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"SOME EFFECTS OF AERATION ON ANAEROBIC DIGESTION"

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by

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SUMMARY

The anaerobic contact process over the year has been found effective for the treatment of meat work waste water. The process is made possible by separating the sludge solids, after which necessary amounts of the concentrated sludge are returned to the digester. Sludge recirculation prolongs solid retention time in the digester. Unfortunately, however, sludge separation by gravitational sedimentation is almost impossible because the sludge tends to rise with the continuous gassing. Therefore treatment of the sludge suspension prior to sedimentation is necessary for effective solid separation, and vacuum and aeration degasification are two common methods used for sludge suspension pretreatment.

In the present study, it was found that the rates of aeration of 0.75 to 1.0 VVM (0.12 to 0.16 cu.ft. of air per gallon of mixed liquor per minute) were optimal for aeration degasification. These aeration rates were not only found sufficient to separate the gases from the sludge solids but also to stop gas production during gravitational sedimentation. They also gave better physical flocculation, improving the maximum reduction of primary particles in the effluent by up to 55 per cent.

In aeration degasification, the air exerts toxic effects on the anaerobic bacteria, and this toxicity has discouraged many people from using aeration degasification for the pretreatment of sludge suspension before sedimentation. The results from this study indicate that with the aeration rate of 0.12 cu.ft. (per gallon per minute) for 15 minutes, the reduction of the daily gas produced was only 5%. The overall reduction of gas was 9.3 litres (per kg. MLSS.kg.COD Loaded) per day for every 10 mg/litre of oxygen absorbed (calculated from sulphite oxidation data in a similar system).

Aeration was not found to affect the gas compositions, which were 65-70 per cent methane and 35-30 per cent carbon dioxide. However, aeration affected the pattern of gas formation; after the sludge suspension was aerated, a lag was observed

of one hour for aeration at 0.12 cu.ft. of air (per gallon per minute) over 15 minutes.

After 22.5 hours digestion of aerated sludge suspension, increases in effluent COD and volatile fatty acids were found. There was an increase in COD by 5.6 ppm for every 10 mg/litre of oxygen absorbed into the sludge suspension. Total volatile acids increased by 15 per cent (for the sparging of 0.12 cu.ft. of air per gallon per minute over 15 minutes).

The effects of air or oxygen on the performance of the anaerobic sludge culture were different from those of the chemical inhibitors such as hemiacetyl of starch and chloral (HSC) and carbon tetrachloride (CCl_4). Oxygen had a direct action on the anaerobes which were probably killed or inactivated, depending upon their sensitivity to air. In contrast, the chemical inhibitors (HSC and CCl_4) selectively inhibited methane bacteria.

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I INTRODUCTION

Anaerobic treatment is a process which has been used extensively for the stabilization of strong biologically degradable organic waste materials. This process is commonly used for stabilization of raw sewage sludge or the sludge produced from aerobic treatment processes. Anaerobic digestion has also been modified and applied to the treatment of some strong industrial wastes, such as meat works wastes. One such process is similar to the activated sludge process and is commonly known as the anaerobic contact process.

An essential feature of the anaerobic contact system is that an appreciable amount of the sludge biomass cells in the effluent is concentrated and recycled to the head of the system where it is mixed with the incoming waste water. In this way, Kirsch and Sykes (2) reported, a high level of cells is retained in the system, and the hydraulic retention time can be shortened to the economical level of approximately 6-12 hours. This system is similar to aerobic activated sludge process and has been often referred to as the anaerobic activated sludge process. The system can be shown diagrammatically as in Figure 1.

The application of anaerobic contact process to the treatment of different types of industrial wastes has been summarized by Kirsch and Sykes (2) as shown in Table I.

The anaerobic contact process has been studied in some detail by the meat-packing industry. The first study was undertaken just on a pilot-scale basis at the Geo. A. Hormell & Co. plant, Minn., under the direction of W.J. Fullen (3) in 1948. The results from the primary study were promising, so the development on pilot-scale was continued by Schroeffer and co-workers (4) (5) (6).

The data obtained by Fullen (3) and Schroeffer et al. (4) (5) (6) were later applied to the design and operation of the full-scale treatment plants for meat packing wastes at the Wilson and Hormel plants at Albert Lea and Austin, Minnesota. Later a similar plant was built to treat the wastes from the Agar Packing Co., in Mokenca, Illinois.

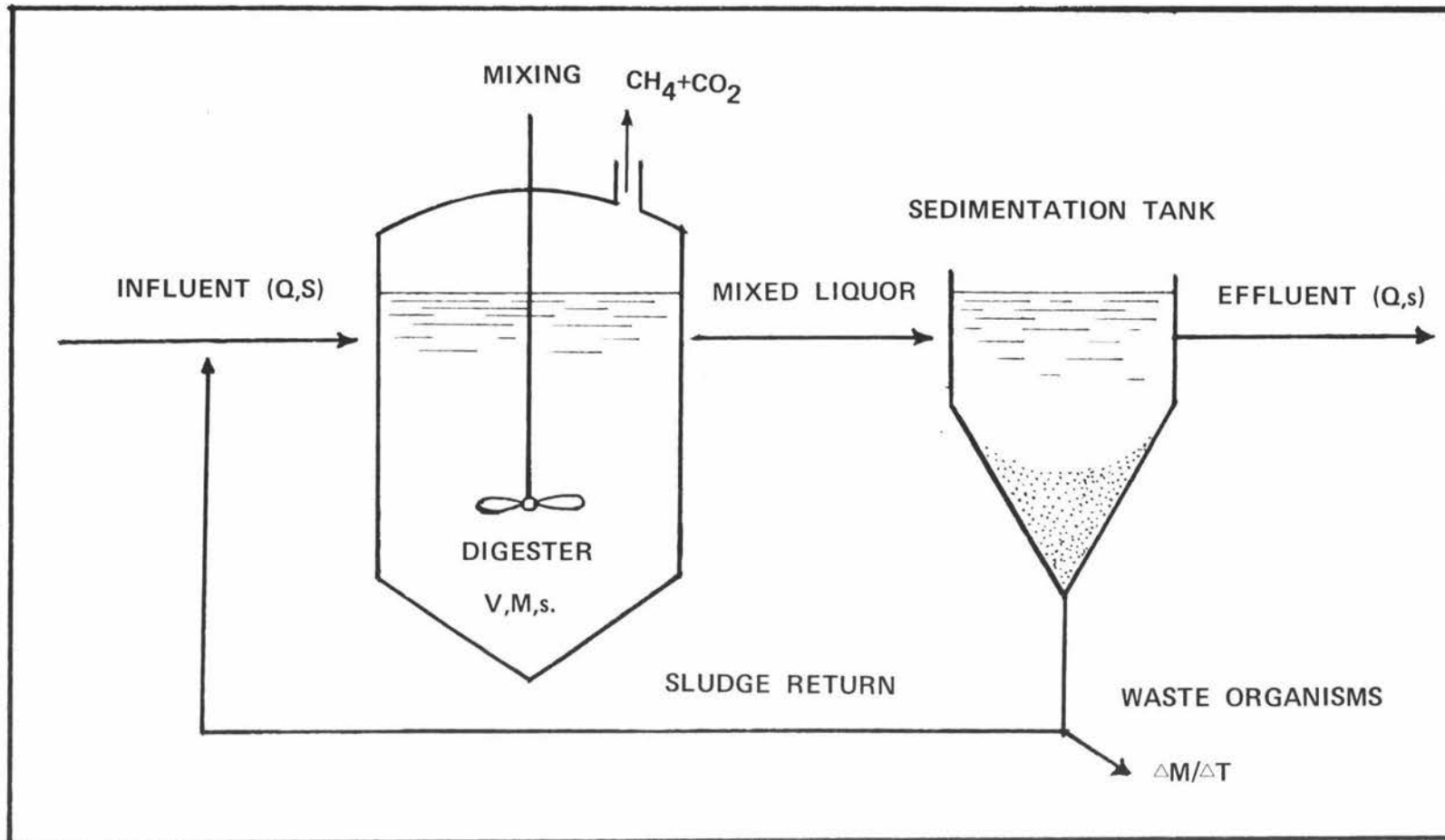


FIGURE 1. A SCHEMATIC DIAGRAM OF ANAEROBIC CONTACT PROCESS
 [After McCarty, P.L. (1).]

TABLE I: Anaerobic Treatment Performance for Systems with Sludge Separation and Return Facilities

Waste	Detention Time (days)	Process Temp. (°F)	B.O.D.		Per cent BOD removal
			Raw Waste (mg/l)	Loading $\frac{\text{lb.}}{\text{ft.}^3 \cdot \text{day.}}$	
Maize starch	3.3	73	6,280	0.110	88
Whiskey dist.	6.2	92	25,000	0.250	95
Cotton kierung	1.3	86	1,600	0.074	67
Citrus	1.3	92	4,600	0.214	87
Brewery	2.3	-	3,900	0.127	96
Starch-gluten	3.8	95	14,000*	0.100*	80*
Wine	2.0	92	23,400*	0.730*	85*
Yeast	2.0	92	11,900*	0.372*	65*
Molasses	3.8	92	32,800*	0.546*	69*
Meat packing	1.3	92	2,000	0.110	95*
Meat packing	0.5	92	1,380	0.156	91*
Fruit canning	0.18	83	800	0.18- 0.43	50-70

* Volatile solids instead of B.O.D.

(AFTER: Kirsch and Sykes, (2).)

The Water Pollution Research Laboratory in England also carried out laboratory and pilot-scale investigations on the anaerobic digestion of meat wastes. This resulted in the construction of two anaerobic treatment plants; one in Northern Ireland and the other in Devon (7).

The major operating problem for this type of plant is solid separation. Gassing of the sludge solids during sedimentation often causes poor solid separation due to sludge rising. Degasification prior to sedimentation has been reported to improve sludge separation. However, the degasifiers must be taken care of; occasional failures of the degasifiers could result in the process washing-out. This problem is quite critical because the solid content will affect the overall efficiency of the process. According to Denmead (7) the suspended solid in the full-scale plants reported above could never be maintained above 10,000 mg/l. Perhaps due to this difficulty no full scale anaerobic contact process has been built since 1962 (7).

Even though the problem of solid separation is so critical for this type of waste treatment, not much study has been done to solve the problem. A review of all the possible methods of solid separation were reported by Steffen and Bedker (8). They include: vacuum degasification, degasification by aeration, and air flotation. Vacuum degasification has been successfully applied to the operation of the full-scale plant at Albert Lea, while aeration is used at Austin in place of vacuum degasification. For air flotation, the application to the full-scale plant has not been reported.

Among all the possible methods, Steffen and Bedker (8) concluded that vacuum degasification is the most economical and practical method of producing gravity separation of the anaerobic solids. From the economic point of view, vacuum degasification is not favorable and so aeration degasification is favoured in New Zealand (9). It has been adopted to solve solids separation problems of the anaerobic digester at the Moerewa Meat Packaging plant at Moerewa, New Zealand. Besides the cost, the performance is easily adjusted to variations in flow and the character of mixed liquor.

Under many circumstances aeration degasification should be considered as a substitute to vacuum degasification. However at present, very little information concerning its mechanism and design is available. So it is considered here that the study on aeration degasification is worthwhile to improve anaerobic contact process technology.

The objects of this study are therefore:

- (1) To observe the flocculation characteristics in the aeration basin in terms of suspended solids removal, relating these to rates and times of aeration.
- (2) To observe, and evaluate, gas formation characteristics; the rates of gas formation and the gas composition.
- (3) To evaluate the process performance in terms of chemical oxygen demand reduction, and production of total volatile acids, as well as individual volatile fatty acids production.

II LITERATURE REVIEW

A. AERATION OR AIR-STRIPPING DEGASIFICATION

Very little information is available on this operation and design of aeration degasification. The only published information available is that reported by Dietz et al (10), Steffen and Bedker (8) and Rands and Cooper (9).

The purpose of aeration is quite similar to that of vacuum degasification which is to scrub the gas from the solid particles so that the sludge can settle down readily in the sedimentation tank. With aeration, the digester effluent is passed through a tank where diffused air is released through the waste material to scrub or air-wash the gas from the solid particles (Dietz et al (10)).

Steffen and Bedker (8), in their pilot-scale study, reported that about 0.1 cu.ft. of air was required per gallon of mixed liquor, and the detention time was 8-10 minutes.

A higher aeration rate and shorter detention time were reported by Rands and Cooper (9). They demonstrated that the most successful conditions for aeration degasification were obtained by using 3.75 psi pressure at the flow rate 0.36-0.37 cu.ft. of air per gallon of mixed liquor. The liquid detention time was reported to be 7.2 minutes.

Steffen and Bedker (8) found that aeration will successfully remove the methane and excess carbon dioxide gases. However, no improvement in sludge concentration was indicated. On the other hand, Rands and Cooper (9) showed that aeration improved sludge settlement. Aeration also inhibited refermentation for a sufficient time to allow the removal of the settled sludge.

B. AERATION FLOCCULATION IN THE TURBULENT REGION

Theoretically, flocculation processes are considered separately under Brownian motion, laminar flow, and turbulent flow regimes. The early theories of flocculation kinetics were developed to express the collision frequencies under Brownian

motion and laminar flow, and the classic flocculation theories for expression of aggregation in these two regimes were developed by Smoluchowski (11, 12) in 1915. Later, the theory of the aggregation developed by Smoluchowski has been adopted to express the flocculation characteristics in turbulent regions.

Camp and Stein (13) were the first to realize that for practical reasons turbulent flocculation was of utmost importance in water treatment. They generalized Smoluchowski's equation to include turbulent flow conditions by defining a root mean square (rms) velocity gradient, G , which they substituted for the well-defined velocity gradient existing in laminar flow. Later, flocculation kinetics in water treatment were carefully studied by Argaman and Kaufman (14, 15). Instead of adopting the term root mean square velocity they expressed a turbulent fluid in terms of a turbulence energy spectrum.

Parker (16) and Parker et al (17) have recently developed the overall flocculation kinetics in the activated sludge process. Their flocculation model was also based on Smoluchowski's classical equation with the incorporation of a turbulent function called eddy scales.

Parker (16) demonstrated that large-scale energy-containing eddies, and even the energy in the microscale region, are not responsible for floc break-up and floc-formation, because very small amounts of energy are dissipated from these two regions. The kinetics of flocculation and floc breaking were assumed to be based solely on the range of eddies termed the "universal equilibrium range". This range has been further divided into a low eddy-size region, "the viscous dissipation subrange", and a larger eddy-size region, the "inertial convection subrange".

The mathematical relationships expressing the overall process of flocculation in turbulent region derived by Parker (16) can be summarized as follows:

$$\frac{n_1^0}{n_1} = \frac{1 + K_A \times G \times T}{1 + K_B \frac{X_0}{n_1} G^m \times T}$$

where:

$$n_1^0 = \text{influent concentration of primary particles, mg/l}$$

n_1	=	effluent and reactor concentration of primary particles, mg/l
X	=	mixed liquor suspended solids (MLSS), mg/l
T	=	mean hydraulic residence time
m	=	a theoretical constant = 2
K_A	=	aggregation rate coefficient
K_B	=	floc breaking rate coefficient
G	=	rms velocity gradient, sec^{-1}
	=	$= \sqrt{\frac{Q' \gamma h}{\mu}}$

where:

Q'	=	unit air supply, cu.ft./sec/cu.ft.
γ	=	liquid specific weight, lbs/cu.ft.
h	=	diffuser depth, ft.
μ	=	viscosity, $\frac{\text{lbs. sec.}}{\text{ft}^2}$

C. ANAEROBIC DIGESTION

Initially the complex organic solids are attacked by bacterial extracellular enzymes. These complex materials will be hydrolyzed to simple soluble compounds which the microorganisms can utilize. The cellulose and starches are hydrolyzed to simple sugars, while the proteins are broken down to amino acids. Only the fatty acids are not attacked by these extracellular enzymes. The hydrolyzed compounds are metabolized leading to the formation of organic acids and other end products. The process of synthesizing bacterial cells, organic acids and other end products is the first phase of anaerobic digestion commonly known as "acid formation or non-methanogenic stage". (McKinney (18)).

As anaerobic digestion processes approach equilibrium, the second group of bacteria which can utilize the metabolic end products from the first phase become more active. The gases hydrogen and carbon dioxide and some volatile fatty acids, especially acetic (Wolfe (19)) are biologically converted, mainly to methane and carbon dioxide. Methane and carbon dioxide are important final end products in anaerobic digestion. This metabolic process is commonly known as "methane formation or the methanogenic phase".

The details of anaerobic digestion have been reviewed by Barker (20), Stadtman (21), Torein and Hattingh (22), Kirsch and Sykes (2) and Wolfe (19).

1. VOLATILE ACIDS PRODUCTION

Volatile acids are the metabolic end products produced from the metabolism of the primary substrates by the non-methanogenic population. Thiman (23) showed that acetic acid could be formed from the fermentation of hydrogen and carbon dioxide by Clostridium acetium. McCarty et al. (24) and Andrews and Peason (25) showed that acetic and propionic acids are also formed as the intermediates in the methane fermentation of the larger chain volatile acids. The biological formation of acetic acid by methane bacteria seems to be doubtful, since it has been reported by Wolfe (19) that no pure culture of methane bacteria can utilize volatile fatty acids except Methanosarcina barkeri, and M. barkeri can only ferment acetic acid to methane and carbon dioxide.

Volatile fatty acids are derived from proteins, carbohydrates and lipids. Acetic acid was reported by McCarty et al. (24) to be the most abundant intermediate in the fermentation of carbohydrates, proteins and lipids. It was also formed as an intermediate in the methane fermentation of propionic and butyric acids. Propionate was formed primarily from carbohydrates, but also was produced in the fermentation of proteins. Butyrate was, on the other hand, produced from proteins and fats. Several other acids were normally present but never as the predominant acids. During unbalanced digestion conditions, acetic and propionic acids were frequently found as the major volatile acids.

Kaplevsky (26) found in anaerobic decomposition of yeast waste mixtures and slaughterhouse wastes that acetic, propionic, butyric and valeric acids were present.

In anaerobic digestion of synthetic wastes, Andrews and Peason (25) demonstrated that acetic, propionic, butyric, valeric and caproic acids were produced. The major acids were acetic and propionic. Butyric and valeric acids were present in moderate amounts, while only small amounts of caproic were detected.

Andrews and Peason (25) also demonstrated that the distribution of individual volatile fatty acids was a function of the residence time. The peak of all the acids, except propionic acid,

was at the residence time of 2.4 days, then the concentration of the acid dropped gradually when the residence time was increased. At the detention time of 23 days acetic and propionic acids were the only acids detected.

Organic loading was reported by Etzel and Pollard (27) to have an effect on the concentration of both total and individual volatile fatty acids, especially acetic and propionic. At the loading rate of 0.115 lbs volatile solid/cu.ft., the total volatile acid was between 2250 to 2500 ppm. The concentrations of acetic and propionic acids were 3.5 to 6.7 ppm, and 65 to 67.4 ppm respectively. When the organic loading was increased to 0.234 lbs volatile solid/cu.ft., the total volatile acid increased to 6000 to 7000 ppm. Acetic acid increased up to 52 to 67 ppm, while propionic was only 15 to 21 ppm.

2. GASEOUS END PRODUCTS

Gas is the end product from both non-methanogenic and methanogenic digestion processes. The gases are composed mainly of methane and carbon dioxide with trace amounts of NH_3 , H_2S , H_2 and N_2 .

Gases from a well-digested sewage sludge mixture will contain 25-35 per cent methane, depending on the composition of the sewage sludge. In the case of the anaerobic digestion of meat packing wastes, the gas was reported by the Eimco Corporation (28) to be composed of 88-90 per cent methane and 10-12 per cent of carbon dioxide.

Normally, methane and carbon dioxide constitute most of the gases in the methanogenic stage, and CO_2 and H_2 are the gases in the non-methanogenic stage. In the balanced condition hydrogen is scarcely detected, but when methane gas is inhibited by CCl_4 and other methane analogue compounds, Thiel (29) and Sykes and Kirsch (30) demonstrated that hydrogen accumulated in large quantity.

In their mixed culture study, Sykes and Kirsch (31) found that hydrogen and carbon dioxide were detected almost immediately after the digestion was started. Hydrogen accounted for most of the gas produced during the first three days of incubation, and the amount of hydrogen was reduced once methane began to accumulate.

Their results were confirmed by studies on pure culture. Sykes and Kirsch (31) illustrated that all the non-methanogenic bacteria isolated produced carbon dioxide. A total of 19 of 75 isolates produced hydrogen gas and none of them produced methane.

The quantity of gas produced during normal sludge digestion varies only slightly. The average gas yield was reported by Sawyer and Grumbling (32) to be 16.5 to 17.1 cu.ft. per lb volatile matter destroyed. Dea (33) also found that the gas yield was 16.83 to 18.25 cu.ft. per pound of volatile matter destroyed.

In terms of volatile matter added, the gas yield was reported by Nash and Chasick (34) to be 8.1 to 8.5 cu.ft. per lb of volatile

matter of sewage sludge added. In anaerobic digestion of meat packing wastes only 7 cu.ft. of gas was observed per pound of organic matter added (28).

3. THE COURSE OF GAS FORMATION

Detailed observations on gas formation in a batch type anaerobic digestion process were first reported by Fair and Moore (35) in 1932. Thirty-six years later, Buraczewski (36), in his study on anaerobic digestion of individual volatile acids, compared his results with those reported by Fair and Moore (35). The results were not exactly identical, but they were very similar, and the mathematical relationships developed by Fair and Moore (35) were reported to be applicable.

Fair and Moore (35) also tried to formulate mathematical relationships explaining the course of gas formation. They found that the rate curve is very similar to that of an autocatalytic reaction in chemical engineering, but that this type of equation could not be accurately applied to the total prediction of the course of gas formation. They finally divided the curve into two parts and assumed that both of these obeyed the first order reaction. Fair and Moore (35) demonstrated that the two-stage process provided the best fit to the results they obtained.

The mathematical formulation can be summarized as follows:

First stage:

$$\frac{dG}{G} = k_1 dt$$

Second stage:

$$\frac{dL_t}{L_t} = -k_2 dt$$

where:

- G = amount of gas produced in time t
 L = total amount of gas generated during digestion
 L_t = L - G = amount of gas remaining to be produced
 k₁, k₂ = constants

D. INHIBITION OF METHANE FORMATION

Recent investigations have indicated that methanogenesis

by mixed populations of bacteria from rumen and anaerobic digesters can be selectively inhibited. A wide range of these chemical inhibitors has been reported in the literature.

Inhibition of methane formation by Methanobacillus omelianskii has been reported by Wolin et al. (37). Low concentrations of methyl or benzyl viologen (5.2×10^{-3} and 1.6×10^{-3} mM of benzyl and methyl viologens respectively) give 100 per cent inhibition of methane, whilst hydrogen, which is normally formed from ethanol, accumulates in greater quantities.

Slyter and Wolin (38) reported the inhibition of methane formation of rumen mixed culture in the presence of CuSO_4 , benzyl viologen and oxygen. CuSO_4 inhibited methane formation which was accompanied by the production of H_2 and ethanol and an increase in propionic formation. Benzyl viologen caused a similar fermentation shift but with an increase in butyrate rather than propionate.

Demeyer and Henderick (39) reported that low concentration of C_{18} - unsaturated fatty acids inhibited rumen methanogenesis with the simultaneous accumulation of hydrogen, and a concomitant production of propionic and lactic acids.

Extremely low concentrations of chloroform and carbon tetrachloride and somewhat larger concentrations of methylene chloride were reported to inhibit methane formation by Bauchop (40). The inhibition function was reported to be selective and accumulation of hydrogen was reported when methane formation was inhibited.

Rufener and Wolin (41) confirmed Bauchop's results. They found that higher amounts of CCl_4 (13 μM) were necessary for effective inhibition, and the total volatile fatty acids production was not disturbed by CCl_4 treatment, although the proportions of propionic and butyric acids increased while acetic acid decreased.

A study of inhibition of methanogenesis in anaerobic digestion by chlorine-containing analogues of methane was first reported by Thiel (29). He showed that 0.96 mg/l chloroform, 2.2 mg/l carbon tetrachloride and 100 mg/l methylene chloride caused 50 per cent inhibition of methane formation. The subsequent accumulation of hydrogen was also reported.

Recently, in the study on the inhibition of methane formation

with CCl_4 of the naturally occurring, anaerobic cultures digesting sewage sludge, Sykes and Kirsch (30) demonstrated that hydrogen gas is produced during the anaerobic digestion, but it is not readily detectable unless methane production is disrupted. The accumulated hydrogen was also reported to inhibit the production of acetate, propionate and hydrogen itself.

Hemiacetyl of starch and chloral (HSC) was claimed by Trei (42) of Smith Kline and French Laboratories, U.S.A. to be able to inhibit methane formation in rumen systems. Approximately 90 per cent of methane gas was inhibited in the presence of 12 ppm HSC. Consequently, hydrogen was accumulated, but the hydrogen gas was less than the expected value.

Oxygen inhibition on methane production by Methanobacterium strain M.o.H. was reported by Robertson and Wolfe (43). With an aeration rate of 1.55 ml per minute, only 90 minutes were needed for 90 per cent reduction of methane gas. The ATP levels decreased as methane formation was inhibited by oxygen, but no hydrogen was reported to accumulate.

Later the effect of oxygen on anaerobic digestion was reported by Fields and Agardy (44). Complete inhibition was observed after 69 hours of continuous oxygen addition at the rate of 1 ml per hour. The final concentration of the oxygen bled into the mixed liquor was 1,330 ppm.

Under the influence of oxygen addition the volatile acids showed a 51.7 per cent increase during the initial 19 hours. The other parameters, such as methane, carbon dioxide, pH and total gas production were reduced from their stabilized condition by 11 to 40 per cent.

