)(11) suggested a loading of 30 lb. BOD/day/1000 cft. aeration tank. Logan et al (12) felt that a BOD loading of 0.22 to 0.4 lb. BOD/day/lb. activated sludge is permissible if the Sludge Volume Index (SVI) is to be maintained under 100.

The amount of solids concentration and the length of aeration required will have considerable effect in the design of treatment plants. Several investigators have studied the effect of initial solids concentration on the rate of oxygen utilization. Many of the results (13)(14)(15)(16)(17)(18)(19)(20) are in excellent agreement. In general it has been found that there will be a high initial rate of oxygen utilization which falls off slowly with time. It has also been stated that duration of the high initial rate will depend upon the strength of sewage and type of sludge. Ingols (21) mentioned that the use of high initial solids concentration induces a high rate of oxygen utilization and shortens the period of high rate consumption after the exogenous substrate has been removed.

The quantities of oxygen necessary for any aerobic biological treatment process should be given primary consideration. The conventional activated sludge process generally operates on a 6-to-8 hour detention time, and the quantities of oxygen supplied should be adjusted. Jenks et al (22) have discussed the theoretical rate of the oxygen requirements of the activated sludge process. They have stated that the rate of air supply should be adjusted to the diminishing demand as substrate is being removed. According to their calculations for a 5-hour detention period, 60 percent of the air is required for substrate oxidation in the first hour, 24 percent during the second hour, and 16 percent during the last 3 hours. Ruchhoft et al (23) indicated that the air requirements for a total detention period of 6 hours are as follows: 50 percent of the oxygen required for substrate oxidation during the first 2 hours of aeration period, about 28 percent during the second 2 hours, and 22 percent during the third 2-hour period. These findings permit the possibility of considering the "tapered aeration process" at treatment plants. However, Grant et al (24) have stated that in the presence of large amounts of activated sludge the rate of oxygen utilization would not diminish rapidly; their findings show a linear relationship between the rate of oxygen utilization and activated sludge concentration.

The rate of substrate removal or the activity of a biological sludge has also been studied in terms of its oxygen utilization rate in the absence of nutrients. Ruchhoft et al (23)(25) have stated that if a sludge shows an endogenous respiration rate above 8 mg O_2/hr/gm-sludge during the first hour, it may be treated as having a poor biological activity. According to them a sludge of oxygen utilization rate above 10 mg O_2/hr/gm-sludge during the first hour, measured in the absence of nutrients, was considered a poor sludge obtained as a result of overloading. Logan (12) observed an endogenous respiration rate of 6.0 to 14.0 mg O_2/hr/gm-sludge for a sludge which had been loaded in the range of 0.2 to 0.5 lb. BOD/day/lb-activated sludge. Okun (6) has observed endogenous respiration rates of 10 to 40 ppm/hr/1000 ppm-volatile solids for sludges which were developed at still higher loadings. Sawyer and Nichols (26) have reported that the oxygen utilization rate varies from 1.9 to 9.8 mg O_2/hr/gm-sludge for domestic sewage in 24 hours, without nutrient addition.

Extensive work has been done in regard to the synthesis of cells with carbohydrate wastes. Sawyer (27) reported that 44 to 64 percent carbohydrate substrates were channeled into synthesis. Plack et al (28) reported 65 to 85 percent; calculations from Gaudy's data (29) show 63 percent. In general, several investigators are in good agreement that carbohydrates result in high amounts of sludge. Smith (30) gave an empirical formula to measure the amount of sludge produced as follows:

$$Y = -0.001 + 0.70\ X$$

Where Y = Pounds of sludge produced daily
X = Pounds of 5 day BOD removed daily

The significance of acclimation has been studied extensively by several investigators (28)(31)(32)(33)(34)(35). The increased rate of glucose removal by activated sludge with repeated feedings of glucose was first demonstrated by Smith (1). Englebrecht and McKinney (36) found that there was no significant difference in the efficiency of substrate removal when sludge was acclimatd for 16 to 23 days. Ruchhoft et al (31) have demonstrated that glucose was removed from solution much more rapidly by activated sludge than by domestic sewage. In their further investigation (32) the rapid removal was attributed to the fact that adaptive enzymes are formed. Similarly, other investigators (28)(33)(34) (35) have also attributed the fact of rapid substrate removal by activated sludge to the formation of adaptive enzymes.

The other important factors that affect the efficiency of removal of any waste in any aerobic biological treatment process are: dissolved oxygen (DO), pH, temperature, sludge age, and volatile matter.

Hicks et al (37) have shown that the oxygen utilization was not affected when dissolved oxygen in the activated sludge mixed liquor was maintained between 1 to 6 ppm. Smith (38) found that there was no effect on the oxygen utilization rate at dissolved oxygen concentrations of 0.2 to 0.6 mg/l. Gaudy et al (39) concluded that "the values of oxygen tension which affect metabolic rate lie below 0.5 mg/l of dissolved oxygen." Okun (40) reported that differences up to 13 ppm of dissolved oxygen in a range of 5 to 25 ppm do not have a significant effect on the oxygen utilization rate. However, he pointed out that "the very nature of the activated sludge process will not permit eliminating the period of zero DO for the biological floc. Nor is it yet possible to permit this period from occurring when maximum food is available." Gaudy et al (39) have also observed low DO values at the beginning of aeration for short-term experiments, and gradual increase of DO with time.

Several investigators have studied the effect of pH, and all are in general agreement that 6.5 to 7.5 is the optimum pH for aerobic biological treatment processes (21)(41)(42). However, Gehm (43) reported that Kraft pulping wastes can be treated by the activated sludge process with a pH as high as 9.8.

Extensive study has been made on the effect of temperature on the efficiency of aerobic treatment processes, and several investigators (44)(45)(46) concluded that 20 to 25°C. is an optimum temperature for an economical and efficient design.

The percentage of volatile matter of the sludge has been used as an indication of efficiency of waste treatment in aerobic biological treatment processes (47)(48)(49). However, it has been realized that volatile matter is a poor index of biochemical activity of microorganisms (50).

Another factor which warrants consideration is sludge age. Several definitions have been given for this term in the literature. Gellman et al (51) defined it as the ratio between the final volatile sludge concentration at the end of one-day aeration and the volatile solids produced during the same period. They have shown that there was no significant effect on the purification or oxidation of a waste for 1.5 to 4.1 days of sludge age using their definition. Wilbur and Chasick (52) defined sludge age as the weight of the dry suspended solids in the aeration tank divided by the weight of the dry suspended solids in the sewage or primary effluent added daily.

Above all, the nutrient requirements should be given primary consideration for successful operation of any aerobic biological treatment process. Extensive work has been reported in the literature on this aspect. Sawyer (28) suggested an optimum BOD to N ratio of 17 to 1, and BOD to P ratio of 90 to 1. This is in general accordance with the findings of other investigators.

Purpose and Scope of the Present Investigation

This study was conducted for the following purposes: (1) to determine the amount of mixed liquor suspended solids present at the beginning and end of aeration in a 15 liter batch activated sludge unit fed on a 24 hour cycle, (2) to gain a better understanding of the substrate removal capacity of the sludge under prolonged aeration, (3) to establish a relationship between initial biological solids concentration and rate of substrate removal, (4) to examine the possibility of release of intermediates for various initial biological solids concentrations, and (5) to study the partition between synthesis and respiration for various initial biological solids concentrations.

Fragmentary data on some of these aspects are available in the literature; however, an integrated study of the magnitude and scope herein reported has not previously been made.

CHAPTER II

MATERIALS AND METHODS

Activated Sludge Batch Control Unit

In order to obtain sludge for studying the kinetic relationship between synthesis and respiration, a batch activated sludge unit was maintained using glucose at 5000 mg/l as the sole source of carbon. A 20 liter aeration vessel was designed to maintain 15 liters of mixed liquor under vigorous conditions of aeration (see Fig. 1).

A sewage seed was obtained from the primary effluent of the Stillwater municipal sewage treatment plant. The constituent chemicals of the standard synthetic waste used in the study are shown in Table I.

TABLE I

Constituent	Concentration	
Glucose	5000	mg/l
$(NH_4)_2\ SO_4$	2500	"
Mg $SO_4 \cdot 7H_2O$	500	"
$Fecl_3 \cdot 6H_2O$	2.5	"
$MmSO_4 \cdot H_2O$	50	"
$CaCl_2 \cdot 2H_2O$	37.5	"
Trace elements (tap water)	100	ml/l
Phosphate buffer pH 7.0	60	ml/l
KH_2PO_4 - 52.7 g/l) pH 6.8		
K_2HPO_4 -107 g/l)		

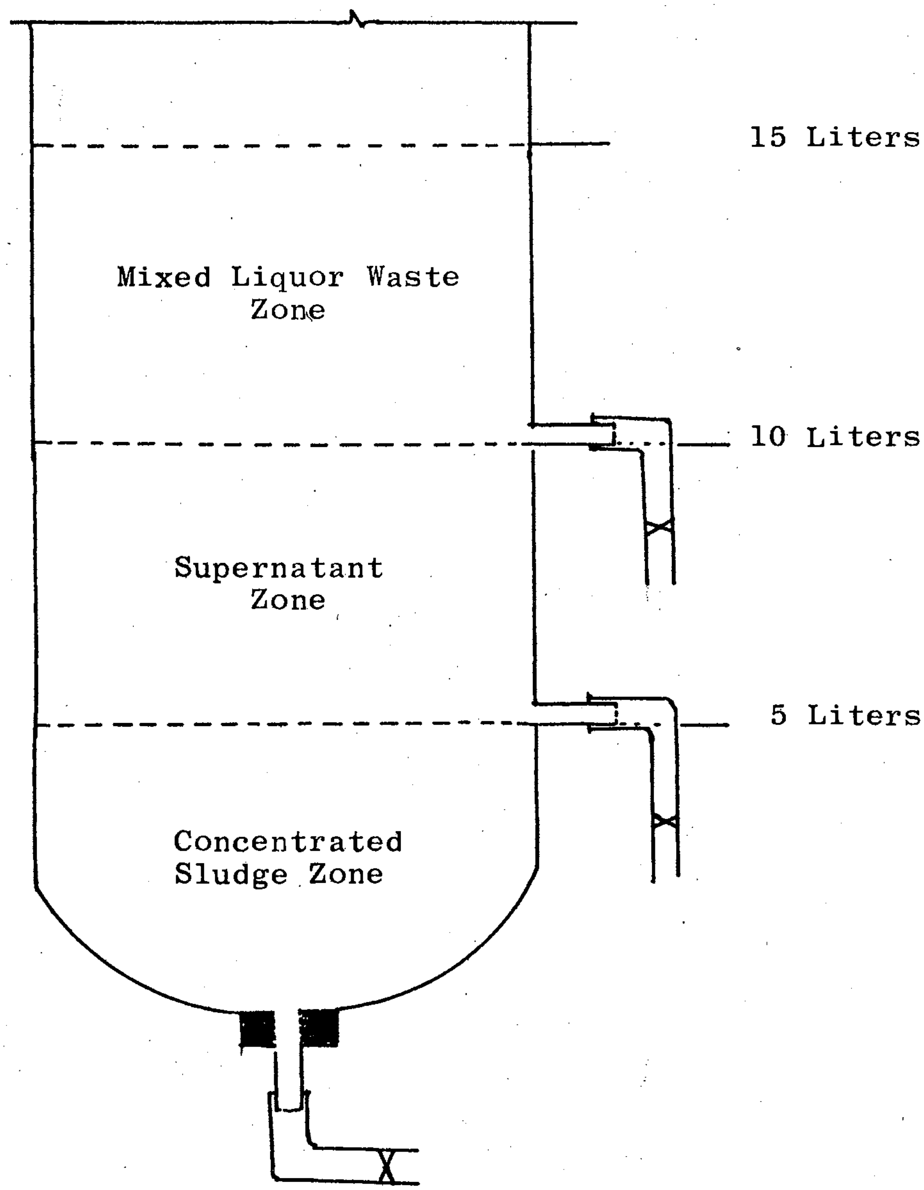

Fig. 1 15 Liter Activated Sludge Batch Unit

The system was maintained as follows: daily 15 liters of mixed liquor were aerated for 23 hours; then 5 liters were wasted. The remaining portion was allowed to settle for 1 hour and 5 liters of supernatant were wasted. The volume was then made up to 15 liters with distilled water and the synthetic waste constituents to yield standard feed concentrations shown in Table I.

Analytical Techniques

I Substrate Determination

(a) Chemical Oxygen Demand (COD)
(b) Anthrone (Total Carbohydrate)

II Biological Solids

(a) Optical Density
(b) Membrane Filter Technique

III Settling Test

(a) Sludge Volume Index (SVI)
(b) Sludge Volume Index and Settleability using diluted sludge

IV Oxygen Utilization (Warburg Technique)

I Substrate Determination

(a) Chemical Oxygen Demand (COD) Test

Even though the biochemical oxygen demand (BOD) test is generally employed to measure organic pollution, the present trend in the pollution control field is to correlate these results to the COD test because of the ease and time-saving aspect of the test. Also, in recent years the value of the BOD test has been subjected to criticism because of discontinuity in the kinetic expression of carbonaceous oxygen demand, This aspect is currently under investigation in the Bio-Engineering laboratories of Oklahoma State University (53)(54)(55)(56)(57). Until more information concerning the kinetics of the BOD test becomes known it would seem best to follow the technical philosophy expressed by Gaudy (58) that the BOD test should be the subject of research rather than the means. For these reasons the COD test was chosen as the major parameter for measuring total substrate removal. In the present study the COD technique was used to measure substrate remaining in solution after passing the mixed liquor through a membrane filter. Detailed procedure for running the COD test is given in Standard Methods (59).

(b) Anthrone (Total Carbohydrate)

Carbohydrate remaining in solution was also measured by the anthrone test using the procedures given by Gaudy (58). This test for total carbohydrate was run in order to obtain information on the possible release of metabolic intermediates during the purification of the synthetic waste. This was accomplished by comparing the results of COD and anthrone analysis on identical samples.

II Biological Solids

(a) Optical Density

To facilitate the estimation of the desired initial biological solids concentration, a calibration curve of biological suspended solids vs optical density (OD) was made. The results are shown in Fig. 2. Glucose-acclimated cells were harvested and washed twice with 0.05 M phosphate buffer solution. The

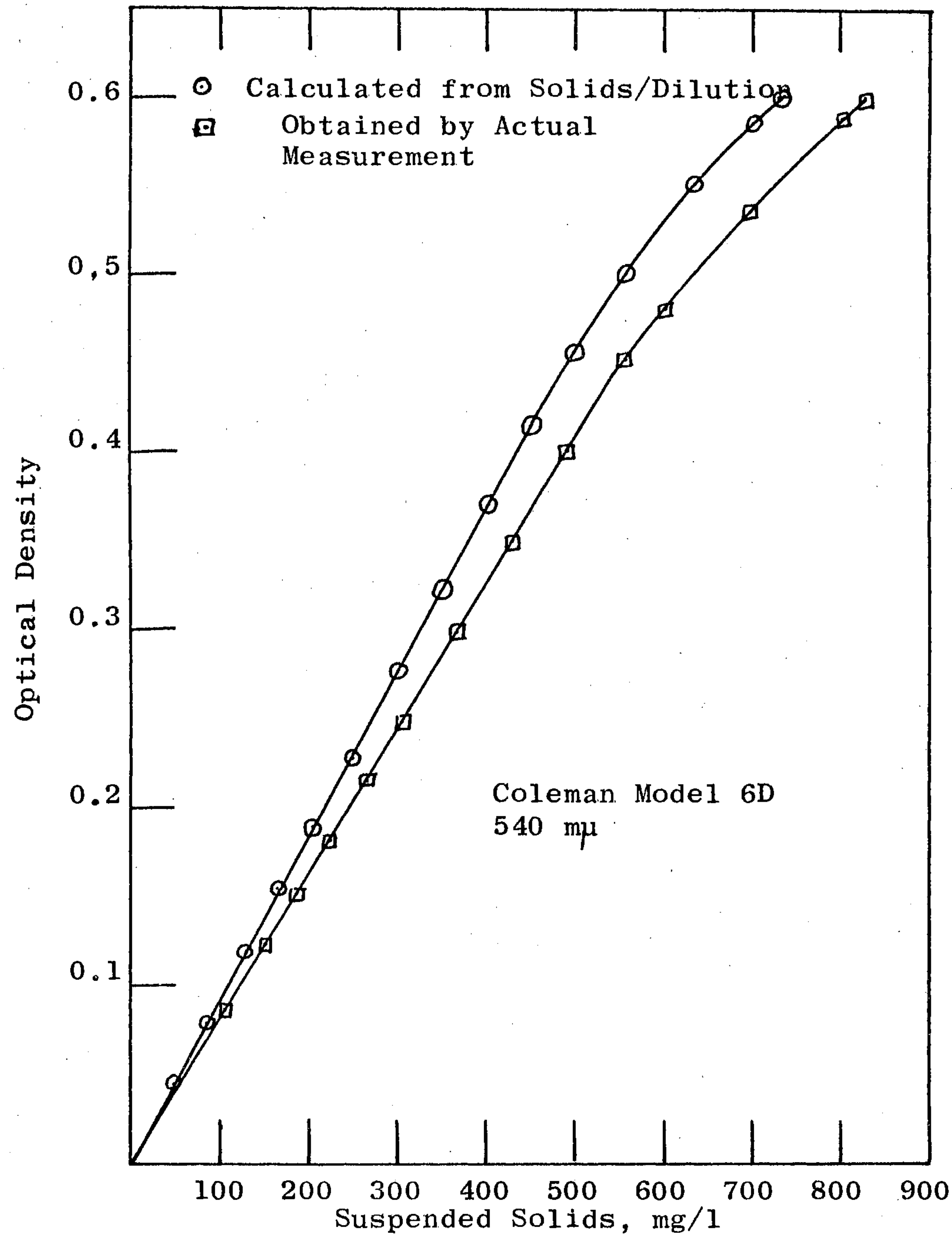

Fig. 2 SUSPENDED SOLIDS vs OD FOR HETEROGENEOUS SEED

washed cells were suspended in distilled water and the amount of biological solids concentration of this suspension was measured by the membrane filter technique (59). The suspension was diluted to obtain various solids concentrations and the optical densities were measured at 540 mμ on the Coleman Colorimeter, Model 6D. Knowing the initial biological solids concentration at zero dilution, the concentration of biological solids at other dilutions was computed. The biological solids concentrations at different optical densities were also measured at various times to check the accuracy of the dilution technique. Both the curves are shown in Fig. 2.

(b) Membrane Filter Technique

The substrate assimilated or the synthesis of biological solids was determined by direct measurement of biological solids using the membrane filter technique as outlined in Standard Methods (59).

III Settling Test

(a) Sludge Volume Index (SVI)

A 1000 ml volumetric cylinder was used to measure the sludge volume index, and sludge was allowed to settle for 30 minutes. This test is often used as an index of settleability of biological sludge. The test procedure and calculations were made in accordance with Standard Methods (59).

(b) Sludge Volume Index and Settleability using Diluted Sludge

Since high solids concentrations may prevent settling, the sludge was diluted. The sludge volume index and sludge settling rates at various solids concentrations were determined by measuring the subsidence of the sludge zone in a 1000 ml graduated cylinder at various time intervals.

IV Oxygen Utilization Rate (Warburg Technique)

Oxygen uptake was measured on a Warburg respirometer using 40 ml of reaction fluid and 1.5 ml KOH(20%) in the center well. The system was maintained at 25°C. using a shaker rate of 104 osc/min. A ten-minute equilibration period was allowed before the manometers were closed. In general readings were taken at 10 or 15 minute intervals during the period of rapid oxygen uptake, and at half-hour time intervals during the remainder of the experimental period. The detailed techniques and calculations used were those given in Standard Methods and Manometric Techniques (59)(60).

Experimental Protocol

1. Sludge Growth and Substrate Removal Studies in the Batch Unit

In order to determine the biological solids concentration in the unit, samples were taken on alternate days before the start of an aeration cycle and at the end of the aeration cycle. The filtrate was used to determine the initial amount of substrate present and the amount of substrate removed at the end of aeration (23 hours) by the COD technique. In addition to these studies, samples were taken in the batch unit during the period of rapid removal of substrate to determine the rate of removal and the maximum amount of sludge produced. Such studies were made at intervals of 8 to 10 days. From these studies the sludge yield in the batch unit was calculated as follows:

$$\text{Sludge Yield} = \frac{\text{Maximum amount of sludge produced}}{\text{Total amount of substrate removed at the time of maximum sludge production}}$$

2. <u>Partition between Synthesis and Respiration under Batch Operation for Various Initial Biological Solids Concentrations</u>

The sludge for these experiments was obtained from the batch unit. Cells were harvested and washed twice with 0.05 M phosphate buffer solution. The soluble organic feed used in all these studies was glucose at an initial concentration of 2000 mg/l; the inorganic salts used were the same as those given in Table I; however, the concentration was adjusted by the ratio 2:5. An aeration vessel of 2.5 liters capacity was used; aeration was maintained at approximately 1200 ml/min/l by means of corborundum diffusing stones. The aeration liquor was made up to 1.5 liters with the necessary inorganic salt solutions and washed sludge. The desired initial solids concentrations were estimated from the optical density-biological solids concentration calibration curve (Fig. 2), and the actual amount of solids concentration present was measured by the membrane filter technique. Before feeding the carbon source 40 ml of cell suspension was placed in a calibrated Warburg flask to measure endogenous respiration. The carbon source was then added to the remaining aeration liquor to give a final concentration of approximately 2000 mg/l. Forty ml of this feed suspension were placed in a calibrated Warburg flask to measure respiration during substrate removal. Another 40 ml sample was taken to measure the actual initial biological solids concentration in the mixed liquor, and the filtrate was used to determine the initial substrate concentration by the COD technique. The percent theoretical oxygen demand was calculated as the accumulated oxygen uptake at the time of substrate removal divided by the initial amount of substrate multiplied by 100. Thus, synthesis and respiration studies were made simultaneously. With the higher initial solids concentrations samples were taken at intervals of 15 to 30 minutes to determine biological solids and substrate remaining during the active period of substrate removal. In the case of low initial biological solids concentrations, samples were taken at intervals of 1 to 1.5 hours during the active period of substrate removal. Filtrate samples were saved to determine the amount of carbohydrates present by the anthrone test.

3. Energy and Material Balances

To check the experimental techniques, two separate experiments were made. The techniques and other parameters used were the same as those described above, except that the anthrone determination was not made but the COD of the cells was determined. The techniques employed were those embodied in an energy balance method which has been recently validated by Gaudy, Bhatla and Gaudy (61). In this method the actual COD of cells in each sample is measured. In addition to the analysis proposed by Gaudy, Bhatla and Gaudy, the actual COD of the mixed liquor was determined each time a sample was taken. In addition to these separate energy balance experiments material balances were made for all experiments using the substrate balance technique described by Gaudy and Engelbrecht(62).

CHAPTER III

RESULTS

Operational Characteristics of the 15 Liter Batch Activated Sludge Control Unit

The concentration of biological solids present at the beginning and end of aeration is shown in Fig. 3. Biological solids concentration varied from 4500 to 5300 mg/l at the beginning of aeration, and the glucose concentration as measured by COD varied from 5000 to 5600 mg/l. The biological solids concentration varied from 6500 to 8300 mg/l at the end of aeration; however, it should be noted that the maximum sludge growth was greater than this concentration, as will be seen later in this chapter. The COD removal data indicates that 92 to 98 percent efficiency of treatment was achieved. The pH was maintained between 6.6 to 6.8. The temperature in the unit varied between 22 to 24^{o}C. No attempt was made to measure the air supplied to the biological population; however, it should be noted that sufficient air was supplied to maintain aerobic conditions. Anaerobic odors were never observed in the batch unit.