III MATERIALS AND METHODS

A. ANALYTICAL TECHNIQUES

The methods and the description of the analytical techniques employed in this study are discussed in the following sections.

1. Chemical Oxygen Demand (COD)

The method is based on the fact that most types of organic material are oxidized by a boiling mixture of chromic and sulphuric acids. The sample of raw wastes or effluent liquor is refluxed with known amounts of potassium dichromate and sulphuric acid and then the excess dichromate is titrated with ferrous ammonium sulfate solution. The amount of oxidisable organic matter, measured as oxygen equivalent, is proportional to the potassium dichromate consumed. The method is described in Standard Methods (45).

2. Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand of raw waste and effluent supernatant was determined by the method described in Standard Methods (45).

Since the raw waste was not completely homogeneous, the dilution step in Standard Methods (45) was modified. The samples were directly pipetted into the previously graduated BOD bottles and then the standard dilution water was carefully siphoned into the bottles.

3. Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen includes ammonia and organic nitrogen. The method using mercuric sulphate as a catalyst, converts organically bound nitrogen with sulphuric acid to which potassium sulphate has been added to raise the boiling point to 345°C to 370°C . After dilution the solution is made alkaline with sodium hydroxide and the ammonia distilled into dilute formic acid solution or the distillate is collected for nesslerization. The ammonia borate is titrated with standard acid using mixed indicators.

This method is described in Standard Methods for the Determination of Meat Wastes and receiving Wastes (46).

4. Total Suspended Solids

Total suspended solids were determined by the method described by Melbourne (47) with the following modifications:

- (a) Glass-fibre filter papers grade GF/A were used instead of GF/C which was recommended by Melbourne (47). The main reason was that the other grades of glass-fibre filter papers, besides GF/A, were not available on the New Zealand market. Also, Melbourne's experimental results showed that this grade (GF/A) was quite satisfactory for total suspended solids determination, and it could be concluded from observation that the results obtained from using this grade of glass-fibre paper were far more satisfactory than those of the standard Gooch crucible recommended in Standard Methods (45).
- (b) The papers were dried at about 104°C for 60 minutes and cooled for 15 minutes.

5. Total Volatile Acids

Volatile acids are the lower molecular weight acids classified as soluble and are the only ones that can be distilled at atmospheric pressure. These volatile acids can be removed from aqueous solution by steam distillation, in spite of high boiling points, because of their high vapour tension.

Total volatile acids of the sludge liquor were determined by the method described in Standard Methods (45) with the following modifications:

- (a) 40 ml sample
- (b) 0.2 ml of FeCl_3 solution, and
- (c) 2 gm filter aid.

With the above modifications it was found experimentally that 93.3 per cent of volatile fatty acids were recovered.

6. Individual Volatile Fatty Acids Analysis

The methods used for the determination of individual volatile fatty acids were the same as those used for rumen liquor

by Bandaranayaka (48).

After titration of the total volatile acids, a further 1 ml of $N/10$ sodium hydroxide solution was added, then transferred to a 250 ml beaker, and evaporated to dryness in an oven. The sodium salts were redissolved in 1 ml of distilled water and from this 1-2 drops were taken for the gas chromatographic analysis. The volatile fatty acids were separated in a Varian Aerograph Series 1200 gas chromatograph.

Details of equipment and the conditions for running the gas chromatograph are as follows:

- (a) Detector: Flame ionization Varian Aerograph series 1200
- (b) Column: A stainless steel column 6 ft. x $\frac{1}{8}$ in. packed with 20% FFAP on 60/80 mesh chromasorb W.
- (c) Conditions:

Oven temperature	- 145°C
Injector temperature	- 170°C
Detector temperature	- 170°C
Hydrogen flow	- 10 ml/11-13 sec.
Nitrogen flow	- 5 ml/11-13 sec.

The gases were bubbled through a solution of formic acid prior to entry into the column.

- (d) Recorder: Disc chart integrator, model 207, series D; manufactured by Leeds & Northrop Speedoware H.

Volatile fatty acids chromatogram is shown in Appendix 1.

7. Gas Analysis

Gas analysis was determined by using a gas chromatograph, model GLC/1, designed and manufactured by Townson & Mercer New Zealand Ltd.

- (a) Detector: Thermal conductivity detector, 2 volts, 1 amp. D.C.
- (b) Column: A copper column 5 ft. x $\frac{1}{8}$ in. packed with Porapak Q (supplied by Waters Association Inc., Framington, Mass., U.S.A.)
- (c) Conditions:

Temperature	- 50°C
Carrier gas N_2	10 ml/16-17 sec.

(d) Chart speed: 1 inch/minute

(e) Recorder: Sargent Recorder, model SRG.

A typical chromatogram showing the separation of hydrogen, oxygen, methane and carbon dioxide is shown in Appendix 2.

B. SYNTHETIC MEAT WORK WASTES

It was important for these experiments that the variables must be kept down to a minimum. Among these variables, one of the most difficult to control was the waste water itself. It was realized that the actual waste water from the freezing works would not be consistent enough for this purpose, if the waste was collected daily or weekly. If the actual meat waste were used it could possibly be stored by collecting large quantities of the waste water and keeping it frozen, but because of the quantities involved this seemed to be impossible. Finally, after careful thought, it was decided to make synthetic meat wastes.

The main components of meat work wastes are carbohydrates, proteins and fats. Most of the carbohydrates come from paunch liquor while blood and fatty meat contribute to most of the protein and fats respectively. Therefore, synthetic meat wastes can be simply made by mixing paunch content, blood and tallow in appropriate ratios. Later, it was found experimentally that fat was difficult to incorporate in the other components, and so the idea of adding tallow into the synthetic waste was abandoned.

Blood was collected directly from the killing floor into 1.5 per cent calcium citrate solution. For the paunch content, the mutton paunch was preferred because it was easier to collect and to separate. The paunch content was also collected directly from the killing floor.

The blood with the presence of anticoagulant could be directly mixed with other components, while the paunch content underwent solid and liquid separation. About 20 per cent v/v of water was added, then the paunch solids, mainly grasses, were separated in a perforated basket centrifuge. The paunch solid was discarded while the liquor was collected for further analysis.

The analytical results of blood and paunch liquor were as follows:

	<u>COD (ppm)</u>	<u>N (ppm)</u>
Paunch liquor	16,500 - 17,100	800 - 850
Blood	300,000 - 316,000	25,000 - 30,000

In the preliminary investigations, 200 ml of paunch liquor was mixed with 5 ml of 100 per cent blood. The mixture was then diluted with tap water to 1 litre. The analytical and calculated results of this synthetic waste are as shown below:

	<u>COD (mg/lit.)</u>	<u>Nitrogen (mg/lit.)</u>
Paunch liquor	17,080	845
Blood	316,000	25,200
Synthetic waste (calculated)	4,916	295
Synthetic waste (analyzed)	4,220	216

It was found that the above synthetic waste was quite satisfactory for the purpose in terms of strength and composition, and above all the bacteria could grow satisfactorily. Therefore, large quantities of mutton paunch and blood were collected, mixed and stored.

Analytical results showed that the strength in terms of COD of the paunch liquor when collected in large quantities was more dilute than the expected. To correct for this the ratio of paunch liquor to blood was therefore altered to 220:7 volume per volume. A litre each of the concentrated mixture was filled into a plastic bag and stored in the deep freeze, and prior to use a bag of concentrated waste was thawed and diluted to 3½ litres.

Analytical results of the synthetic wastes are shown in the table below.

TABLE II: Analytical Results of Synthetic Meat Wastes

Items	Total Waste	Centrifugal Waste*
Total solids (mg/l)	6060	4770
Suspended solids (mg/l)	1617	-
COD (mg/l)	6150	4600
Kjedhal N. (mg/l)	340	300
BOD (mg/l)	2500	2148

*Note: The raw waste was centrifuged at 3800 rpm for 30 minutes.

IV EXPERIMENTAL PROCEDURE

The laboratory investigations described in this thesis included the operation of a laboratory pilot and bench-scale completely mixed fermenters under controlled laboratory conditions. The pilot-scale anaerobic digestion system was operated at 25°C, while the bench-scale digester was operated at 37.5°C.

The original plan was to run the experiments with the pilot-scale unit, so that it could be operated continuously with the accessory equipment available. Soon it was found that the digester had too large a capacity for the quantity of synthetic waste which could be prepared and so the bench-scale anaerobic digestion system was constructed. The small digestion system was operated throughout most of the study while the pilot-scale unit was used for building up an actively metabolising sludge.

A. DESCRIPTION OF APPARATUS

1. Pilot-Scale Unit

The laboratory pilot-scale anaerobic digestion unit is shown in Figure 2. Details of the components are described below.

a) Digester

The digester was an open-top unit fitted with a stirrer of 73 rpm velocity. The digester was constructed from a 44 gallon oil drum specially painted to protect it from rust. Since the digester was an open-top system, a $\frac{1}{2}$ " thick styrofoam sheet was used to cover the liquid and air interface.

b) Feed Tank

The feed tank was also constructed from a 44 gallon oil drum cut into two. The stirrer was mounted on top of this feeding unit to ensure that a homogeneous feed was obtained.

c) Heating Unit

A copper coil of $\frac{1}{2}$ " I.D. was used as a heat source and the liquor temperature was controlled at 25°C.

d) Sedimentation Tanks

The primary sedimentation tank was constructed from a

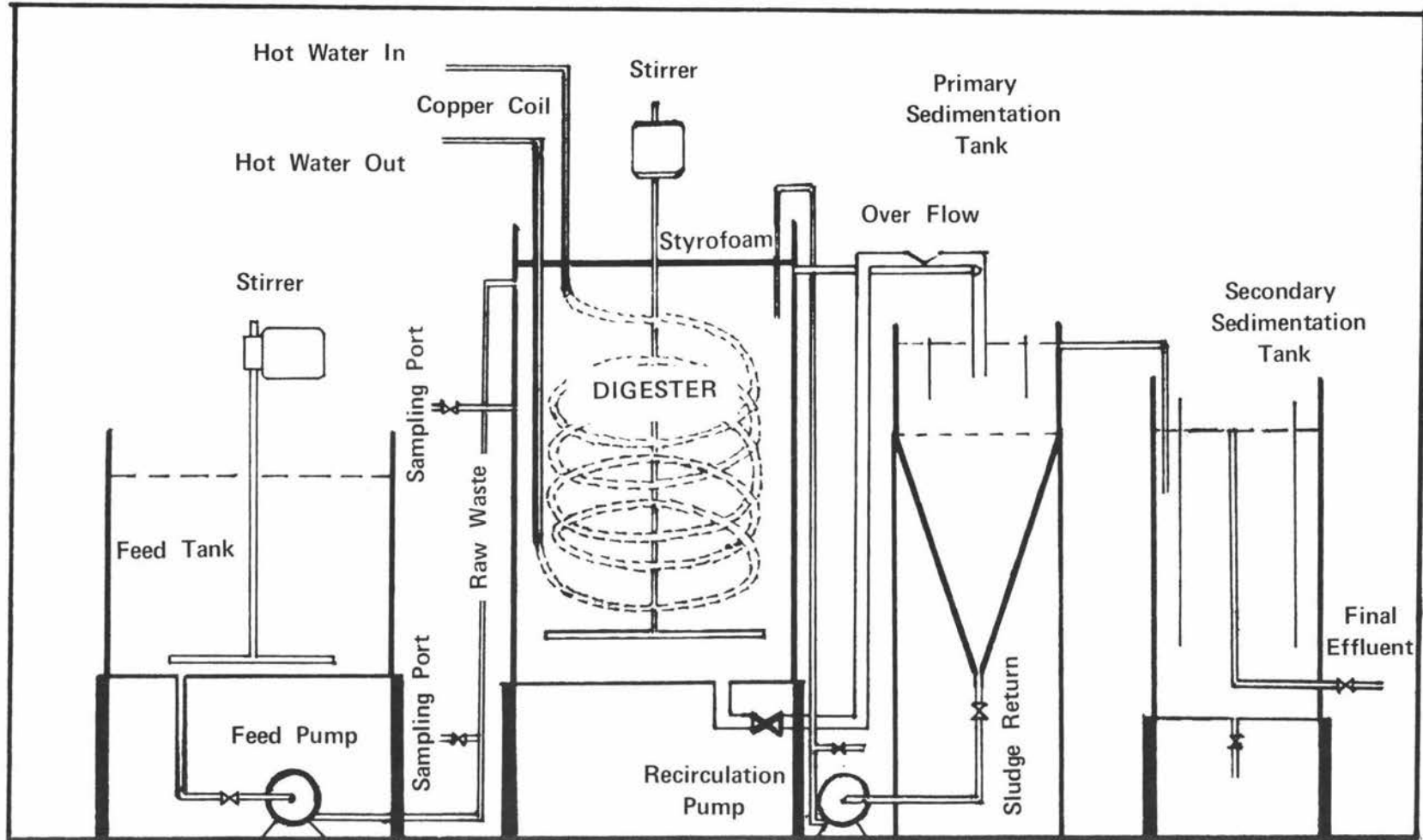


FIGURE 2. LABORATORY PILOT-SCALE ANAEROBIC DIGESTION SYSTEM.

stainless steel cone used for determining floatable fat in meat works waste water. The secondary sedimentation tank was constructed from a steel cylindrical vessel.

e) Pumps

Pumps were needed for feeding and recirculating the settled sludge solids, so that the ratio of recirculation rate and feeding of 2:1 could be obtained.

Two Sigmamotor pumps, Model T6SH, marketed by Zero-max Corp., Minneapolis, Minn., U.S.A. were used for this purpose.

2. Bench-Scale Anaerobic Digestion Systems

Two identical fermenters were installed and run in parallel so that one fermenter could be used as a control. The digesters were constructed from 10 litre narrow-mouth Pyrex aspirators with suitable fittings to allow feeding of the raw waste to the units, the withdrawal of waste or digested sludge, liquid and gas sampling points, and measuring of the quantity of the gases produced. The unit is shown diagrammatically in Figure 3.

A magnetic stirrer was used for mixing the sludge. The two magnetic stirrers were driven by a belt from a $\frac{1}{8}$ H.P. motor. In these circumstances, identical mixing could be obtained in both fermenters.

Gas volume was determined by displacing an acid-salt solution (2% H_2SO_4 and 10% NaCl) in a 5 litre aspirator. The amount of the acid-salt solution displaced gave the volume of gas obtained from the fermentation.

B. START-UP OF ANAEROBIC TREATMENT SYSTEMS

1. Pilot-Scale Digestion System

The pilot-scale unit was started by seeding the digester with actively digesting municipal sewage sludge to provide the active biological culture. The seed sludge was obtained from the sludge digester at the Sewage Works of Palmerston North City.

The seed sludge was screened through a wire mesh to remove any large particles which might clog the digester tubing. The seed sludge was then diluted with tap water so that about 1-1.2 per cent mixed liquor suspended solids (MLSS) could be obtained.

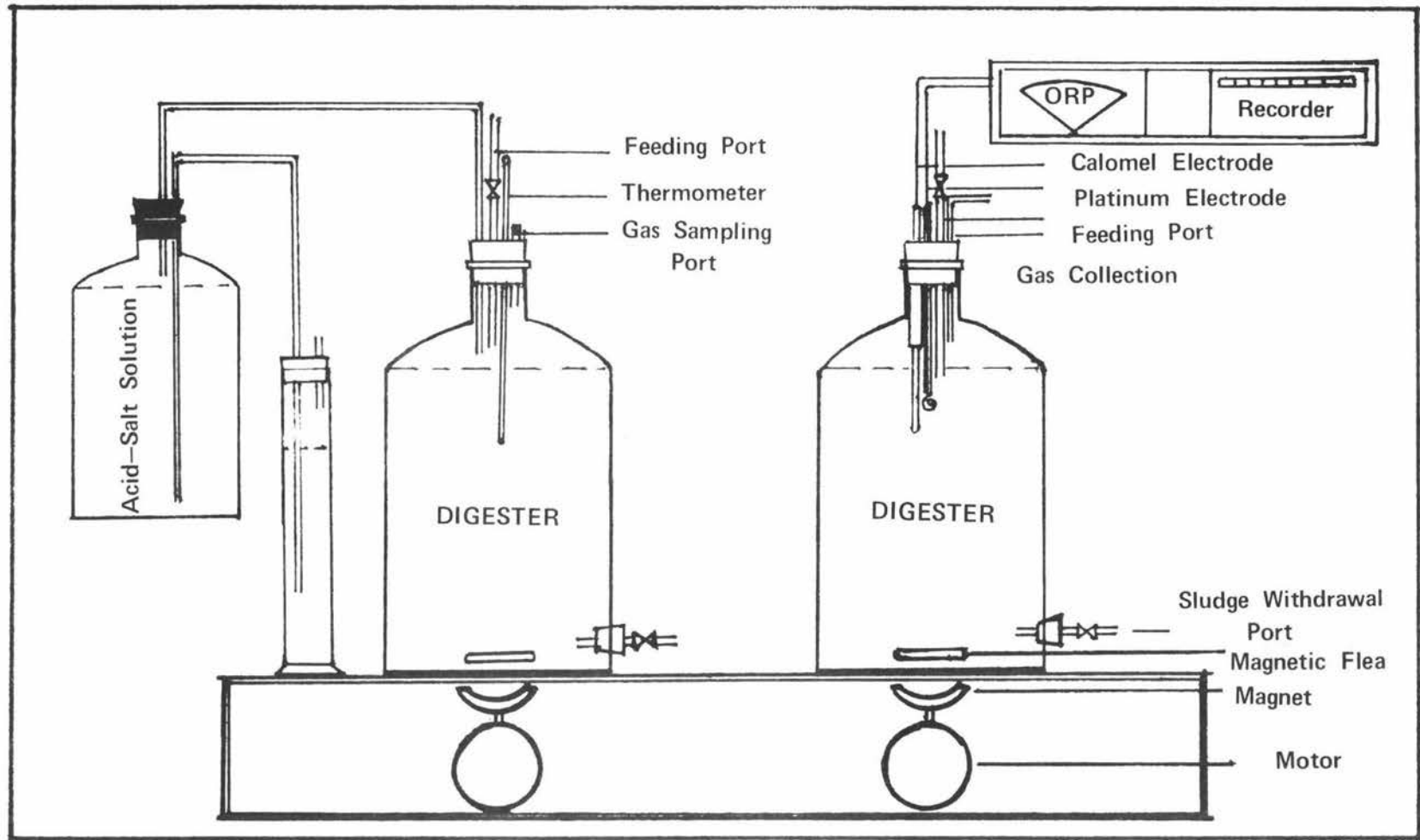


FIGURE 3. LABORATORY BENCH-SCALE ANAEROBIC DIGESTION SYSTEM.

The digester was then filled up with the sludge to the 40 gallon mark.

The digester content was allowed to stand overnight; then about 20 gallons of the supernatant were siphoned out, and the same amount of the synthetic meat waste was added. The digester was fed in this way every other day for 14 days. It should be noted at this stage that the heating unit and the stirrer were operated during the day-time only. The temperature was controlled at 25°C.

After 2 weeks the digester was continuously fed. According to the plan the loading was 0.0775 lb BOD (cu.ft.day), which was claimed by Schroepfer et al. (4) to get 90% BOD reduction. After about one week of operation some problems arose and the plan had to be abandoned.

The main problem was the losses of MLSS due to the continuous gasification in the sedimentation tank. After 7 days of operation the MLSS decreased to 7000 ppm. Also, it was not found practicable to prepare such a great volume of the synthetic waste. Thereafter, the fermenter was fed batchwise at a very low loading rate, a quarter of the design figure. The stirrer was operated just during the day-time and, as mentioned previously, this large fermenter was thereafter used for growing active sludge for the bench-scale laboratory fermenters.

2. Bench-Scale Anaerobic Digestion System

Two lots of ten litres of the stabilized sludge were drawn from the pilot-scale fermenter and each lot filled up one of the laboratory bench-scale fermenters. The sludge liquor was then allowed to stand overnight and 3.33 litres of the supernatant were siphoned out. The digester was then filled up with the raw waste. The magnetic stirrer was switched on, and the stabilization process was in operation.

The effective digestion period was 22.5 hours. After this, the whole content was drawn to the sedimentation vessel which was another 10 litre glass aspirator, and the sludge solids were allowed to settle down for one hour. At the end of the hour, 3.33 litres of the supernatant were siphoned out. Then the same amount of the raw waste was added and mixed with the sludge solids,

and this mixture was returned to the fermenters. This whole process was repeated daily to give a mean liquid detention time of about 67.5 hours.

The chemical oxygen demand of the raw waste water was 6150 ppm, and the organic loading was 20.5 gms COD per day, thus giving 0.384 lb COD (cu.ft.day).

V EXPERIMENTAL RESULTS AND DISCUSSION

A. AERATION FLOCCULATION

1. Materials and Methods

At first, the aeration flocculation was investigated in a one litre glass measuring cylinder using a 10 ml pipette as an air sparger. The assembly of the equipment is shown diagrammatically in Figure 4.

The results obtained from the preliminary investigation were quite promising. It was then decided to use a more effective air diffuser. Therefore, for the second series of tests, the pipette was replaced by a ceramic air diffuser of the type normally used for a fish pond. A two litre glass measuring cylinder was used as a flocculation chamber. The assembly of the equipment is shown diagrammatically in Figure 5.

In the tests, 1000 or 2000 ml of mixed liquor were drawn from the laboratory-scale fermenters, and transferred into the flocculation chamber. Then the required amount of air was sparged into the mixed liquor. At the end of aeration, 1000 ml of the sludge were transferred to a one litre beaker for sedimentation. After 30 minutes, 150-200 ml of the supernatant were decanted and stored for further analysis.

The performance of the flocculator was evaluated in terms of suspended solids and COD reduction. The performance parameter of n_1^0/n_1^1 was used to determine the efficiency of the flocculation chamber, where n_1^0 and n_1^1 are the influent concentration of suspended solids (or COD) and the effluent concentrations respectively.

2. Results

The experiments were carried out in a one litre flocculation chamber, following four different aeration times of 7, 12, 20 and 30 minutes respectively. For each aeration time, aeration rates of 0.5, 1.25, 2.25 and 3.25 $\frac{\text{volume}}{\text{volume}\cdot\text{minute}}$ (VVM) were operated.

The anaerobic sludge suspension entering the flocculator is

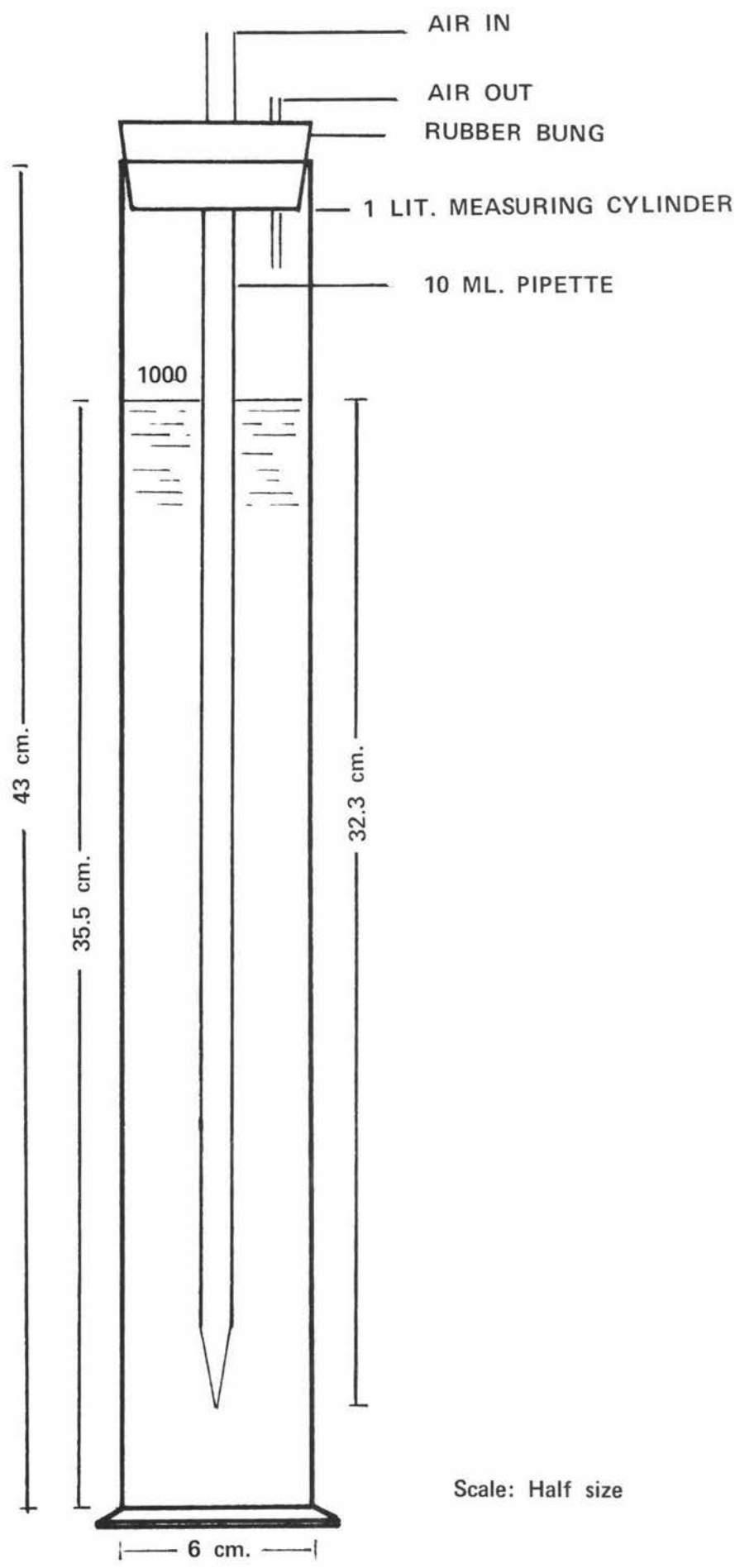


FIGURE 4. FIRST AERATION FLOCCULATION SYSTEM.