Sludge growth and Substrate Removal Studies in the 15 Liter Batch Activated Sludge Control Unit

The results of studies made to determine the rate of sludge

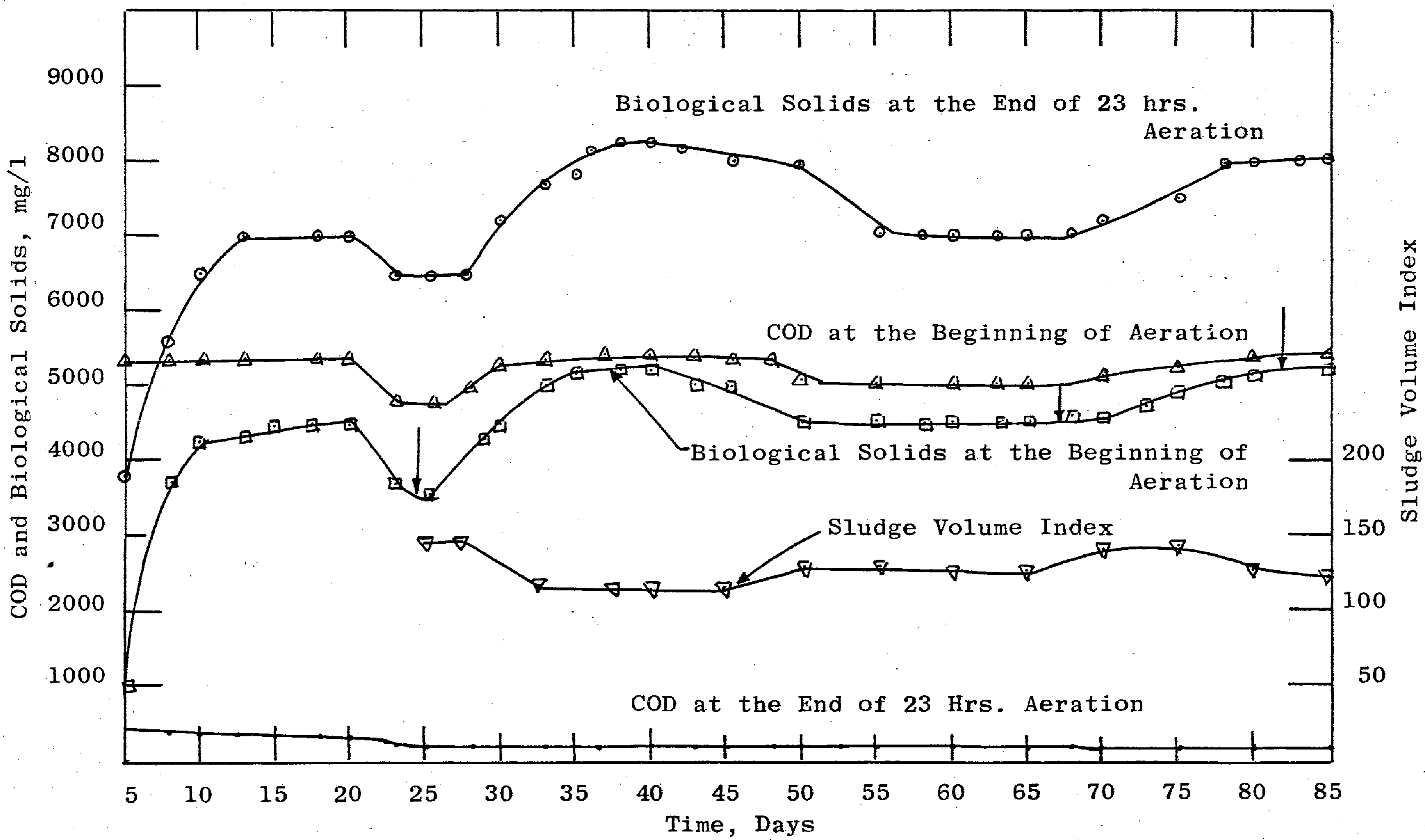

Fig. 3 OPERATIONAL CHARACTERISTICS - FIFTEEN LITER BATCH ACTIVATED SLUDGE CONTROL UNIT

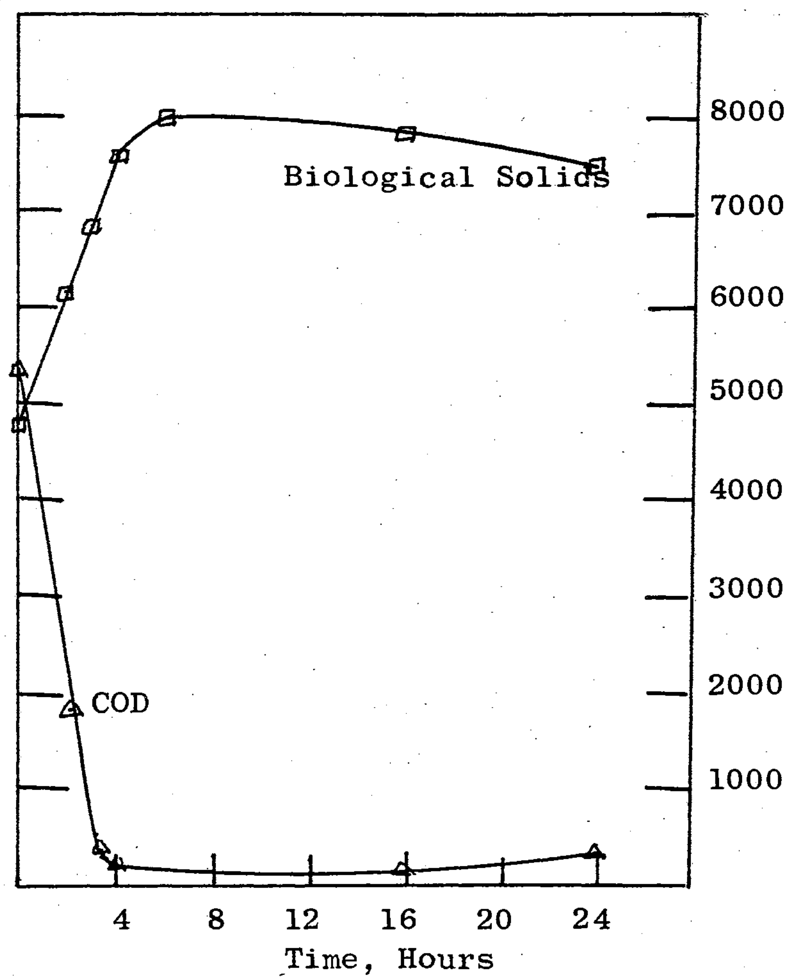

Fig. 4 SLUDGE GROWTH AND COD REMOVAL STUDY ON THE 15 LITER BATCH UNIT AFTER 15 DAYS OF OPERATION

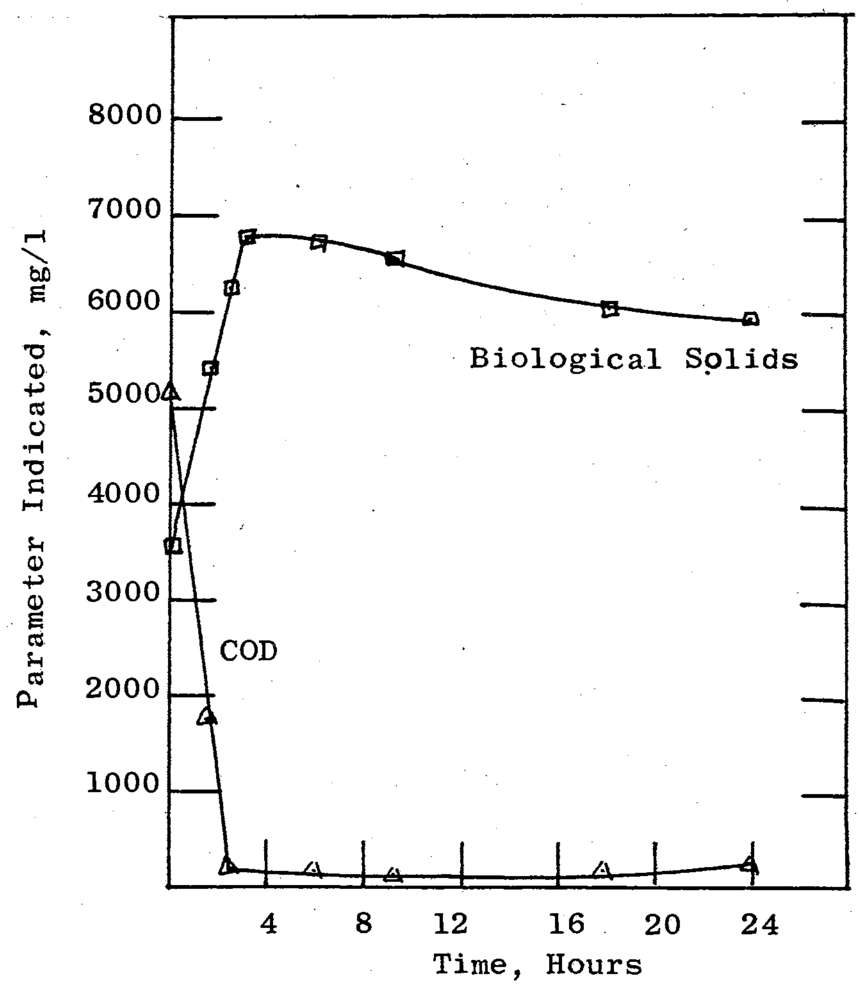

Fig. 5 SLUDGE GROWTH AND COD REMOVAL STUDY ON THE 15 LITER BATCH UNIT AFTER 25 DAYS OF OPERATION

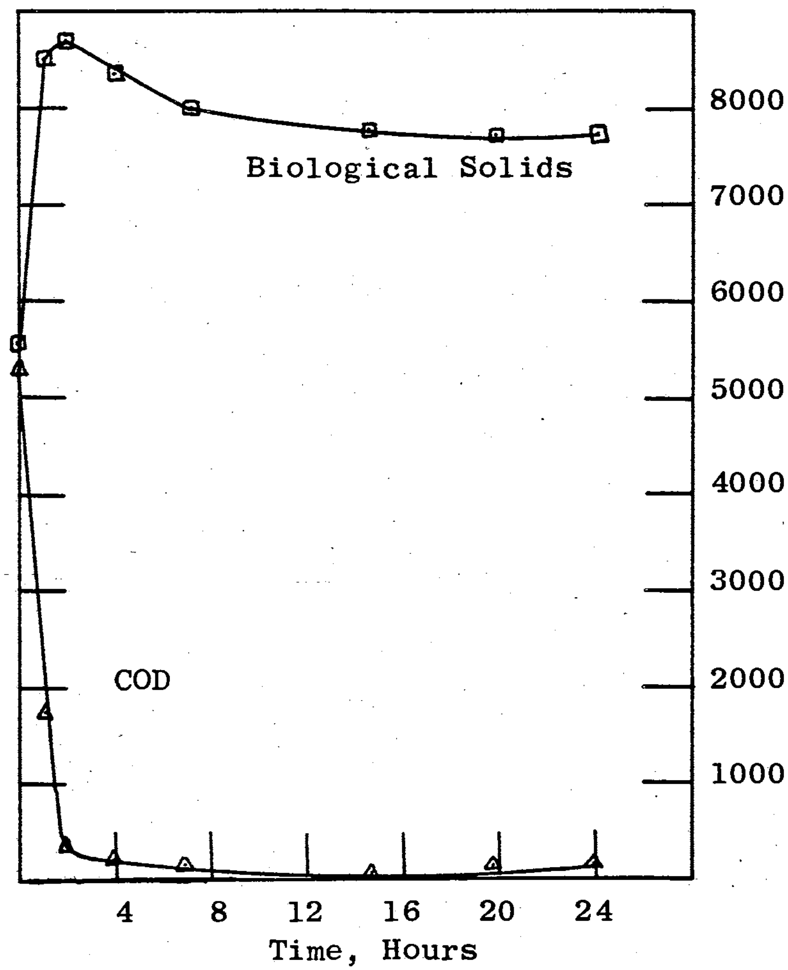

Fig. 6 SLUDGE GROWTH AND COD REMOVAL
STUDY ON THE 15 LITER BATCH UNIT
AFTER 37 DAYS OF OPERATION

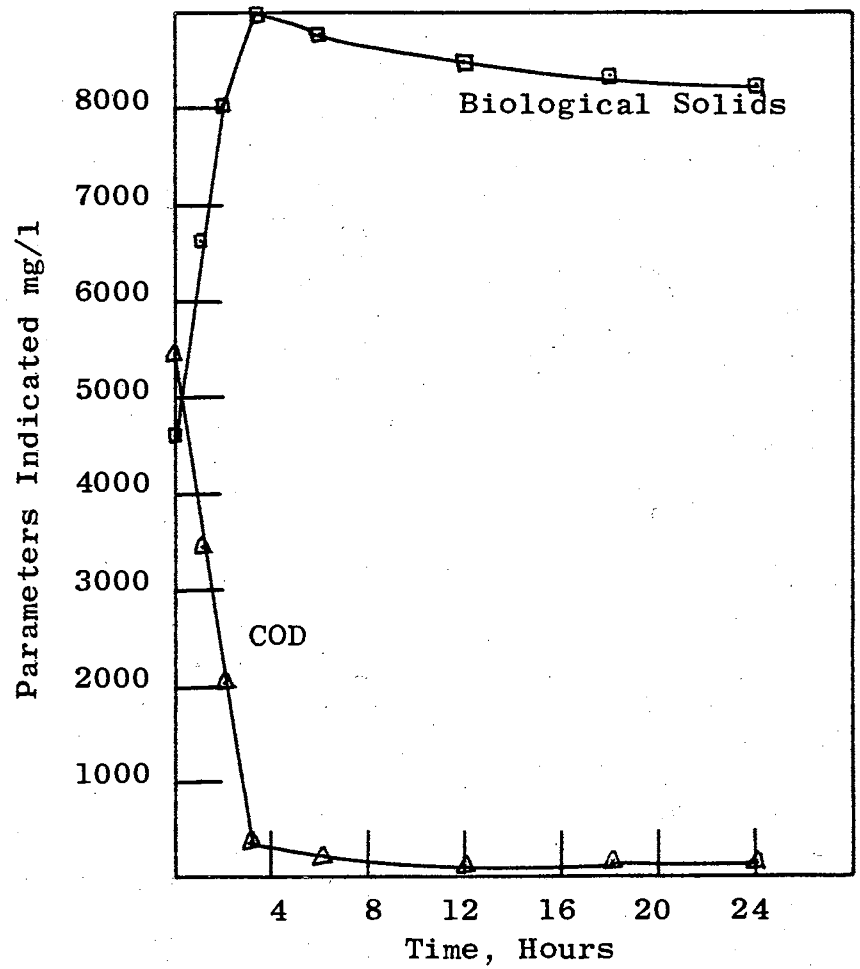

Fig. 7 SLUDGE GROWTH AND COD REMOVAL
STUDY ON THE 15 LITER BATCH UNIT
AFTER 50 DAYS OF OPERATION

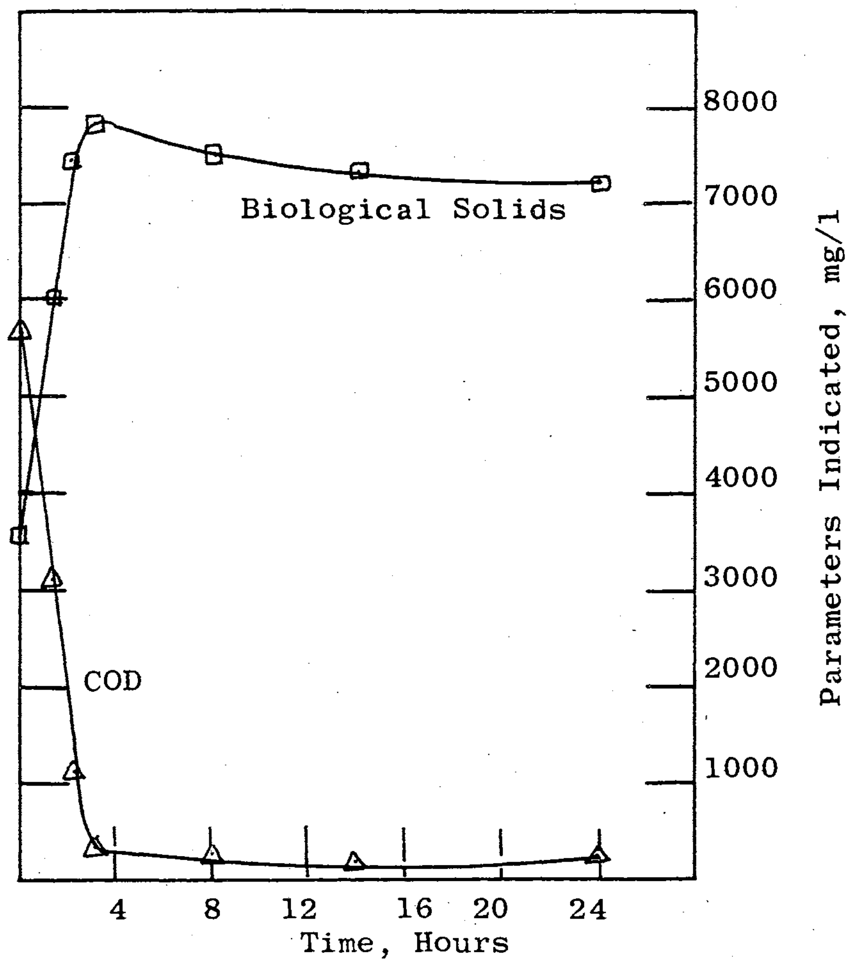

Fig. 8 SLUDGE GROWTH AND COD REMOVAL STUDY ON THE 15 LITER BATCH UNIT AFTER 64 DAYS OF OPERATION

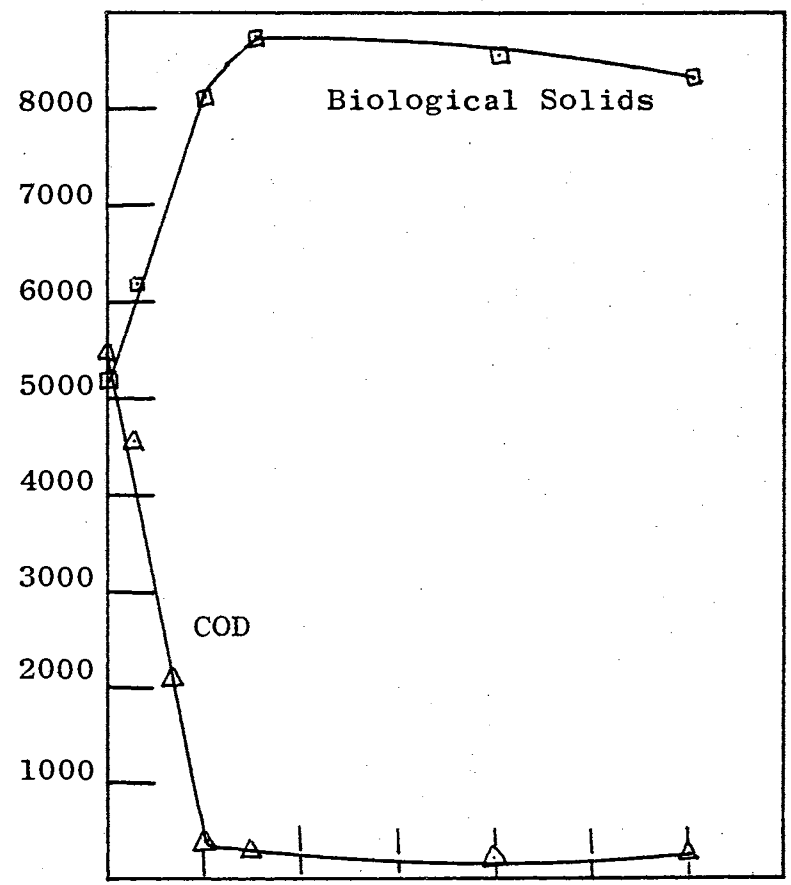

Fig. 9 SLUDGE GROWTH AND COD REMOVAL STUDY ON THE 15 LITER BATCH UNIT AFTER 75 DAYS OF OPERATION

Fig. 10 Microphotograph of the Sludge after 39 Days Operation of the Batch Unit (X430)

Fig. 11 Microphotograph of the Sludge after 50 Days Operation of the Batch Unit (X430)

production and substrate removal in the control unit are shown in Figures 4 through 9. The results shown in Fig. 4 were obtained at 15 days from the start of the control unit. Ninety-eight percent of COD removal was achieved in 4 hours, and sludge yield of 63 percent was observed. The results shown in Fig. 5 were obtained 25 days from the start of the control unit. At this time the sludge showed poor settling properties, and the low concentration of biological solids present at this time can be seen in Fig. 3. A 95 percent COD removal was achieved in 2.5 hours, and a sludge yield of 80 percent was observed. The results shown in Fig. 6 were obtained 39 days from the start of the control unit. At this time the sludge changed from its normal brown appearance and became slightly dark in color. Microphotographs representative of the general sludge appearance were taken, and it can be seen from Fig. 10 that large floc particles together with protozoa, bacteria and fungi were present. A 95 percent COD removal was achieved in 2.5 hours, and an 80 percent sludge yield was observed. The results shown in Figures 7 and 8 were obtained 50 to 64 days from the start of the control unit. In both cases 96 percent substrate removal was achieved in 3.5 hours, and sludge yields of 80 and 85 percent were observed. During this period the sludge changed completely from its normal brown appearance to a deep black color. Microphotographs of the sludge were taken at this time. It can be seen from the microphotograph (Fig. 11) that chain-forms of microorganisms were predominating; however, these forms

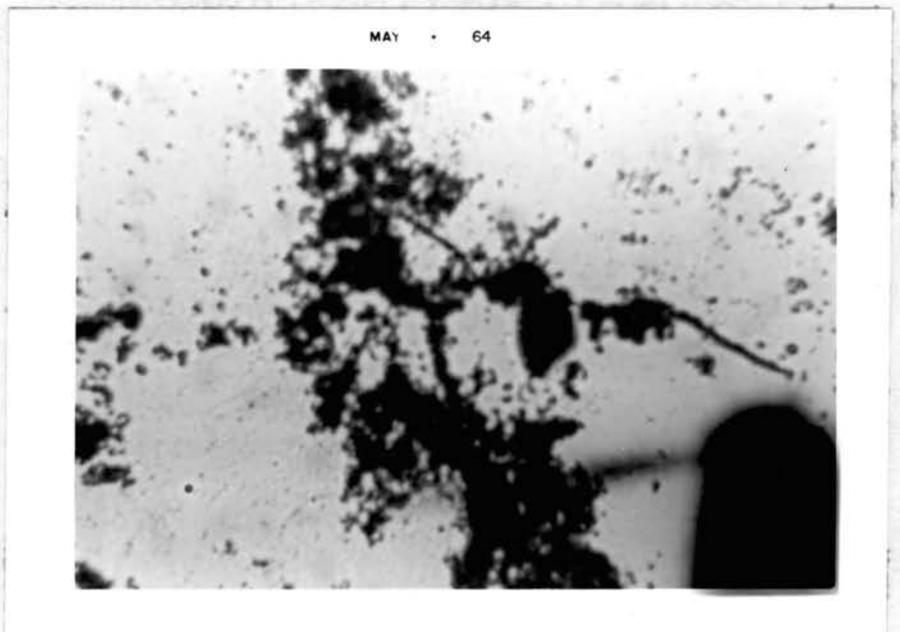

Fig. 12 Microphotograph of the Sludge after 70 Days Operation of the Batch Unit (X430)

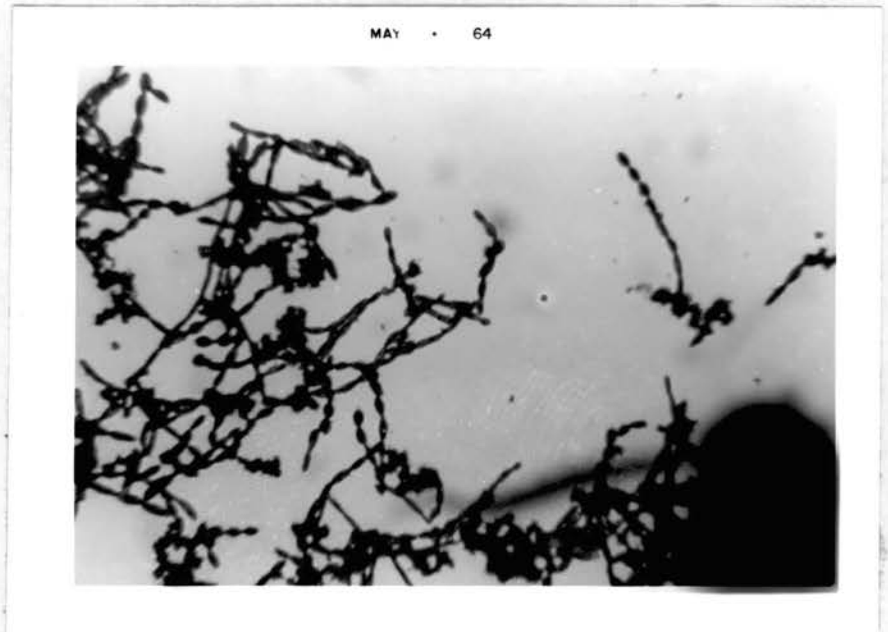

Fig. 13 Microphotograph of the Sludge after 80 Days Operation of the Batch Unit (X430)

do not correspond with descriptions of Sphaerotilus, which generally predominates at higher concentrations of carbohydrate wastes. At 70 days from the start of the control unit the sludge had regained its normal brown appearance, and microphotographs taken at this time (see Fig. 12) showed that large floc particles together with protozoa, bacteria and other filamentous organisms were predominating. This is the normal appearance of activated sludge. At 75 days from the start of the control unit a sludge growth and substrate removal study was made, and the results are shown in Fig. 9. Ninety-three percent substrate removal was achieved in 4 hours, and a sludge yield of 72 percent was observed. At 80 days from the start of the control unit the sludge returned to the dark appearance, and microscopic examination (Fig. 13) indicated the predominance of chain-forms of microorganisms similar in appearance to those shown in Fig. 11. It is interesting to note that cyclic changes in predominating species of microorganisms appear to be unavoidable, even though operational conditions are maintained precisely constant.

Sludge Volume Index (SVI) Studies on the 15 Liter Activated Sludge Control Unit

The sludge volume index was determined at various times during the present investigation; the results are shown in Fig.3. In general whenever good settling occurred in the batch unit a SVI of approximately 120 was observed, and when sludge settled poorly a SVI of approximately 150 was observed.

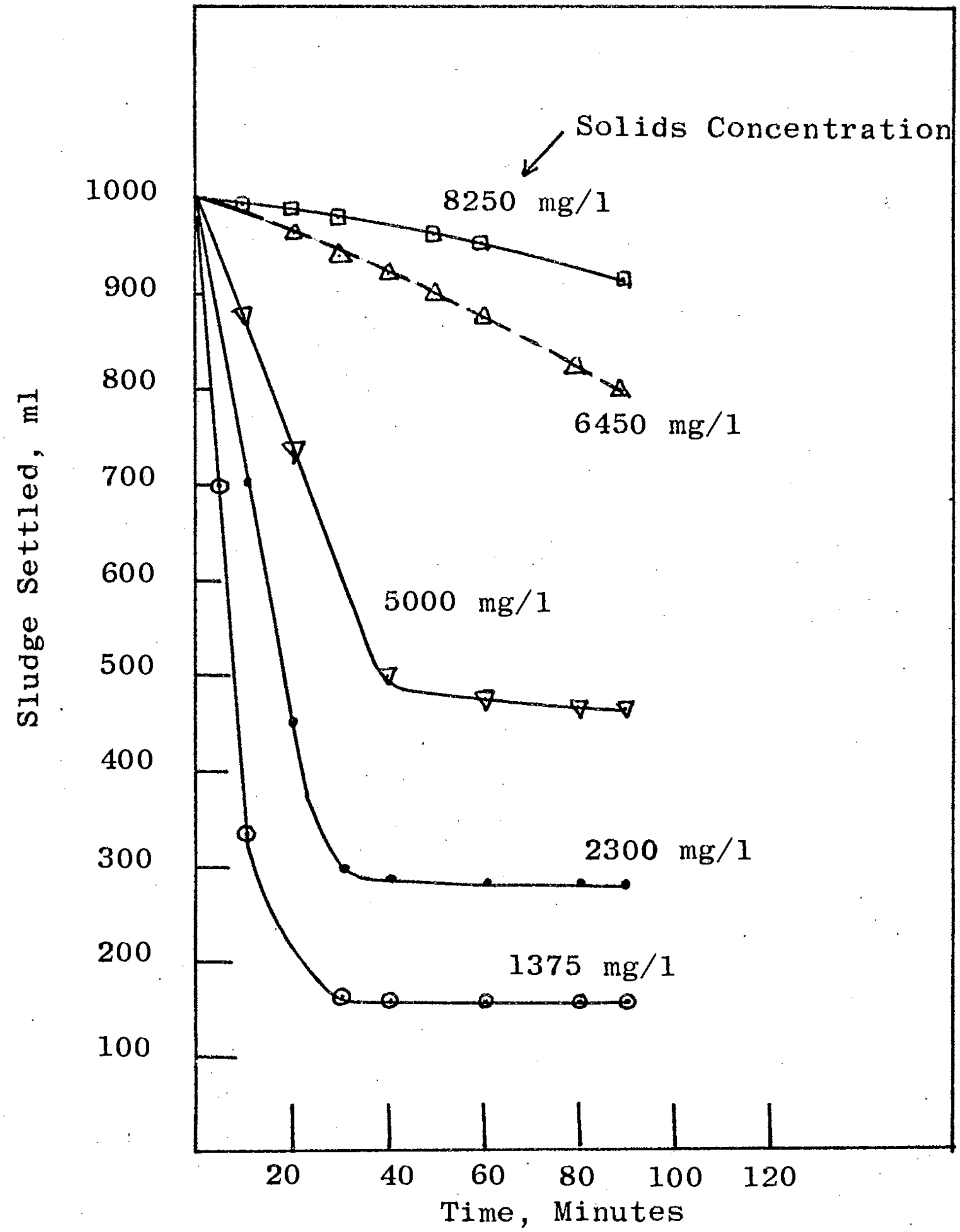

Fig. 14 SETTLING CURVES FOR VARIOUS SOLIDS CONCENTRATIONS, mg/l (Test performed in a 1000 ml Graduated Cylinder)

Sludge Volume Index and Settleability using Diluted Sludge

At various times throughout the study settling tests were made using sludge from the control unit. Some of these were run at times when excellent settleability was observed in the batch unit; others were made when the sludge showed poor settling characteristics. Typical settling curves are shown in Fig. 14. From this figure it can be seen that only 50 ml of settling occurred in the one liter graduated cylinder in an hour when a solids concentration of 8250 mg/l was used. However, at this time the settleability of the sludge in the batch unit was excellent. It was felt that high concentration of sludge was the probable cause for poor settling in the graduated cylinder; accordingly the sludge was diluted to give solids concentrations of 5000, 2300, and 1375 mg/l, and settling tests were made. Results for these dilute concentrations are also shown in Fig. 14. It can be seen that diluting the sludge enhanced its settleability. In all cases the same sludge volume index was observed.

The dotted line settling curve shown in Fig. 14 was obtained at a time when poor settling was observed in the batch unit. From these data it can be seen that the standard procedure for obtaining sludge volume index (SVI =

$$\frac{\text{ml settled sludge in 30 minutes}}{\text{mg/l suspended matter}} \times 1000)$$

can yield erroneous results when the test is interpreted as an index of settleability. This aspect is discussed in the following section (see Discussion).

Partition between Synthesis and Respiration under Batch Operation for Various Initial Biological Solids Concentrations

Eighteen experiments were performed on sludge harvested from the activated sludge control unit in order to gain an insight into the partition of substrate between synthesis and respiration at various initial biological solids concentrations. Pertinent data for all experiments are summarized in Tables IIA and IIB. The experiments are listed chronologically, and are arranged in groups according to studies made using identical sludges. All columns of data are identified and explained in the key accompanying each Table. The data for Experiments 19 and 20 were obtained by Krishnan (63).

After 18 days of operation of the batch unit Experiments 1, 2 and 3 (Group I) were made with initial solids concentrations of 104, 316 and 470 mg/l. The changes in system parameters observed during substrate removal are shown in Figures 15, 16 and 17. The percent theoretical oxygen demand exerted at 97 and 98 percent substrate removal was 29 and 30 for initial biological solids concentrations of 316 and 470 mg/l respectively, whereas 31 percent theoretical oxygen demand was exerted at 60 percent substrate removal at the termination of the experiment with an initial biological solids concentration of 104 mg/l. The percent theoretical oxygen demand exerted at the point of glucose (COD glucose) removal was 31, 26 and 20 for initial biological solids concentrations of 104, 316 and 470 mg/l. The cell yield was calculated as the maximum amount of biological

TABLE II A

SUMMARY OF THE EXPERIMENTS ON PARTITION BETWEEN SYNTHESIS AND RESPIRATION STUDIES

1	2	3	4	5	6	7
1	10/26/63	I	104	2100	30 at 61	31
2	10/26/63	I	316	2000	30 at 97	25
3	10/26/63	I	470	2140	29 at 98	21
4	11/ 1/63	II	410	2020	24 at 96	18
5	11/ 1/63	II	1100	1900	18 at 97	10
6	11/ 1/63	II	1200	1800	16 at 99	10
7	11/13/63	III	435	2020	28 at 95	24
8	11/13/63	III	926	1920	28 at 98	23
9	11/13/63	III	2050	1880	15 at 98	8
10	11/19/63	IV	152	2274	31 at 97	28
11	11/19/63	IV	515	2214	30 at 98	22
12	11/22/63	V	700	2010	27 at 79	26
13	11/22/63	V	1200	1960	20 at 85	20
14	11/29/63	VI	375	2220	27 at 63	
15	11/29/63	VI	750	2070	29 at 98	26
16	11/29/63	VI	1450	1990	20 at 97	17
17	12/11/63	VII	580	1800	22 at 76	21
18	12/11/63	VII	1200	1760	20 at 98	14
19	12/13/63		1967	1760	12 at 94	9
20	12/13/63		1532	1760		

KEY

Column No.	Description
1	Experiment No.
2	Date
3	Group of the Experiment
4	Initial Biological Solids Concentration, mg/l
5	Initial COD, mg/l
6	Percent Theoretical Oxygen Demand at Indicated percent COD Removal
7	Percent Theoretical Oxygen Demand Exerted at Glucose Removal

TABLE II B

SUMMARY OF THE EXPERIMENTS ON PARTITION BETWEEN SYNTHESIS AND RESPIRATION STUDIES

1	8	9	10	11	12	13	14
1	81	5.3	3.90	18.0	90	3.25	-
2	55	12.5	4.64	7.6	125		39.6
3	53	16.0	8.30	8.9	180		38.6
4	59	22.0	8.35	10.2	220		53.5
5	62	45.0	19.60	8.9	410		37.2
6	68	48.0	22.40	9.5	520		43.3
7	75	9.2	9.00	10.3	170		39.6
8	84	19.3	24.00	13.0	310		30.0
9	68	60.0	30.00	7.3	660		32.2
10	61	9.0	3.27	10.7	120	3.50	-
11	67	12.0	10.00	9.7	170		33.0
12	88	10.0	23.40	16.7	190		27.2
13	72	20.0	37.50	12.5	280		23.3
14	80	6.0	14.60	18.9	105	6.50	-
15	84	15.0	23.30	15.5	180		24.0
16	76	21.5	43.80	15.5	344		23.7
17	73	16.0	9.38	8.1	190		32.8
18	69	33.0	19.80	8.2	410		34.2
19	66	57.0	32.00	8.2	-		-
20	66	42.5	-	-	-		-

KEY

Column No.	Description
1	Experiment No.
8	Cell Yields, Percent
9	Percent COD Removal per Hour during the Linear Phase of Substrate Removal
10	Endogenous O_2 Uptake, mg/l, during the First Two Hours
11	Endogenous O_2 Uptake, mgO_2/hr/gm-Sludge
12	Solids Increase, mg/l per Hour
13	Biological Solids Doubling Time, Hrs.
14	Solids Increase per Hour (percentage)

solids produced divided by the amount of substrate utilized to produce the maximum cell concentration. Whenever experiments terminated without attaining a peak in sludge concentration, cell yields were calculated during the linear phase of substrate removal as the cell growth during the linear phase of substrate removal divided by the substrate utilized to produce cells at that time. The sludge yield was found to be 53 and 55 percent for initial solids concentrations of 470 and 316 mg/l, and unit activity of the sludge in both cases was 8 mg O_2/hr/gm-sludge. The cell yield was observed to be 81 percent for initial solids concentration of 104 mg/l; however, unit activity of the sludge for this solids concentration was 18 mg O_2/hr/gm-sludge. Thus, the high sludge yield for this solids concentration may be correlated to the higher unit activity of the sludge.

After 24 days of operation of the batch unit Experiments 4, 5 and 6 (Group II) were performed. The results of these experiments are shown in Figures 18, 19 and 20. 24, 18 and 16 percent of the theoretical oxygen demand was exerted at 96, 97 and 99 percent of substrate removal, with initial solids concentrations of 410, 1100 and 1200 mg/l respectively. Sludge yield varied from 59 to 68 percent; unit activity of the sludge varied from 9 to 10 mg O_2/hr/gm-sludge. The percent theoretical oxygen demand exerted at the time of glucose (COD glucose) removal was 18 for initial solids concentration of 410 mg/l and 10 for initial solids concentrations of 1100 and 1200 mg/l.

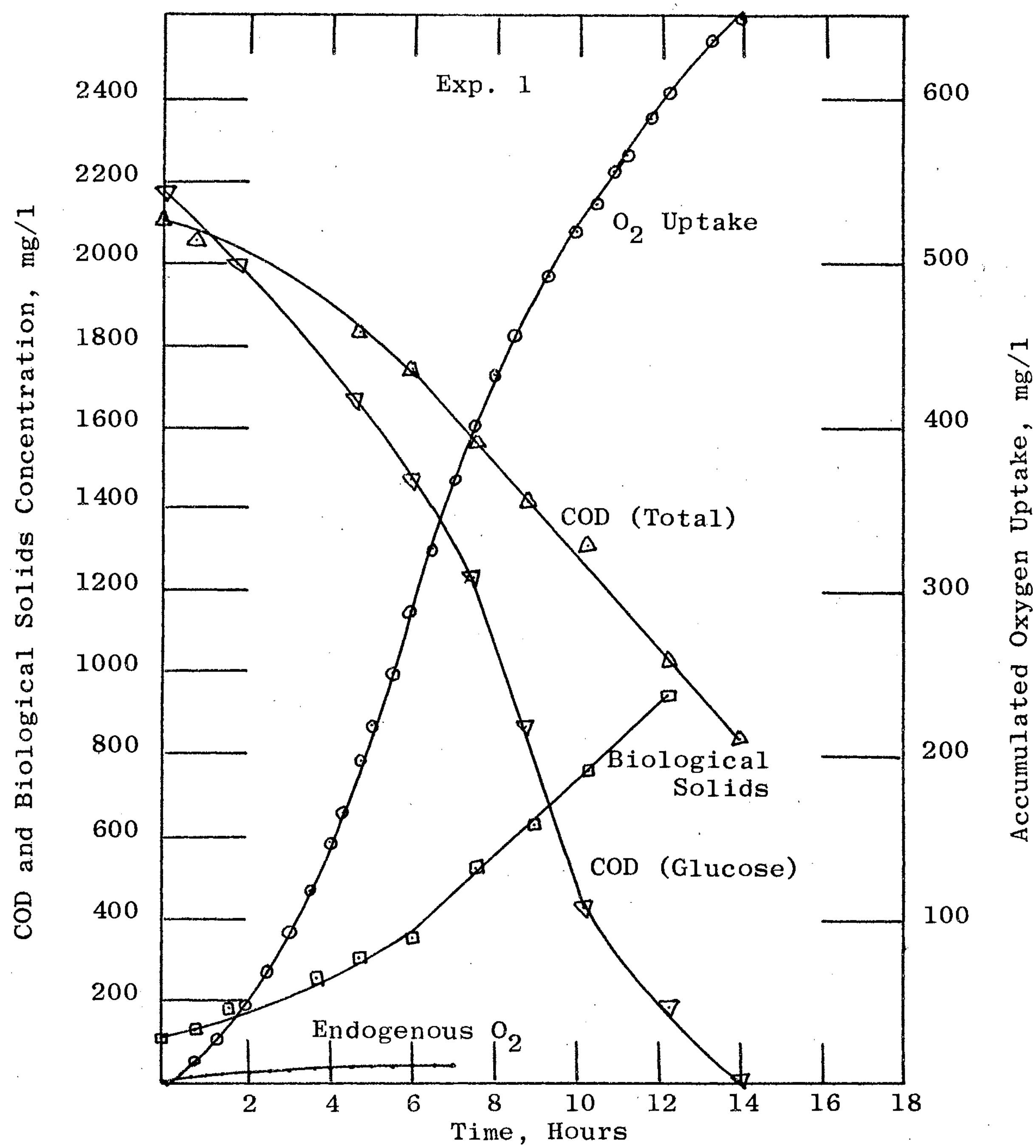

Fig. 15 METABOLISM OF GLUCOSE ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 104 mg/1

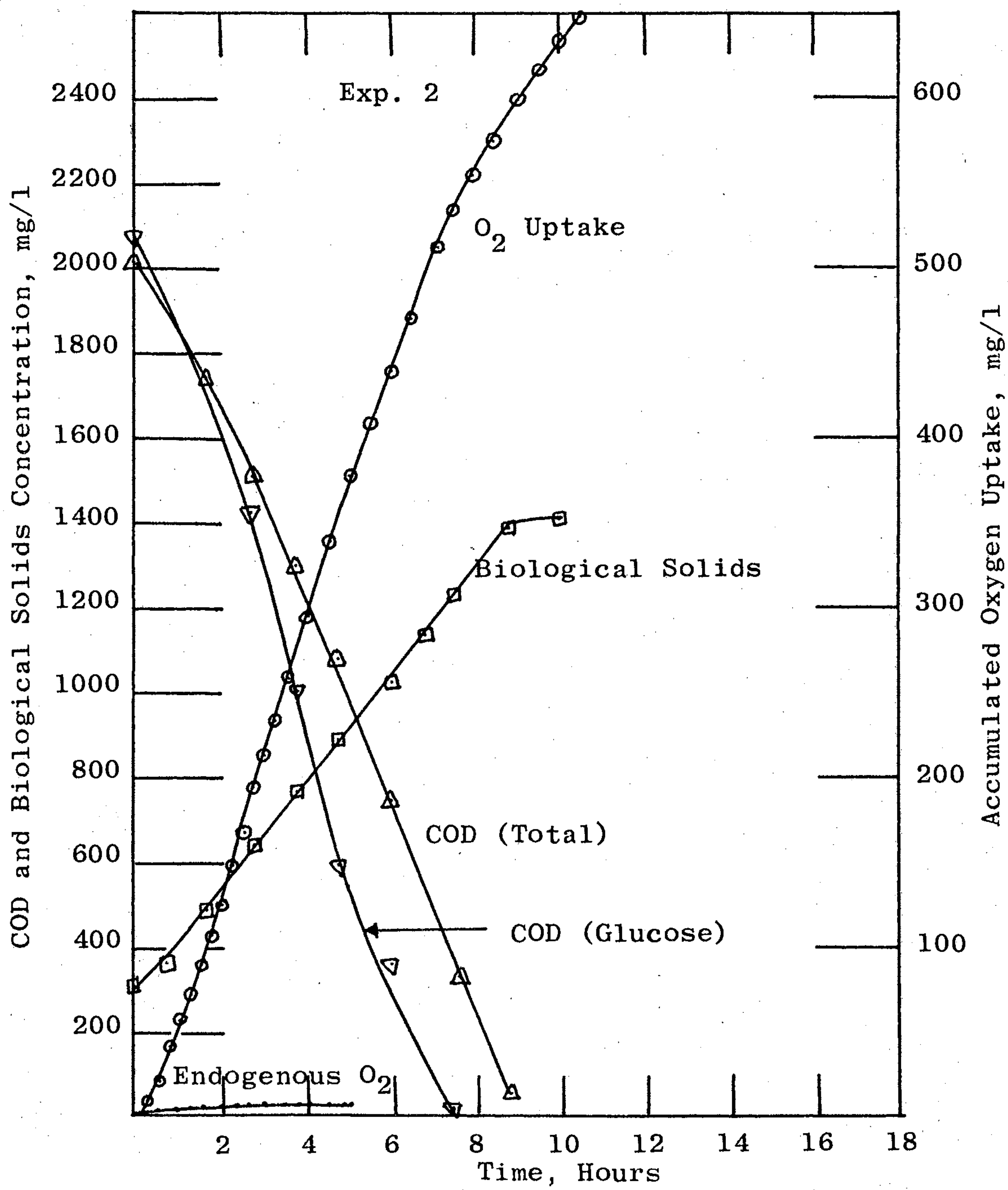

Fig. 16 METABOLISM OF GLUCOSE ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 316 mg/l

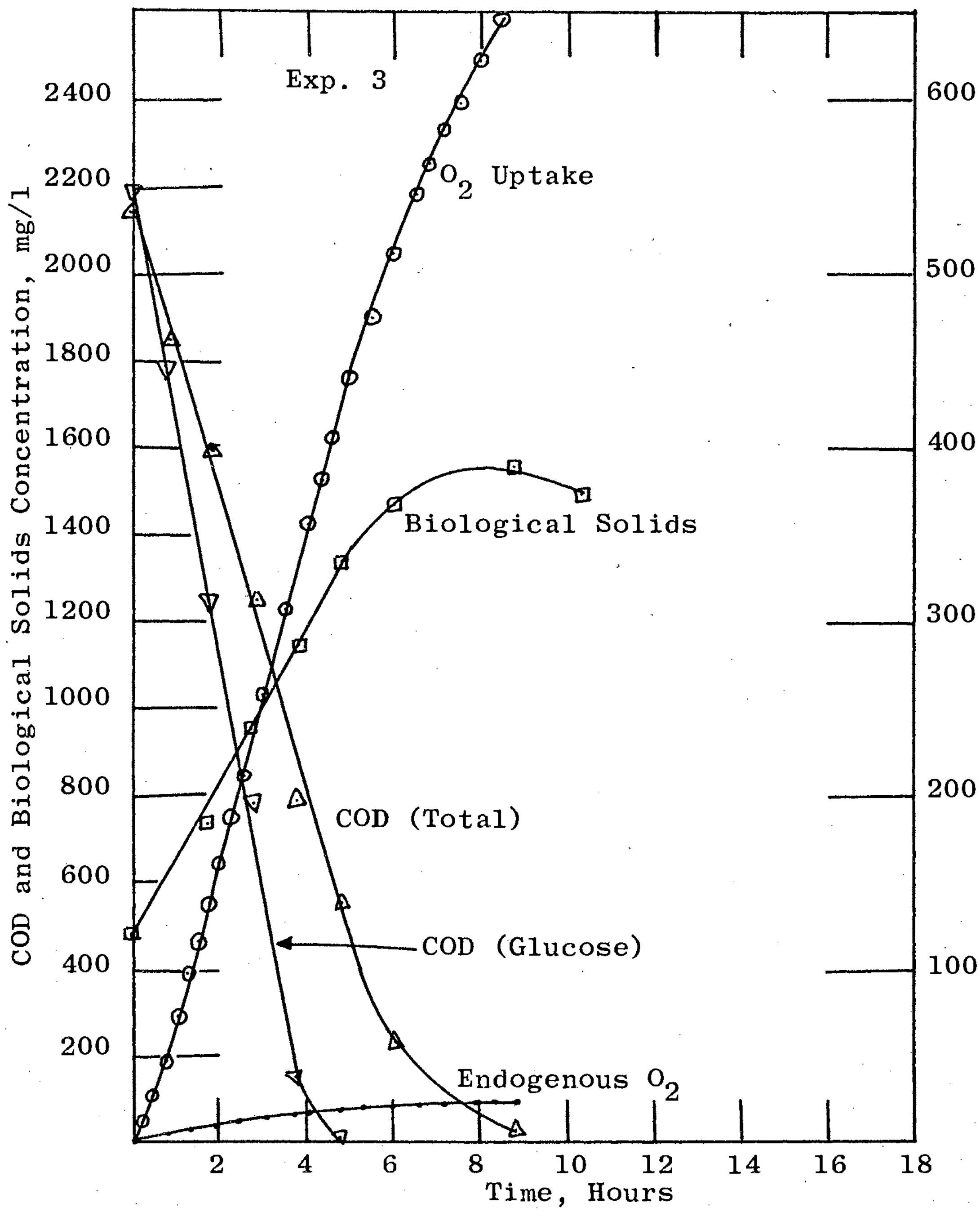

Fig. 17 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 470 mg/l

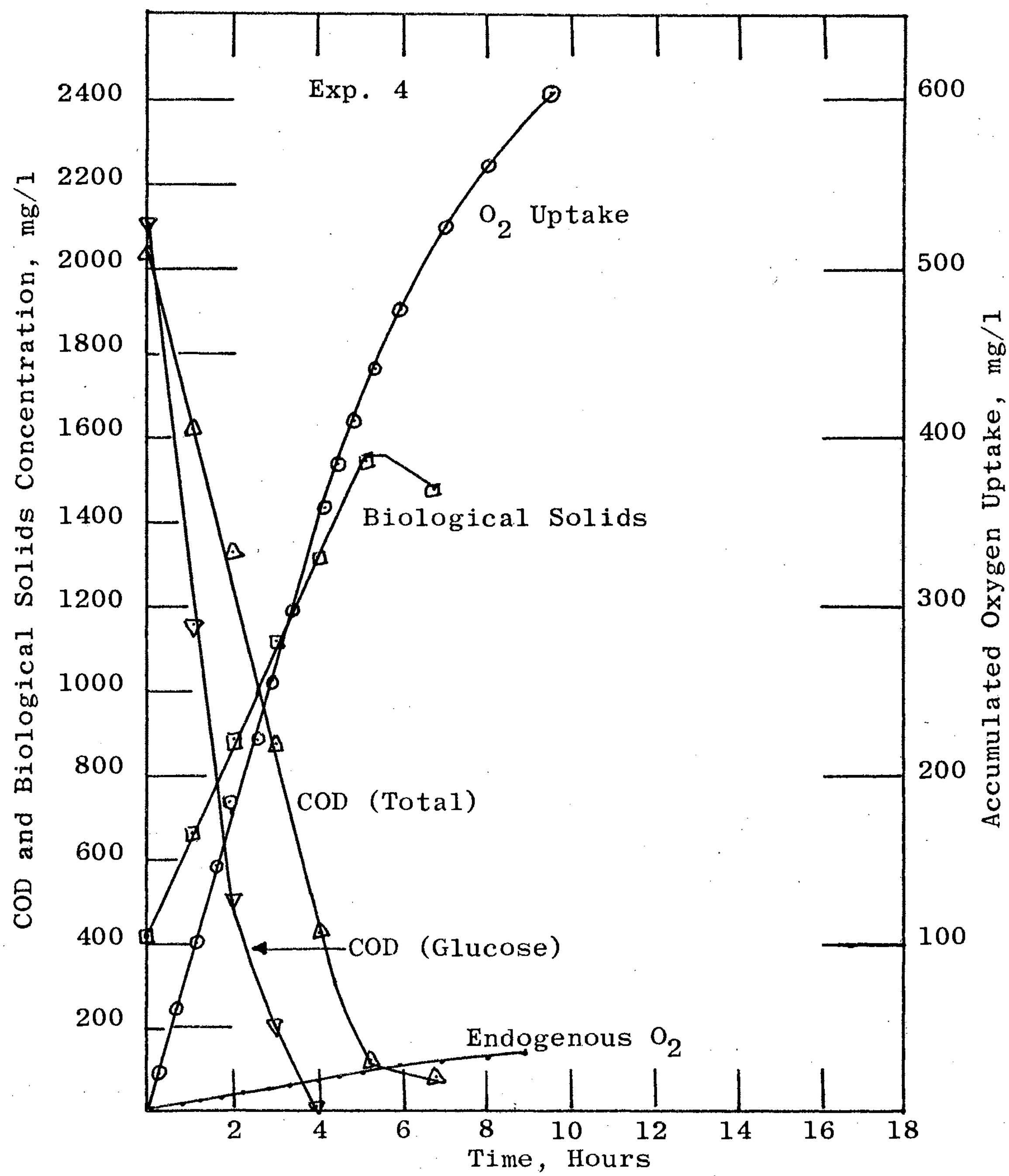

Fig. 18 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 410 mg/l

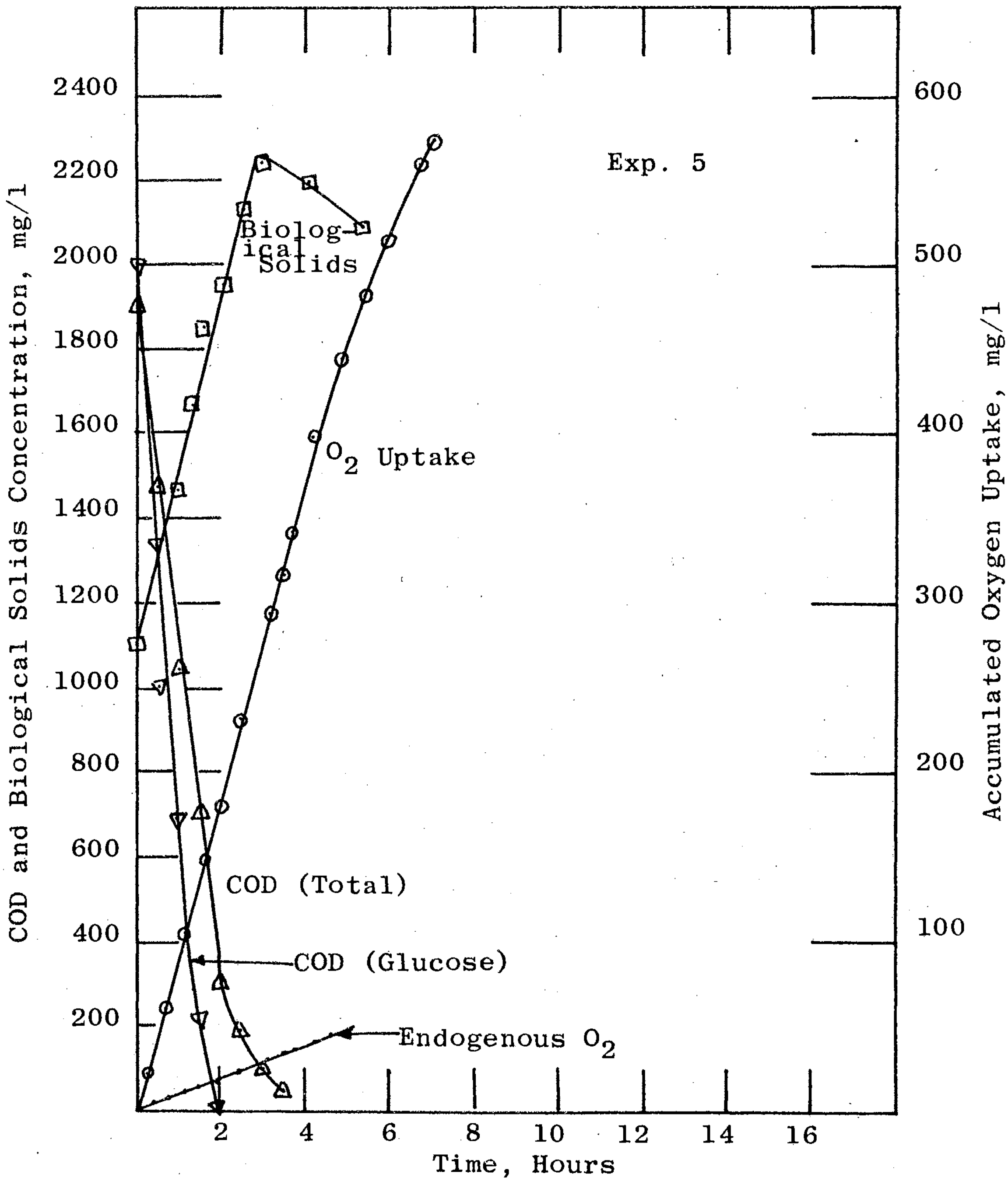

Fig. 19 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 1100 mg/l

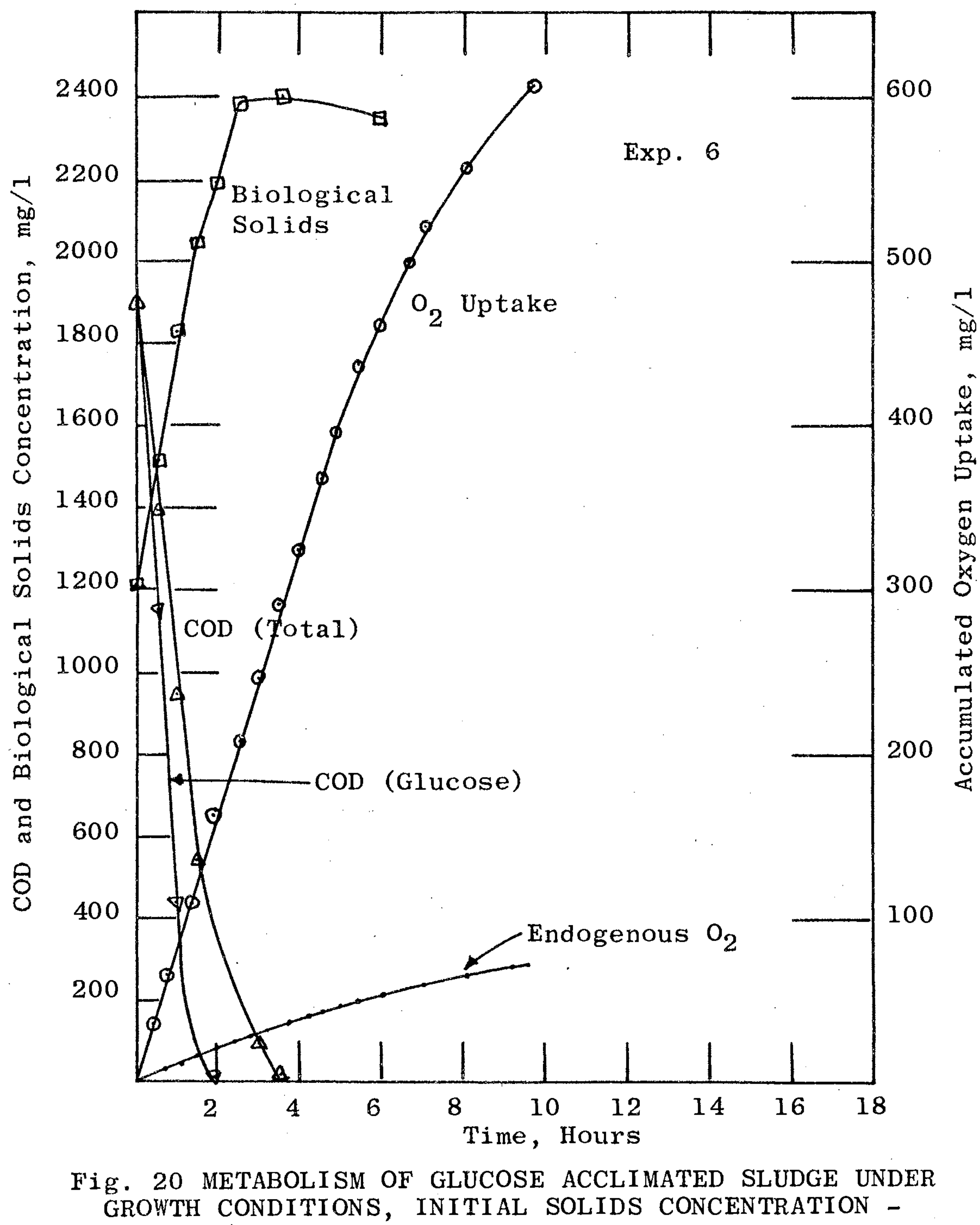

Fig. 20 METABOLISM OF GLUCOSE ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 1200 mg/l

After 37 days of operation of the batch unit, Experiments 7, 8 and 9 (Group III) were made. The results are shown in Figures 21, 22 and 23. In these experiments initial solids concentrations of 435, 926 and 2050 mg/l were used. At this time the appearance of the sludge in the batch unit had changed slightly from its normal brownish color and had become slightly dark in appearance; however, bacteria and protozoa were dominant at this period. The percent theoretical oxygen demand exerted was 15 at 98 percent substrate removal for initial solids concentration of 2050 mg/l, whereas 28 percent theoretical oxygen demand was exerted at 95 and 98 percent substrate removal with initial solids concentrations of 435 and 926 mg/l. The percent theoretical oxygen demand exerted was 8 for initial biological solids concentration of 2050 mg/l at the time of glucose (COD glucose) removal, whereas it was 24 and 23 for initial biological solids concentrations of 435 and 926 mg/l. Even though the same sludge was used for all experiments, a unit activity of 13 mg O_2/hr/gm-sludge was observed for initial solids concentrations of 926 mg/l, whereas unit activities of 10.3 and 7.3 mg O_2/hr gm-sludge were observed for initial solids concentrations of 435 and 2050 mg/l respectively. The sludge yield was observed as 68 and 75 percent for initial solids concentrations of 2050 and 435 mg/l, whereas 84 percent sludge yield was observed for initial solids concentration of 926 mg/l.