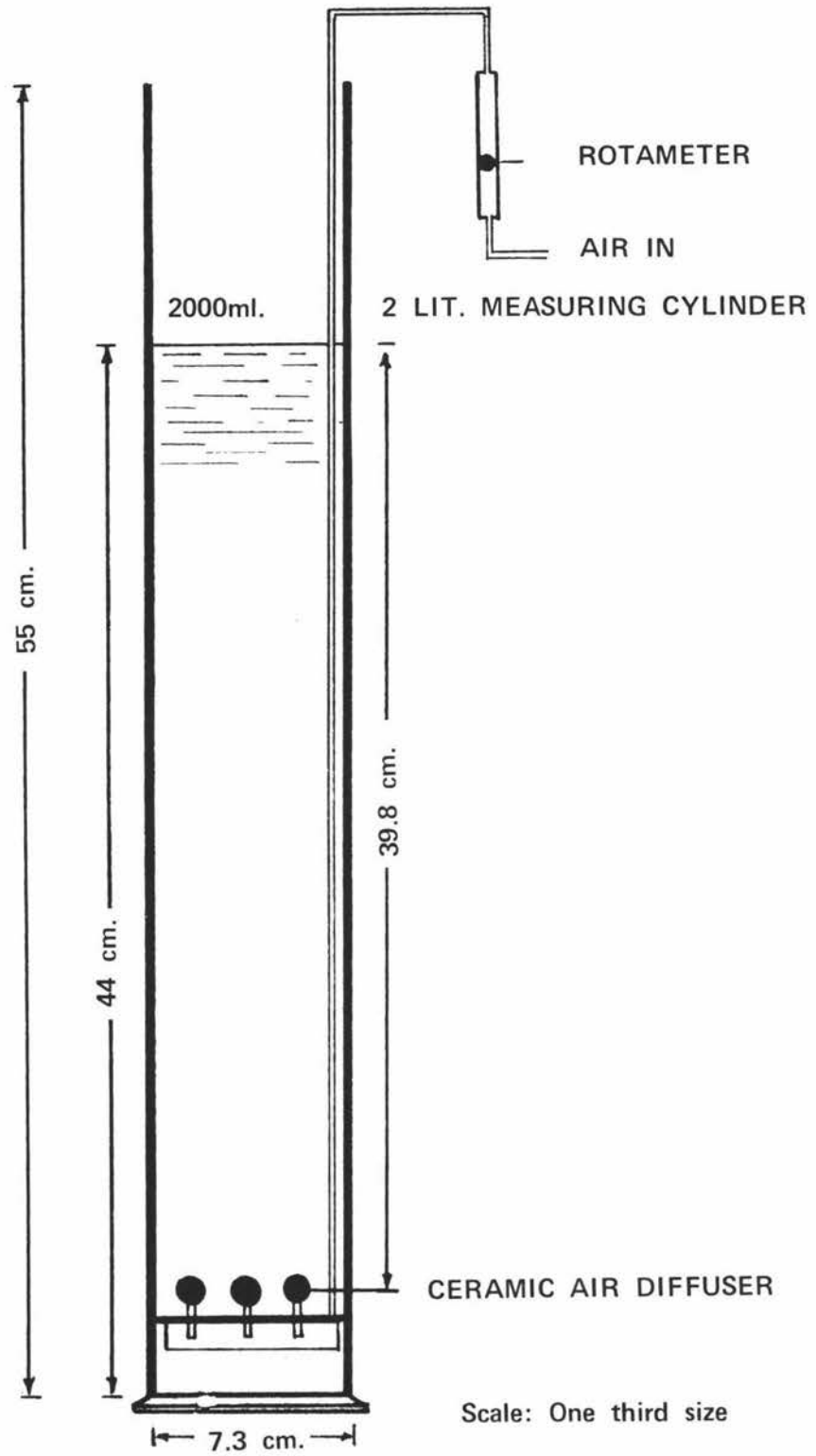


FIGURE 5. SECOND AERATION FLOCCULATION SYSTEM.

similar to that of activated sludge described by Parker et al. (17), consisting of dispersed primary particles and preformed flocs. The dispersed primary particles may be individual bacteria or other particulate matter. These primary particles presumably arise mainly from floc breakup, but it is possible that they may be dispersed particles that have never been incorporated into floc. The floc itself results from microbial growth, and aggregation of dispersed particles is limited in size by breakup in the turbulent digester.

Effluent suspended solids (before aeration) 710 ppm
 Effluent COD (before aeration) 4050 ppm

With the diffuser depth (h) of 1.11 ft., the following values of rms velocities (G) were obtained.

Aeration (lit/lit.min)	0	0.5	1.25	2.25	3.25
G, sec ⁻¹	0	123.8	194.5	262.5	315.5

Note: Root mean square velocity gradient (G) can be calculated from the formular developed by Parker et al. (16).

$$G = \sqrt{\frac{Q' \times h}{u}}$$

Where:

- u = viscosity, $\frac{\text{lb. sec.}}{\text{ft}^2}$
 Q' = unit air supply, $\frac{\text{ft}^3}{\text{sec. ft}^3}$
 \times = liquid specific weight, lb/ft³
 h = diffuser depth, ft.

$$\begin{aligned} \text{The unit of G} &= \sqrt{\frac{\text{ft}^3 \cdot \text{ft}^2 \cdot \text{ft. lb}}{\text{sec. ft}^3 \cdot \text{sec. lb. ft}^3}} \\ &= \text{sec}^{-1} \end{aligned}$$

The results are summarized in Figures 6, 7 and 8. Figure 6 shows the reduction of suspended solids after flocculation, while the decrease of effluent COD is demonstrated in Figures 7 and 8. The results indicate that flocculation of the anaerobic sludge is governed by two factors; flocculation intensities, and time

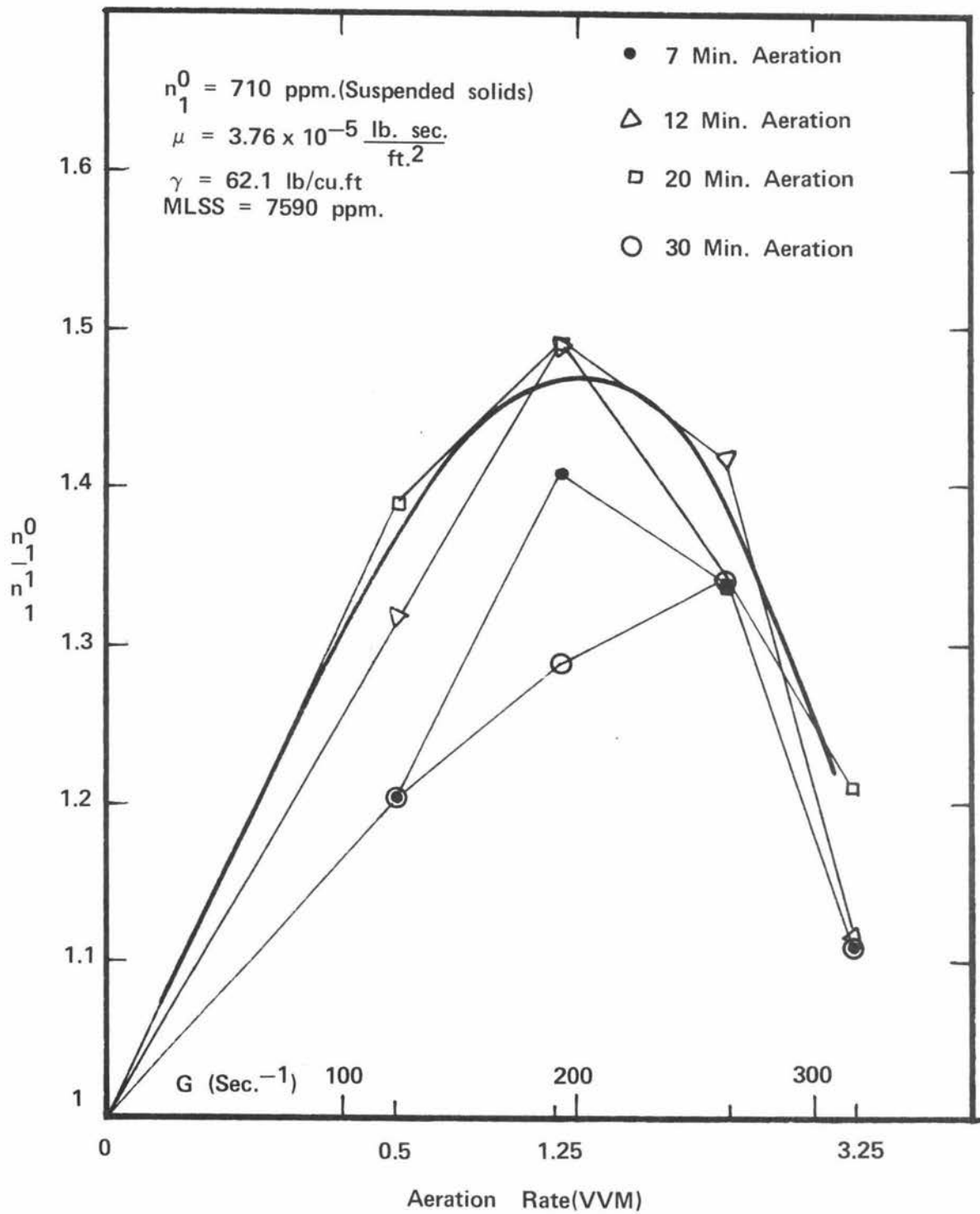


FIGURE 6. PLOT OF FLOCCULATOR PERFORMANCE (SUSPENDED SOLIDS) WITH VELOCITY GRADIENT OR AERATION RATE. (1 Lit. Flocculator)

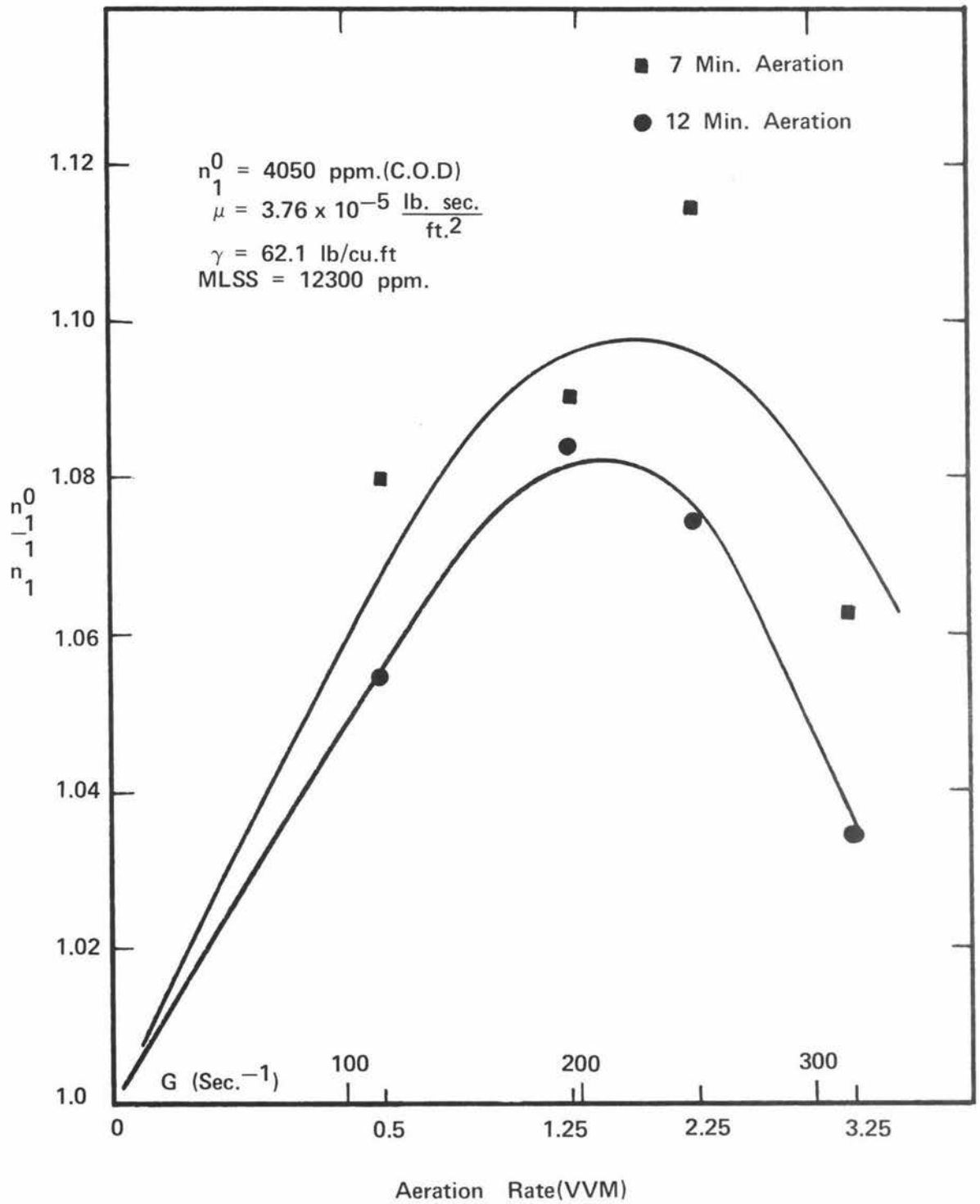


FIGURE 7. PLOT OF FLOCCULATOR PERFORMANCE (AS C.O.D.)
 WITH VELOCITY GRADIENT OR AERATION RATE.
 (1 Litre Flocculator)

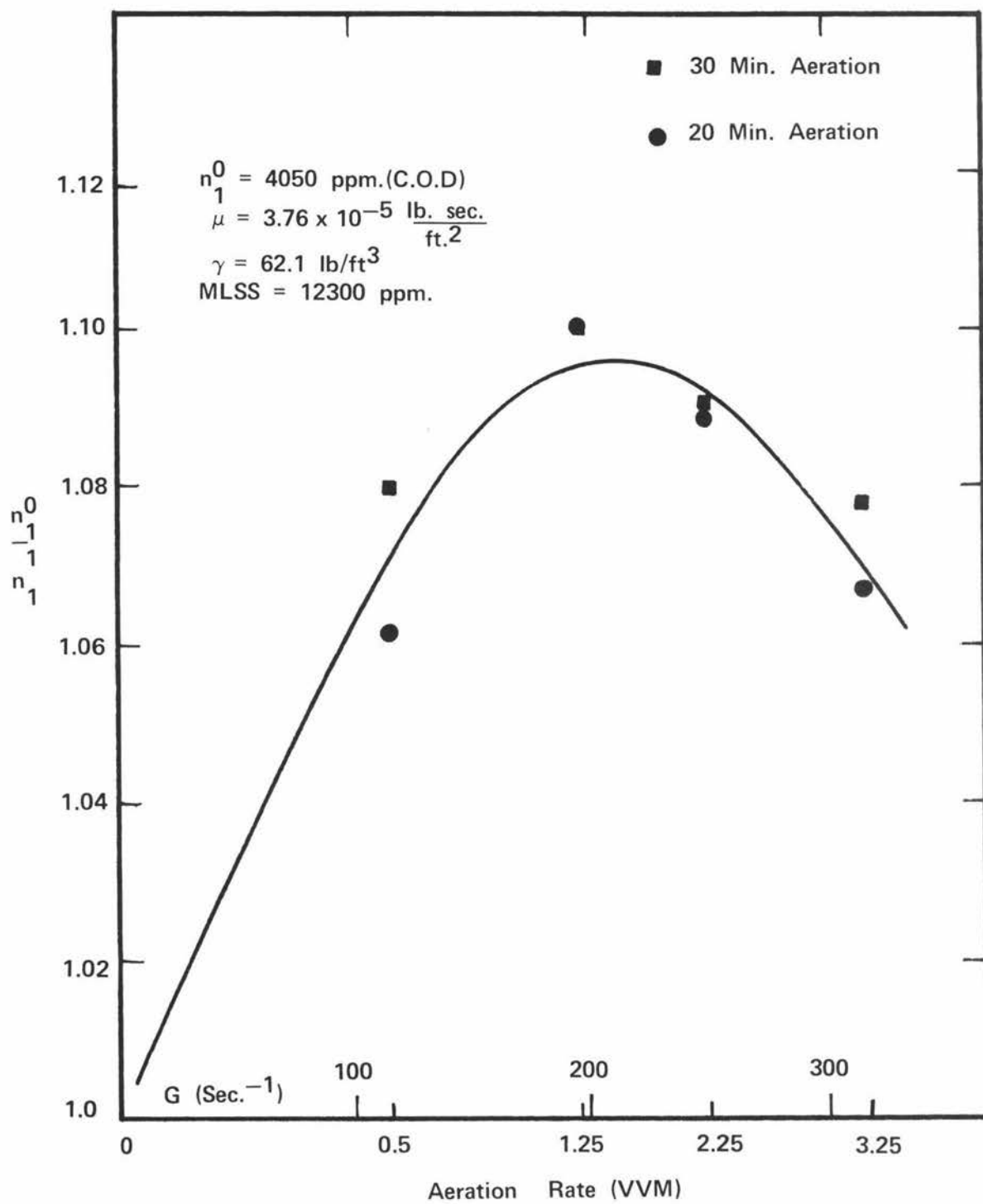


FIGURE 8. PLOT OF FLOCCULATOR PERFORMANCE (AS C.O.D.)
 WITH VELOCITY GRADIENT OR AERATION RATE.
 (1 Litre Flocculator)

needed for completion of flocculation. It can be concluded from Figures 6, 7 and 8 that the optimum flocculation time was between 12 to 20 minutes. The turbulent intensity, in terms of root mean square velocity gradient, was about 200 sec^{-1} .

The flocculation of the sludge from the laboratory-scale fermenters was also investigated in a two litre flocculation chamber. The results are summarized in Figures 9 and 10. Reduction of suspended solids after 15 minutes flocculation is demonstrated in Figure 9. The maximum efficiency was observed at approximately 200 sec^{-1} rms "velocity" gradient as in the earlier tests in the smaller vessel. COD reduction (Figure 10), on the other hand, reached maximum efficiency at the higher velocity gradient (G) of about 250 sec^{-1} .

It was realized that flocculation of the sludge must also depend upon the properties of the sludge mixed liquor, apart from physical conditions. An investigation was therefore undertaken with sludge obtained from the pilot-scale fermenter operated at 25°C . The results (Figure 11) indicate that after 15 minutes flocculation, the maximum reduction of suspended solids was obtained at a velocity gradient value of approximately 100 sec^{-1} .

The rates of sedimentation of the sludges after aeration from both laboratory and pilot scale fermenters were also observed. Sedimentation was investigated in a two litre measuring cylinder. The results are summarized in Figures 12, 13 and 14.

The height of the sludge interface as a function of time of the sludge from a laboratory-scale fermenter is shown graphically in Figure 12. It indicates that after different rates of aeration, the settling rates of the sludge were reduced drastically. On the other hand, the settling characteristic of the sludge from the pilot-scale fermenter was slightly improved after the sludge suspension was subjected to aeration prior to sedimentation.

3. Discussion

Experimental results indicated that aeration flocculation could be employed, as an alternative to a low velocity paddle stirrer, to improve physical flocculation of the primary particles in the mixed liquor. The result was that suspended solids and chemical oxygen demand (COD) of the supernatant liquor decreased

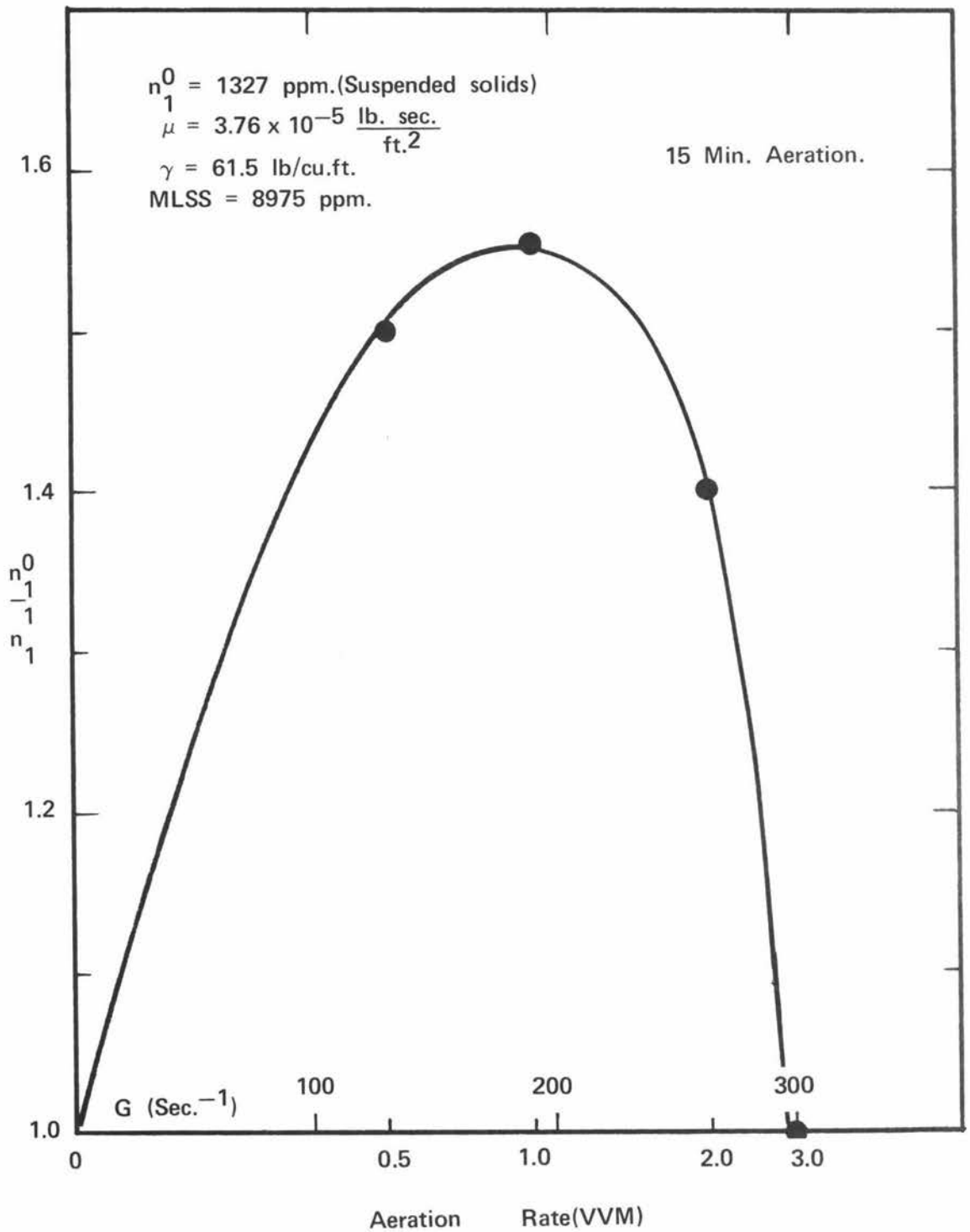


FIGURE 9. PLOT OF FLOCCULATOR PERFORMANCE (SUSPENDED SOLIDS) WITH VELOCITY GRADIENT OR AERATION RATE. (2 Lit. Flocculator.)

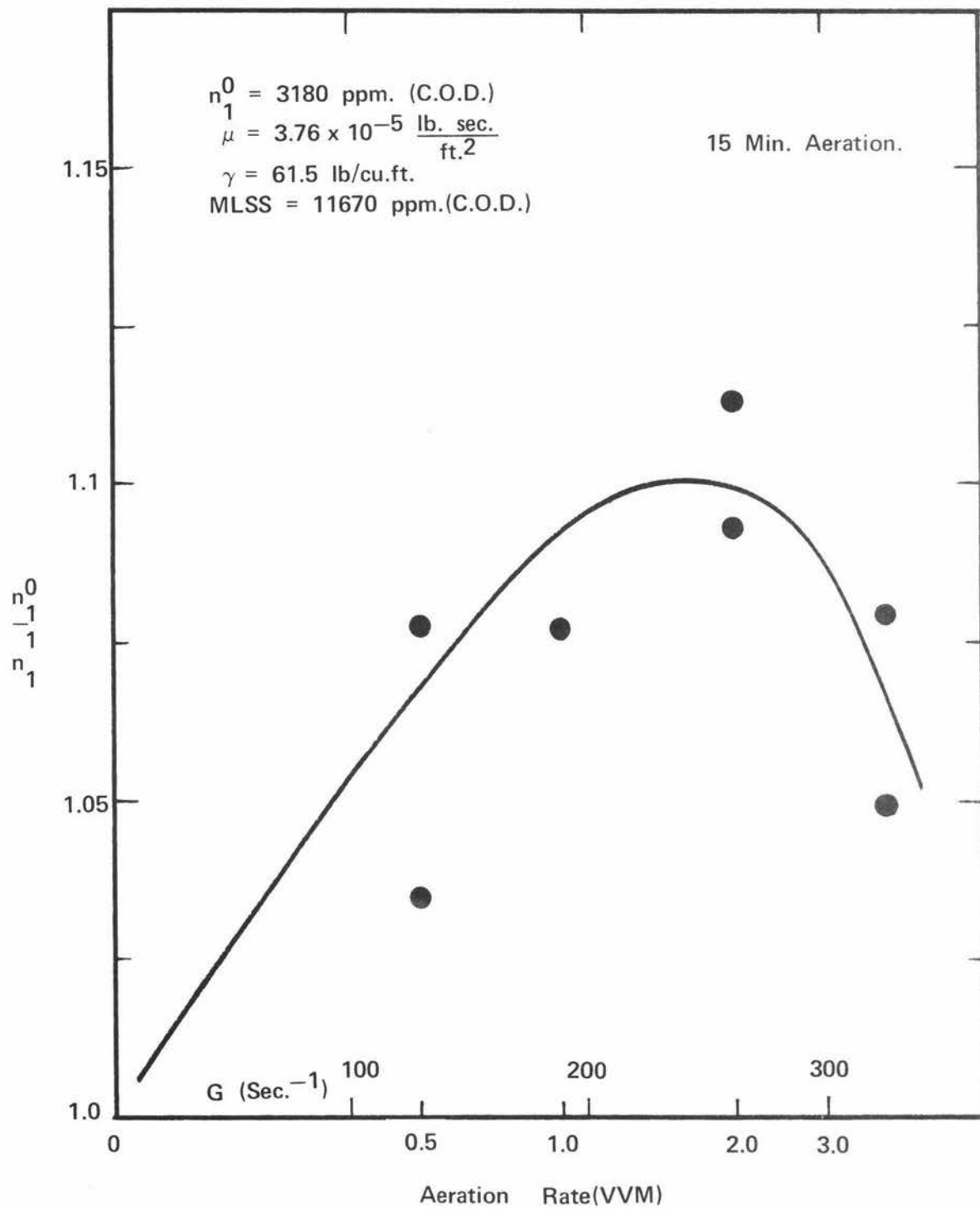


FIGURE 10. PLOT OF FLOCCULATOR PERFORMANCE (C.O.D.)
 WITH VELOCITY GRADIENT OR AERATION RATE.
 (2 Lit. Flocculator.)

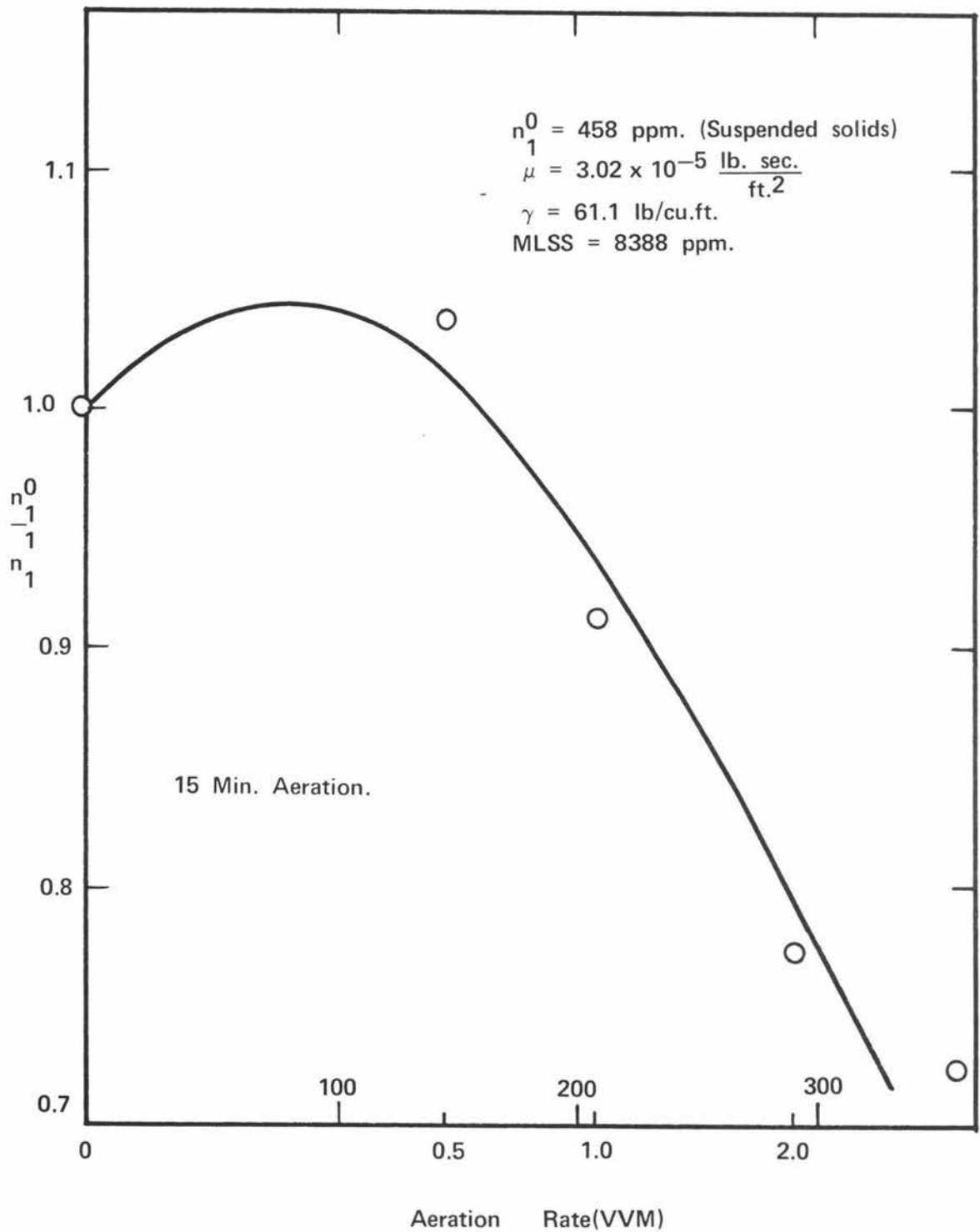


FIGURE 11. PLOT OF FLOCCULATOR PERFORMANCE (SUSPENDED SOLIDS) WITH VELOCITY GRADIENT OR AERATION RATE.
 (2 Lit. Flocculator.)

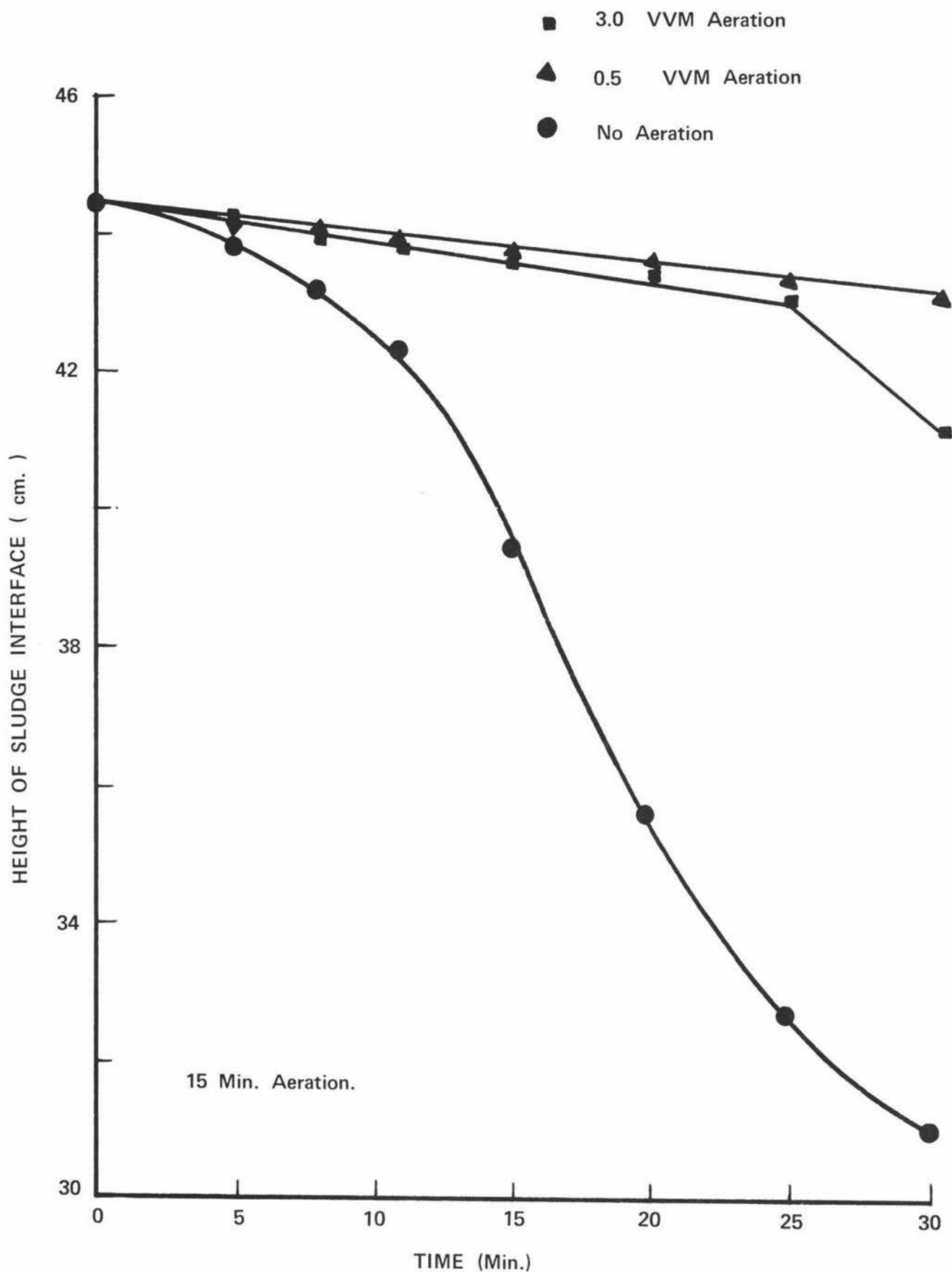


FIGURE 12. PLOT OF HEIGHT OF SLUDGE INTERFACE AND SETTLING TIME.
(Sludge from the laboratory-scale fermenter.)

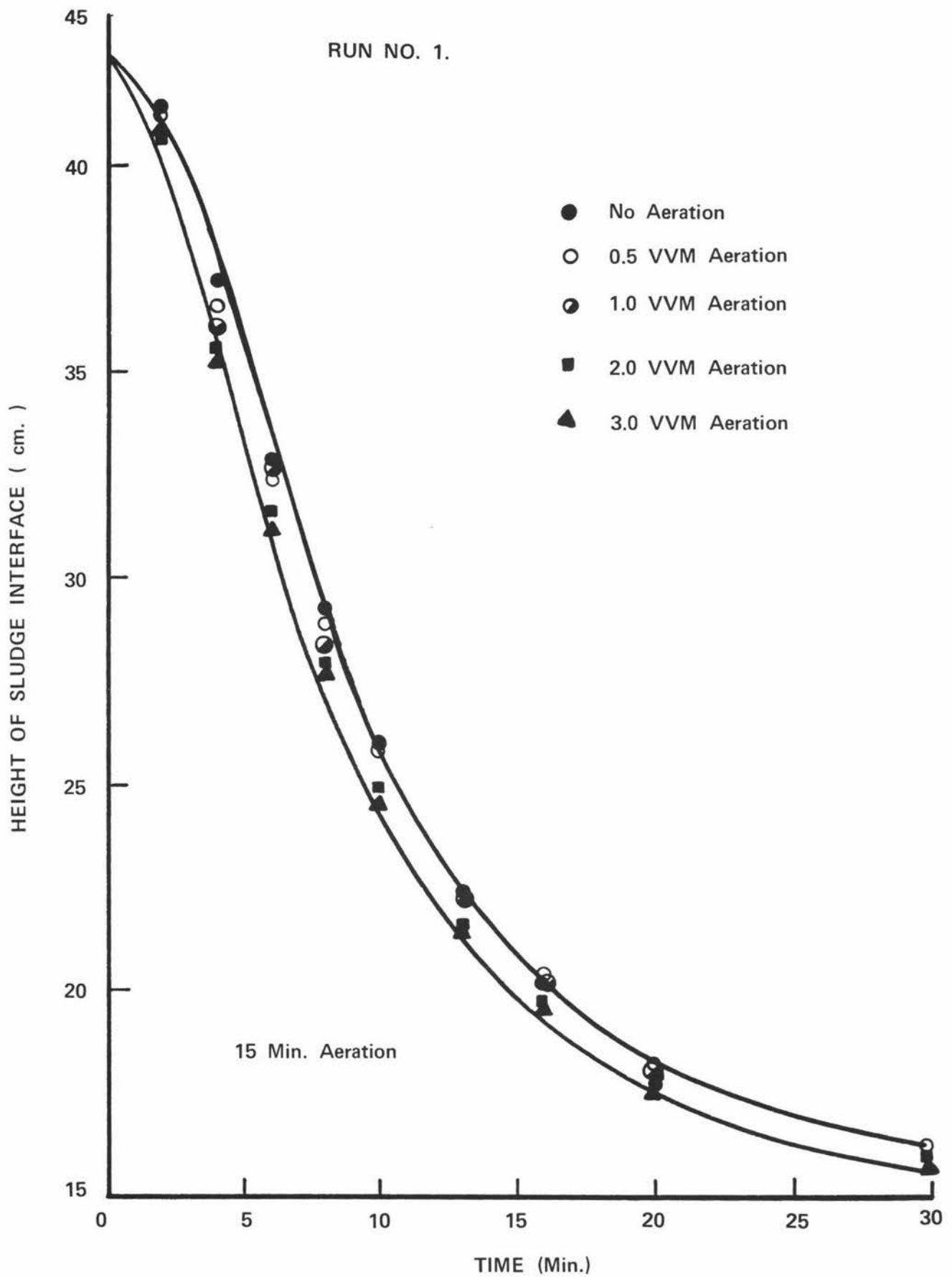


FIGURE 13. PLOT OF HEIGHT OF SLUDGE INTERFACE AND SETTLING TIME.
(Sludge from the pilot-scale fermenter.)

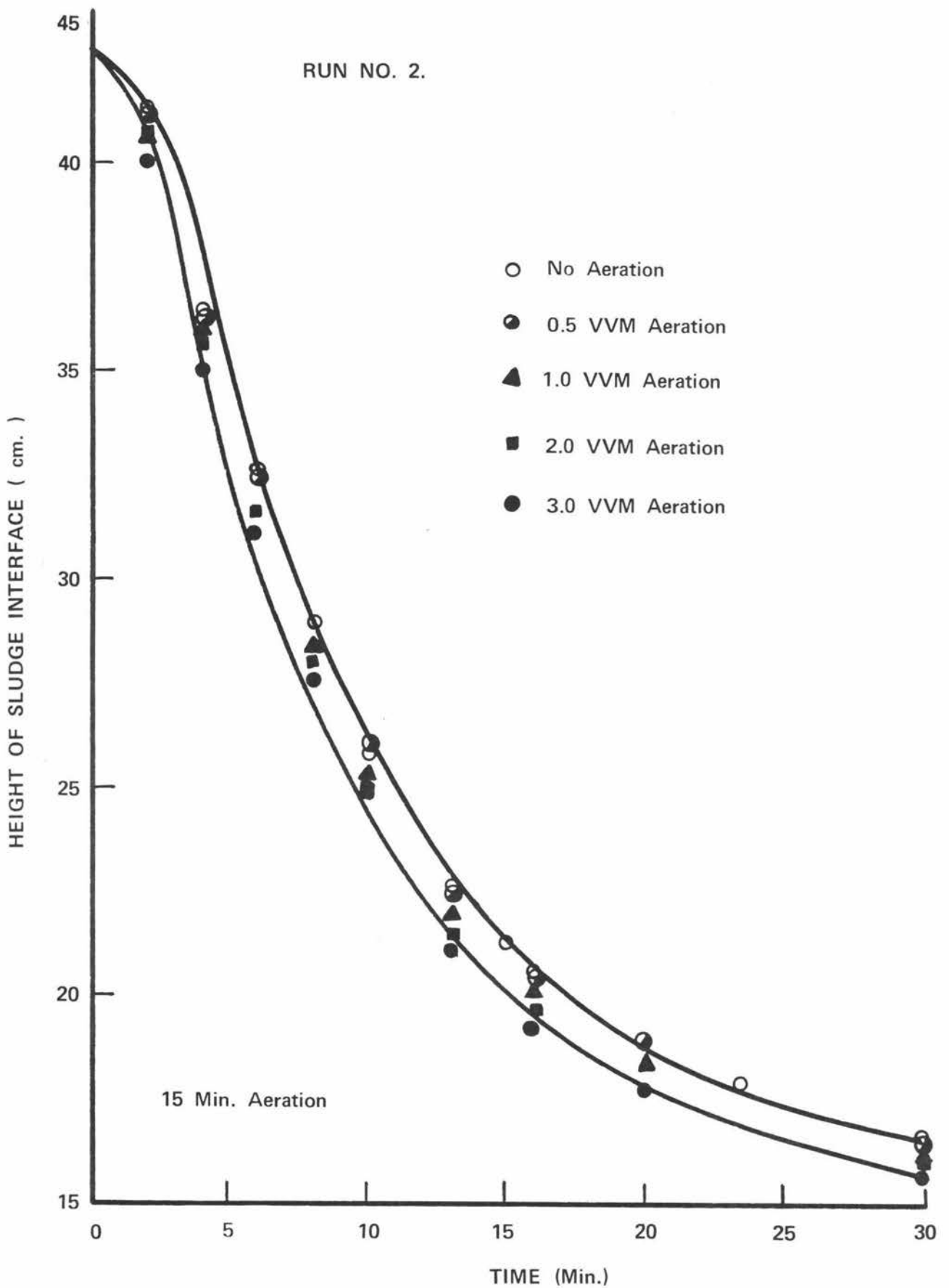


FIGURE 14. PLOT OF HEIGHT OF SLUDGE INTERFACE AND SETTLING TIME.
(Sludge from the pilot-scale fermenter)

to a certain extent, depending upon the characteristics of the sludge flocs and the operational conditions. It was not known whether flocculation in anaerobic cultures was purely physical, or whether any natural polymers produced by bacteria played a role in the flocculation.

It has been reported by many workers that poly-beta-hydroxybutyric acid (PHB) plays an important role in flocculation of activated sludge. The mechanisms and production of PHB were recently reviewed by Boyle et al. (49) and it is indicated in the report of Parker (15) that PHB plays a significant role in the flocculation of activated sludge. Thus it might be possible that PHB or similar substances are produced by the anaerobes in anaerobic digestion. This is referred to in the statement made by McKinney (18) - "bioflocculation occurs by similar process in both aerobic and anaerobic systems". Mild stirring and aeration serve to increase the collisions of the primary particles and the bacterial flocs.

The formula previously developed by Parker (15) to express turbulent intensity in terms of rms velocity gradient (G) in activated sludge reactors was found satisfactory for the calculation of G in these flocculation chambers.

The performance of the flocculation chamber was expressed in terms of both suspended solids and COD after 30 minutes of quiescent settling. As shown in Figures 6 to 10, suspended solids seem to be a better parameter to use to express the performance of the flocculation chambers. A direct count of primary particles was recommended by Parker (16) but suspended solids' determination by fibre glass filter papers was found more satisfactory in this study.

The optimal velocity gradient for the maximum reduction of suspended solids was in the range of 100 to 200 sec^{-1} after 15 minutes aeration. The lowest value of velocity gradient (100 sec^{-1}) was found in flocculation tests of the sludge obtained from a pilot-scale fermenter. These figures are high in comparison with the optimum values of 40 sec^{-1} , and 10-60 sec^{-1} reported by Parker et al. (17) for physical flocculation of activated sludges and water treatment respectively. Values of G of the

magnitude observed in this investigation have been commonly reported in aerobic activated sludge fermenters ($G = 136 \text{ sec}^{-1}$).

The data indicate that the physical flocculation characteristics reported in the case of activated sludge and water treatment, are substantially different from those found with sludge from the anaerobic contact process. The sludge concentration and the different sludge characteristics are probably the main factors. The sludge concentration in anaerobic contact processes (7,500-12,000 ppm) is several times higher than those of activated sludge, which was reported by Parker (16) to be about 1522 ± 311 ppm. Consequently, the concentration of primary particles in the anaerobic process would be expected to be high (457.5-710 ppm), higher than that of activated sludge (53.1-12) (16). This might account for the higher G values observed in the flocculation of anaerobic sludge.

The concentration of primary particles has been demonstrated by Parker et al. (16) to affect the flocculation (K_A) and floc breakup (K_B) coefficients. They found that under the same operating conditions, the higher the concentrations of primary particles, the higher the values of flocculation and floc breakup coefficients. With the concentration of primary particles of 53.1 and 12 mg/litre, values of K_A were 2.8×10^{-6} and 0.79×10^{-6} , and K_B were 113×10^{-8} and 4.7×10^{-8} respectively.

This would probably explain the variations in primary particles reduction (flocculator performance) found in the different tests. The percentage reductions of 55% and 48% were found with the initial primary particle concentrations of 1327.5 and 710 mg/litre respectively, while only 4.5 per cent reduction was reported in the sludge with initial primary particle concentration of 457.4 mg/litre.

It was reported by Steffen and Bedker (8) that no improvement in sludge concentration was found when the sludge was aerated prior to sedimentation. However, Rands and Cooper (9) found that aeration enhanced settlement of the sludge solids. The relationships between aeration and sludge settlement reported in the literature are thus conflicting and inconclusive. Figure 12 shows that after the aeration of 0.5 and 3 VVM for 15 minutes, the settlement of sludge obtained from a laboratory-scale fermenter

was reduced drastically. After 30 minutes of quiescent settling the heights of the sludge interface had fallen only to 96.2, 92.4 and 69.7 per cent of the total height of the measuring cylinder where the sludge was previously aerated at 0.5 VVM, 3 VVM and no aeration respectively. However, the sludge obtained from a pilot-scale fermenter showed a slight improvement in sedimentation when the sludge suspensions were subjected to aeration at 0.5 to 3.0 VVM (Figures 13 and 14).

The reason for this would probably lie in the operating conditions, in the fermentation, perhaps in temperature. The fermenter reported by Randa and Cooper (9) was operated at ambient temperature (of about 20°C) while the pilot-scale reactor was operated at 25°C. Also, the active sludge concentrations from both cases would probably be lower than those of the laboratory-scale fermenter and lower than those reported by Steffen and Bedker (8).

This explanation is supported by the results of Schroeffer and Ziemke (6) who found that the settlement of the sludge from a fermentation operated at 35°C was slower than that from a fermentation operated at 25°C.

B. GAS PRODUCTION

1. Materials and Methods

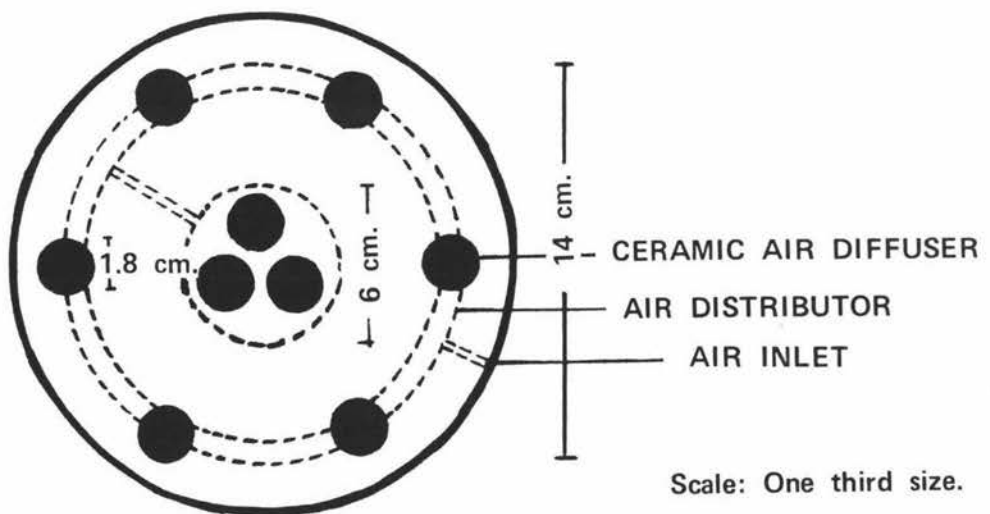
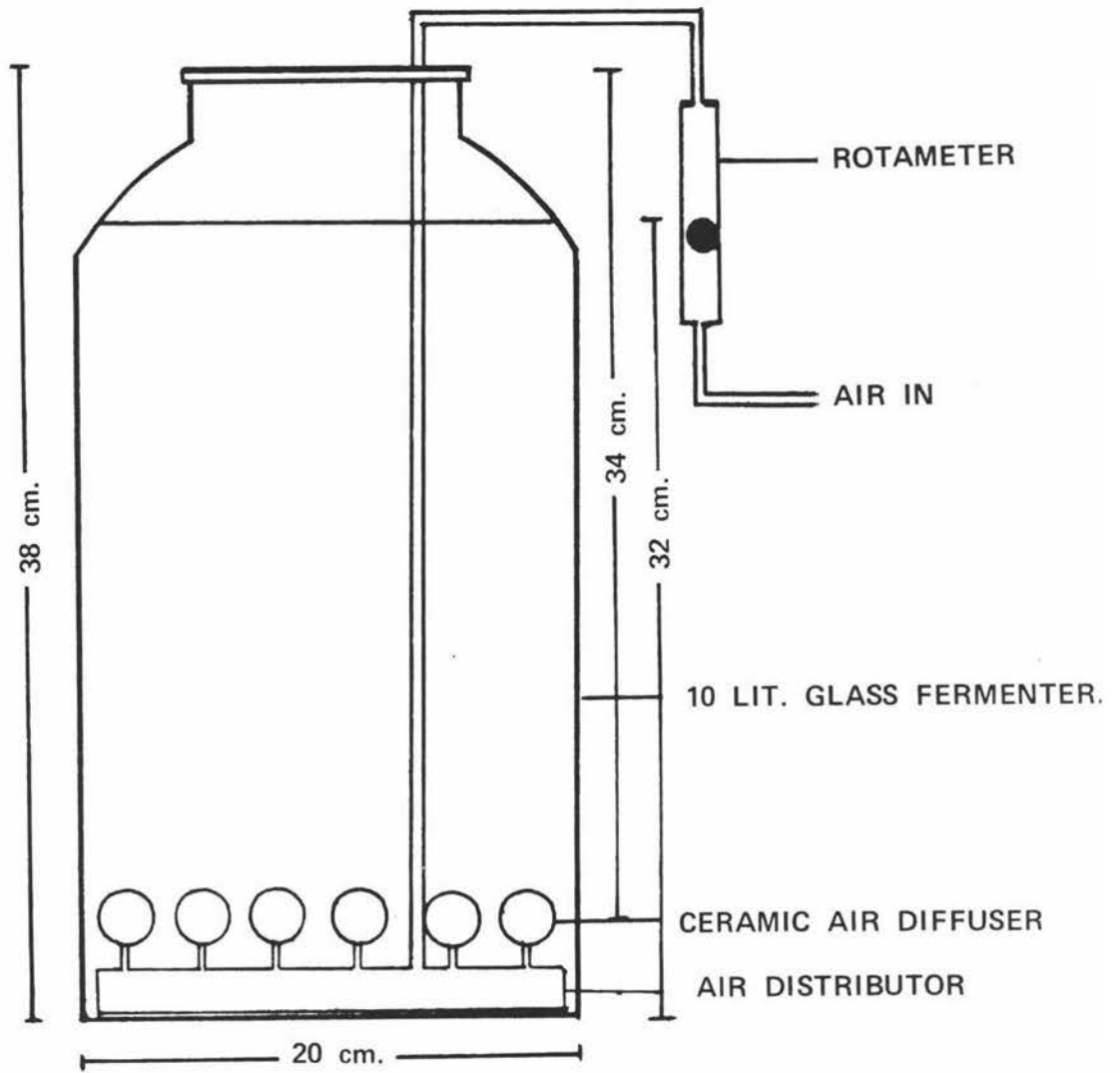
The operation was generally the same as that described in the operation section for the laboratory-scale fermenters, except that the sludge solids were aerated before they were allowed to settle.