After 43 days of operation of the batch unit Experiments 10 and 11 (Group IV) were made with initial solids concentra-

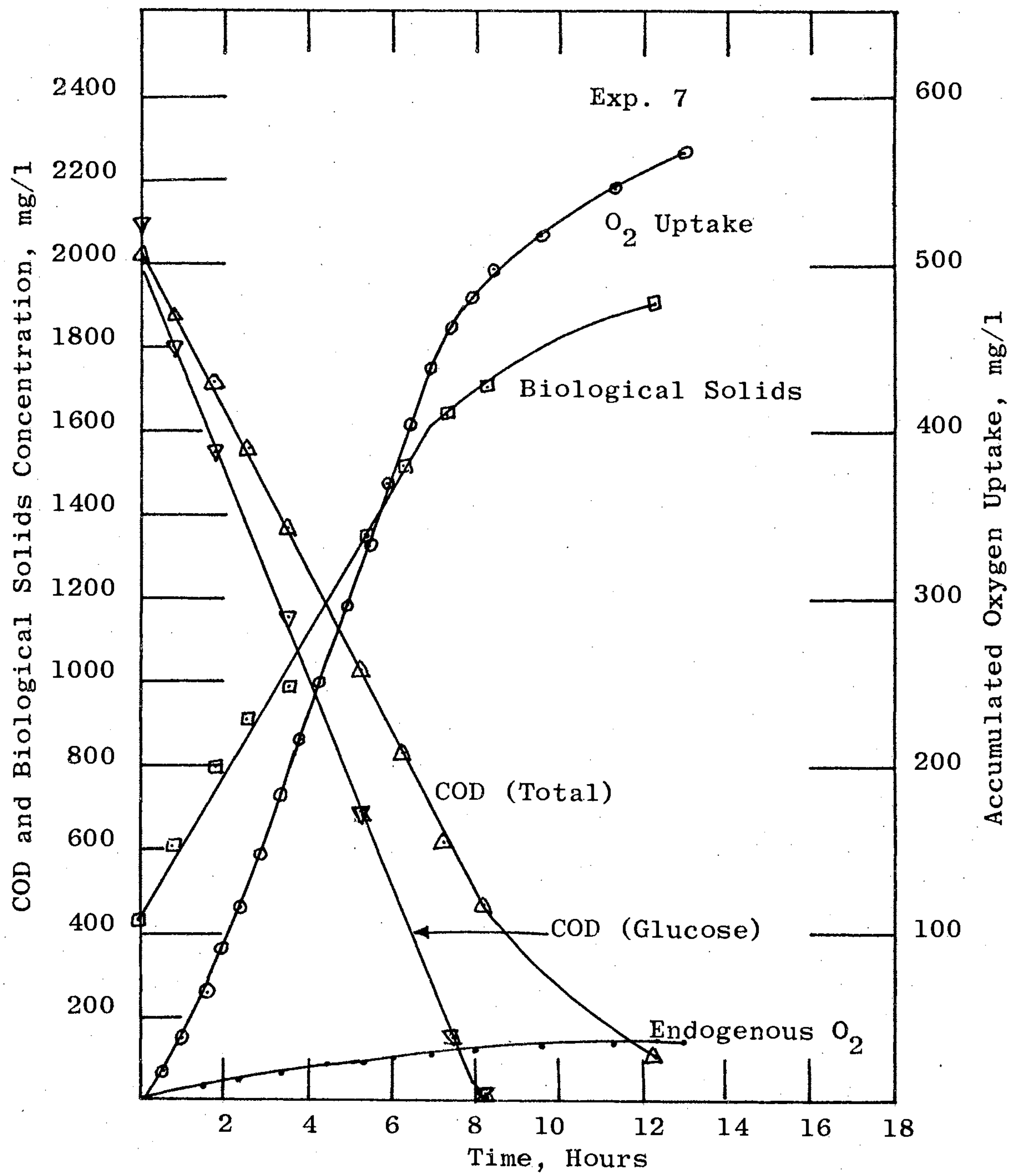

Fig. 21 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 435 mg/l

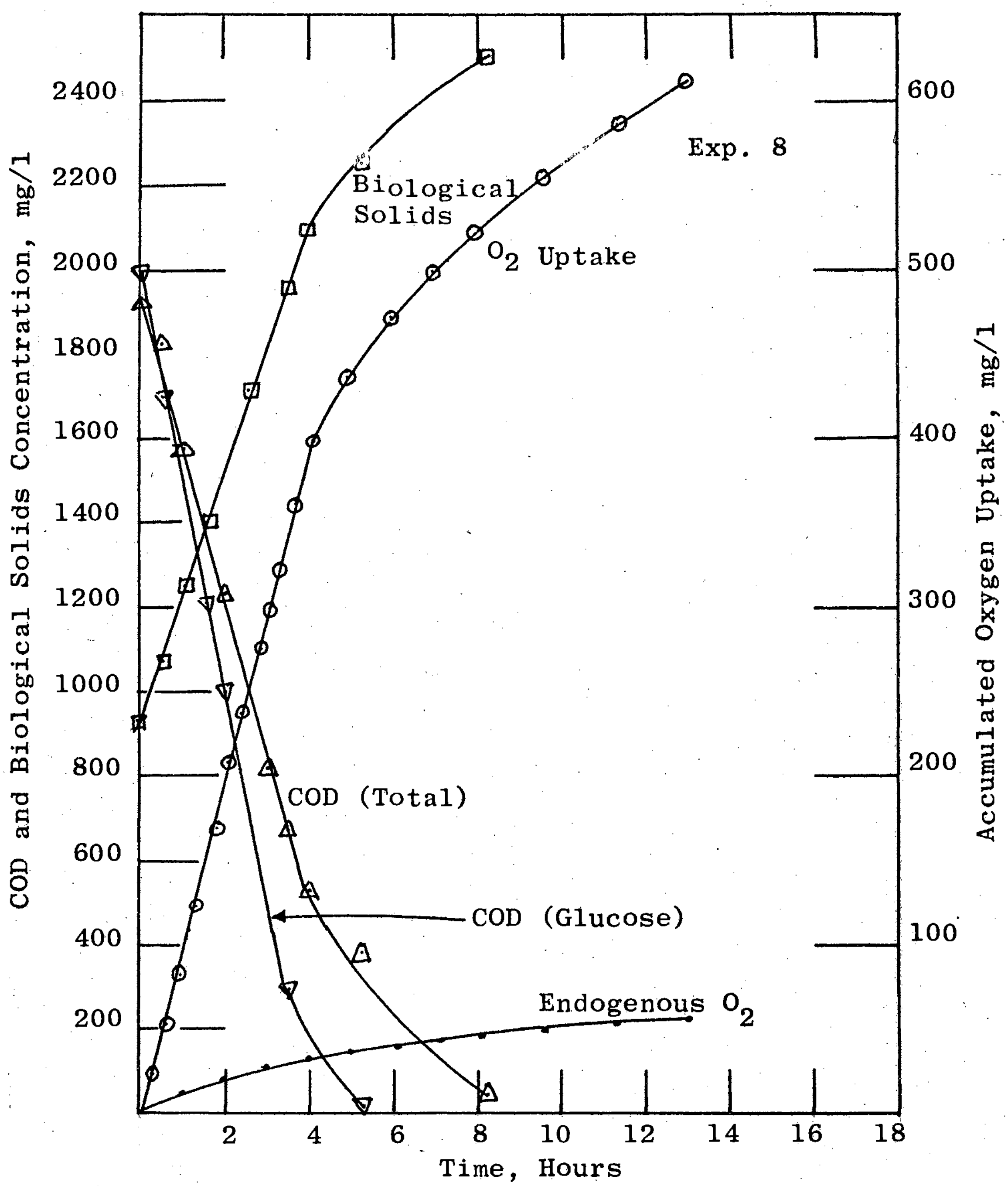

Fig. 22 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 926 mg/1

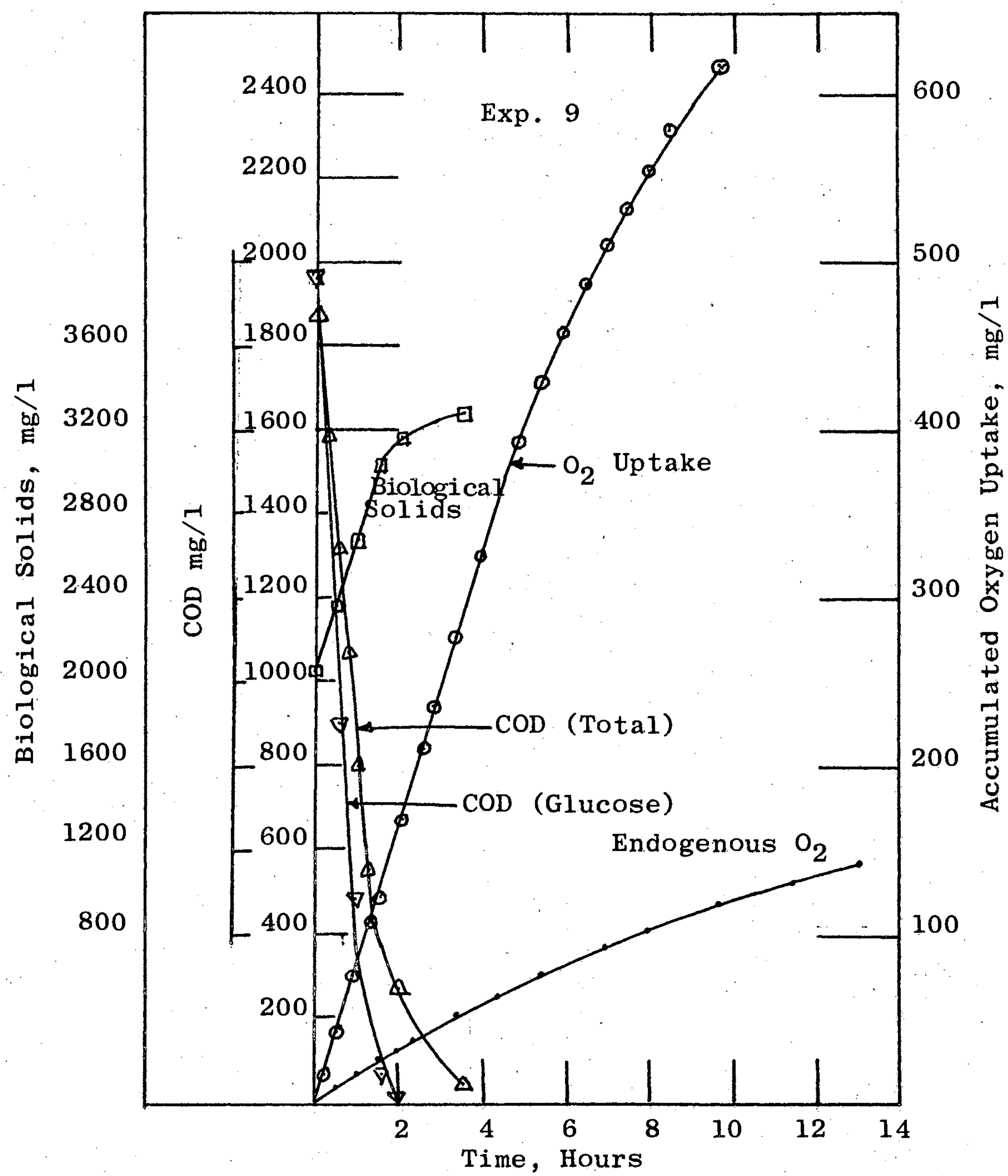

Fig. 23 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 2050 mg/1

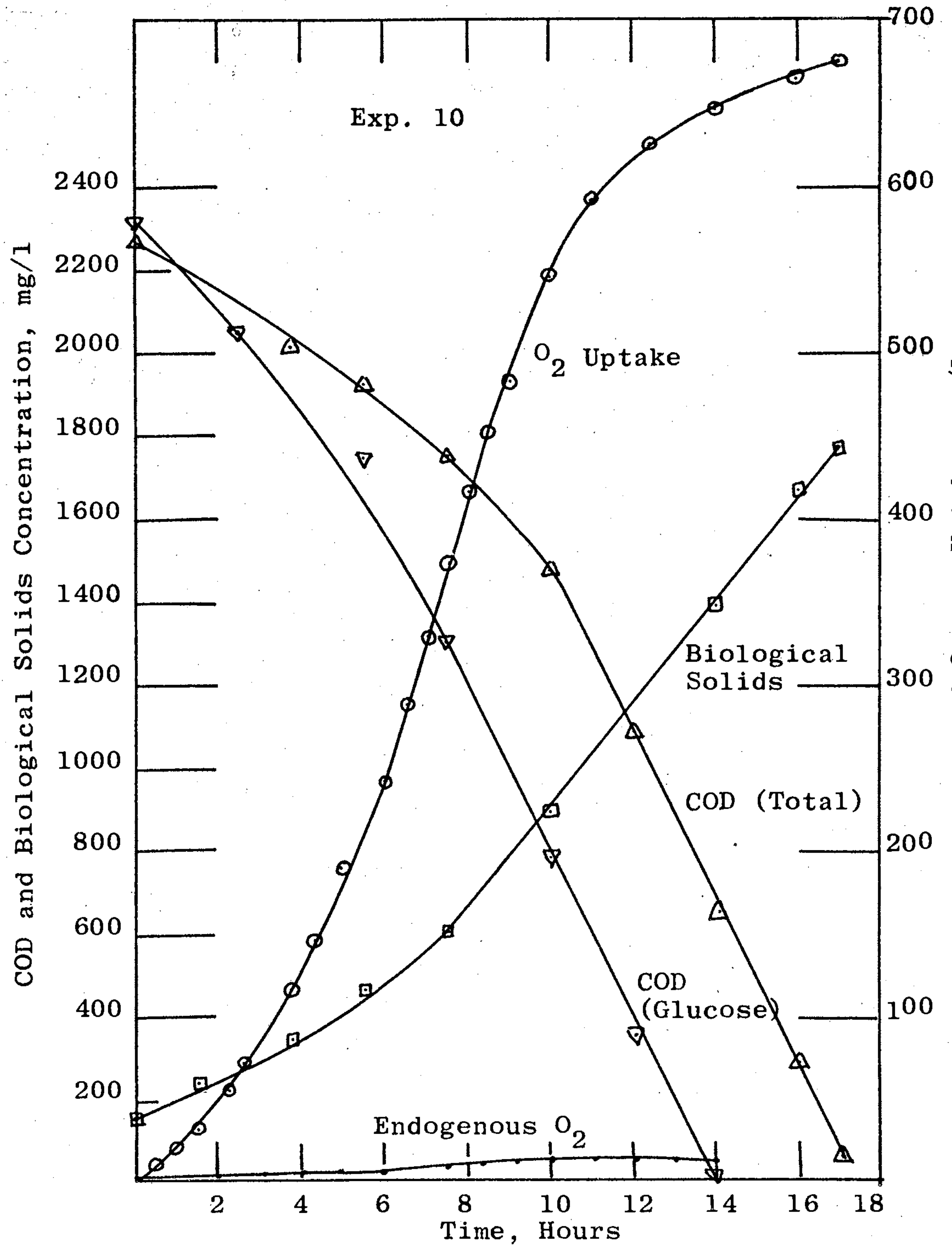

Fig. 24 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 152 mg/l

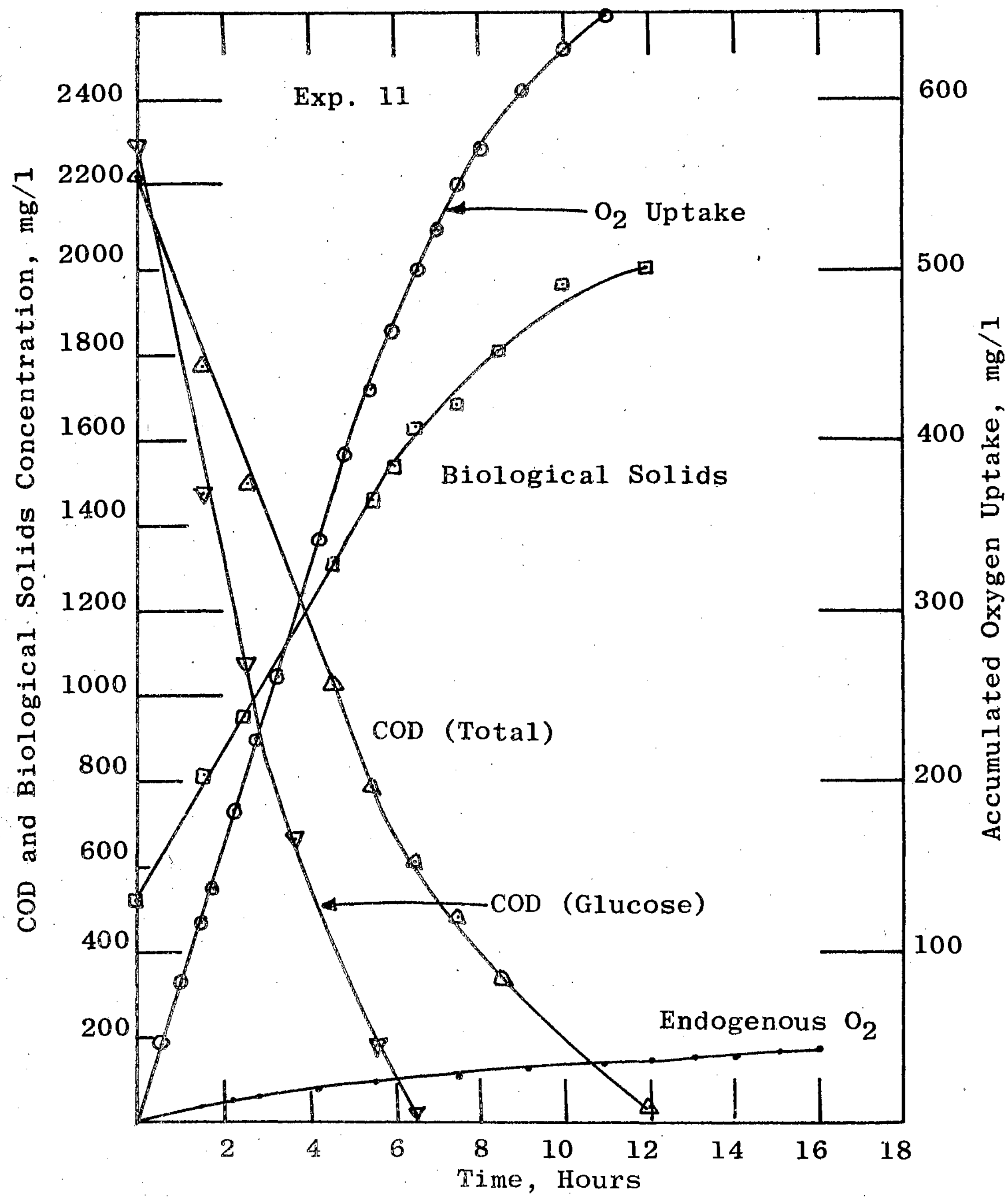

Fig. 25 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 515 mg/1

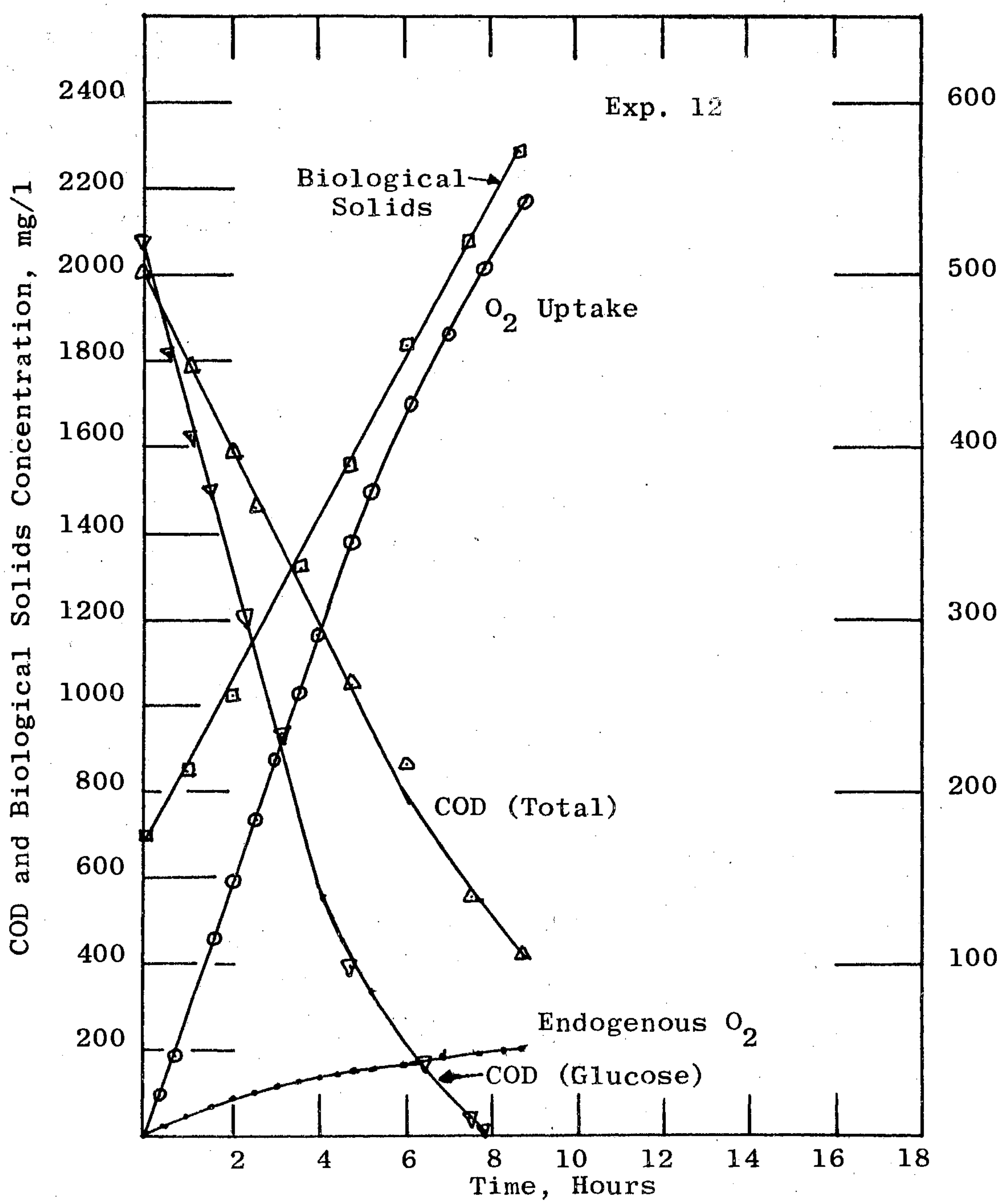

Fig. 26 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 700 mg/1

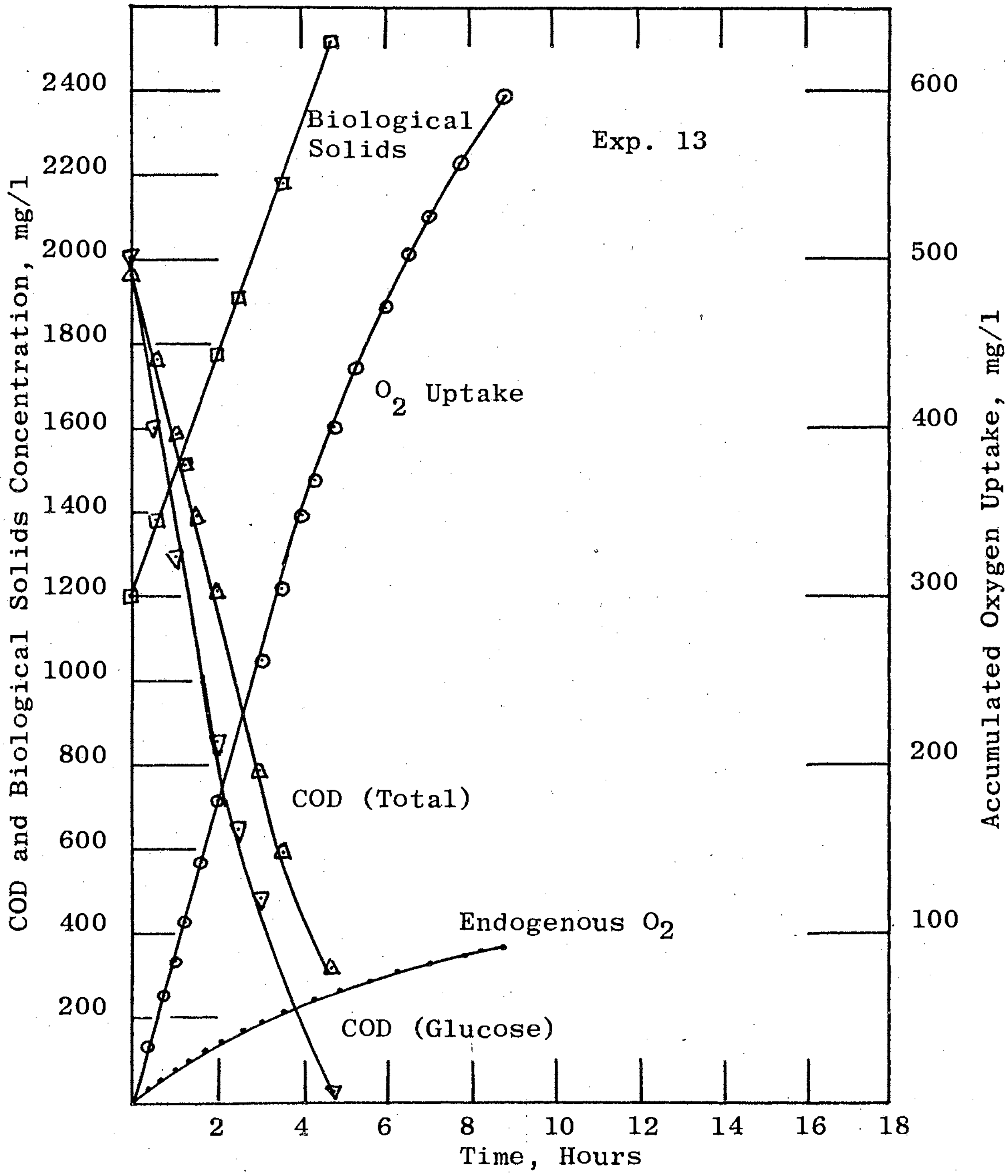

Fig. 27 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 1200 mg/l

tions of 152 and 515 mg/l; these results are shown in Figures 24 and 25. The percent theoretical oxygen demand exerted was 31 and 30 at 97 and 98 percent substrate removal for initial solids concentrations of 152 and 515 mg/l. The percent theoretical oxygen demand exerted was 28 and 22 at the time of glucose (COD glucose) removal for initial solids concentrations of 152 and 515 mg/l. The sludge yield was observed to be 61 percent for initial solids concentration of 152 mg/l, as calculated during the linear phase of substrate removal, and the sludge yield was 67 percent for initial solids concentration of 515 mg/l. The unit activity of the sludge was 9.7 and 10.7 mg O_2/hr/gm-sludge.