Aeration was carried out in a 10 litre glass fermenter, with the air blown in through nine ceramic air diffusers. These were connected together in such a way that three lay in the middle and the remainder in the outer ring. The air tube was connected through a rotameter to the air compressor. The assembly of the equipment is shown diagrammatically in Figure 15.

2. Results

a) Sulphite Oxidation of the Aerating System

Sulphite oxidation is one of the methods generally accepted for the determination of oxygen transfer coefficients in fermenters.



Scale: One third size.

FIGURE 15. A SCHEMATIC DIAGRAM OF AERATION CHAMBER
(10 Lit. Capacity)

The method was well described by Cooper et al. (50).

Figure 16 illustrates the results of the oxygen transfer coefficient ($k_L a$) at 35°C in the 10 litre aerating chamber. The mathematical relationships between $k_L a$ and aeration rate can be written following Steel and Brierly (51) as:

$$k_L a = k G^{0.9115}$$

Where:

- $k_L a$ = mass transfer coefficient, hr^{-1}
 k = constant
 G = air flow rate (VVM)

The relationships between aeration rates and mass transfer coefficient are shown in Table III.

TABLE III: Mass Transfer in the Aeration Vessel

Aeration rates (VVM)	0.25	0.5	0.75	1.0	1.25
$k_L a$ (hr^{-1})	76	144	198	269	320.6
Rates of oxygen absorption $\frac{mg}{litre.hr}$	560	1060	1460	1980	2360

b) Rates of Gasification

Both laboratory-scale fermenters were used for the determination of the rate and total amount of gas formation, one as a control, and the other for the aeration tests. Before the aeration tests were started the fermenters (Fermenter no. I and Fermenter no. II) were brought to a steady state condition. The steady state condition was observed over seven days. Daily gas production for the period of 7 days indicated that the performance of both fermenters was identical. Statistical analysis of daily gas production for the period mentioned above (Appendix 3) showed that the performance of both fermenters was insignificantly different at the 95 per cent level.

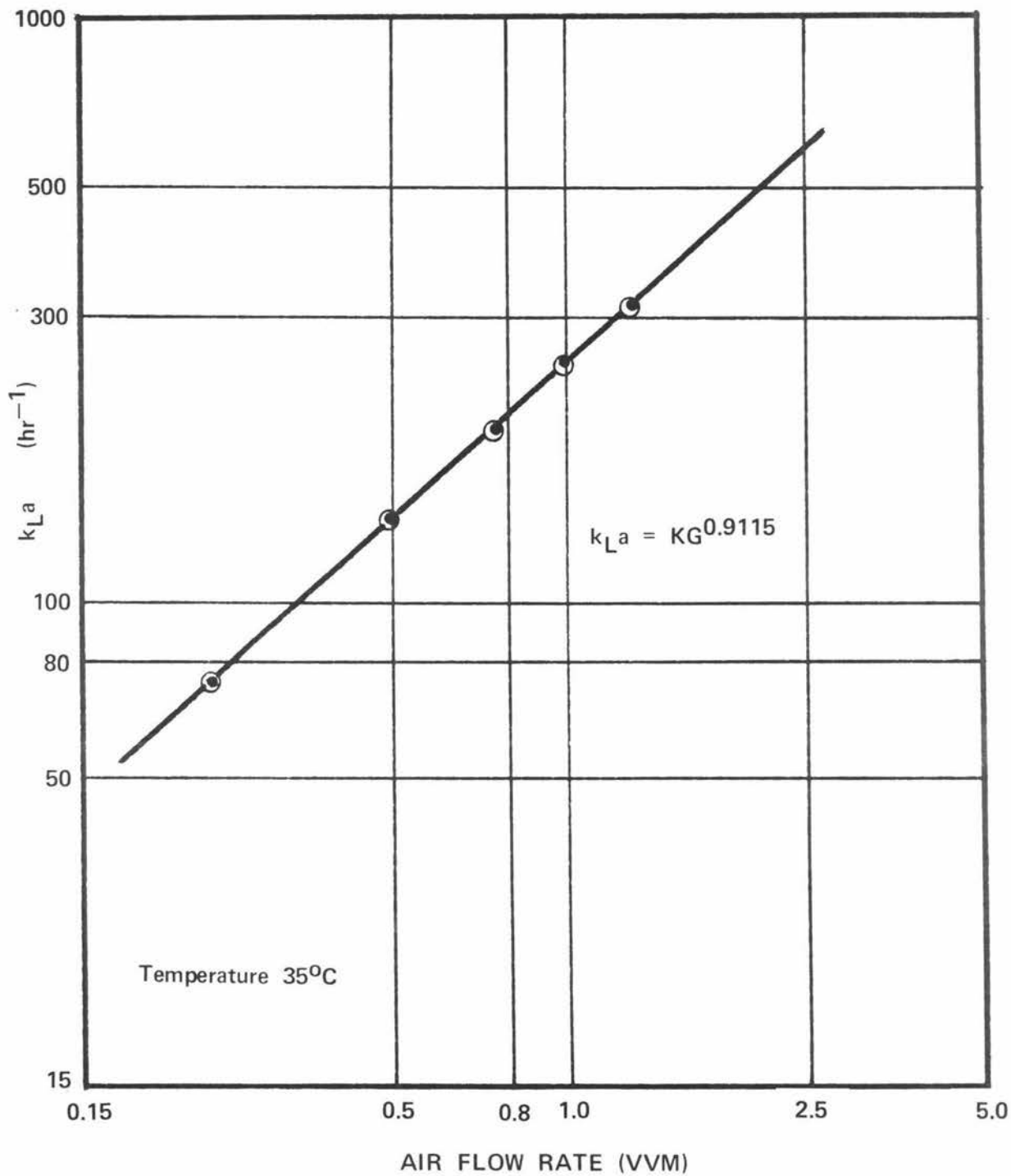


FIGURE 16. PLOT OF OXYGEN TRANSFER COEFFICIENT AGAINST AIR FLOW RATES.

The fermenters were aerated at rates of $0.75 \frac{\text{Vol.}}{\text{Volume.Min}}$ (VVM) which was found to be the optimal level for flocculation (Section V.A.). The time allowed for aeration was varied from 15 to 60 minutes, and at each level of aeration the test was operated on two to three consecutive days. The results are summarized graphically in Figure 17.

It is shown in Figure 17 that without aeration constant rates of gas formation were observed for a period after the fermenter was started and the rates then fell off after 10 hours of fermentation. When the mixed liquor was aerated the pattern of gas formation altered. There was a lag at the beginning of fermentation, after which, for a time, constant rates were observed. The longer the aeration, the longer the lag period was observed. When aeration was increased to one hour the rates of gas formation were altered and the volumes of the gases produced were reduced drastically.

In the final stage of gas formation, after the constant rates period, the rates gradually decreased after 10 to 14 hours of digestion. However, gas production did not cease even after a relatively long period of digestion without feeding. This is shown in Figure 18, when the digesters were operated for 42 hours after the sludge solids were contacted with air at the rate of 0.75 VVM for 30 minutes. At the end of the second day the gases still continued to evolve. After 24 hours of digestion the rate of gas formation was virtually constant at about 6 per cent of the maximum rate of gas formation.

After the end of the third day of 60 minutes aeration (0.75 VVM), Fermenter no. II was allowed to recover by feeding it daily (as described previously) and without further aeration. The results (Figure 19) show that after the digester had been aerated the gas formation rates took a considerable time to return to their former values, but after a period of time (about 13 to 14 days in the case of 60 minutes aeration) they appeared to recover almost completely.

When 3650 mg of oxygen (measured by the sulphite oxidation technique) were absorbed (0.75 VVM aeration for 15 minutes), about four days were needed to bring the efficiency of the fermenter back to normal. The results are shown in Figure 20.

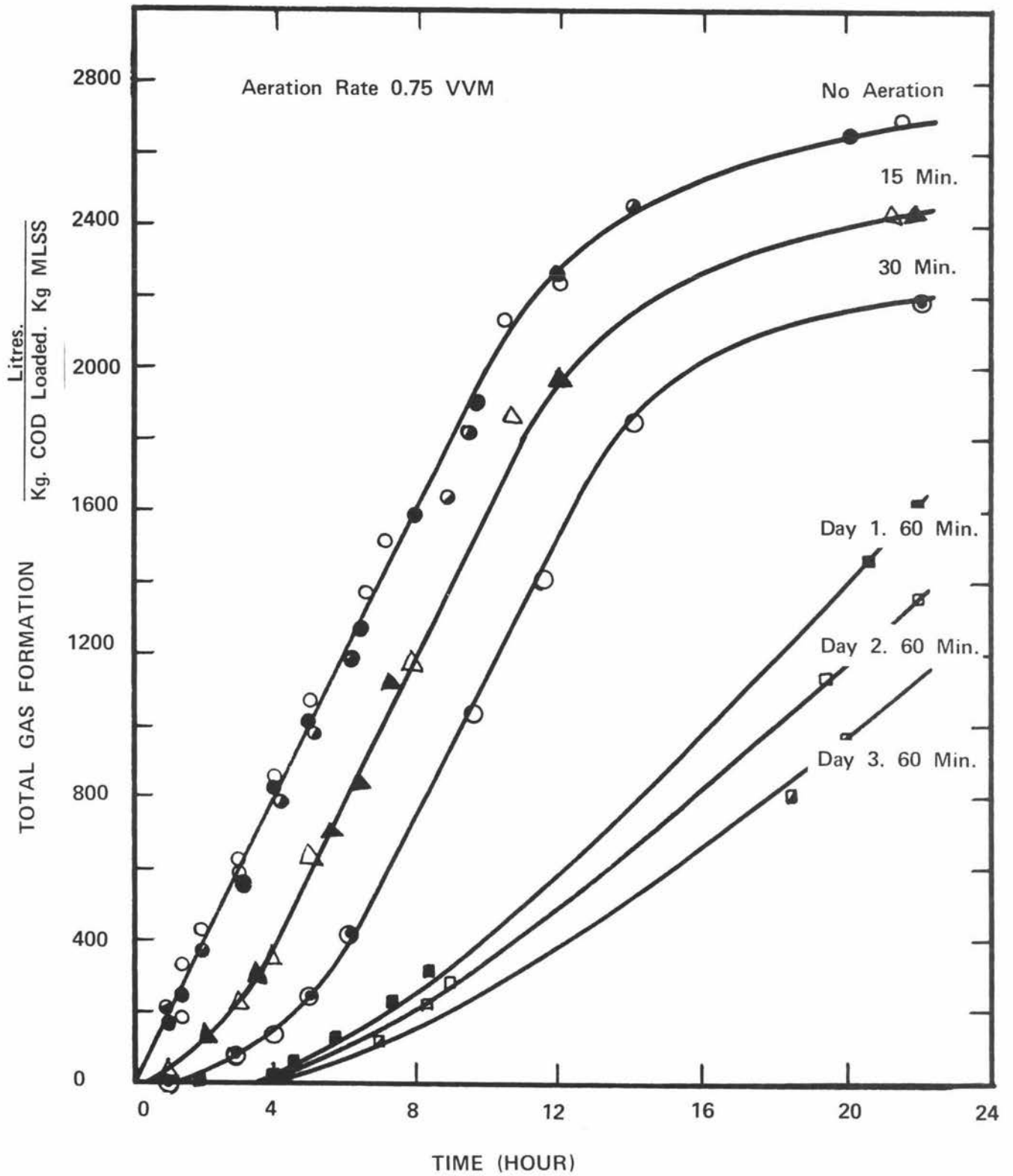


FIGURE 17. AERATION AND GAS FORMATION

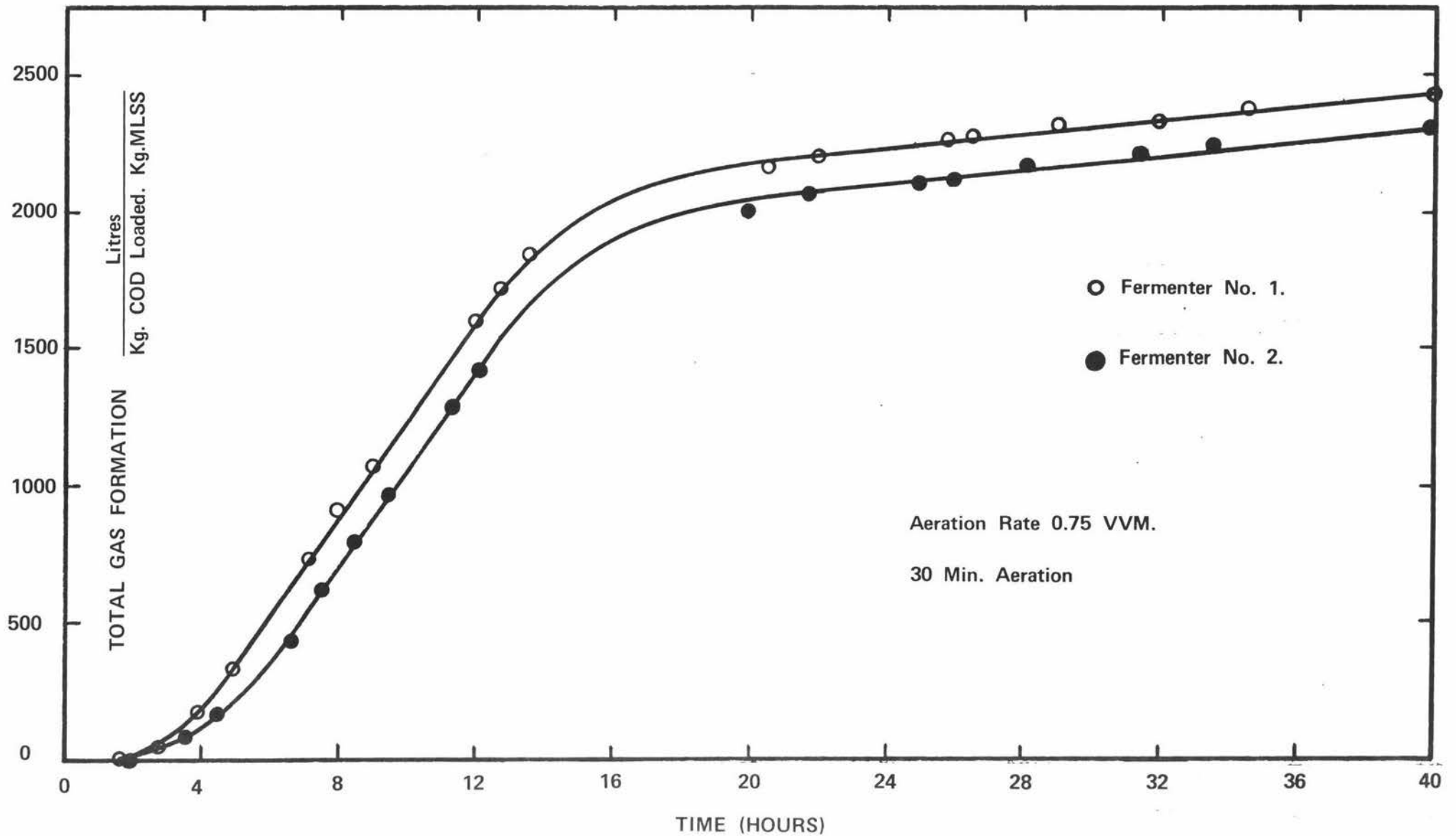


FIGURE 18. PATTERNS OF GAS FORMATION

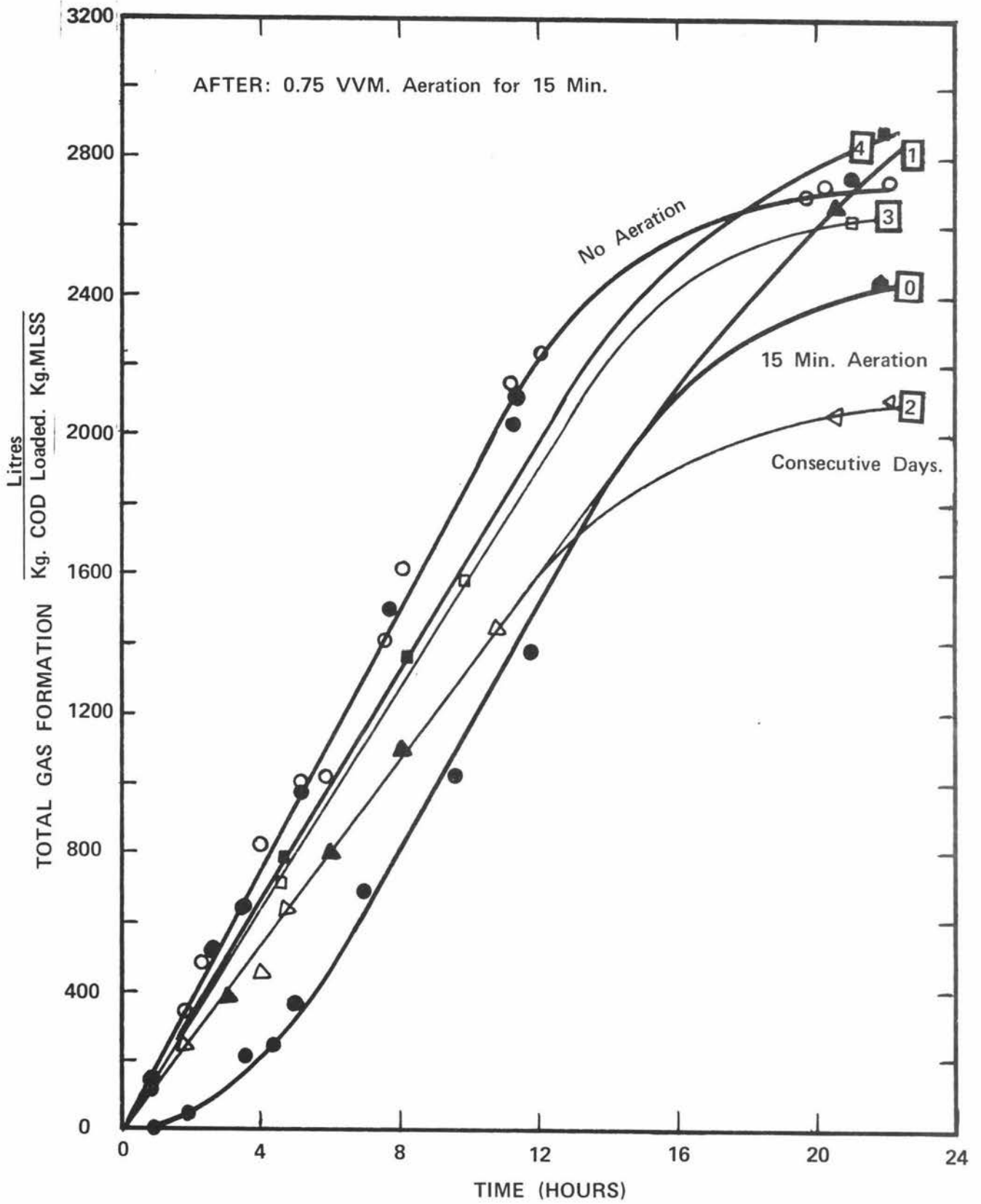


FIGURE 20. GAS FORMATION AND FERMENTER RECOVERY

The length of time needed for recovery of the digester depends upon the degree to which the anaerobic sludge was previously inactivated. The more oxygen absorbed, with consequent damage of bacterial activity, the longer the time needed for recovery of the digester.

It is interesting to note that when the fermenters were fully recovered no lag was found. As soon as the aeration was stopped and normal feeding resumed almost the same pattern of gas formation as the control was observed. Constant rates of gas formation were observed for a period after the fermenter was started and the rates then fell off after a certain period of digestion. The constant rates were lower than those of control on the first day of digester recovery, but with time the gas production rates (constant rates) gradually increased. Besides, during the eight days of normal feeding with no aeration (Figure 19), no falling rates of gas formation after the period of constant rates were observed. It could be postulated that during this period of digestion the dissolved COD substrates present in the digester were so high that they did not limit the rate of gas formation in 22.5 hours of digestion. It is shown in Figure 28 that when the digester was aerated for 60 minutes the digested supernatant was found to contain COD of about 2700 ppm, while only 1400 ppm of COD was normally analysed in the effluent from the digester without aeration.

The total oxygen or air absorbed into the mixed liquor was governed by two factors: the rate of aeration, and time. It was not certain whether the time factor would have any effect on inactivation of the anaerobes independently of the oxygen absorption. An experiment was, therefore, carried out with the two fermenters stabilized so that their rates of gas formation indicated that their performances were identical, and a t-test showed there was no significant difference between them at 95 per cent level (Appendix 4). Fermenter no. I was then aerated for 15 minutes at 0.25 VVM aeration and then for 30 minutes, and Fermenter no. II for 15 and then 30 minutes at 0.5 VVM. Under these conditions the quantities of oxygen transferred were calculated for the sulphite oxidation test to be 1400, 2800, 2650, and 5300 milligrams respectively.

The results are shown in Figures 21 and 22. They appear to indicate that the quantity of oxygen transferred, rather than the time of aeration, affected the performance of the digesters. The overall inactivation of the anaerobes was indicated by reduction in total gas formation and the rates of gas formation.

The effect of oxygen-free-nitrogen and pure oxygen on the performance of anaerobic digestion was also investigated. The graphs (Figure 23) indicate that nitrogen has the same general effect on the pattern of gas formulation as air and pure oxygen. Total gas reduction at the end of 22.5 hours digestion was also observed.

Under the same aerating conditions (0.5 VVM aeration for 15 minutes), it was found that the effect of nitrogen on the activity of anaerobic bacterial culture was less severe compared with that of air and pure oxygen. A short period of lag of three hours occurred when the sludge was in contact with nitrogen, while the longer lags of four and five hours were observed when the sludge suspension was aerated with air and pure oxygen respectively. After these treatments the quantity of gas produced (from the sludge which was previously in contact with nitrogen, air and pure oxygen) was quite constant. A constant rate of about 230 litres (per kg. COD loaded. kg. MLSS) per hour was found. For a period the rates of gas production fell off after 10, 10.5 and 11 hours with the sludge being aerated with pure nitrogen, air and pure oxygen respectively until the end of the digestion period.

Both pure nitrogen and oxygen, as well as air, had the same effect on the reduction of the daily gas produced. After 22.5 hours of digestion the total amounts of gas produced were 2880, 2600, 2400 and 2320 litres (per kg. COD loaded. kg. MLSS) from the digesters with no aeration, and aeration with pure nitrogen, air and pure oxygen respectively accounted for 10, 17 and 21 per cent reduction of the total gas formation.