After 46 days of operation of the batch unit Experiments 12 and 13 (Group V) were made with initial solids concentrations of 700 and 1200 mg/l; the results are shown in Figures 26 and 27. At this time sludge taken from the batch unit was very dark in appearance, and microscopic examination of the sludge showed that chain-forms of microorganisms predominated. The percent theoretical oxygen demand exerted was 27 at the point of 79 percent substrate removal for initial solids concentration of 700 mg/l and 20 percent of the theoretical oxygen demand was exerted at 85 percent substrate removal for initial solids concentration of 1200 mg/l. The percent theoretical oxygen demand exerted was 26 and 20 at the point of glucose (COD glucose) removal for initial solids concentrations of 700 and 1200 mg/l. The sludge yields were observed as 88 and 72 percent for initial solids

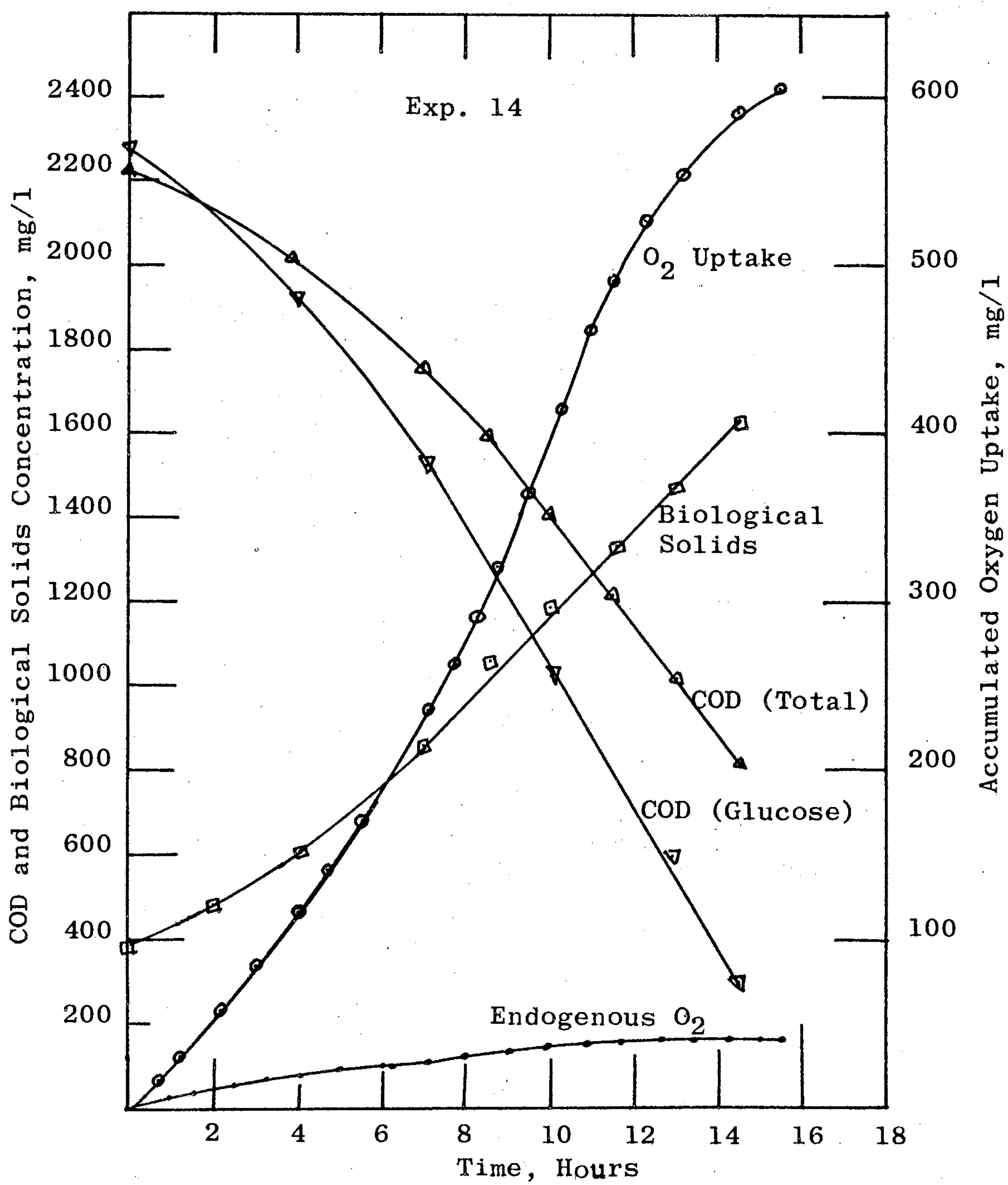

Fig. 28 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 375 mg/1

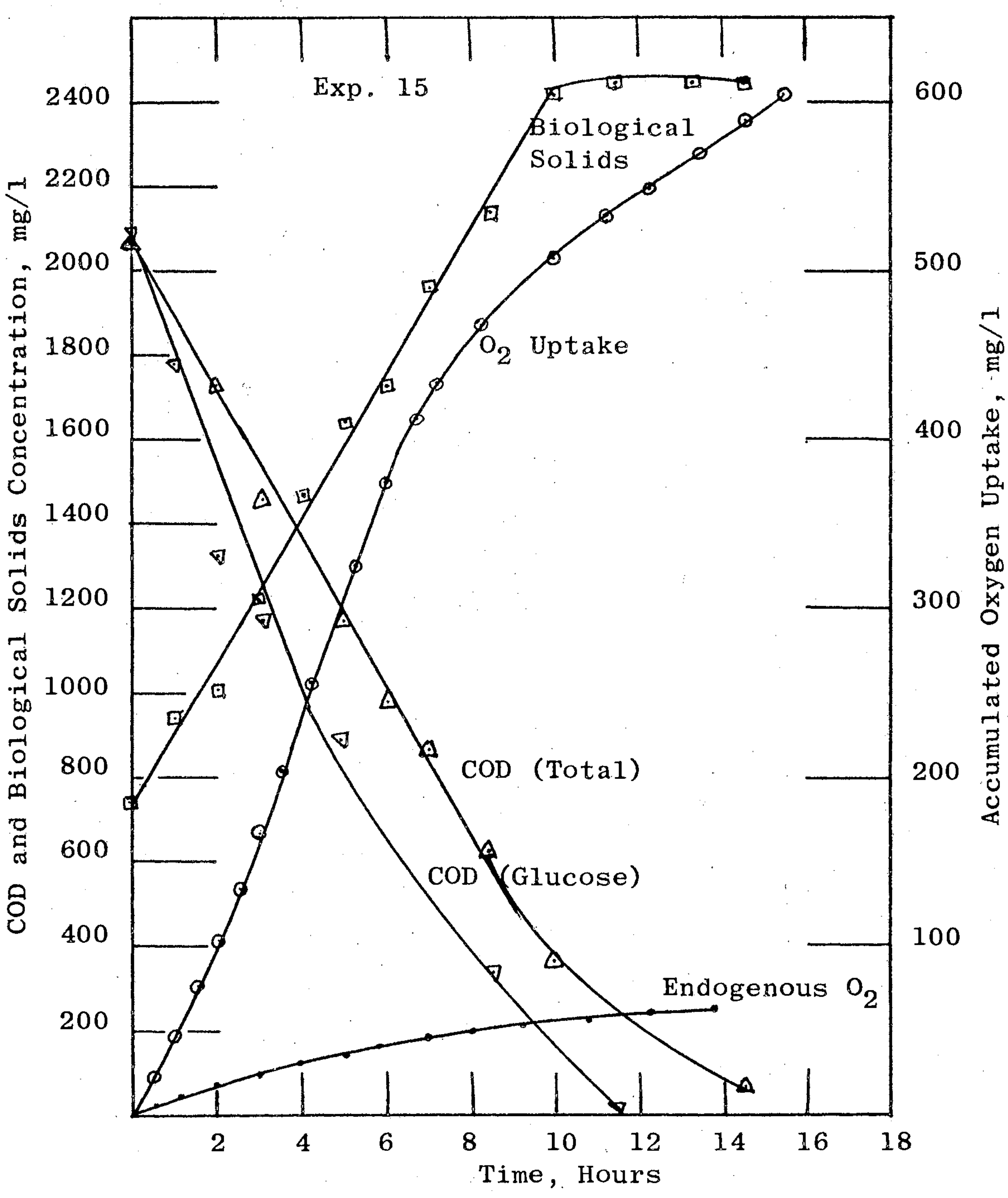

Fig. 29 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 750 mg/l

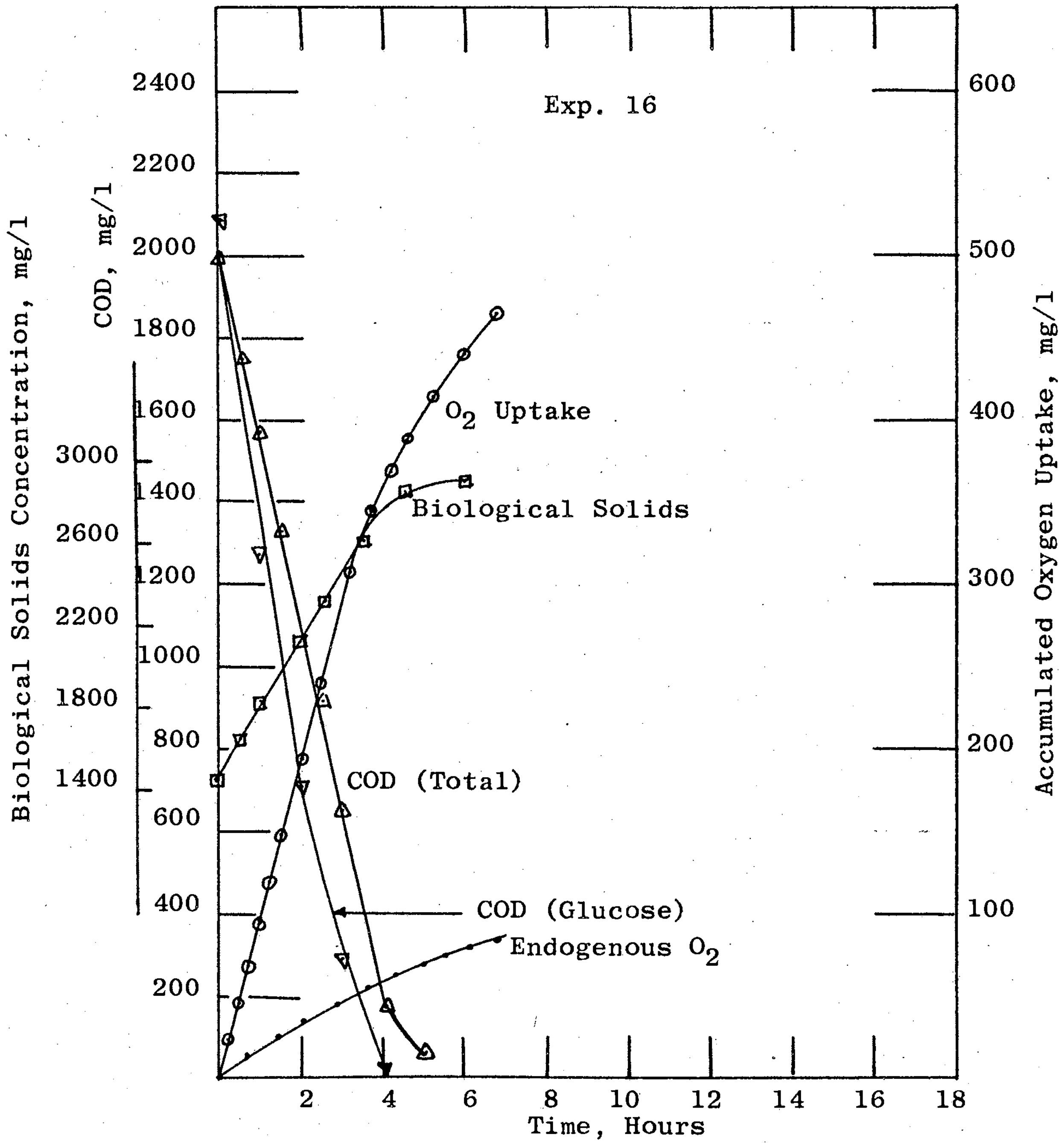

Fig. 30 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 1450 mg/l

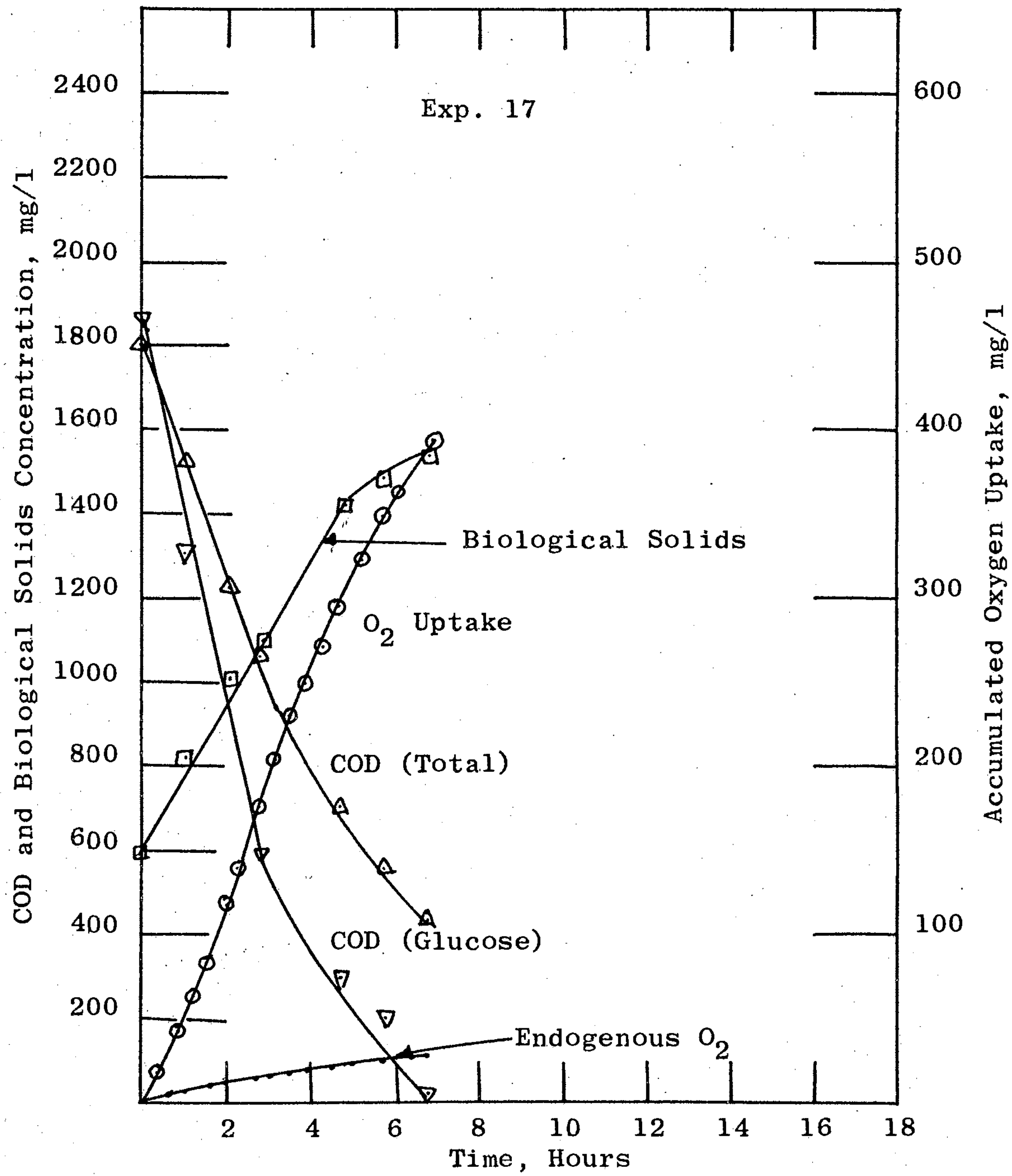

Fig. 31 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 580 mg/1

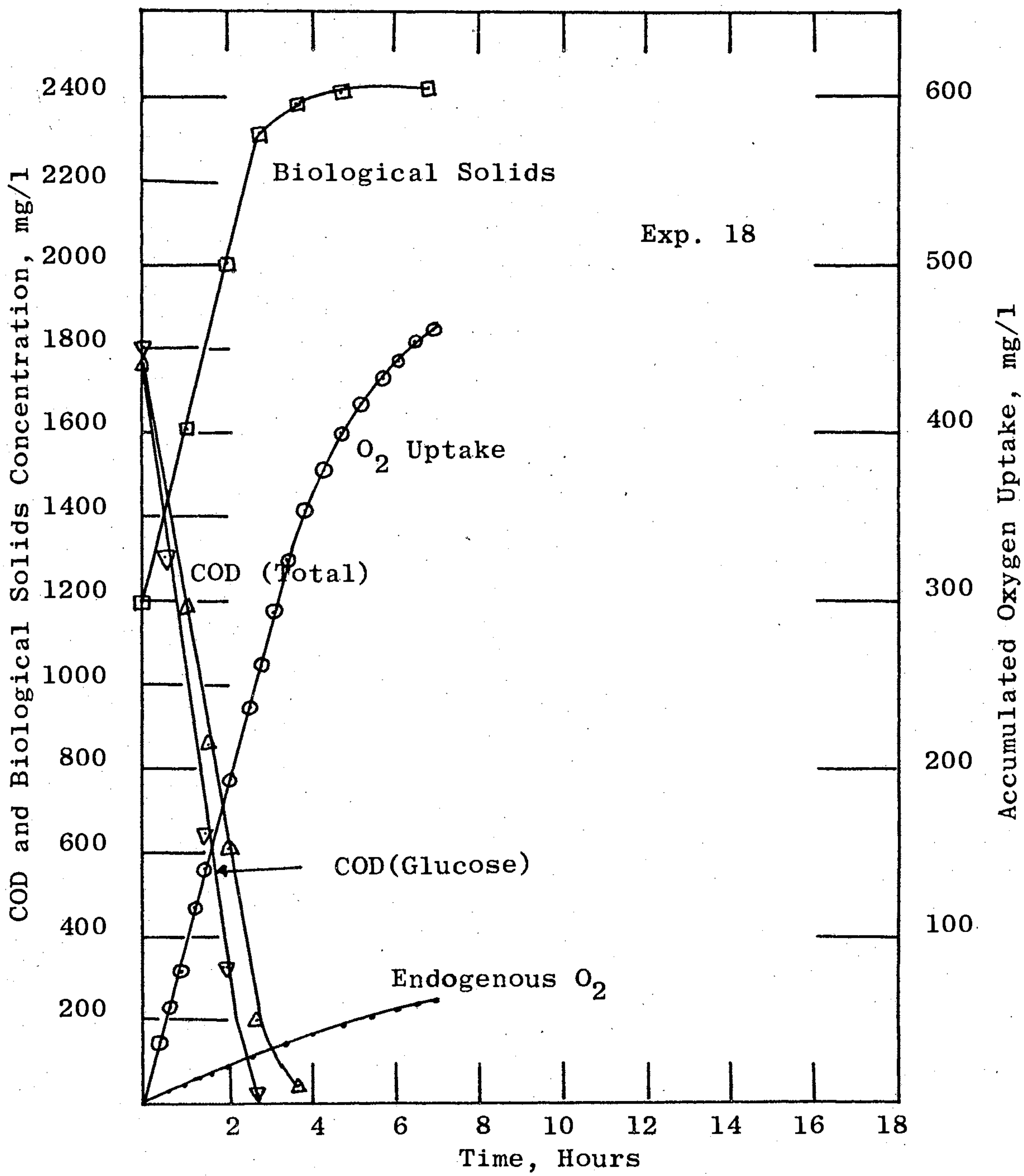

Fig. 32 METABOLISM OF GLUCOSE-ACCLIMATED SLUDGE UNDER GROWTH CONDITIONS, INITIAL SOLIDS CONCENTRATION - 1200 mg/1

concentrations of 700 and 1200 mg/l respectively. The unit activity of the sludge was observed as 16.7 and 12.5 mg O_2/hr/gm-sludge for initial solids concentrations of 700 and 1200 mg/l.

After 53 days of operation of the batch unit Experiments 14, 15 and 16 (Group VI) were made with initial solids concentrations of 375, 750 and 1450 mg/l. At this time chain-forms of microorganisms were still predominating in the batch unit. The results of these experiments are shown in Figures 28, 29 and 30. The percent theoretical oxygen demand exerted was 27, 29 and 20 at 63, 98 and 97 percent substrate removal for initial solids concentrations of 375, 750 and 1450 mg/l. The percent theoretical oxygen demand exerted was 26 and 17 at the time of glucose (COD glucose) removal for initial solids concentrations of 750 and 1450 mg/l; the sludge yield varied from 76 to 84 percent. The unit activity of the sludge varied from 15.5 to 18.9 mg O_2/hr/gm-sludge.

After 66 days of operation Experiments 17 and 18 (Group VII) were made with initial solids concentrations of 580 and 1200 mg/l; the results are shown in Figures 31 and 32. At this time the sludge had regained its normal brown appearance and microphotographs revealed that protozoa and bacteria were the predominating organisms rather than the chain-forms of microorganisms which had predominated when the sludge was dark in appearance. The unit activity of the sludge was 8.0 mg O_2/hr/gm-sludge; the sludge yield was observed to be 73 percent for the initial solids concentration of 580 mg/l and 69 percent for the initial solids concentration of 1200 mg/l. The percent theoretical oxygen demand exerted at the time of glucose (COD glucose) removal was 21 and 14 for initial solids concentrations of 580 and 1200 mg/l.

Relationship between Substrate Removal and Initial Biological Solids Concentration

An attempt was made to see if a general relationship existed between initial biological solids concentration and the rate of substrate removal. Substrate removals (calculated on a percentage basis) for all 18 experiments are given in Table III. The percent substrate removal vs time for different initial biological solids concentration is plotted in Figures 33 through 35. It can be seen from these figures that the rate of substrate removal within each group was greater as initial biological solids concentration increased.

A plot of initial biological solids concentrations vs rate of substrate removal (during the linear phase) expressed on a percentage basis is shown in Fig. 36. From this figure it is seen that percent substrate removal was of increasing order with increasing solids concentration (104 to 2050 mg/l), and that no simple straight line relationship existed for all experiments. However, four different straight line relationships were observed when sludges were grouped in accordance with the major changes in predominating microorganisms observed during the experimental period. Curve 1 represents the relationship for sludge when it exhibited its normal brown color. At this time

TABLE III

PERCENT COD REMOVAL FOR VARIOUS INITIAL BIOLOGICAL SOLIDS, EXPERIMENTS 1 THROUGH 18

Exp.	Initial Solids Concentration mg/l	Time, Hours * / Percent COD Removal, Indicated Time								
1	104	1.75	4.75	6.0	7.5	8.75	10.25	12.25	14.0	*
		4.75	12.8	17.2	25.2	32.4	37.4	51.0	60.0	⊙
2	316	2.75	3.75	4.75	6.0	7.5	8.75			
		24.5	35.0	45.5	62.5	83.4	97.25			
3	470	1.75	2.75	3.75	4.75	6.0	7.5	8.75		
		25.5	41.5	63.2	74.2	89.8	96.0	98.0		
4	410	1.0	2.0	3.0	4.0	5.25	6.75			
		19.8	34.0	56.5	79.0	94.0	95.5			
5	1100	0.5	1.0	1.5	2.0	2.5	3.0	3.5		
		22.6	45.2	62.5	84.5	90.2	95.5	97.0		
6	1200	0.5	1.0	1.5	2.5	3.0	3.5			
		22.8	47.5	70.0	92.5	95.5	99.8			
7	435	1.75	2.5	5.25	7.25	8.25	12.25			
		14.8	22.8	49.0	69.5	77.0	95.0			
8	926	1.0	2.0	3.0	4.0	5.25	8.25			
		18.0	36.0	57.5	74.0	80.4	98.0			
9	2050	0.5	1.0	1.5	2.0	3.5				
		29.8	57.5	80.0	85.2	97.5				
10	152	2.5	5.5	8.5	10.0	12.0	14.0	16.0	17.0	
		9.7	15.4	27.3	35.2	53.2	71.2	87.4	97.5	
11	515	1.5	3.75	5.5	7.5	8.5	10.0	12.0		
		20.0	44.0	64.0	78.0	85.0	94.5	98.0		
12	700	1.0	2.0	3.5	6.0	7.5	8.75			
		11.0	21.0	35.4	57.4	72.5	78.5			
13	1200	1.0	2.0	3.0	3.5	4.75				
		19.4	38.3	56.5	70.0	84.0				
14	375	1.0	4.0	7.0	10.0	11.5	13.0	14.5		
		2.7	8.6	20.7	36.5	45.0	54.0	63.0		
15	750	1.0	2.0	3.0	5.0	7.0	8.5	11.5	13.0	
		9.7	16.5	30.4	43.5	58.4	69.5	89.5	98.0	
16	1450	1.0	2.0	3.0	4.0	5.0				
		21.0	47.0	67.5	91.0	96.5				
17	580	1.0	2.0	2.75	5.75	6.75				
		15.0	32.0	41.0	69.0	76.0				
18	1200	0.5	1.0	1.5	2.0	2.75	4.75			
		16.5	33.0	51.0	65.0	87.0	93.0			