It is interesting to note that in terms of the oxygen absorption value calculated from the sulphite oxidation test, the oxygen transfer coefficient ($k_L a$) obtained from oxygen at the same rate of aeration would be approximately five times as much as that from air. Consequently, the oxygen absorbed from oxygen gas should be five times as much in comparison with that from using the atmospheric air. The results (Figure 23) of the total gas

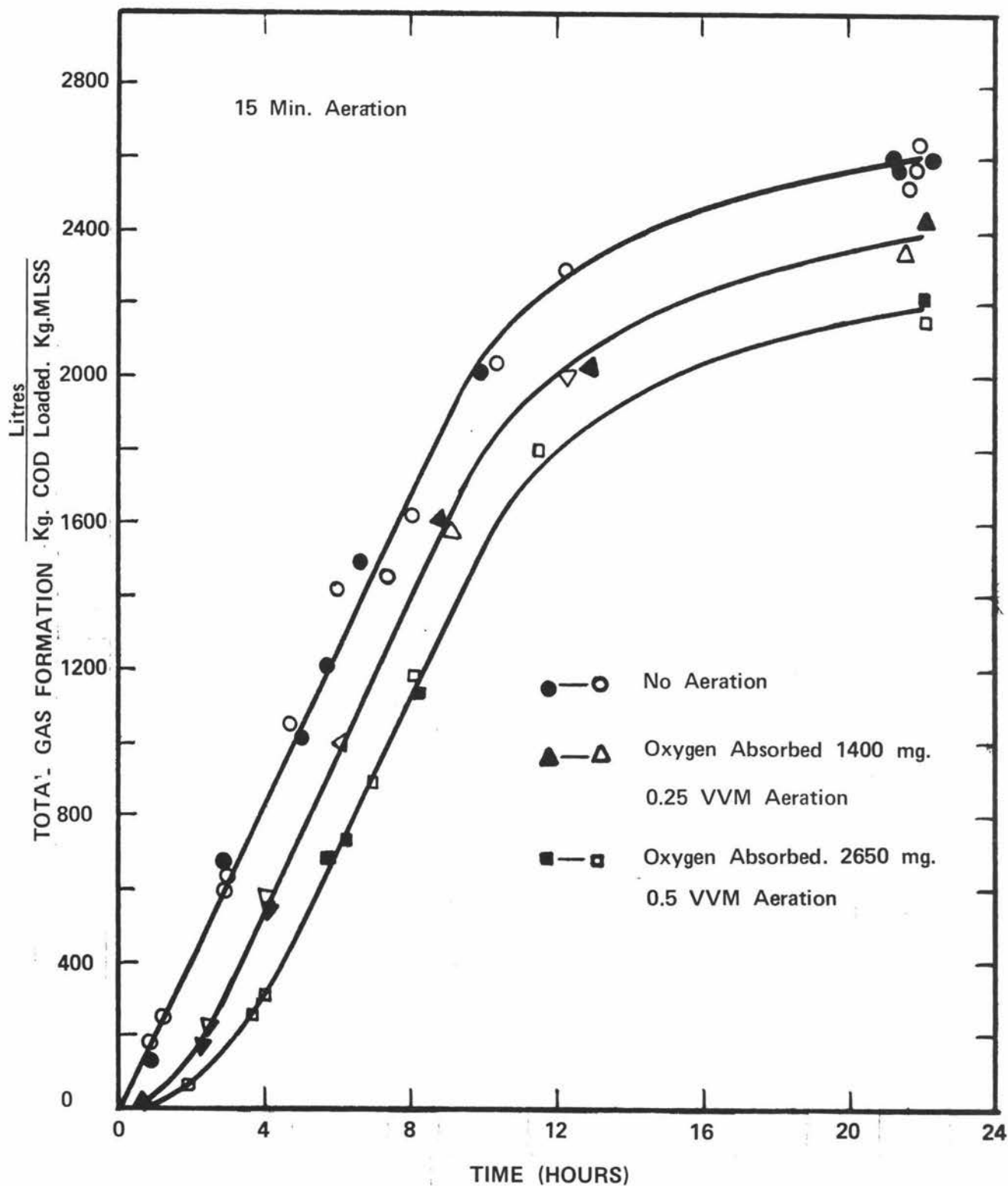


FIGURE 21. AERATION AND GAS FORMATION.

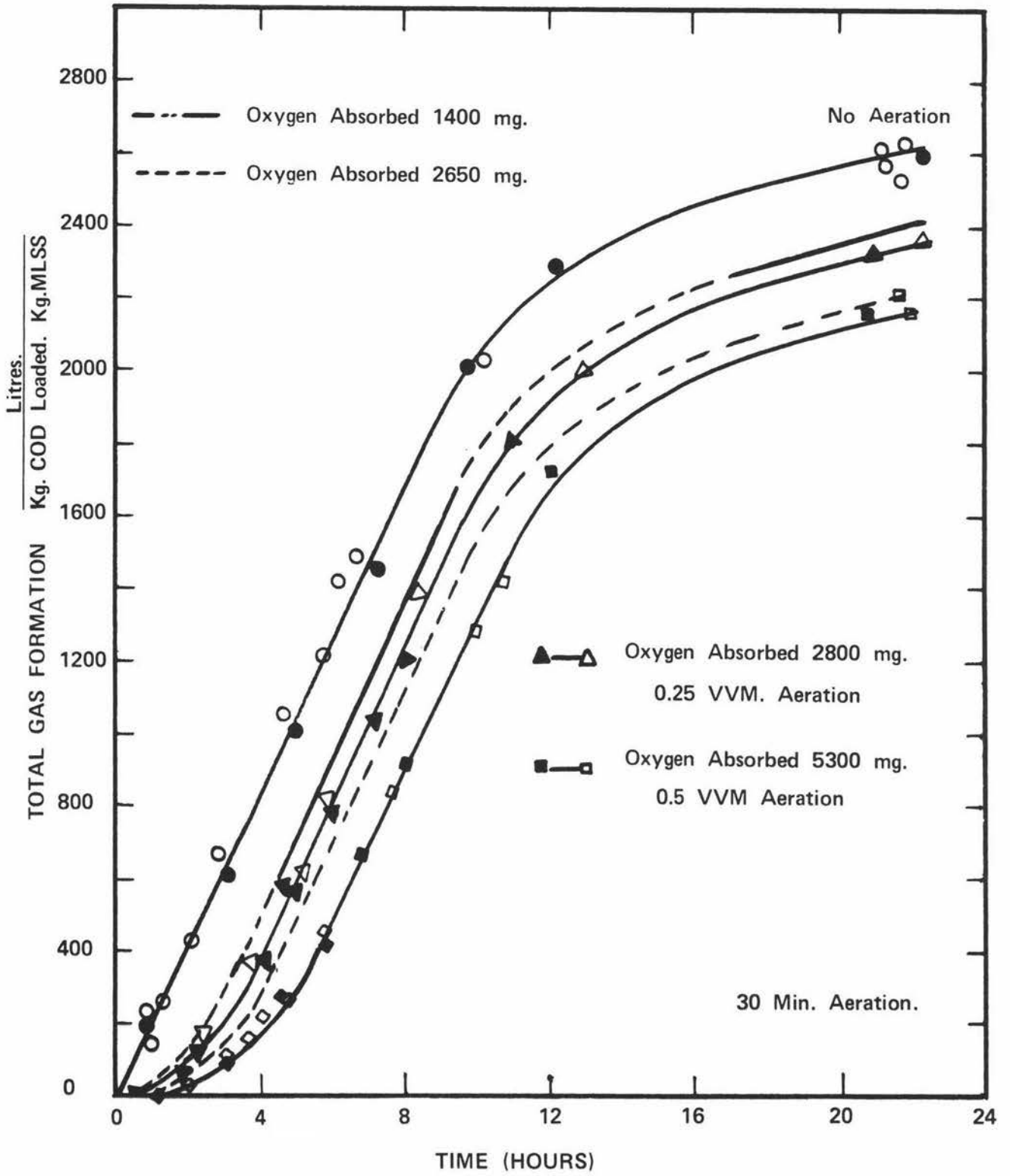


FIGURE 22. AERATION AND GAS FORMATION

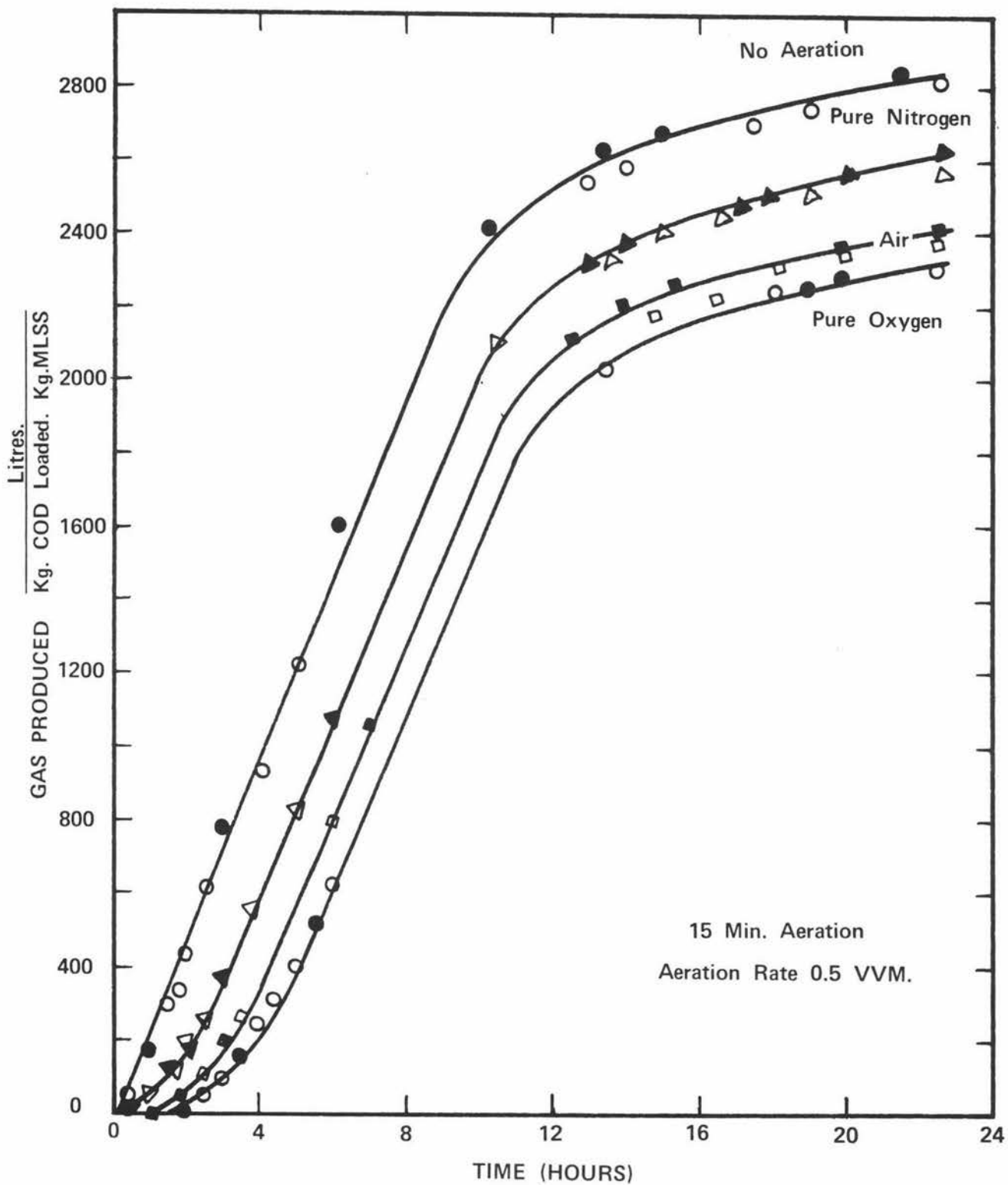


FIGURE 23. GAS PRODUCTION IN THE PRESENCE OF AIR, PURE NITROGEN AND OXYGEN IN THE DIGESTER.

formation contradict the above assumption. There was only 3.3 per cent difference in the total gas formation. The reasons behind this are not known and further investigations are indicated.

The daily (22.5 hours) volumes of gas formation in the first experiment (0.75 VVM aeration) are shown in Figure 24. The graphs (Figure 24) indicate that during the stabilization period for the digesters, the difference between the mean daily gas production was not significant at 95 per cent confidence limits (Appendix 3).

Once the aeration (0.75 VVM aeration for 15 minutes) was started in Fermenter no. II the daily volume of gases being formed decreased from 6500 to 6100 ml. With 15, 30 and 60 minutes aeration of 0.75 VVM the volumes of the gases obtained under comparable conditions averaged from three days after aeration were 6100, 5550 and 3600 ml respectively, while the results obtained from Fermenter no. I (control) were fairly constant with an average volume of 6400 ml per day.

It appears that the amount of gas produced is inversely related to the amount of oxygen absorbed into the mixed liquor prior to sedimentation. The correlation between gas production and the oxygen absorbed into the sludge liquor calculated from the sulphite oxidation test is shown in Figure 25.

The patterns of gas formation over different digestion periods are different. There was hardly any gas produced when the oxygen absorbed was higher than 10,000 mg (1,000 mg/lit.) in the first three hours after aeration. At the digestion periods below 12 hours the curves are concave and the degree of concavity is less when the digestion period becomes longer and longer. After 12 hours digestion a linear relationship between the gas produced and the oxygen absorbed was observed.

The gases produced were mainly methane and carbon dioxide, and trace amounts of others. The gas compositions were found to be 60 to 65 per cent methane, and 40 to 35 per cent carbon dioxide. Methane composition was fairly constant, even when the sludge was subjected to aeration.

It was found that after aeration there was a change in the total volatile fatty acids, and this alteration might have had an effect on the composition of carbon dioxide.

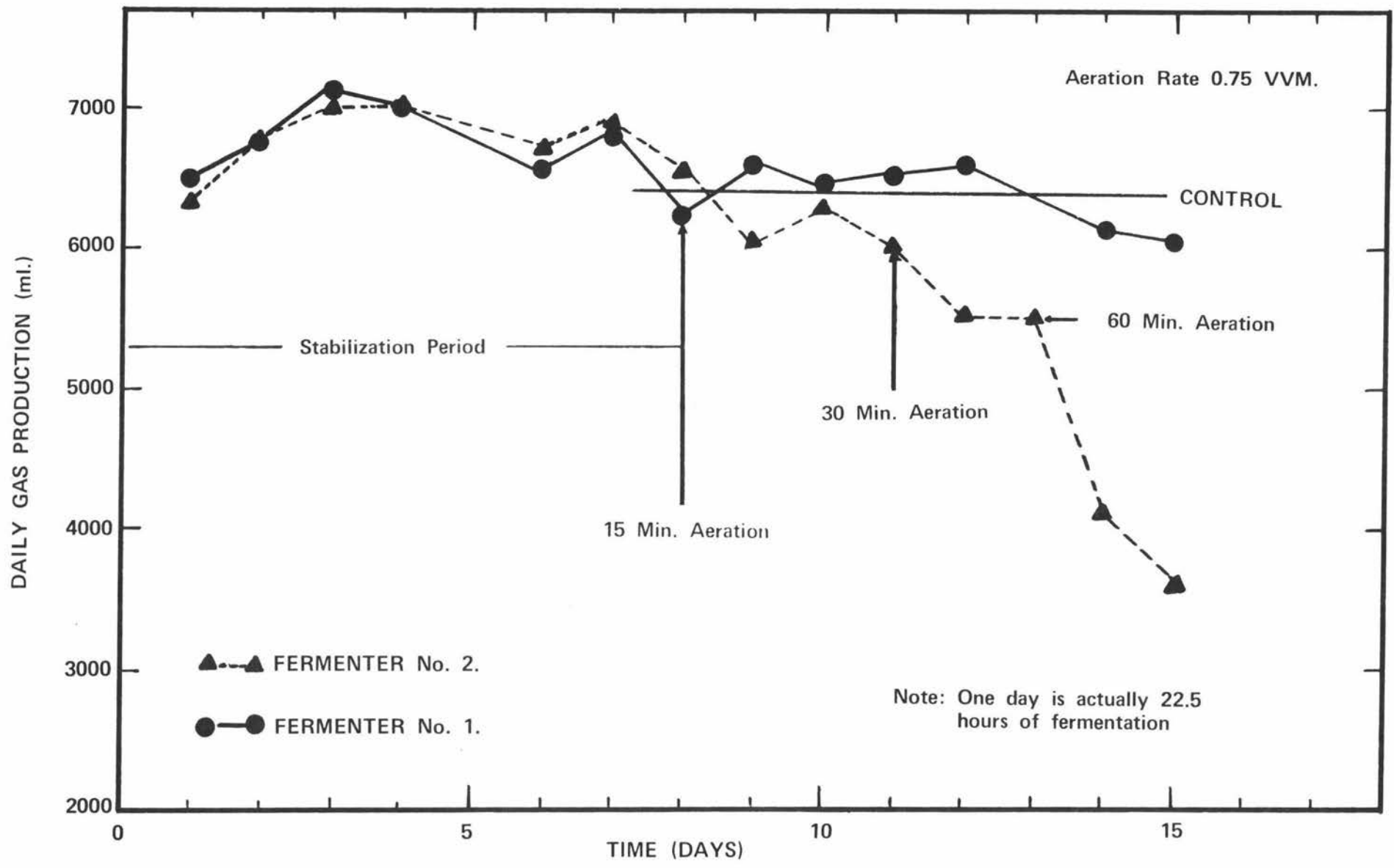


FIGURE 24. PLOT OF GAS PRODUCED PER DAY AGAINST DAYS OF OPERATION.

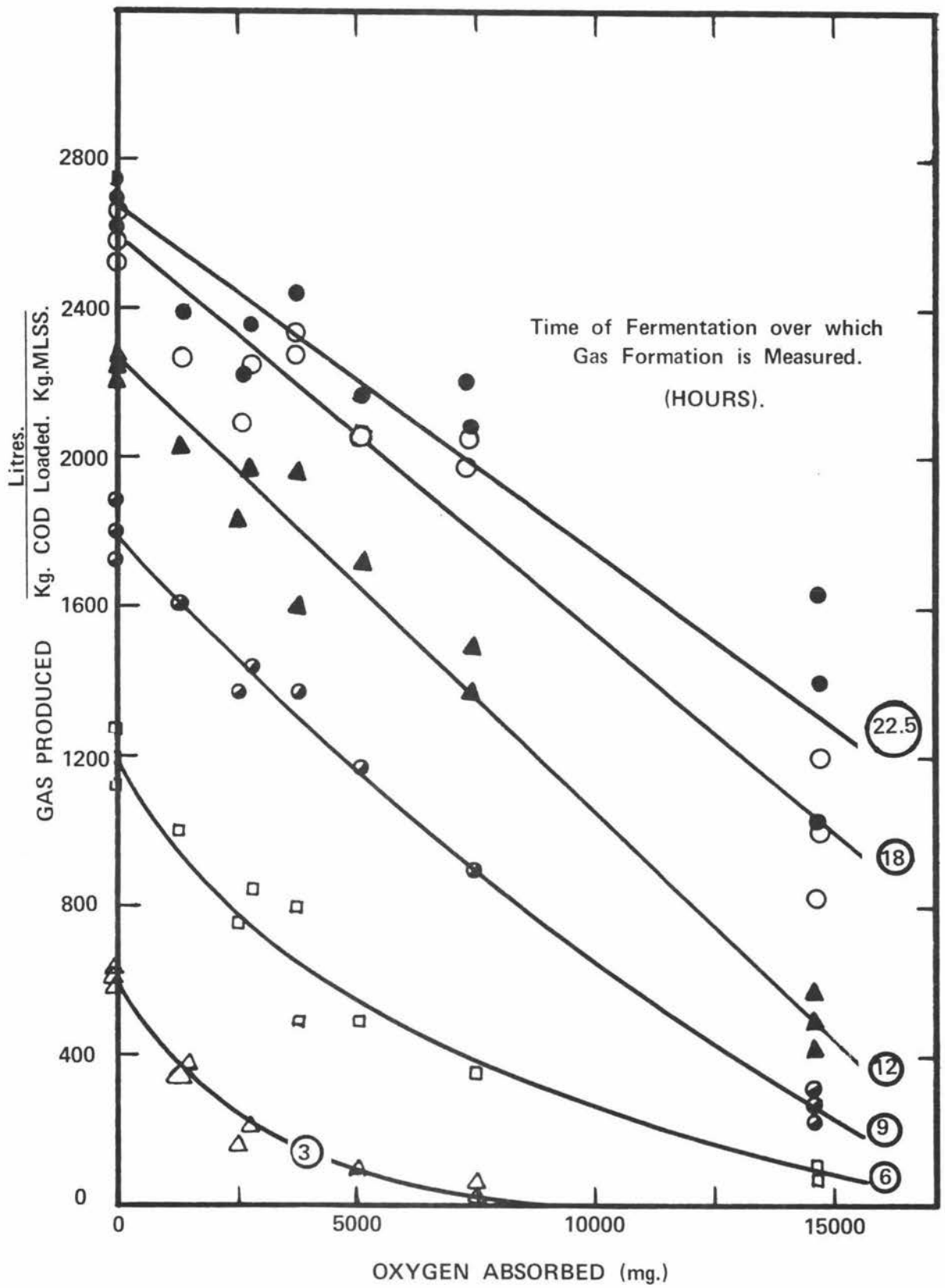


FIGURE 25. GAS PRODUCTION AND OXYGEN ABSORPTION.

The results are shown in Table IV, showing that there was a change in carbon dioxide when the sludge liquor was aerated.

TABLE IV: Gas Compositions and Aeration

CONDITIONS	PERCENTAGE OF CO ₂
Control	42.2
0.5 VVM (15 min.) N ₂	36.2
0.25 VVM (15 min.) Air	35.4
0.5 VVM (15 min.) O ₂	36.2

c) Performance of Anaerobic Digestion

The performance parameters of the anaerobic digestion process which are of interest, besides gas production, include chemical oxygen demand, suspended solids, total and particular volatile fatty acids and gas compositions.

The change in chemical oxygen demand in relation to the gas production is illustrated in Figure 26. Once the digestion started, the effluent COD was reduced, rapidly at first, then more gradually. On the other hand the gas was produced at constant rates. No lag was found in gas production or in COD reduction. After six hours of digestion 2850 ml of gas were evolved, while 8000 mg dissolved COD was destroyed from the original concentration of 2,400 ppm, then COD was gradually reduced while the rate of gas formation remained constant. The soluble COD was reduced to 1320 ppm and 5,500 ml of gas evolved after 12 hours of digestion. At the end of digestion (24 hours) the total gas of 7,000 ml was produced from 11.5 gm dissolved COD.

The amount of 11.5 gm of dissolved COD was a part of the organic material contributing to gas formation. The rest of the gas was produced from the solid waste which contributed 1290 ppm COD in raw wastes.

Chemical oxygen demand of the supernatant (after centrifuging the total digester liquid at 3800 rpm for 30 minutes) during

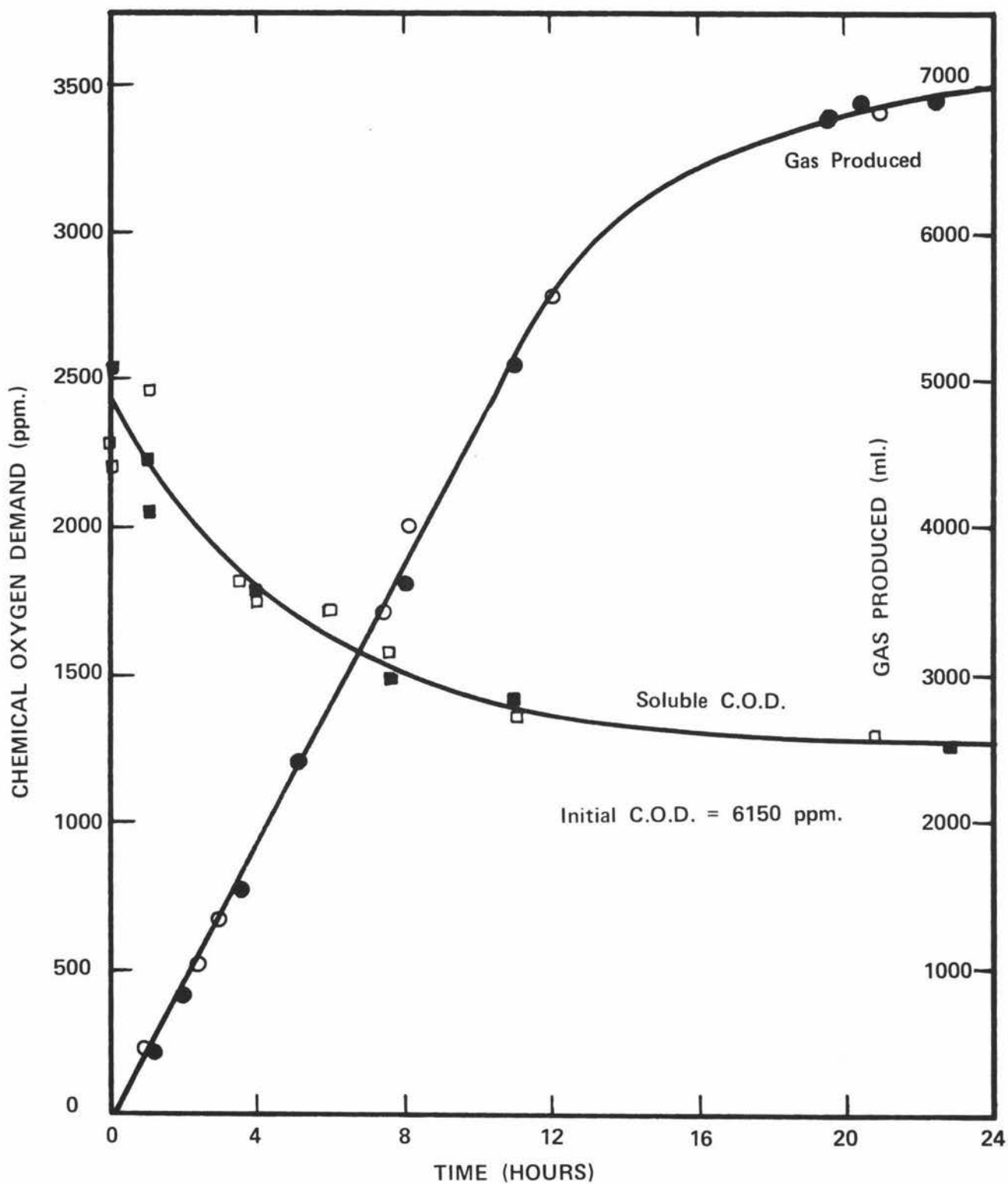


FIGURE 26. THE GRAPH SHOWING THE RELATIONSHIPS BETWEEN GAS PRODUCTION AND THE CHEMICAL OXYGEN DEMAND.

the whole period of fermentation is shown in Figures 27, 28 and 29. Figure 27 shows the level of COD from the start to the end of the aeration tests. After aeration was started the chemical oxygen demand of the supernatant rose gradually while that of the control (Fermenter no. 1) was constant at an average of 1320 ppm. The chemical oxygen demand remaining in the effluent rose with the amount of oxygen absorbed into the mixed liquor as the fermenter activity diminished. At the end of digestion the COD of the effluent was 1470, 1520 and 2150 for the aeration of 0.75 VVM for 15, 30 and 60 minutes indicating the absorption of 3650, 7300 and 14600 mg of oxygen (365, 730 and 1460 mg/lit.) in the sludge mixed liquor.

When the aerated digesters had recovered, it was found that the efficiency of the digesters was gradually restored. After three days, with aeration each day, the digester was then operated without aeration and was found to have gas production rates the same as those of the control which had no aeration at all. The daily gas produced increased to the maximum and consequently the effluent COD was reduced to minimum. The values of the effluent COD after the recovery of the aerated digesters are illustrated in Figures 28 and 29.

Figure 28 shows that after the mixed liquor was in contact with 14,600 mg or 1,460 mg/lit. of oxygen (0.75 VVM aeration for 60 minutes) at least twelve days were required to bring the effluent COD back to the normal level. After twelve days of recovery the effluent COD decreased from about 2,700 ppm to 1,400 ppm. When a lesser amount of oxygen was absorbed into the sludge mixed liquor, a shorter period of time was needed for the recovery of the digester. It was found that only five days were needed to bring the upset digester (365 mg/lit. oxygen absorption) to almost full efficiency. These results indicated that lesser amounts of the bacterial culture were killed or inactivated in the latter case. The bacterial activity remaining in the digester was indicated by the effluent COD. The effluent COD was 1,680 ppm from the sludge after absorption of 365 mg/lit. oxygen and 2,700 ppm from the sludge after absorption of 1,460 mg/lit. oxygen.

With the average mixed liquor suspended solids (MLSS) of

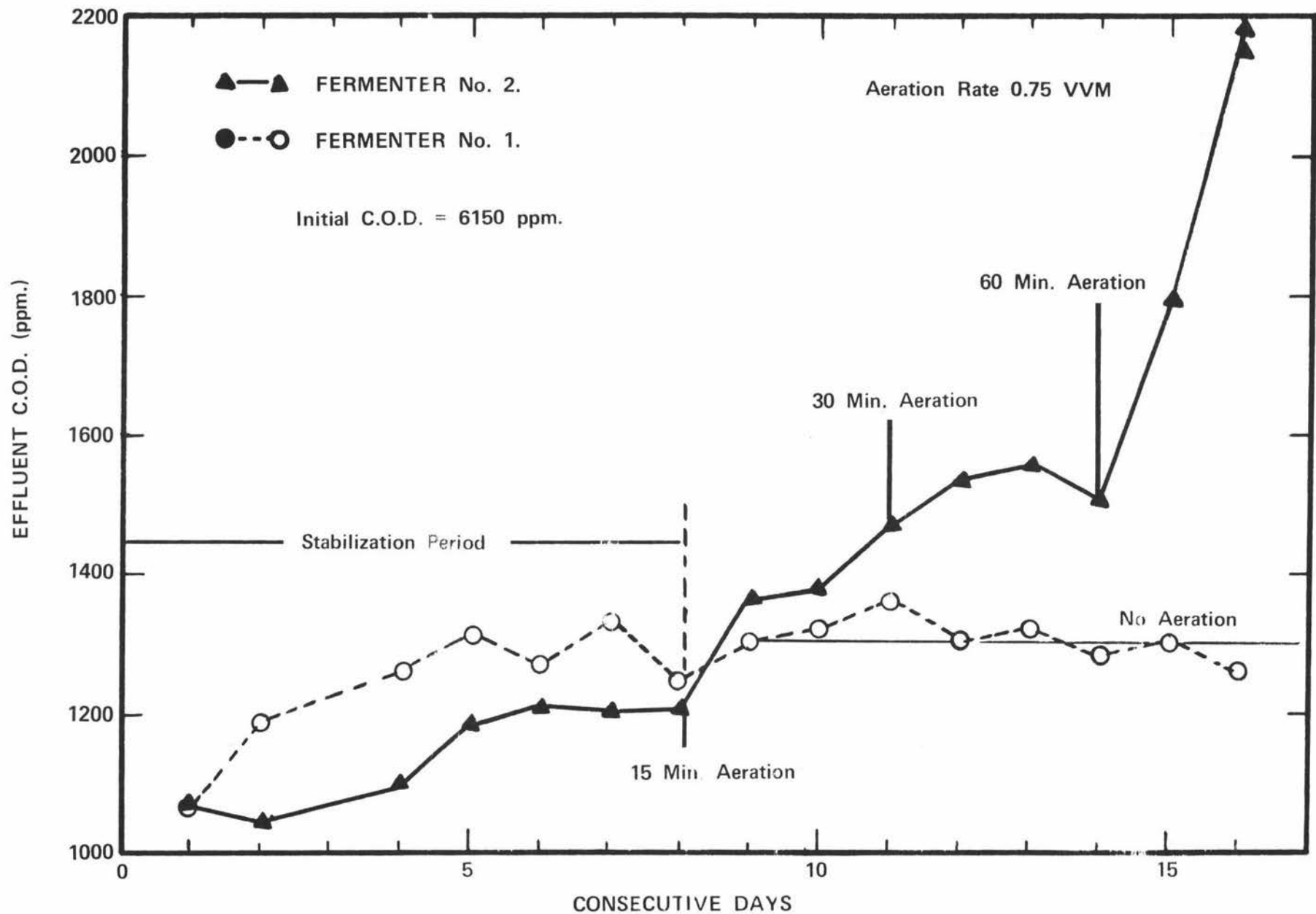


FIGURE 27. EFFLUENT CHEMICAL OXYGEN DEMAND RELATIVE TO AERATION.

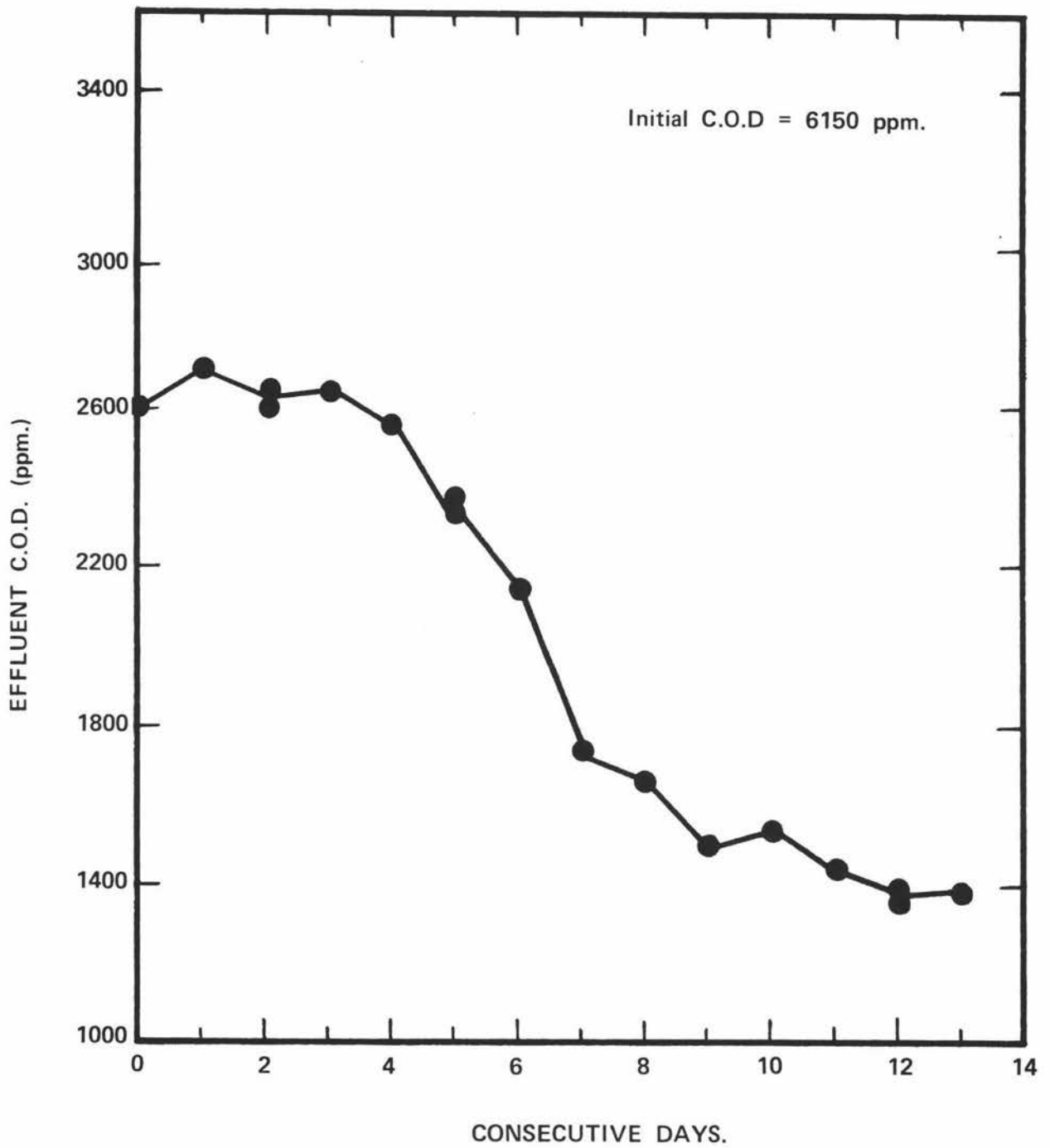


FIGURE 28. EFFLUENT C.O.D. DURING THE RECOVERY PERIOD OF THE DIGESTER.

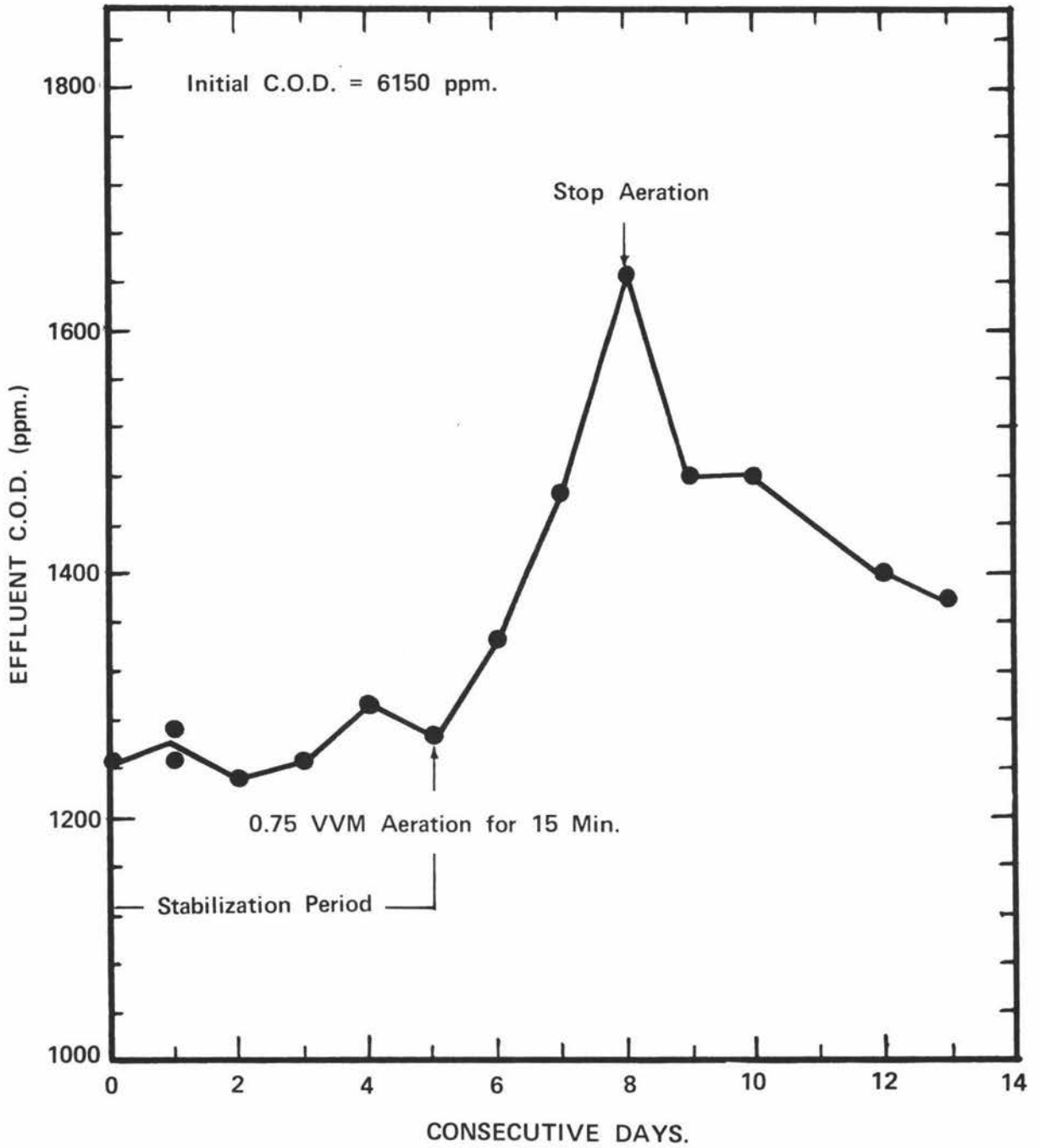


FIGURE 29. EFFLUENT CHEMICAL OXYGEN DEMAND BEFORE AND AFTER AERATION.

12,175 ppm the relationship between the effluent COD and the amount of oxygen applied into the digester is illustrated diagrammatically in Figure 30. The graph shows that the greater the amount of oxygen absorbed into the digester, the greater the amount of COD remaining in the effluent liquor. The relationships were almost a straight line.

Suspended solids concentration of the effluent during the whole period of digestion is demonstrated graphically in Figure 31. The results show that with 15 minutes aeration (0.75 VVM aeration) the suspended solids were only 280 ppm, compared with the control of 320 ppm. Once the aeration was extended to 30 and 60 minutes, the concentration of suspended solids rose to 580 and 750 ppm respectively. The increase of the suspended solids with 30 and 60 minutes aeration was probably due to the mechanical breakdown of the flocs. It was reported by Parker et al. (17) that flocculating time, besides the turbulent intensity, had an effect on the effluent suspended solids.

Results of total volatile fatty acids are shown graphically in Figure 32. The graph indicates that the total volatile fatty acids are directly correlated with the amount of oxygen added into the sludge liquor. After 15, 30 and 60 minutes of aeration (0.75 VVM), there was an increase of total volatile fatty acids of 14, 35 and 48 per cent respectively. Presumably, the increase in the accumulation of the fatty acids was due to inhibition of the methane formers but had little effect on the fatty acids production. It was reported by Amberg et al. (52) that the optimal aeration rate of $1.58 \frac{\text{ft}^3}{\text{lb. MLSS}}$ enhanced the production of volatile fatty acids.

Individual volatile acids also showed the same trend. Analysis in Figure 33 indicates that there was an increase of individual volatile fatty acids, particularly acetic and propionic acids. The higher the absorption of oxygen, the higher the amount of acetic and propionic acids accumulated. Of the other volatile acids such as butyric, valeric and caproic, only very small amounts were detected.

Attempts were made to measure the oxidation reduction potential (ORP) in three cases; no aeration, aeration with nitrogen and actual aeration for 15 minutes (0.5 VVM). The results (Figure 34)

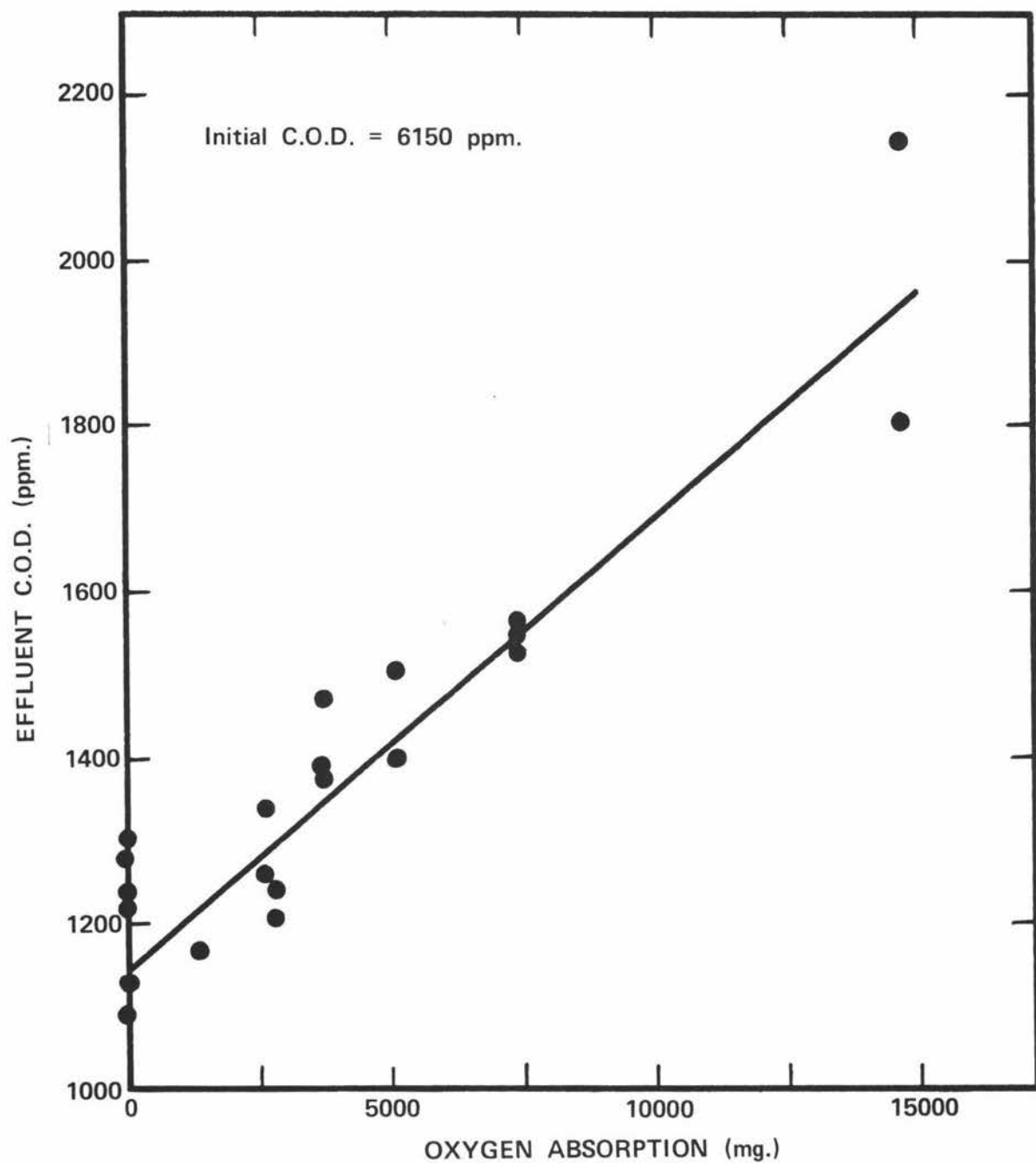


FIGURE 30. EFFLUENT CHEMICAL OXYGEN DEMAND AFTER ONE DAY DIGESTION RELATIVE TO OXYGEN ABSORPTION.

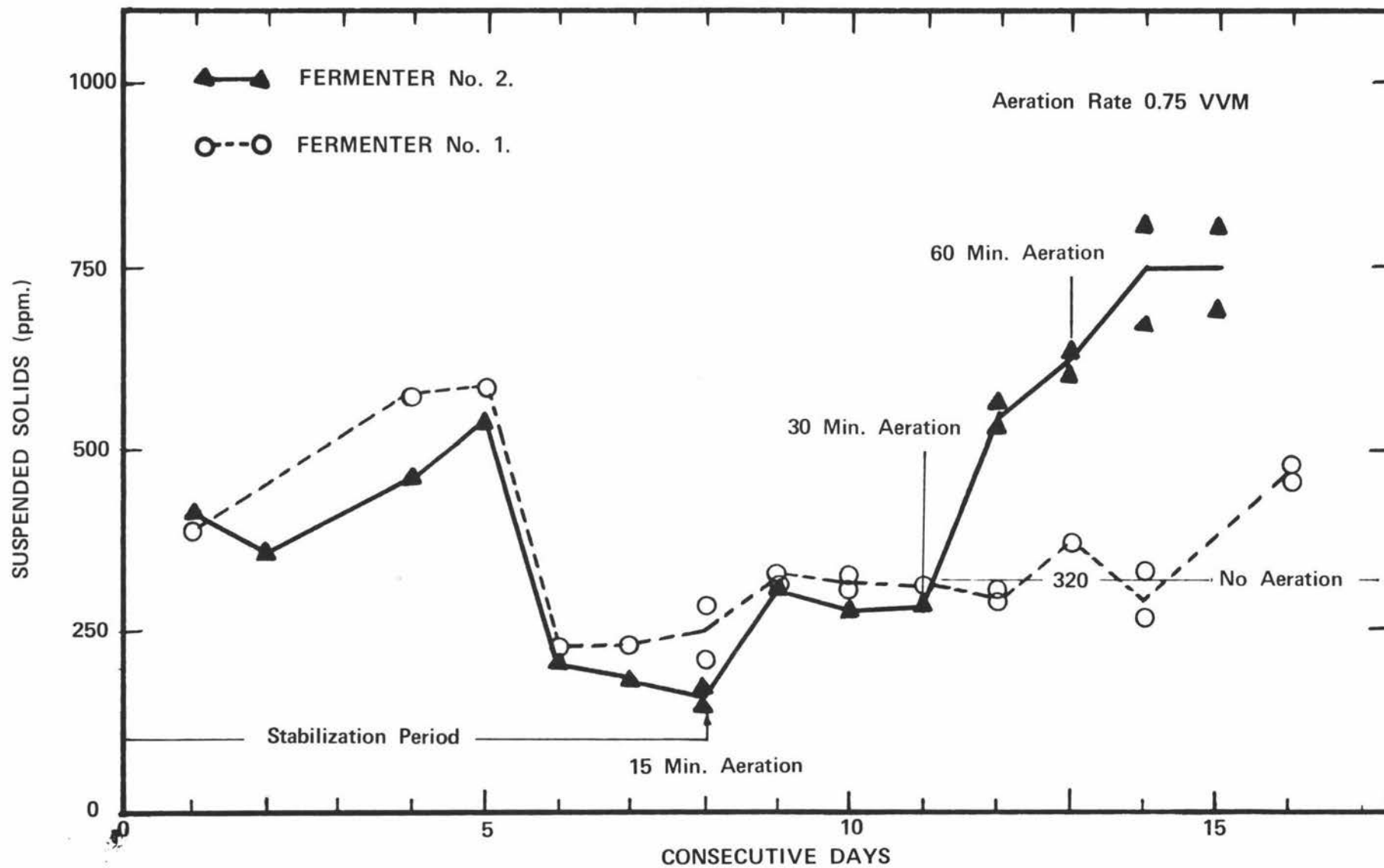


FIGURE 31. CHANGES IN EFFLUENT SUSPENDED SOLIDS WITH AERATION.

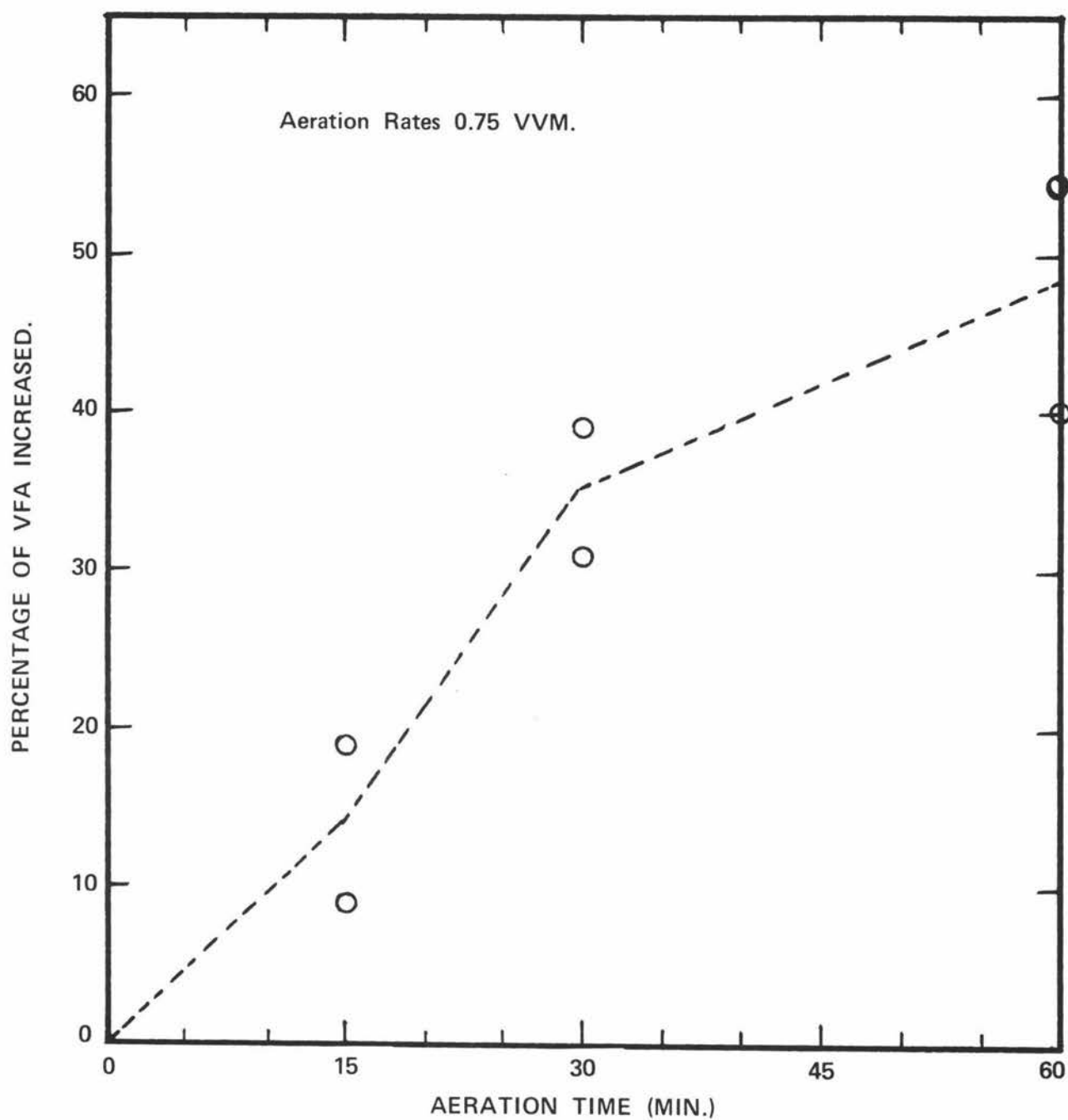


FIGURE 32. ACCUMULATION OF VOLATILE FATTY ACIDS RELATIVE TO AERATION.