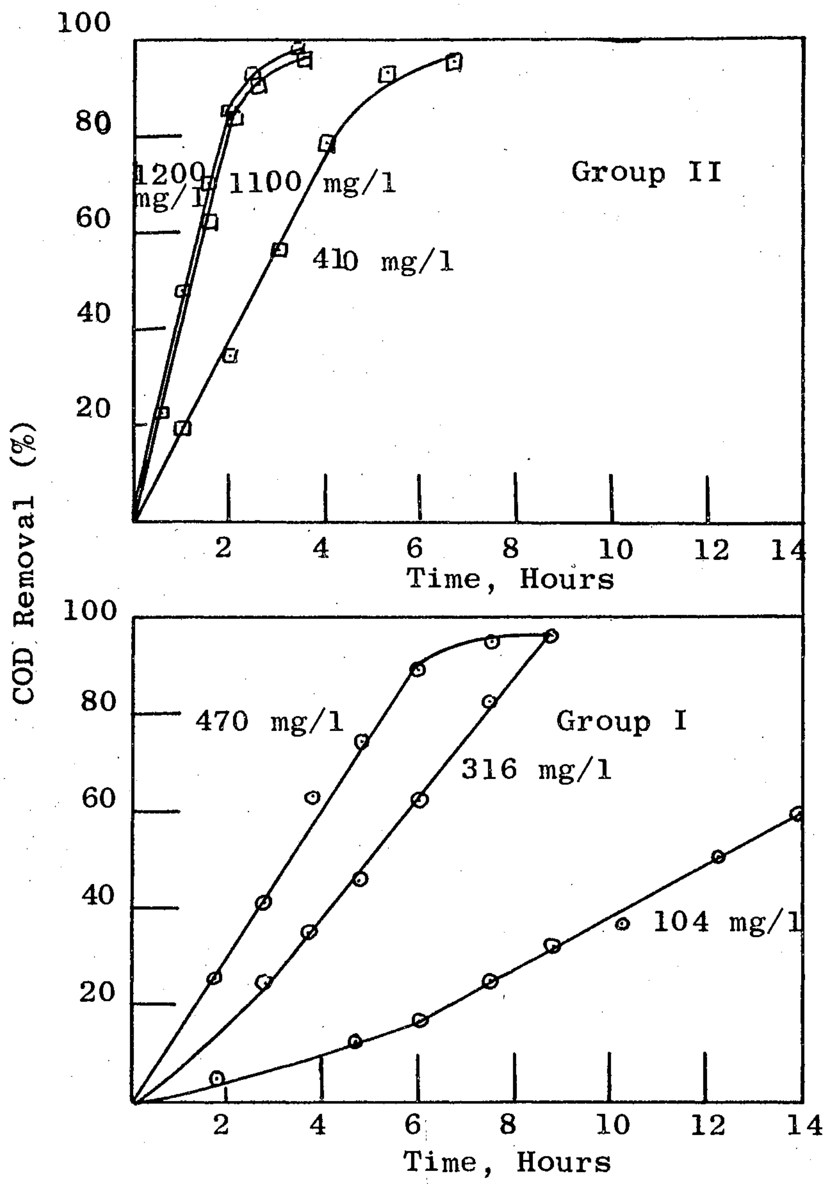

Fig. 33 RELATIONSHIP BETWEEN COD REMOVAL AND BIOLOGICAL SOLIDS CONCENTRATION EXPERIMENTS IN GROUPS I and II

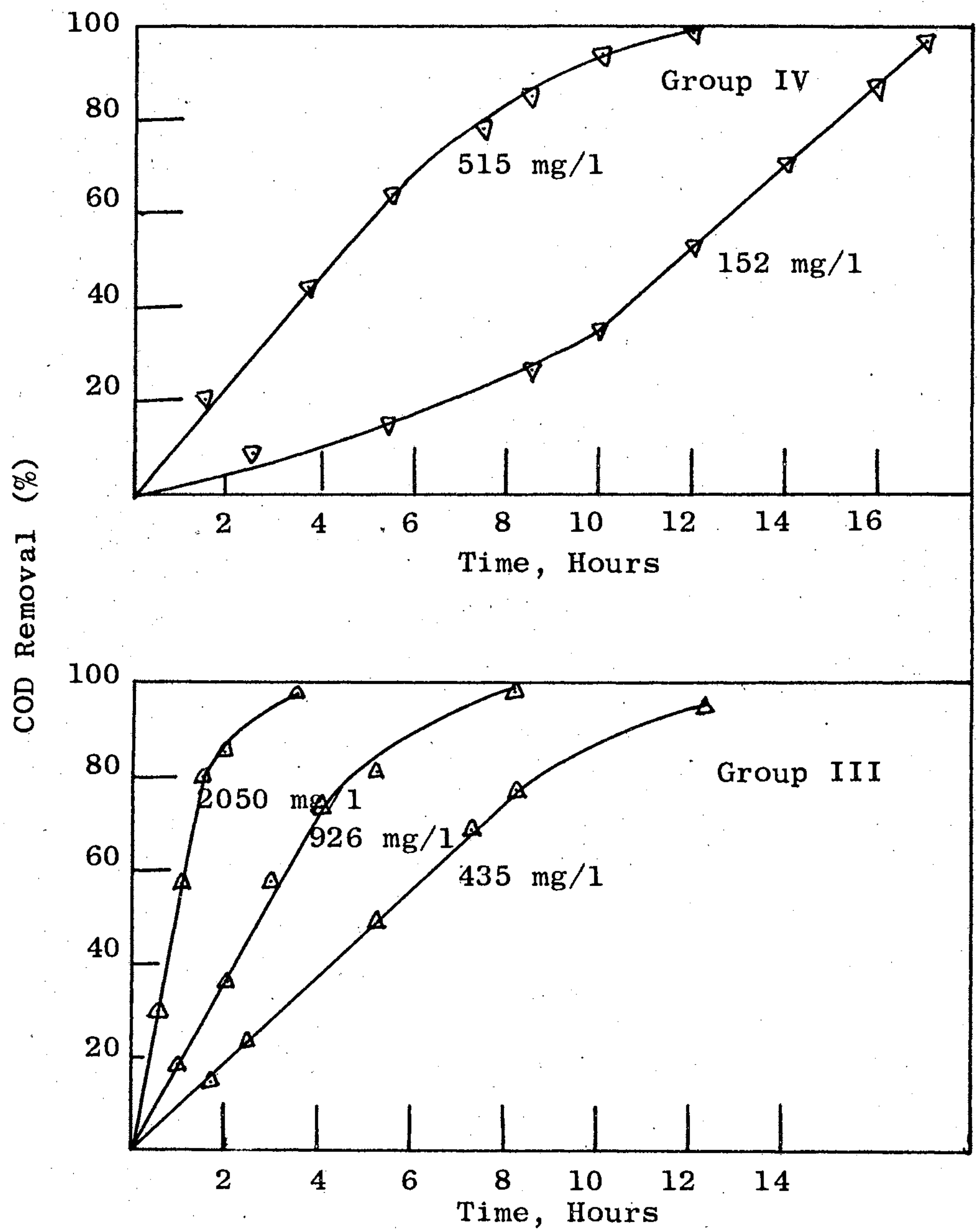

Fig. 34 RELATIONSHIP BETWEEN COD REMOVAL AND INITIAL BIOLOGICAL SOLIDS CONCENTRATION FOR EXPERIMENTS IN GROUP III and GROUP IV

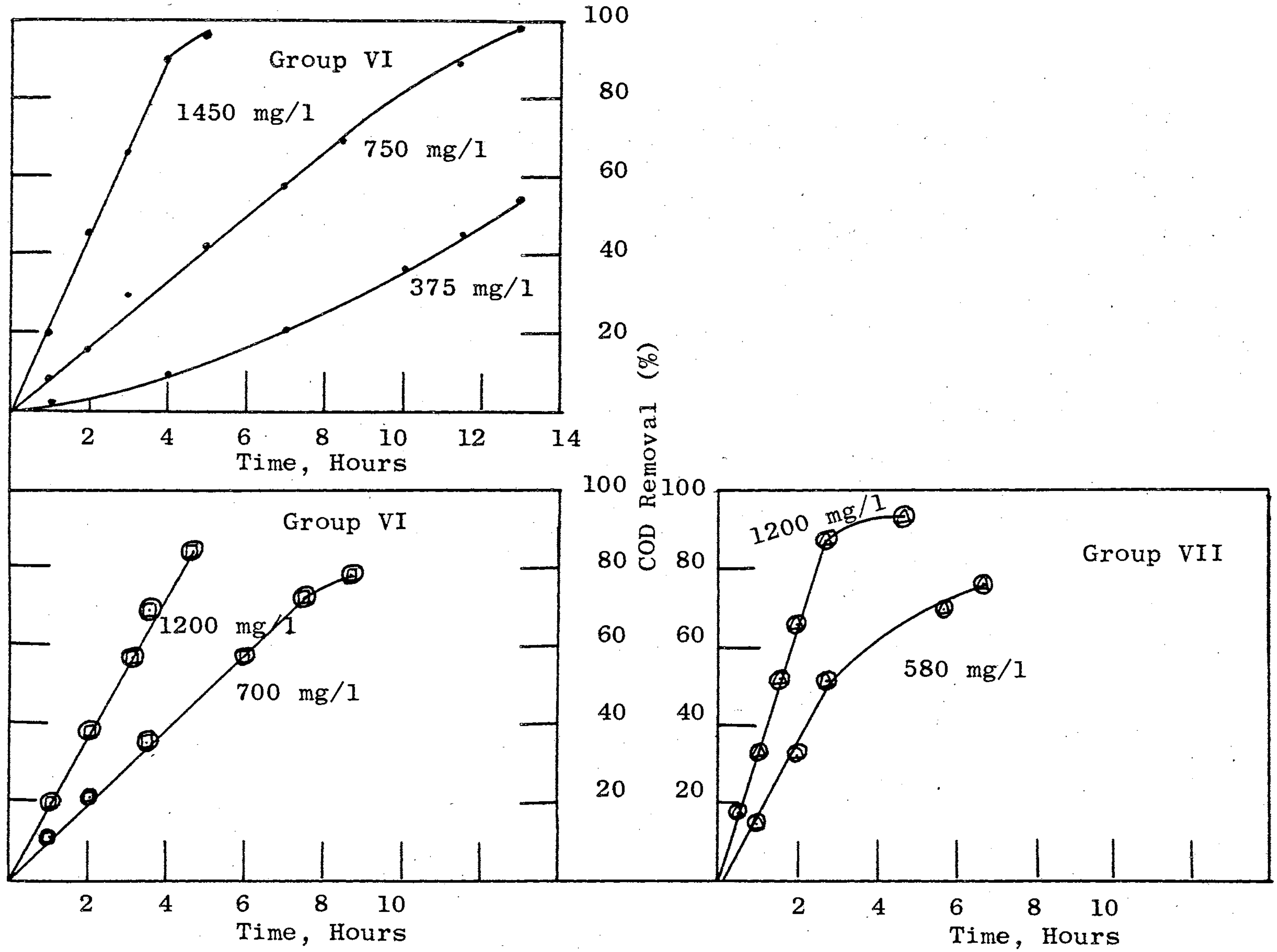

Fig. 35 RELATIONSHIP BETWEEN COD REMOVAL AND INITIAL BIOLOGICAL SOLIDS CONCENTRATION FOR EXPERIMENTS IN GROUPS V, VI and VII

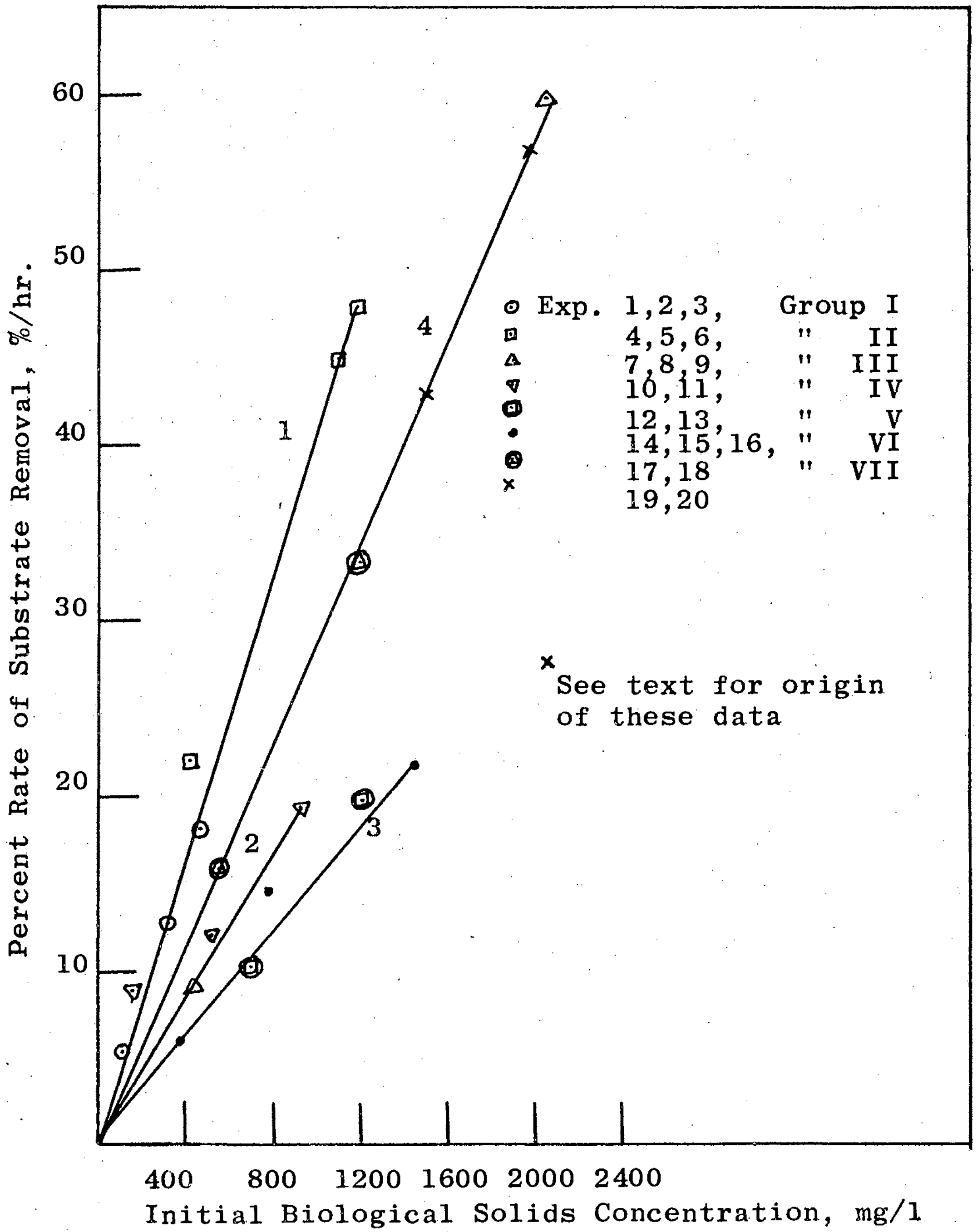

Fig. 36 RELATIONSHIP BETWEEN INITIAL BIOLOGICAL SOLIDS CONCENTRATION AND PERCENT RATE OF SUBSTRATE REMOVAL (Curve 1 Expts. No. 1,2,3,4,5,6, and 10
Curve 2 " 7,8,11, and 15
Curve 3 " 12,13, and 16
Curve 4 " 17,18,19, and 20)

bacteria, protozoa and yeasts were predominating. The sludge in these experiments was taken after 18 and 24 days of operation of the batch unit (Experiments 1 through 6). The unit activity of the sludge during this time varied from 8 to 10 mg O_2/hr/gm-sludge except for Experiment 1 which showed a unit activity of 18 mg O_2/hr/gm-sludge. Curve 2 represents the relationship obtained for sludge used when it exhibited a slightly dark appearance; however, bacteria and protozoa were still predominating. The sludge in these experiments was taken after 37 and 43 days of operation of the batch unit (Experiments 7 through 11). The unit activity of the sludge during this time varied from 10 to 13 mg O_2/hr/gm-sludge, except for Experiment 9, which showed a unit activity of 7.6 mg O_2/hr/gm-sludge. Curve 3 represents the relationship for sludge used when it exhibited a dark appearance; at this time chain-forms of microorganisms were predominating. This relationship was obtained when the sludge was taken after 46 to 53 days of operation of the batch unit (Experiments 12 through 16). The unit activity of the sludge during this period varied from 13 to 19 mg O_2/hr/gm-sludge. Curve 4 represents the relationship obtained for sludge when it had regained its normal brown appearance; bacteria, protozoa and yeasts were again predominating. The sludge in these experiments was taken after 68 and 70 days of operation of the batch unit. The data for two experiments (19 and 20) was obtained by Krishnan (63). The unit activity of the sludge during this time was 8.0 mg O_2/hr/gm-sludge. The data for Experiment 9 also fits this relationship; however, it should be noted that its unit activity was 7.6 mg O_2/hr/gm-sludge.

Relationship between Cell Yield and Initial Biological Solids Concentration

The cell yield calculated on a percentage basis is plotted vs initial biological solids concentration in Fig. 37. A wide variation of cell yield (53 to 88 percent) was observed for various initial biological solids concentrations; however, in general sludges in the same group (identical sludge) gave nearly constant cell yields. This aspect will be further discussed in the next chapter. The data for Experiments 19 and 20 were obtained by Krishnan (63).

Relationship between Biological Solids Production and Initial Biological Solids Concentration

A plot was made to determine if a general relationship existed between rate of solids increase per hour and initial biological solids concentration. The results are shown in Fig. 38. Sludge production was measured on a weight per hour basis since growth followed zero order kinetics for the experiments plotted in Fig. 38. It is also interesting to note that for these experiments substrate removal also followed zero order kinetics. The dotted line was plotted from Experiments 13 through 16 (Groups V and VI) when the sludge exhibited poor substrate removal capacity and chain-forms of microorganisms were predominating. A plot was also made between initial biological solids concentration and rate of solids increase per hour calculated on a percentage basis; the results are shown in

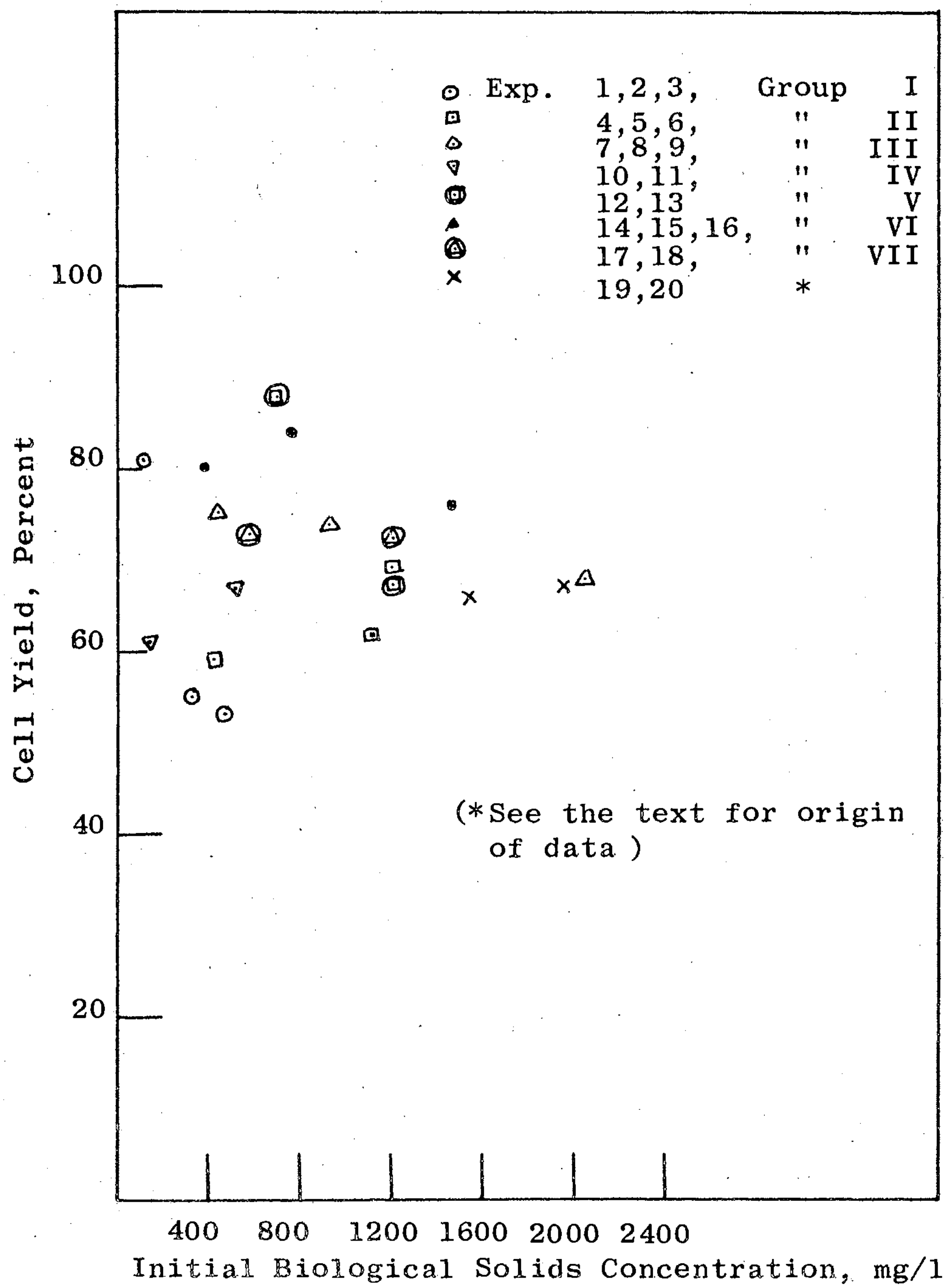

Fig. 37 RELATIONSHIP BETWEEN INITIAL BIOLOGICAL SOLIDS CONCENTRATION AND CELL YIELD (PERCENT)

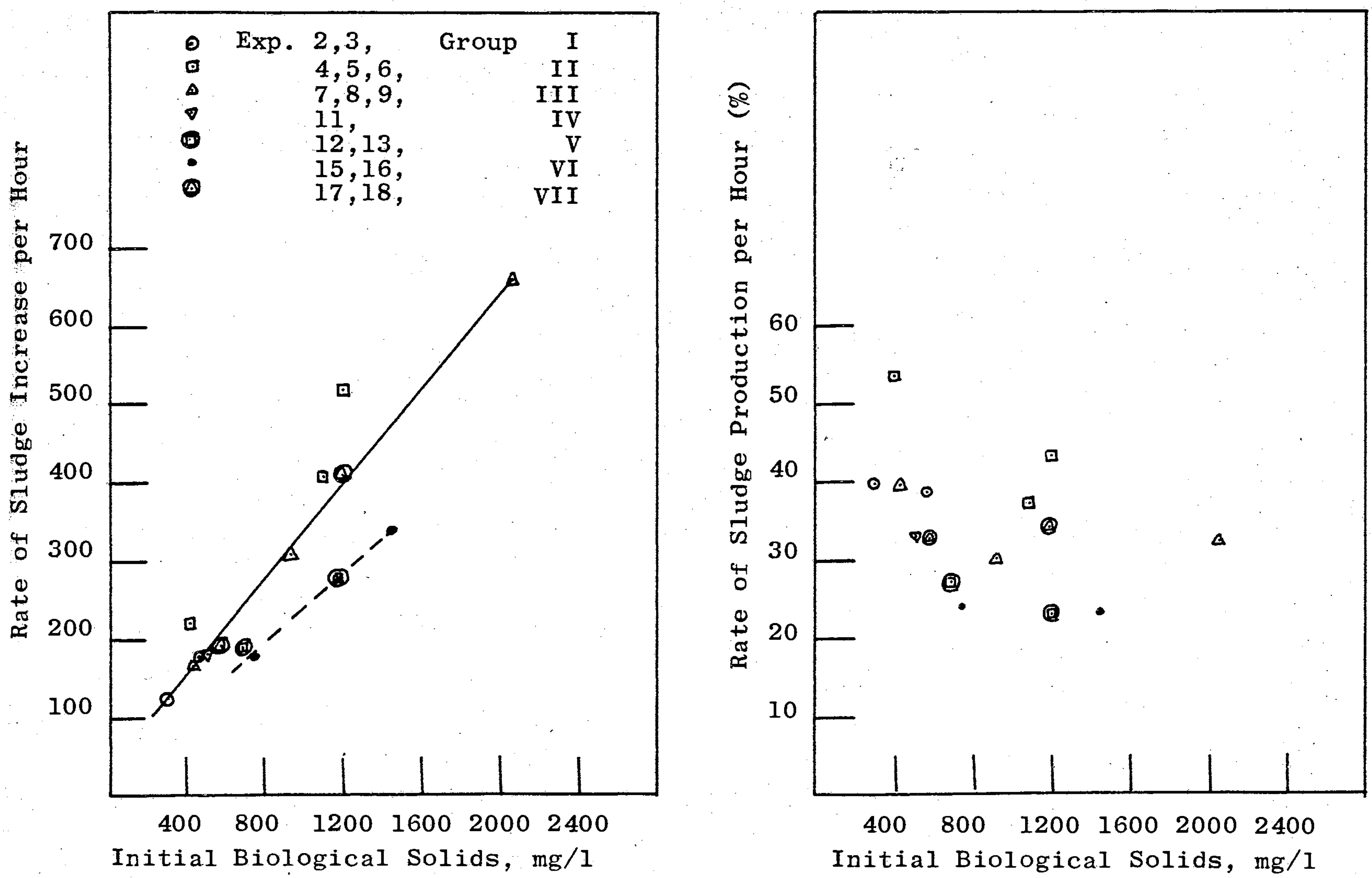

Fig. 38 RELATIONSHIP BETWEEN BIOLOGICAL SOLIDS PRODUCTION AND INITIAL BIOLOGICAL SOLIDS CONCENTRATION

the same figure. It is seen from this figure that no definite relationship existed between the rate of solids increase and initial solids concentration.

Experiments 1, 10 and 14 were the only ones which followed first order kinetics. Accordingly, biological solids doubling times for these experiments were calculated and are reported in Table IIB. It is seen that doubling times for initial solids concentrations of 104, 152 and 375 mg/l were 3.25, 3.50 and 6.50 respectively. It should be noted that chain-forms of microorganisms were predominating when the experiment was made with an initial biological solids concentration of 375 mg/l and a low efficiency of substrate removal was observed at this time. The higher doubling time for this experiment may be correlated to the predominating species present at this time.

Relationship between Substrate channeled into Respiration and Initial Biological Solids Concentration

A plot of percent substrate respired at the point of 95 to 98 percent substrate removal for various initial biological solids concentrations is shown in Fig. 39. The percent substrate respired was taken as the percent theoretical oxygen demand exerted at the time of substrate removal. The data from which this particular plot was made are given in Table IIA. From this figure it can be seen that, in general, lower amounts of substrate were respired at higher initial biological solids concentrations. To cite an example, at the time of substrate removal 16 percent theoretical oxygen demand was exerted for

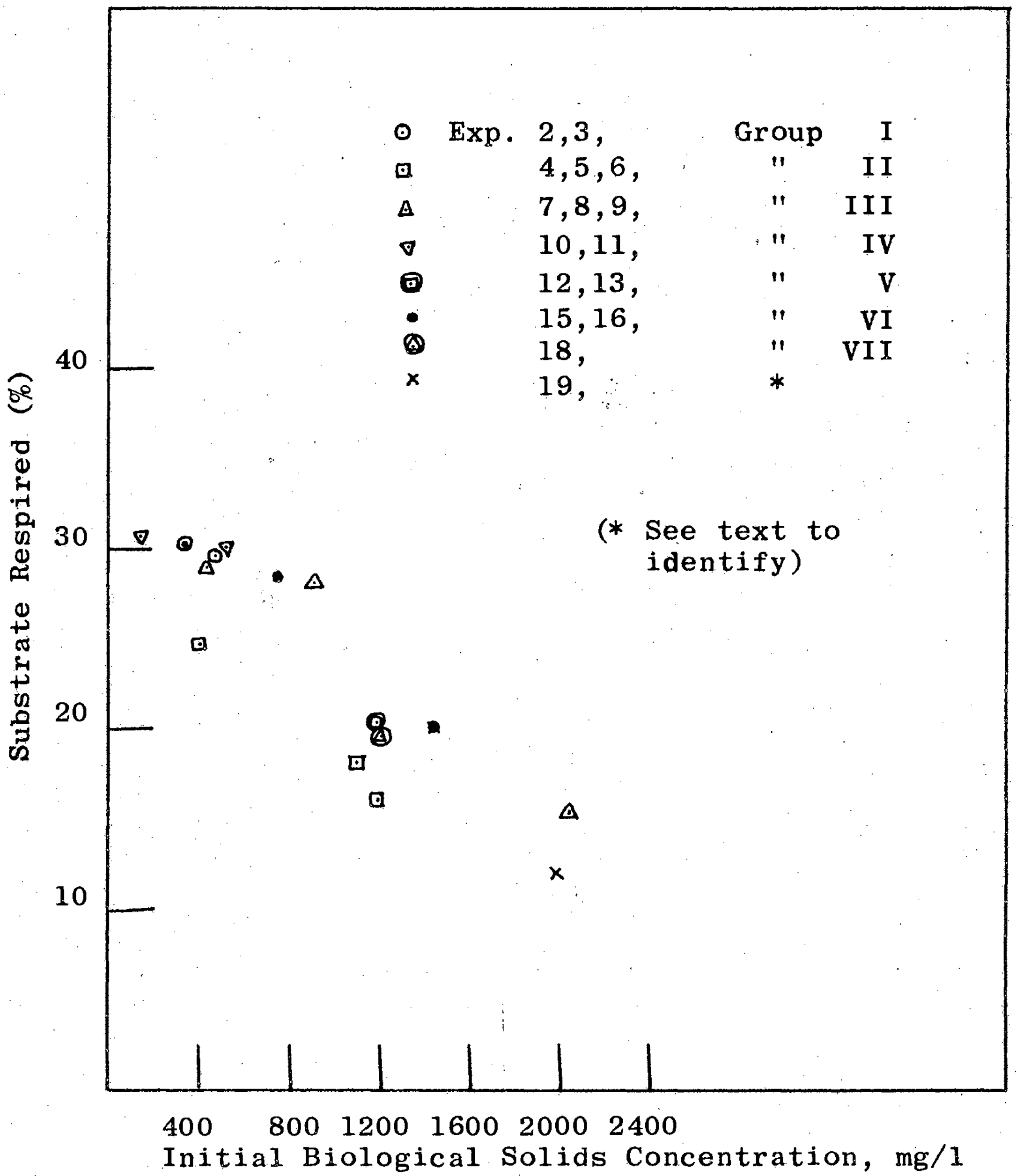

Fig. 39 RELATIONSHIP BETWEEN INITIAL BIOLOGICAL SOLIDS CONCENTRATION AND SUBSTRATE RESPIRED AT 95 to 98% SUBSTRATE REMOVAL

an initial biological solids concentration of 1200 mg/l, whereas 25 percent theoretical oxygen demand was exerted for initial biological solids concentration of 410 mg/l. It is emphasized that this trend holds good, especially when identical sludges are considered. The data for Experiment 20 were obtained by Krishnan (63).

Substrate Recoveries

(a) Material Balance Calculations

The percent recoveries for all experiments were calculated on a weight basis (61) and tabulated in Table IV. Percent recoveries as high as 150 to 180 were observed for some experiments (see Experiments 1 and 10 in Table IV). However, it should be noted that the high percent recoveries were observed only during the initial hours of the experiments. Also, in Experiment 14 (see Table IV) with initial solids concentration of 375 mg/l, percent recoveries as high as 150 to 180 were observed. It should be noted biological solids production in this experiment followed first order kinetics. Percent recoveries as low as 70 were observed for high initial solids concentrations (Experiments 5 and 9; see Table IV); however it was not a general occurrence because 100 percent recoveries were observed in most of the experiments at high initial solids concentrations (Experiments 6, 13, 16 and 18; see Table IV). It may be seen from Table IV that high or low percent recoveries can be correlated with the cell yields. It is also seen from Table IV that cell yields obtained with identical sludge were constant (within

TABLE IV

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/l	Accum. Decrease in COD mg/l	COD cal. as Substrate mg/l *	Solids mg/l	Accum. Increase in Solids mg/l	Accum. O_2 Uptake mg/l	Substrate Respired mg/l *	% Recovery	Cell Yields Percent
Exp. 1									
0	2100	-		104	-	-	-	-	-
1.75	2000	100	94	184	80	37.5	35	122	80
4.75	1830	270	253	305	201	200	187	153	75
6.0	1740	360	337	361	257	285	267	156	72
7.5	1565	535	502	595	491	402	376	173	92
8.75	1420	680	636	687	583	472	442	151	86
10.25	1315	785	735	800	696	528	495	152	89
12.25	1025	1075	1010	941	837	605	567	129	88
14.00	840	1260	1180	-	-	-	-	-	-

* Substrate = oxygen consumption multiplied by $\frac{180}{192}$

TABLE IV (Cont).

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/l	Accum. Decrease in COD mg/l	COD cal. as Substrate mg/l*	Solids mg/l	Accum. Increase in Solids mg/l	Accum. O_2 Uptake mg/l	Substrate Respired mg/l*	% Recovery	Cell Yields Percent
Exp. 2									
0.0	2000	-	-	316	-	-	-	-	-
2.75	1510	490	460	645	329	192	180	110	72
4.75	1090	910	852	895	579	355	333	107	64
6.0	750	1250	1170	1030	714	455	426	98	57
7.5	332	1668	1565	1230	914	528	495	94	55
8.75	55	1945	1820	1390	1074	588	552	90	56
Exp. 3									
0.0	2140	-	-	470	-	-	-	-	-
2.75	1250	890	835	932	462	235	220	82	52
3.75	788	1352	1265	1140	670	330	309	78	50
4.75	552	1588	1490	1340	870	425	398	85	55
6.0	236	1904	1780	1470	1000	510	478	83	53
7.5	87	2053	1920	1490	1020	600	562	83	50
Exp. 4									
0.0	2020	-	-	410	-	-	-	-	-
1.0	1620	400	375	660	250	85	80	88	63
2.0	1335	685	642	885	475	180	169	100	70
3.0	878	1142	1070	1120	710	265	249	90	62
4.0	422	1598	1500	1310	900	345	324	82	57
5.25	121	1899	1780	1540	1130	425	399	86	60

*Substrate = oxygen consumption multiplied by $\frac{180}{192}$

TABLE IV (Cont.)

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/l	Accum. Decrease in COD mg/l	COD cal. as Substrate mg/l*	Solids mg/l	Accum. Increase in Solids mg/l	Accum. O_2 Uptake mg/l	Substrate Respired mg/l *	% Recovery	Cell Yields Percent
Exp. 5									
0.0	1900	-	-	1100	-	-	-	-	-
0.5	1470	430	403	1335	235	45	42	69	55
1.0	1040	860	805	1460	360	95	89	56	42
1.5	702	1198	1125	1850	750	140	131	79	63
2.0	297	1603	1500	1955	855	195	183	69	53
3.0	89	1811	1695	2240	1140	285	267	83	63
Exp. 6									
0.0	1800	-	-	1200	-	-	-	-	-
0.5	1390	410	385	1505	305	35	33	88	75
1.0	945	855	802	1830	630	75	70	88	74
1.5	540	1260	1180	2040	840	115	108	80	67
2.5	138	1662	1560	2380	1180	205	192	88	71
3.0	81	1719	1610	2350	1150	250	234	86	67
Exp. 7									
0.0	2020	-	-	435	-	-	-	-	-
1.75	1720	300	282	795	360	72	68	152	-
2.50	1560	460	432	916	481	125	117	138	-
3.50	1370	650	610	990	555	195	183	121	85
6.25	832	1188	1155	1520	1085	390	366	126	90
8.25	468	1552	1460	1700	1265	490	460	118	82
12.25	111	1909	1790	1915	1480	555	520	112	78

* Substrate = oxygen consumption multiplied by $\frac{180}{192}$

TABLE IV (Cont.)

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/l	Accum. Decrease in COD mg/l	COD cal. as Substrate mg/l*	Solids mg/l	Accum. Increase in Solids mg/l	Accum. O_2 Uptake mg/l	Substrate Respired mg/l*	% Recovery	Cell Yields Percent
Exp. 8									
0.0	1920	-	-	926	-	-	-	-	-
1.0	1575	345	324	1260	334	85	80	128	-
1.5	1350	570	535	1400	474	130	122	110	84
2.0	1230	690	648	1490	564	185	174	114	82
3.0	816	1104	1035	1850	924	295	277	116	84
3.5	666	1254	1175	1960	1034	350	328	116	83
5.25	380	1540	1445	2250	1324	450	422	121	86
Exp. 9									
0.0	1880	-	-	2050	-	-	-	-	-
0.5	1320	560	525	2370	320	40	38	68	57
1.0	800	1080	1010	2690	640	80	75	70	59
1.5	380	1500	1410	3060	1010	120	113	75	68
2.0	278	1602	1500	3180	1130	160	150	79	70
3.5	48	1832	1715	3300	1250	280	262	88	68
Exp. 10									
0.0	2274	-	-	152	-	-	-	-	-
2.5	2054	220	206	287	135	70	66	98	62
5.5	1924	350	328	468	316	215	202	158	90
7.5	1754	520	488	605	453	370	347	164	87
8.5	1654	620	582	632	480	450	422	155	78
12.0	1064	1210	1135	1095	943	615	576	134	78
16.0	298	1976	1850	1670	1518	660	620	116	77
17.0	63	2211	2080	1760	1608	675	632	108	73

* Substrate = oxygen consumption multiplied by $\frac{180}{192}$

TABLE IV (Cont.)

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/1	Accum. Decrease in COD mg/1	COD cal. as Substrate mg/1*	Solids mg/1	Accum. Increase in Solids mg/1	Accum. O_2 Uptake mg/1	Substrate Respired mg/1*	% Recovery	Cell Yields Percent
Exp. 11									
0.0	2214	-	-	515	-	-	-	-	-
1.5	1774	440	412	816	301	120	113	101	69
3.75	1239	975	915	1180	665	305	286	104	68
5.5	792	1422	1330	1460	945	430	404	104	66
7.5	490	1724	1615	1685	1170	540	506	104	68
10.0	121	2093	1960	1970	1455	625	585	104	70
12.0	38	2176	2040	2008	1493	665	625	104	69
Exp. 12									
0.0	2010	-	-	700	-	-	-	-	-
1.0	1790	220	206	855	155	75	70	109	71
2.0	1590	420	394	1020	320	145	136	116	76
3.50	1300	710	665	1320	620	255	239	129	87
6.0	860	1150	1080	1840	1140	425	398	142	95
7.50	558	1452	1360	2080	1380	490	460	135	95
8.75	432	1578	1480	2280	1580	535	501	140	100
Exp. 13									
0.0	1960	-	-	1200	-	-	-	-	-
1.0	1580	380	356	1445	245	90	85	93	65
2.0	1210	750	704	1570	370	175	164	76	50
3.0	790	1170	1100	2050	850	265	248	100	73
3.5	590	1370	1285	2180	980	305	286	99	72
4.75	318	1642	1540	2520	1320	400	375	110	81

* Substrate = oxygen consumption multiplied by $\frac{180}{192}$

TABLE IV (Cont.)

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/l	Accum. Decrease in COD mg/l	COD cal. as Substrate mg/l*	Solids mg/l	Accum. Increase in Solids mg/l	Accum. O_2 Uptake mg/l	Substrate Respired mg/l*	% Recovery	Cell Yields Percent
Exp. 14									
0.0	2220	-	-	375	-	-	-	-	-
4.0	2030	190	178	600	225	110	103	182	118
5.50	1960	260	244	706	331	165	155	198	127
8.50	1600	620	580	1100	725	310	290	157	117
11.50	1220	1000	760	1330	955	485	455	151	96
13.00	1015	1185	1110	1470	1095	550	515	145	93
14.50	820	1400	1310	1620	1245	590	552	137	89
Exp. 15									
0.0	2070	-	-	750	-	-	-	-	-
2.0	1730	340	319	1010	260	105	98	112	77
4.0	1260	810	760	1475	725	235	220	124	90
6.0	980	1090	1020	1730	980	380	356	131	90
8.5	630	1440	1350	2140	1390	475	445	132	97
10.0	363	1707	1600	2440	1690	510	478	129	99
11.5	226	1844	1730	2500	1750	540	506	130	95
Exp. 16									
0.0	1990	-	-	1450	-	-	-	-	-
0.5	1745	245	239	1650	200	50	47	103	82
2.0	1060	930	872	2110	660	195	183	97	71
3.0	645	1345	1260	2440	990	285	267	100	74
4.0	177	1813	1698	2800	1350	360	338	100	75
5.0	73	1913	1790	2870	1420	405	380	100	75

* Substrate = oxygen consumption multiplied by $\frac{180}{192}$

TABLE IV (Cont.)

MATERIALS BALANCE FOR METABOLISM OF GLUCOSE BY GLUCOSE-ACCLIMATED SLUDGE, EXPERIMENTS 1 THROUGH 18

Hrs.	COD mg/l	Accum. Decrease in COD mg/l	COD cal. as Substrate mg/l*	Solids mg/l	Accum. Increase in Solids mg/l	Accum. O_2 Uptake mg/l	Substrate Respired mg/l*	% Recovery	Cell Yields Percent
Exp. 17									
0.0	1800	-	-	580	-	-	-	-	-
1.0	1525	275	258	826	246	50	47	113	90
2.0	1225	575	540	1020	440	125	117	103	77
2.75	1060	740	695	1100	520	180	169	99	71
4.75	700	1100	1030	1420	840	300	281	109	77
5.75	555	1245	1165	1490	910	352	330	107	73
6.75	432	1368	1285	1540	960	395	370	103	70
Exp. 18									
0.0	1760	-	-	1200	-	-	-	-	-
0.5	1470	290	272	1420	220	40	38	95	76
1.0	1180	580	545	1610	410	90	85	91	71
2.0	610	1150	1080	2000	800	190	178	91	70
2.75	196	1564	1465	2310	1110	270	253	93	71
4.75	122	1638	1540	2410	1210	400	375	102	74

* Substrate = oxygen consumption multiplied by $\frac{180}{192}$

10 percent regardless of initial biological solids concentration. This is especially noticeable whenever 100 percent recoveries were observed.

(b) Energy balance calculations

The results of these experiments are shown in Figures 40 and 41. This method has been proposed recently by Gaudy, Bhatla and Gaudy (61). In this method the COD removed in any given time is compared with the summation of oxygen uptake and the COD of the cells produced during this time period. In addition to these parameters, the COD of the mixed liquor was also determined in the present study. The COD of the mixed liquor in any given time period is compared with the summation of COD remaining in the filtrate and the COD of the cells produced during this time period. In these experiments "old" cells were used. The designation of the "cell age" is the same as described by Gaudy, Komolrit and Bhatla (64). The sludge used for the experiment shown in Fig. 40 was obtained from the batch activated sludge unit after 70 days of operation. The microscopic examination of the sludge indicated the presence of bacteria and protozoa as the predominating organisms. In the experiment shown in Fig. 41 the sludge was obtained after 85 days of operation of the batch unit, at which time chain-forms of microorganisms were predominating. Various types of material and energy balances were made for these experiments (see Table V). Percent recoveries calculated on the basis of weight calculations and energy balance methods gave better percent recoveries

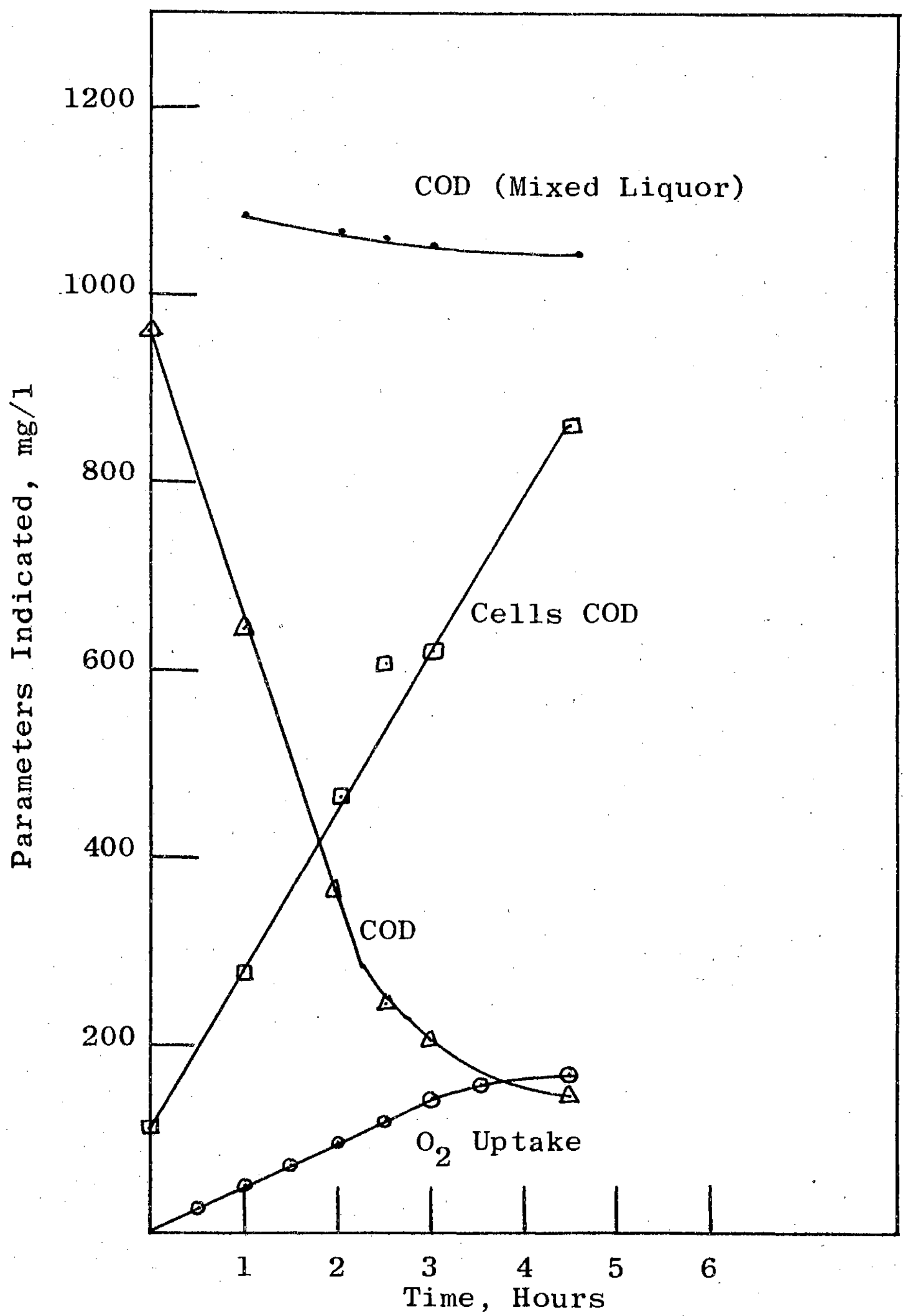

Fig. 40 ENERGY BALANCE ON GLUCOSE ACCLIMATED CELLS after 70 Days' Operation of the Batch Unit

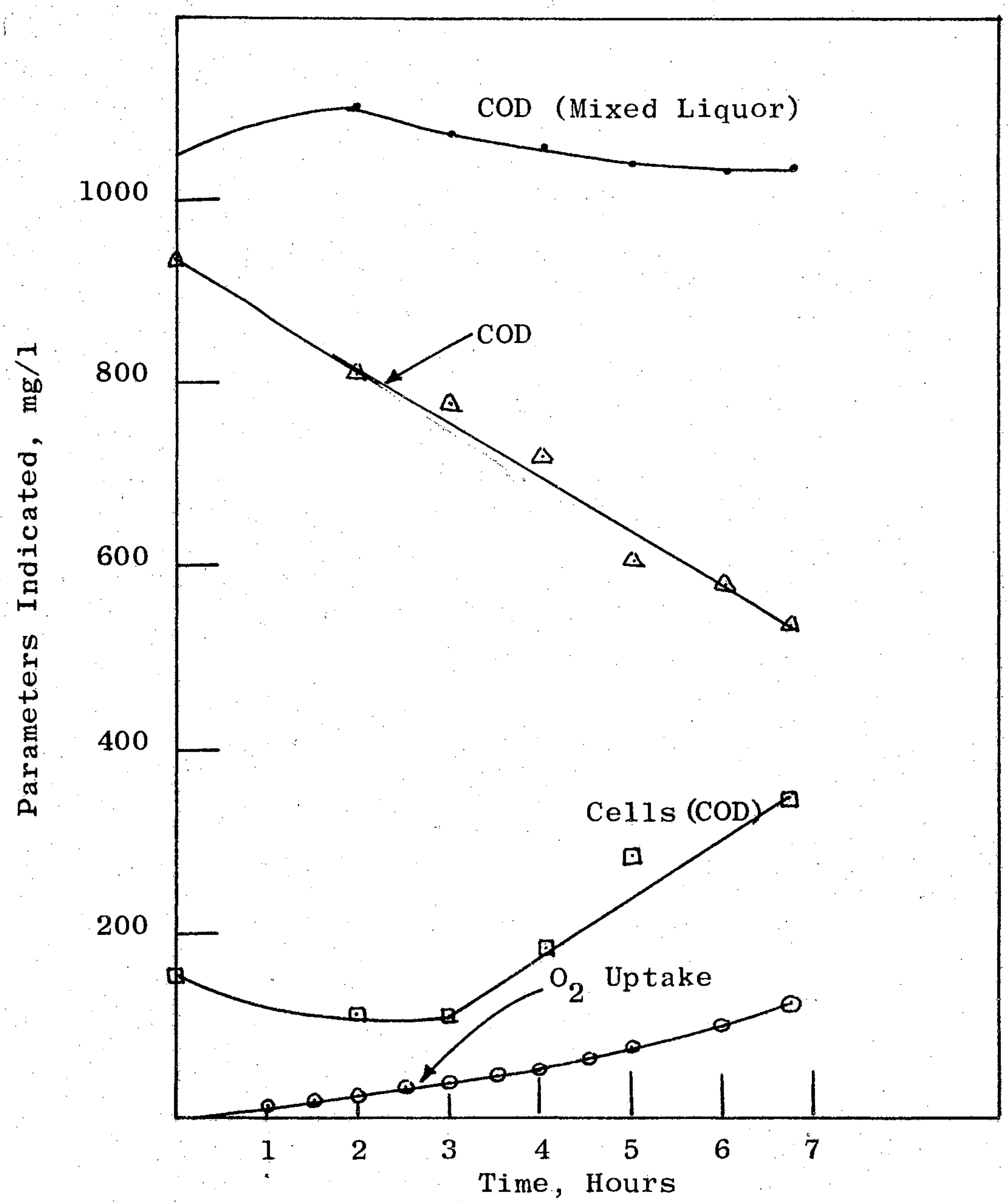

Fig. 41 ENERGY BALANCE ON GLUCOSE ACCLIMATED CELLS after 85 days of operation of the batch unit

TABLE V

SUBSTRATE RECOVERIES USING VARIOUS METHODS OF COMPUTATION
(GLUCOSE-ACCLIMATED CELLS)

Time Hrs.	COD Remaining mg/l	Cells mg/l	O_2 Uptake mg/l	COD of Cells mg/l	Δ COD mg/l	Δ Cells mg/l	Δ COD Cells mg/l	COD MLSS	Calculated Substrate Recovery % I	II	III	IV	V
0.0	965	396	-	100	-	-	-		-	-	-	-	-
1.0	642	600	45	372	323	204	272	1080	82	100	102	98	86
2.0	369	816	95	566	596	420	466	1070	91	112	115	95	78
2.5	242	936	120	705	723	540	605	1060	96	118	122	100	80
3.0	200	960	140	715	765	554	615	1050	96	117	121	99	77
4.5	144	972	175	960	821	576	860	1040	97	117	121	124	97
0.0	930	126	-	152	-	-	-	1050	-	-	-	-	-
2.0	810	218	20	378	120	92	113	1100	98	120	125	110	86
3.0	780	250	35	370	150	124	109	1070	111	135	140	95	84
4.0	720	283	50	525	210	157	187	1060	103	125	129	111	86
5.0	608	330	78	740	322	204	294	1040	92	110	116	113	86
6.0	586	405	100	570	344	279	209	1030	108	129	142	91	87
6.75	536	422	115	850	394	296	349	1040	102	123	135	115	86

I Materials balance, weight calculation
II Materials balance, carbon calculation
III Energy balance, based on empirical formula for composition of activated sludge
IV Energy balance, based on measurement of cell COD
V Energy balance, based on measurement of mixed liquor COD

than calculations based on empirical formulae and carbon balance methods. It can be seen from Table V that calculations based on mixed liquor COD gave somewhat low percent recoveries; however, the range of 80 to 85 percent is acceptable for many experimental applications, and this new method of making an energy balance for aerobic systems seems worthy of further investigation.

CHAPTER IV

DISCUSSION

High concentrations of carbohydrate wastes have been thought to permit the growth of the filamentous organism Sphaerotilus and cause sludge bulking. The existence of a bulking sludge is usually manifested by enormous increase in Sludge Volume Index. The problem involved is that the sludge does not settle in the final clarifiers; however, the results herein presented show that a COD loading of 1.06 lb COD/day/lb mixed liquor suspended solids can be accommodated without bulking. This represents a high carbohydrate loading. Ingols and Heukelekian (4)(5) pointed out that at high glucose concentrations, when excessive amounts of suspended solids concentrations were present in relation to feed concentration, and with abundant supply of nitrogen source, sludge bulking may not occur for a relatively long period. They claim that Sphaerotilus can predominate with an insufficient supply of nitrogen, whereas Zoogleal forms must have an adequate nitrogen source to build up new protoplasm with the glucose present. Thus, the presence of an abundant source of nitrogen offers the possibility of overcoming filamentous organisms. In the present study a COD-to-N ratio of 10:1 and the solids-to-feed ratio of approximately 1:1 was maintained. Therefore, the findings herein reported are in general agreement with the observations of Ingols and Heukelekian with respect to bulking.

In the present study a Sludge Volume Index of 120 to 150 was observed throughout the experimental period. However, the present study provides information that Sludge Volume Index is not a good measure of settling characteristics of sludge. It is seen from Fig. 3 (see arrows on the biological solids curve at the beginning of the aeration cycle), that the sludge exhibited poor settling characteristics on some days, and the settling tests made at this time indicated that 140 ml of sludge settled in an hour in the one liter measuring cylinder. A settling test was also made when the sludge showed excellent settling in the batch control unit; but only 50 ml of sludge settled in an hour in the one liter measuring cylinder used for the settling test. It should be noted that the diameter of the batch control unit was approximately four times the diameter of the one liter measuring cylinder. Clifford and Windridge (65) stated that the diameter of the settling vessel did not affect the settling rate of various solids concentrations; however, they mentioned that activated sludge settles more rapidly in wide than in narrow cylinders, which was assumed to be due to surface effects on the wall. They found that conditioning sludge with tap water enhanced the settling characteristics of sludge. Rudolfs and Lacy (66) noted retarded settling rates when the solids concentration increased from 800 to 5000 mg/l, and they also observed that the diameter of the vessel did not affect the percent volume occupied by a given sludge after 30 minutes of settling. The results of the settling curves for various solids concentrations which were obtained by diluting with tap water (see Fig. 14) indicate that retarded settling occurs with increased solids concentrations as observed by Rudolfs and Lacy (66). The results of the present study also indicated that the vessel diameter may affect the settling rate of the sludge in agreement with Clifford and Windridge (65). However, it should be noted that whenever severe bulking occurred, neither the Sludge Volume Index nor the vessel diameter could be related to the settling properties of the sludge. A similar observation was made by Isenberg and Heukelekian (67), and they emphasized that sludge density is an important factor in assessing the settling characteristics of sludge. The phenomenon of bulking can be neither estimated by Sludge Volume Index nor correlated to vessel diameter, but may be due to either a change in the predominance of the species or rapid buildup of the polysaccharide which causes the sludge to bulk temporarily, as stated by Kraus (7).

Higher BOD loadings have been thought to reduce the efficiency of treatment (9)(10)(11) in activated sludge treatment processes; however, in the present study it can be seen from Figures 3 through 8 that 95 to 98 percent COD removal was achieved with a loading of 1.06 lb COD/day/lb mixed liquor suspended solids. It is emphasized that in the present study any loss in COD removal efficiency due to nonsettleability of the sludge was not measured since effluent COD was measured only on membrane filtrate. An enormous increase in sludge growth for carbohydrate wastes was pointed out by Ruchhoft (28). In the present study sludge yields of 65 to 85 percent were observed (see Figures 3 through 8).

The significance of acclimation has been emphasized by several investigators (28)(31)(32)(33)(34)(35). It was seen from Figures 3 and 4 that 95 percent efficiency of substrate removal was achieved in 4.0 hours after 15 days of operation of the batch unit, and 96 percent efficiency of substrate removal was achieved in 2.5 hours after 25 days of operation of the batch unit. However, acclimation over a relatively long period can bring about tremendous changes in predominance of the species as measured by both physical appearance and biochemical activities of the sludge. The sludge showed the normal brownish appearance for the beginning 35 days of operation, and became dark in appearance after 45 days of operation of the batch unit. Smith (1) observed the same phenomenon employing high glucose concentrations, and could overcome it by stopping the loading (omission of glucose) temporarily. In the present study the glucose loading was never stopped, and the sludge regained its normal brown appearance after 60 days of operation and again turned dark after 75 days of operation of the batch unit. The pH of the sludge was maintained between 6.6 to 6.8. The results may be considered as simply due to natural changes of predominance of the species until further search of the causes of these changes are investigated.

The results obtained in the partition between synthesis and respiration studies may be analyzed in light of the studies of several investigators. McWhorter and Heukelekian (68) have recently investigated the effect of varying seed and substrate concentrations on substrate removal and cell yields, using glucose as the soluble organic waste. Even though the cell yields they observed were relatively low compared to those reported in the literature, cell yields remained consistent for varying seed and substrate concentrations. In the present study seed concentration varied from 104 to 2050 mg/l, using glucose concentration of approximately 2000 mg/l. The cell yields were shown in Table IIB. It was seen that they are fairly consistent when identical sludge was considered. A wide variation in cell yields for carbohydrate wastes has been observed by several investigators. Sawyer (27) reported 44 to 64 percent, and Plack et al (28) observed 65 to 85 percent for carbohydrate wastes. In the present study high cell yields are correlated to higher unit activity of the sludge. A sludge of unit activity below 11 mg O_2/hr/gm-sludge gave a cell yield of 53 to 75 percent, and a sludge of unit activity above 12 mg O_2/hr/gm-sludge gave a cell yield of 72 to 88 percent. These results emphasize that cell yields mainly depend upon the biochemical activities of the sludge. When dealing with heterogeneous populations the biochemical activities of the predominating species should be considered in making any comparison of cell yields for any particular waste.

The cell yields were constant during the growth phase, and are shown in Table IV. These results are in general accord with the findings of McWhorter and Heukelekian (68).

The amount of substrate channeled into respiration was one of the main interests of the present study. The percent theoretical oxygen demand exerted for various initial biological solids was shown in Fig. 39 (see Results chapter). It was seen from this figure that percent theoretical oxygen demand exerted was above 25 at the point of substrate removal for initial biological solids concentrations below 900 mg/l, whereas it was below 20 for initial solids concentrations of above 1100 mg/l. A similar observation was made by McWhorter and Heukelekian (68). They reported that 27.5 percent theoretical oxygen demand was exerted using an initial activated sludge seed of 364 mg/l and 21 percent theoretical oxygen demand for an initial activated sludge seed of 1010 mg/l. The low amount of theoretical oxygen demand exerted, using high initial activated sludge seed concentrations may be accounted for as either storage or absorption, since adsorption is not observed for glucose, according to Rucchoft et al (32), and Gaudy and Englebrecht (62). These results further indicate that in the presence of high concentrations of activated sludge seed the demand of oxygen will be exerted even after the exhaustion of substrate, confirming the findings of Grant et al (24).

It is interesting to note that in all 18 experiments performed to study partition between synthesis and respiration for various initial biological solids concentrations, 95 to 98 percent COD removal was achieved. Recently McWhorter and Heukelekian (68) reported that a constant percent (5 to 15) of residual COD remained in the supernatant. They felt that this finding indicated the presence of dissolved organic matter. The present study emphasizes that 95 to 98 percent COD removal can be achieved employing initial biological solids concentrations as high as 2100 mg/l (studies made on partition between synthesis and respiration), and 5000 mg/l (batch activated sludge control unit).

The percent theoretical oxygen demand exerted at the time of glucose removal was also studied recently by McWhorter and Heukelekian (68). They showed that 18 percent theoretical oxygen demand was exerted regardless of initial seed concentration. To the contrary, the results of the present study indicated a definite relationship between percent theoretical oxygen demand and initial biological solids concentrations as shown in Fig. 42. Curve 1 represents the results of Group III experiments. It can be seen that 18 percent theoretical oxygen demand was exerted at the time of glucose removal for initial solids concentrations of 410 mg/l, whereas 10 and 9 percent theoretical oxygen demand was exerted at the time of glucose removal for initial solids concentrations of 1100 and 1200 mg/l. Curve 3 represents the results of Group V and VI experiments, at which time the substrate removal capacity of the sludge was affected tremendously due to the presence of chain-forms of microorganisms. Again, it can be seen that 26 percent theo-

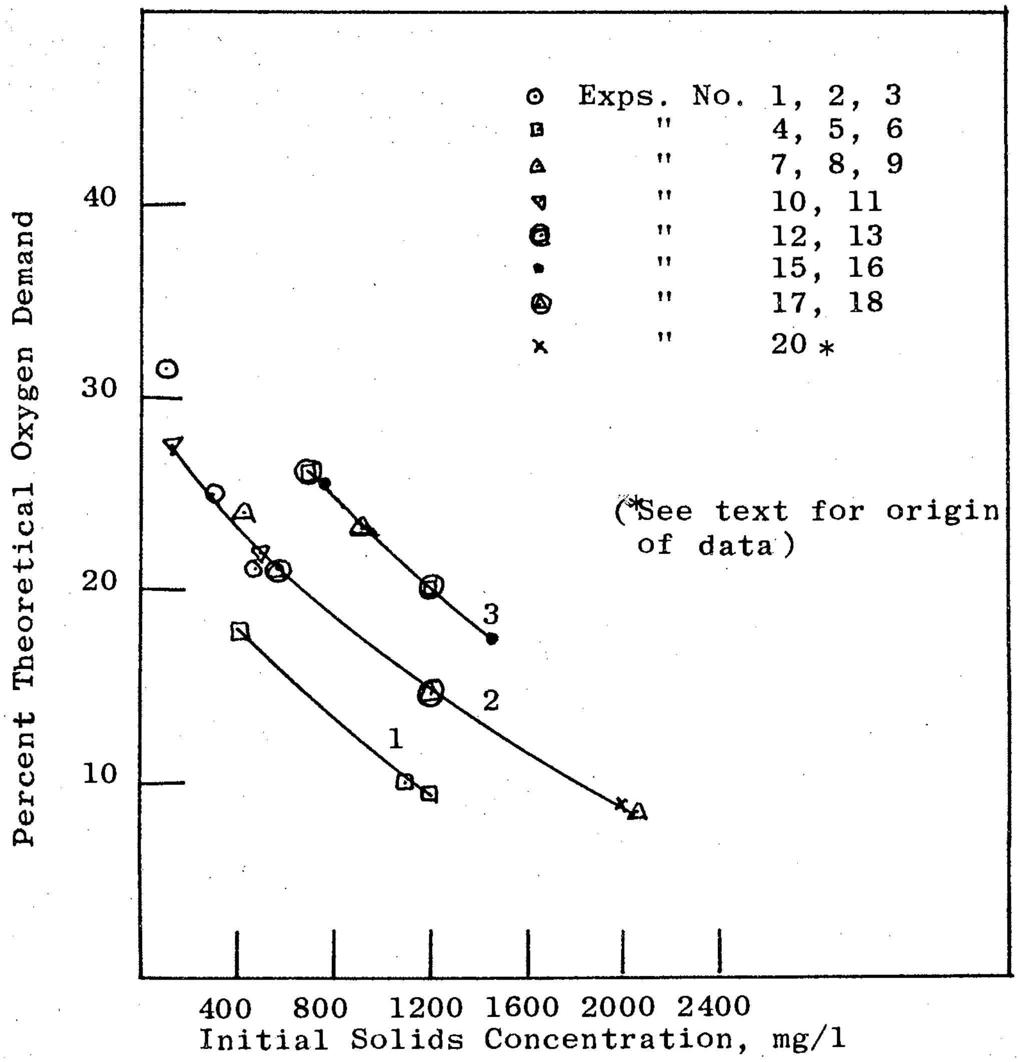

Fig. 42 RELATIONSHIP BETWEEN INITIAL BIOLOGICAL SOLIDS CONCENTRATION AND PERCENT THEORETICAL OXYGEN DEMAND EXERTED AT GLUCOSE REMOVAL

retical oxygen demand was exerted for initial solids concentration of 700 mg/l, whereas 20 and 17 percent theoretical oxygen demand was exerted for initial solids concentrations of 1200 and 1450 mg/l respectively. Curve 2 was obtained for experiments in Groups I, III, IV and VII. Again, the same general trend was observed. The data for Experiment 20 was obtained by Krishnan (63).

Graphs of percent COD removal against time have been shown in Figures 33 through 35 (see Results chapter), and in general it was found that high initial biological solids concentrations remove substrate at a faster rate than low initial biological solids concentrations. Ruchhoft et al (31) observed the similar type of relationship, and they pointed out the possibility of applying the Freundlich adsorption isotherm since increasing rates of glucose removal were observed for increasing quantities of sludge after the first hour of contact. In the present study all of these experiments except Experiments 1, 10 and 14 followed zero order kinetics with respect to substrate removal. The results are in agreement with Eckenfelder's conclusion (50), i.e., when the increase in solids (ΔS) is less than the initial solids concentration (S), zero order kinetics may be applied. The results of Experiments 1, 10 and 14 followed first order kinetics of sludge growth and substrate removal. It is further noted that two distinct phases are seen as pointed out by Eckenfelder (50). The biological solids doubling times were calculated and reported in Table IIB. It can be seen that there was no distinct change in the doubling times for initial biological solids concentrations of 104 and 152 mg/l; however, the doubling time for initial biological solids concentration of 375 mg/l is much greater in comparison to initial biological solids concentrations of 104 and 152 mg/l. This may be attributed to the predominating species present at that time.

Different straight line relationships were observed between the percent rate of substrate removal and initial biological solids concentration (see Fig. 36, Results chapter). These relationships were obtained when sludge was taken from the batch unit at different times to study partition between synthesis and respiration. Ruchhoft et al (31) observed similar relationship for activated sludge using glucose as the carbon source; however, they observed only one simple straight line relationship. This might have been obtained using identical sludge. The different relationships shown in the present study using sludges with different biochemical activities should be considred in present treatment plant design consideration. This type of natural cyclic change in the predominating species should be thoroughly investigated before making any definite conclusions.

A plot of the biological sludge production rate per hour vs initial biological solids concentration (see Fig. 38) indicates that the rate of sludge growth per hour was of increasing order with the initial biological solids concentrations. The percent rate of sludge production for various initial biological solids concentrations plotted in the same figure indicate that there was no definite relationship. From this data it is interesting to see that substrate was either used for new cell production or absorbed in some state, and suggests that there was no maintenance requirement up to initial biological solids concentrations of 2050 mg/l; however, this aspect warrants further study.

In the present studies endogenous respiration was taken as an index of biochemical activities of the sludge. This parameter was also employed by Ruchhoft et al (23)(25). They classified sludge which had an endogenous respiration rate above 10 mg O_2/hr/gm-sludge as having poor biochemical activity. Logan (12) observed an endogenous respiration rate of 6.0 to 14.0 mg O_2/hr/gm-sludge in the loading range of 0.2 to 0.5 lb/BOD/day/lb sludge. The present study does confirm that endogenous respiration rates above 12.0 mg O_2/hr/gm-sludge are a poor index of biochemical activities of the sludge; however, it should be noted that the higher endogenous respiration rates cannot be attributed to the properties of a bulky sludge.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. When a batch unit was operated on a 24-hour feeding cycle an initial biological solids concentration of 4600 to 5300 mg/l was developed, feeding regularly the pure organic compound glucose at a concentration of 5300 to 5600 mg/l using the following conditions: pH 6.6 to 6.8, temperature 22 to 24^{o}C, COD to N ratio 10:1. The changes in predominance of the species which can occur at this loading (1.06 lb COD/day/lb sludge) appears to be tremendous.

2. A wide variation in the cell yields (55 to 88 percent) was observed; however, identical sludges (sludges in the same group) gave almost identical cell yields. The cell yields measured during the growth phase were constant.

3. The percent theoretical oxygen demand exerted was above 25 for initial biological solids concentrations below 900 mg/l; whereas it was below 20 for initial biological solids concentrations above1100 mg/l at the time of substrate removal.

4. The percent theoretical oxygen demand exerted at the time of glucose removal varied over a wide range for various initial biological solids concentrations, and a definite trend was observed. In general 8 to 20 percent theoretical oxygen demand was exerted for initial biological solids concentrations above 1100 mg/l; whereas above 20 percent theoretical oxygen

demand was exerted for initial biological solids concentrations below 900 mg/l at the time of glucose removal.

5. The rate of substrate removal is of increasing order with increasing initial solids concentrations. The kinetics of sludge growth and substrate removal followed zero order with initial biological solids in the range 300 to 2050 mg/l (except in one experiment) when the initial glucose concentration used was in the vicinity of 2000 mg/l.

6. Different linear relationships were observed between percent substrate removal per hour and initial biological solids concentrations. The relationships studied thus far hold good up to an initial biological solids concentration of 2050 mg/l, using an initial glucose concentration in the vicinity of 2000 mg/l.

7. The results of sludge production per hour for varying initial biological solids concentrations (300 to 2050 mg/l) indicated no maintenance requirement of the sludge.

8. The oxygen uptake measured in the absence of nutrients can be taken as a parameter to classify the biochemical activities of the activated sludge. High endogenous respiration rates do not necessarily indicate bulking sludge, but sludges with endogenous respiration rates above 12 mg O_2/hr/gm-sludge, measured in the first two hours, may be considered as having poor efficiency of substrate removal.

9. The substrate recoveries calculated on the basis of energy balance (64) and weight basis (61) gave closer to 100

percent recoveries. The modified method proposed in the present study (COD on the mixed liquor) also gave reasonable percent recoveries to check the experimental techniques.

SUGGESTIONS FOR FURTHER WORK

The different relationships observed between the percent rate of substrate removal and initial biological solids concentrations indicate the need for caution in applying many of the prevalent design calculations employed for designing treatment facilities. It seems that tremendous changes in predominance of the species are invariable when high carbohydrate concentrations are employed. It would be highly desirable to see whether these changes occur at low carbohydrate loadings when sludge is taken at different times to establish relationships between initial biological solids concentrations and the percent rate of substrate removal. At the same time the relationship between synthesis and respiration can also be studied, using sludge developed by high and low concentrations of glucose. Similar studies may also be made in continuous flow systems to gain better understanding of the effect of higher loadings.

A SELECTED BIBLIOGRAPHY

1. Smith, J. S., "A Study of the Conditions Favoring Bulking of Activated Sludge," Sewage Works Jour., 4, 961 (Nov. 1932).

2. Smith, J. S., "Bulking of Activated Sludge. II. On the Causative Organisms," Sewage Works Jour., 6, 1041 (Nov. 1934).

3. Ruchhoft, C. C. and J. H. Watkins, "Bacteriological Isolation and Study of the Filamentous Organisms in the Activated Sludge of the Des Plaines River," Sewage Treatment Works, 1, 52 (Oct. 1928).

4. Ingols, R. S. and H. Heukelekian, "Studies on Activated Sludge Bulking I. Bulking of Sludges by Means of Carbohydrates," Sewage Works Journal, 11, 9-27, (Nov. 1939).

5. Ingols, R. S. and H. Heukelekian, "Studies on Activated Sludge Bulking III. Bulking of Sludge Fed with Pure Substances and Supplied with Different Amounts of Oxygen," Sewage Works Journal, 12, 849 (Sept. 1940).

6. Okun, D. A., "Systems of Bio-precipitation of Organic Matter from Sewage," Sewage Works Journal, 21, 5, (Sept. 1949).

7. Kraus, L. S., "Combined Treatment of Industrial and Domestic Wastes," Sewage and Ind. Wastes, 30, 199, (Feb. 1958).

8. McKinney, R. E. and M. P. Horwood, "Fundamental Approach to the Activated Sludge Process I. Floc Producing Bacteria," Sewage and Ind. Wastes, 24, 117 (Feb. 1952).

9. McKinney, R. E., "Activity of Micro-organisms in Organic Waste Disposal II. Aerobic Processes," Applied Microbiology, 5, 167 (1957).

10. Greeley, S. A., "The Development of the Activated Sludge Method of Sewage Treatment," Sewage Works Journal 17, 1137 (Nov. 1945).

11. Greeley, S. A., "High Rate Biological Sewage Treatment," Sewage Works Journal, 15, 1069 (Nov. 1943).

12. Logan, R. P. and W. E. Budd, "Effects of BOD loadings on Activated Sludge Plant Operation," Biological Waste Treatment, Pergamon Press, New York (1955).

13. Bloodgood, D. E., "Studies of Activated Sludge Oxidation at Indianapolis," Sewage Works Journal, 10, 26 (Jan. 1938).

14. Butterfield, C. T., C. C. Ruchhoft and P. D. McNamee, "Studies of Sewage Purification. VI. Biochemical Oxidation of Sludge Developed by Pure Cultures of Bacteria isolated from Activated Sludge," Sewage Works Journal 9, 173 (March 1937).

15. Goldthorpe, H. H., "Some Experiments on the Oxygen Requirements on Activated Sludge," Inst. Sewage Purification, 1, 142 (1936)

16. Grant, S., H. Hurwitz and F. W. Mohlman, "The Oxygen Demand of an Activated Sludge," Sewage Works Jour. 2, 228 (April 1930).

17. Heukelekian, H. and R. S. Ingols, "Studies on the Clarification Stage of Activated Sludge Process. III. Carbon Dioxide Production during the Clarification and Oxidation Stage of Activated Sludge," Sewage Works Journal, 9, 717 (Sept. 1937).

18. Kessler, L. H. and M. L. Nichols, "Oxygen Utilization by Activated Sludge," Sewage Works Jour. 7, 810 (Sept. 1935).

19. McNamee, P. D., "Oxidation of Sewage by Activated Sludge," Sewage Works Journal, 8, 562 (July 1936).

20. Sawyer, C. N., "Activated Sludge Oxidation. III. Factors Involved in Prolonging Initial High Rate of Oxygen Utilization by Activated Sludge Sewage Mixtures," Sewage Works Journal, 11, 595 (July 1939).

21. Ingols, R. S., "Oxidation-Reduction Enzymes in Activated Sludge," Sewage Works Journal, 12, 862 (Sept. 1940).

22. Jenks, H. L. and Levine, "A Stream Flow Sewage Treatment Process," Eng. News-Record, 100, 808 (Nov. 1928).

23. Ruchhoft, C. C., P. D. McNamee and C. T. Butterfield, "Studies of Sewage Purification. VII. Biochemical Oxidation of Activated Sludge," Pub. Health Reports, 1690 (Sept. 1938).

24. Grant, S., E. Hurtwitz and F. W. Mohlman, "The Oxygen Requirements of the Activated Sludge Process," Sewage Works Journal, 2, 228 (April 1930).

25. Ruchhoft, C. C. and D. C. Smith, "Studies of Sewage Purification IX. Biochemical Oxidation of Activated Sludge," Public Health Reports, 54, 924 (1939).

26. Sawyer, C. N. and M. L. Nichols, "Activated Sludge Oxidation. II. A Comparison of Oxygen Utilization by Activated Sludge Obtained from Four Wisconsin Municipalities," Sewage Works Journal, 11, 462 (May 1939).

27. Sawyer, C. N., "Bacterial Nutrition and Synthesis," Biological Treatment of Sewage and Ind. Wastes, Reinhold Publishing Co., New York (1955).

28. Plack, O. R. and C. C. Ruchhoft, "Studies of Sewage Purification. XVII. The Utilization of Organic Substrates by Activated Sludge," Sewage Works Journal, 19, 423 (May 1947).

29. Gaudy, A. F. Jr., "Biochemical Aspects of Qualitative Shock Loading of Aerobic Waste Treatment Systems," Ph.D. Thesis, University of Illinois, 1959.

30. Smith, D., "Aerobic Biological Stabilization of Organic Substrates," Sewage Works Journal, 24, 107 (Sept. 1952).

31. Ruchhoft, C. C., J. F. Kachmar and W. A. Moore, "Studies of Sewage Purification. XI. The Removal of Glucose from Substrates by Activated Sludge," Sewage Works Journal, 12, 27 (Jan. 1940).

32. Ruchhoft, C. C., J. F. Kachmar and O. R. Plack, "Studies of Sewage Purification. XII. Metabolism of Glucose by Activated Sludge," Sewage Works Journal, 12, 485 (May 1940).

33. Gellman, I. and H. Heukelekian, "Biological Oxidation of Formaldehyde," Sewage and Ind. Wastes, 22, 1321 (Oct. 1950).

34. Busch, A. W. and N. Myrick, "BOD Progression in Soluble Substrates. III. Short-term BOD and Bio-oxidation Solids Production," Presented at the 15th Annual Industrial Waste Conference, Purdue University, (May 1960).

35. Mills, E. J. Jr. and V. T. Stack, "Acclimation of Micro-organisms for the Oxidation of Pure Organic Chemicals," Presented at the 9th Annual Industrial Waste Conference, Purdue University (May 1954).

36. Engelbrecht, R. S. and R. E. McKinney, "Activated Sludge Cultures Developed in Pure Organic Compounds," Sewage Works Journal, 29, 1350 (Dec. 1957).

37. Hicks, R. and G. E. P. Boxe, "Rate of Solution of Air and Rate of Transfer of Sewage Treatment of Activated Sludge Process," Sewage Purification, 1, 271 (March 1939).

38. Smith, D. B., "Measurement of the Respiratory Activity of Activated Sludge," Sewage and Ind. Wastes, 25, 767 (July 1953).

39. Gaudy, A. F. Jr. and B. G. Turner, "Effect of Air Flow Rate on Response of Activated Sludge to Quantitative Shock Loading," Proceedings 17th Industrial Waste Conference, 136, Purdue University (1962).

40. Okun, D. A. and W. A. Lynn, "Preliminary Investigation into the Effect of Oxygen Tension on Biological Sewage Treatment," Biological Waste Treatment of Sewage and Ind. Wastes, Reinhold Publishing Co., New York 1, 192 (1955).

41 Keefer, C. E. and J. Meisel, "Activated Sludge Studies - Efficiency and Performance Under Various Operating Conditions," Sewage Works Journal, 22, 1518 (Dec. 1950).

42. McKinney, R. E., "Microbiology for Sanitary Engineers," McGraw-Hill, New York (1960).

43. Gehm, H. W., "Activated Sludge at High Temperatures and High pH Value," Biological Waste Treatment of Sewage and Ind. Wastes, Reinhold Publishing Co., 1, 352 (1955).

44. Hurley, J., "Winter Difficulties at Sewage Disposal Works," Surveyor, 104, 659 (1945).

45. Sawyer, C. N. and G. A. Rohlich, "Activated Sludge Oxidations. IV. The Influence of Temperature Upon the Rate of Oxygen Utilization by Activated Sludge," Sewage Works Journal, 11, 946 (Nov. 1939).

46. Imhoff, K. and G. M. Fair, "Sewage Treatment," John Wiley & Sons, New York (1940).

47. Okun, D. A., "A System of Bio-precipitation of Organic Matter from Sewage," Sewage Works Journal, 21, 763 (Sept. 1949).

48. Dawson, P. S. S. and S. H. Jenkins, "The Oxygen Requirements of Activated Sludge Determined by Manometric Methods," Sewage Works Journal, 21, 643 (July 1943).

49. Whitehead, H. C. and F. R. O'Shaughnessy, "Improving the Efficiency of Activated Sludge," Surveyor, 89, 407 (1936).

50. Eckenfelder, W. W. and D. J. O'Connor, "Principles of Biological Oxidation," Pergamon Press, New York (1961).

51. Gellman, I. and H. Heukelekian, "Studies of Biochemical Oxidation by Direct Methods. III. Oxidation and Purification of Industrial Wastes by Activated Sludge," Sewage and Ind. Wastes, 25, 1196 (Oct. 1953).

52. Torpey, W. N. and A. H. Chasick, "Principles of Activated Sludge Operation," Sewage and Ind. Wastes, 27, 1217 (Nov. 1955).

53. Gaudy, A. F. Jr., M. N. Bhatla and F. Abu-Niaaj, "Studies on the Occurrence of the Plateau in BOD Exertion. I. Heterogeneous and Pure Culture Seeds with Various Concentrations of Glucose as Carbon Source," Submitted to J.W.P.C.F. (1963).

54. Gaudy, A. F. Jr. and M. N. Bhatla, "Studies on the Occurrence of the Plateau in BOD Exertion. II. Experiments using Different Carbohydrates and Multi-Component Systems," Submitted for Publication.

55. Gaudy, A. F. Jr., M. N. Bhatla and F. Abu-Niaaj, "Studies on the Occurrence of the Plateau in BOD Exertion," 18th Industrial Waste Conference, Purdue University (May 1963).

56. Follett, R. H. and A. F. Gaudy, Jr., "Studies on the Occurrence of the Plateau in BOD Exertion. III. Simple and Complex Wastes with Various Seed and Substrate Concentrations," Presented at the 14th Oklahoma Industrial Waste Conference (Nov. 1963).

57. Bhatla, M. N. and A. F. Gaudy, Jr., "Studies on the Causation of Phasic Oxygen Uptake in High Energy Systems," 19th Purdue Industrial Waste Conference, Purdue University (May 1964).

58. Gaudy, A. F. Jr., "Colorimetric Methods for the Determination of Protein and Carbohydrate Content of Biological Sludges," Ind. Water and Wastes 7, 17 (1962).

59. Standard Methods for the Examination of Water and Wastewater, 11th Ed., A.P.H.A., N. Y., 1960.

60. Umbreit, W. W., R. H. Burris and J. F. Stauffer, "Manometric Techniques," Burgess Pub. Co., Minneapolis, Minnesota (1957).

61. Gaudy, A. F. Jr., M. N. Bhatla and E. T. Gaudy, "The Use of Experimentally Determined COD Values of Bacterial Cells in Making Energy Balances for Waste Water Purification Studies," Submitted for Publication.

62. Gaudy, A. F. Jr. and R. S. Engelbrecht, "Basic Biochemical Considerations during Metabolism in Growing vs Respiring Systems," Proceedings 3rd Conference on Biological Waste Treatment, New York (1961).

63. Krishnan, P., "Unpublished Data," Bio-engineering Laboratories Oklahoma State University (Sept. 1963).

64. Gaudy, A. F. Jr., K. Komolrit and M. N. Bhatla, "Sequential Substrate Removal in Heterogeneous Populations," J.W.P.C.F., 35, 903 (1963).

65. Clifford, W., and M.E.Windridge, "Proc. Inst. Sewage Purification," 115 (1932).

66. Rudolfs, W. and I. O. Lacy, "Settling and Compacting of Activated Sludge," Sewage Works Jour., 6, 647 (1934).

67. Isenberg, E. and H. Heukelekian, "Sludge Volume Index," Water and Sewage Works, 107, R-95 (1960).

68. McWhorter, I. R. and H. Heukelekian, "Growth and Endogenous Phases in the Oxidation of Glucose," Presented at the International Conference on Water Pollution Research, London (Sept. 1962).

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