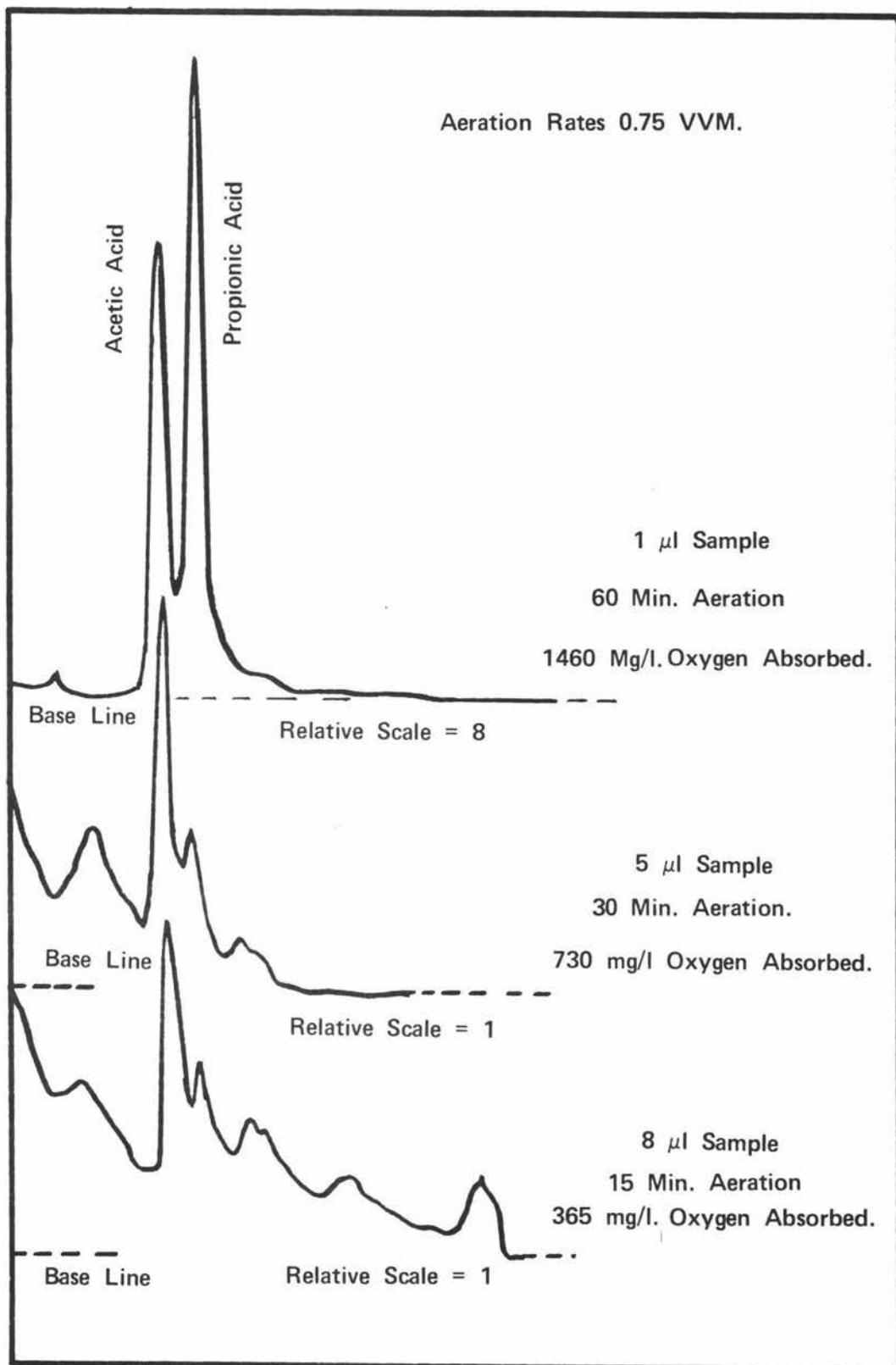


FIGURE 33. CHROMATOGRAMS SHOWING ACCUMULATION OF VOLATILE FATTY ACIDS RELATIVE TO OXYGEN ABSORPTION.

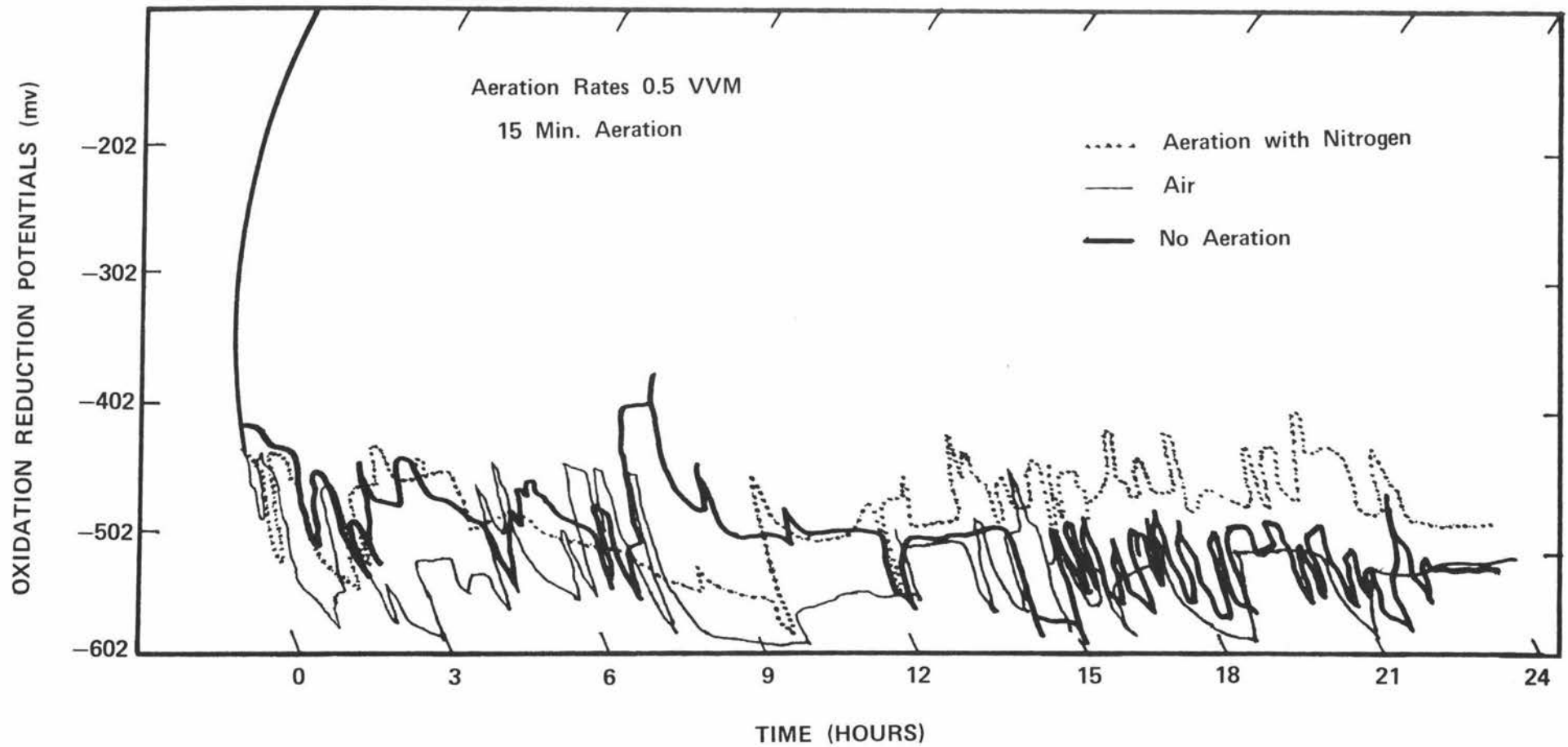


FIGURE 34. THE GRAPH SHOWING THE CHANGES OF OXIDATION REDUCTION POTENTIALS WITH TIME.

were inconclusive. The graph implies that neither nitrogen nor air has any apparent effect on the changes of the oxidation reduction potential (ORP) in anaerobic digestion systems. The ORP is in the range of -450 mV to -550 mV and the results are in general agreement with those reported by Blanc (53).

Hartz and Kountz (54) reported that dissolved oxygen in the feed slurry increased the ORP from the original value of -510 mV to -540 mV to approximately -350 mV, and their digesters returned to their original value within two to three hours. With nitrogen agitated digesters they found that the ORP became more negative.

It was expected that when the sludge was aerated prior to sedimentation, the ORP should become higher than the original value during the first stage of digestion, but this was not observed. Probably all the oxygen absorbed was utilized by the facultative anaerobes during the sedimentation.

3. DISCUSSION

From the observations on gas formation the results showed that rates of gas formation differed from those of Fair and Moore (35) and Buraczewski (36). Almost constant rates, instead of exponential rates, were found in the earlier stages of the digestion and then after about 10 hours of digestion gas production decreased and falling rates of gas formation were observed.

Gas production as a function of time agreed with that found in sewage sludge digestion and reported by Maly et al. (55) and Diets et al. (56). Gas formation patterns, as reported by Fair and Moore (35) and Buraczewski (36) are similar to those obtained in this study when the sludge mixed liquor was aerated. The constant rate of gas formation found in this study was in the range of 200 to 300 litres (per kg.MLSS.kg.COD loaded.hour).

Fair and Moore (35) did not explain the condition of the sludge they used. It is presumed that the lag reported in their study was because the sludge was not properly stabilized or completely mixed. The absence of a lag in the gas production (with no aeration) in this study might have been because the sludge was well adapted to the feed and well mixed.

In the present experiments the tests were continued for up to 48 hours (after 0.75 VVM aeration for 30 minutes followed by

normal feeding). After the falling rates of gas formation from about 22 hours onwards, the gas production rate was slow, but still continued steadily (at about 6 per cent of the maximum rate). The continuous evolution of the gas at low rates was due to substrate limitation. Utilization (slow rate) of solid substrates also contributed to the slow rates of gas production. It is reasonable to presume (from this finding) that the slow and steady rate of gas formation occurred in the digester with no aeration because a reasonably high dissolved COD was still present in the supernatant after 22.5 hours of digestion, and some of the solid substrate COD would also be present.

It was claimed by Burbank et al. (57) that once the anaerobic cultures are exposed to the atmospheric air, the culture will be inactivated within 30 seconds. If this statement is true, all the anaerobes in the digesters in this study should be entirely inactivated when they are exposed to contact with 1400 mg to 14,600 mg (140 mg/lit. to 1,460 mg/lit.) of oxygen (calculated from the sulphite oxidation). In this study it was found that after the sludge was aerated at different conditions prior to sedimentation, comparatively large volumes of gas, including methane, were produced when the sludge was returned to the digester indicating substantial activity remaining in the sludge. In fact, the reduction in the total gas formed, comparing aerated and unaerated sludge, was relatively small, and of the order of only 4.7 and 13.1 per cent when the sludge was aerated at the rate of 0.75 VVM for 15 and 30 minutes respectively. The relationships between the amount of gas accumulated and the oxygen presumably absorbed in the digesters are illustrated graphically in Figure 25. According to these findings a linear reduction between the gas produced and the oxygen absorbed could be assumed after 22.5 hours of digestion. It is shown (Figure 25) that apparently only 9.3 litres (per kg.MLSS.kg.COD.Loaded.day) of the total gas produced were reduced for 10 mg/lit. of the oxygen absorbed.

When the sludge liquor was aerated for 60 minutes at the rate of 0.75 VVM, the presumed oxygen absorbed was 1,460 mg/lit. About 47 per cent reduction of the total gas produced was found after one day's (22.5 hours) digestion. This amount of calculated oxygen absorption (1460 ppm) was higher than that reported by Fields and Agardy (44) to give complete inactivation of the anaerobes

in sewage sludge digestion.

The pattern of gas formation was also affected by aeration. Once the sludge liquor was aerated prior to sedimentation a lag in gas production was observed after the aerated sludge was returned to the digester. The extent of the lag depended upon the amount of oxygen absorbed into the sludge liquor. The higher the concentration of oxygen, the longer the lag became. With 1,460 mg/lit. of oxygen absorbed into the sludge liquor, a lag of four hours was found.

The extent of the lags and oxygen absorption appeared to be linearly related as shown in Figure 35. A lag of 3.1 hours per 10,000 mg of oxygen presumably absorbed into the sludge suspension was observed after the digester had been started.

Thereafter, gas production gradually increased until the constant rate periods were observed. Then the rates of gas production fell off. It was found in this study that when the oxygen absorption was lower than 750 mg/lit. the constant rates of gas formation were observed for a period of time before they started to fall off. When the oxygen presumably absorbed into the sludge liquor was increased to 14,600 mg, the constant rates of gas formation were not found. Also, the falling rates of gas formation did not occur. It was assumed, when a high level of oxygen (1,460 mg/lit.) was presumably absorbed into the sludge, that large numbers of the anaerobes, which are responsible for gas formation, were inactivated. Consequently, the total volumes and the pattern of gas formation were altered.

When nitrogen was used instead of air a smaller effect on gas formation was observed. Nitrogen has been successfully used in combination with carbon dioxide by Sykes and Kirsch (3) to get rid of oxygen in the growth studies of the anaerobes in sewage sludge digesters, and there should be no reason for it to be toxic to the anaerobic cultures in the sludge in this study. Therefore, the effect of the nitrogen on the anaerobes would be expected to be just a physical one. Comparing the effects of sparging with nitrogen, with air and with oxygen, against no sparging, showed reductions in the volume of total gas produced amounting to 10, 17 and 21 per cent respectively.

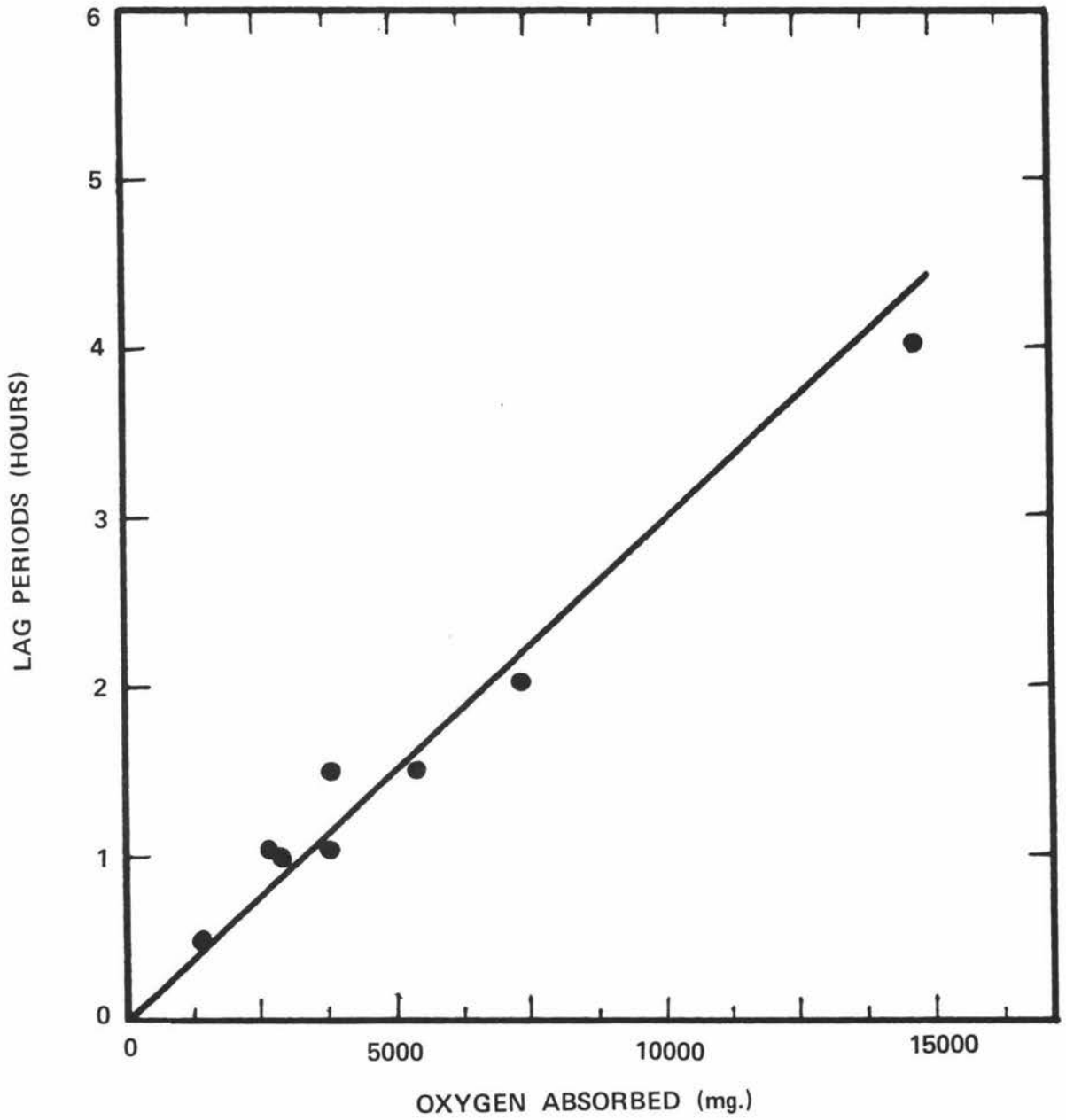


FIGURE 35. PLOT OF LAG PERIOD AND OXYGEN ABSORPTION.

There were insufficient data to show whether, after exposure to the aeration, the anaerobes were killed or whether only their activity was retarded. Probably both effects occurred. The strict anaerobes which are highly sensitive to oxygen would probably be killed, but the activity of the less sensitive anaerobes and facultative anaerobes was apparently only retarded for a certain period of time. The retarding action was indicated by the lag period at the beginning of digestion, then the period of constant rates of gas formation before the rates of gas production started to fall off. The results from this study showing the reduction in daily total gas formation, and increase in dissolved COD in the supernatant after 22.5 hours digestion, indicated that after the anaerobes were in contact with oxygen some of them were permanently inactivated, which resulted in a decrease of the overall bacterial activity in the digesters.

It should be noted here that when the sludge liquor was aerated, and the consequent oxygen presumably absorbed was between 140 to 730 mg/lit., a period of constant rates of gas formation after a lag was found (when the aerated sludge was returned into the digesters). It was also found that when the digesters upset by aeration were allowed to recover no lag occurred. Constant rates of gas formation were observed, but the rates were lower when compared with those of the control, depending on the oxygen presumably absorbed into the sludge liquor. On the first day of the recovery, it was found that after 15 to 16 hours of digestion the total volume of the gas formed was comparable to that of the gas produced from the aerated liquor (Figure 20). This indicated that some permanent inactivation occurred when the bacterial sludge was exposed to oxygen.

It also is probable that the association of the anaerobes in the sludge, besides their nature, enhanced their resistance to oxygen absorbed into the sludge liquor. The anaerobes present in the sludge mixed liquor were in the form of flocs and primary particles which include single celled bacteria. The bacteria which associated together to form flocs would probably have a protective shield (slime) which acted as a barrier for the oxygen transfer. Thus the flocculated anaerobes would have a better chance of survival compared with single cell bacteria or the

primary particles.

In their aeration studies, Steffen and Bedker (7) found that total counts of anaerobes were not reduced. They reported that the total counts of the anaerobes were reduced in the aeration process, but they were then built up again in the sludge separation tank, and counts were comparable to counts taken without aeration. Optimal aeration rates of 0.1 cu.ft. of air per gallon of sludge mixed liquor for 8 to 10 minutes were reported. The minimum aeration rate used in this study was 0.042 cu.ft. of air per gallon of sludge mixed liquor (0.25 VVM aeration) for 15 minutes when a lag and reduction in the total volume of gas production were found. Regardless of the other design parameters of the aeration chamber in both studies, the aeration rates and consequently the oxygen absorbed, reported by Steffen and Bedker (7) were higher than those found with the minimum aeration in this study. Thus, their results indicating no effect on the anaerobes conflict with the present observations and no explanation can be formed further.

Under the operating conditions of 12,175 ppm MLSS, 67.5 hours liquid detention time, and the organic loading of 0.384 lb COD per cu.ft. per day, the chemical oxygen demand of the centrifuged effluent (3700 rpm for 30 minutes) was only 1240 ppm, and this accounted for 80 per cent reduction of COD. Once the mixed liquor was aerated the efficiency of the digester decreased. Consequently, the effluent COD increased to 1385, 1520 and 2150 ppm following the absorption of 3,650, 7,300 and 14,600 milligrams (365, 730 and 1,460 mg/lit.) of oxygen respectively. It was quite obvious that, in terms of the effluent COD, the sludge performance had been affected by oxygen, presumably absorbed into the sludge mixed liquor. The overall linear relationship between the effluent COD and the oxygen absorbed into the sludge mixed liquor is illustrated in Figure 30. It is shown that for 100 milligrams (10 mg/lit.) of oxygen absorbed, there was an increase in 5.6 ppm of COD in the effluent. The increment of COD in the effluent was probably due to the overall reduction in the bacterial activities when the sludge mixed liquor had been aerated prior to quiescent settling.

The overall results from the experiments indicate that

aeration affects gas production, altering the initial lag but not the steady rate and insignificantly reducing the total volume of gas produced at the lower levels of aeration. At higher levels of aeration (about 1460 mg/lit. of oxygen absorbed), the lag, the steady rates and also the total amounts of the gas produced were substantially reduced. Also at the higher levels of aeration some long term effects were found on the sludge, judged from its rate of recovery, whereas the recovery after short periods of aeration appeared rapid. The results with gases other than air indicated a physical effect although there were insufficient data to separate the physical and chemical effects of the added gas. A general correlation was evident between the gas evolution and the fermentation of the wastes as measured by the decreased COD, but because of the complexity of the system this could not be put on a quantitative basis.

C. INHIBITION OF METHANE FORMATION

1. Materials and Methods

Heterogeneous, anaerobic, bacterial cultures were obtained by removing 1200 ml, well-mixed samples of mixed liquor from the laboratory-scale fermenters. The mixed liquor was then cultivated in two 1000 ml Erlenmeyer flasks. Each flask was equipped with a pressure relief valve described in Sykes and Kirsch (30), and the growth was undertaken on a shaker. The incubation temperature was 37°C. Fermentation was continued until steady state conditions were obtained. The operational conditions were the same as those of the laboratory-scale fermenters.

The inhibitors used were hemiacetyl of starch and chloral (HSC), and CCl_4 . HSC is quite readily soluble in hot water, so there was no problem with the adjustment of HSC concentration. The CCl_4 , on the other hand, must be dissolved in ethanol.

The actual inhibition was carried out in a gas-tight 60 ml squat vial. After 50 ml of newly fed mixed liquor were transferred into the vial, pure nitrogen was used to flush the vial for 4-5 minutes. Prior to the vials being placed on the shaker, the required amounts of HSC or CCl_4 were then injected into the mixed liquor. The inhibition test was continued for 24 hours at an incubation temperature of 37°C.

2. Results

Thiel (29) and Sykes and Kirsch (30) recommended that the mixed sludge liquor should be buffered before the inhibition test is started, the buffer being expected to maintain a constant pH of about 7. Even with the buffered sludge liquor the result published by Sykes and Kirsch (30) showed that the pH of the sludge culture after the inhibition fluctuated with the incubation time.

In this investigation it was decided not to buffer the mixed liquor prior to the inhibition test. Figure 36 indicates that pH fluctuation was drastic especially in the inhibition test with CCl_4 . With the presence of 2 ppm CCl_4 , the pH dropped abruptly from 7.4 to 7.05 after 24 hours of digestion. As the concentration of CCl_4 was increased, the pH started to rise again. pH 7.35 was found when CCl_4 was increased up to 16 ppm, and it stayed at 7.35 even when the concentration of CCl_4 was increased to 24 ppm. The reason could be explained by the variations of the concentrations of the accumulated volatile fatty acids which are shown graphically in Figure 39.

The pH of the cultures in the inhibition test with HSC remained reasonably constant at pH 7.4, but it decreased when the HSC addition was over 14 ppm. The final pH was 7.3 when the culture was dosed with 30 ppm HSC.

Gas composition data are shown in Figure 37. The gases produced from the culture dosed with HSC were composed of carbon dioxide and methane. No accumulation of hydrogen was indicated after 24 hours of fermentation. Of the gases, CO_2 constituted about 33% and no change of the percentage of CO_2 with the concentration of HSC occurred. Methane, on the other hand, varied in concentration with the increase in the dose of HSC. As the concentration of HSC was increased, methane gradually decreased. Methane concentration was reduced from 68 per cent to 50 per cent when the sludge liquor was dosed with 30 ppm HSC.

Figure 38 shows the composition of gases produced when the sludge cultures were dosed with CCl_4 . The composition of gases varied with the concentration of CCl_4 present in the sludge liquor. Methane, the main component of the gases' end product in anaerobic

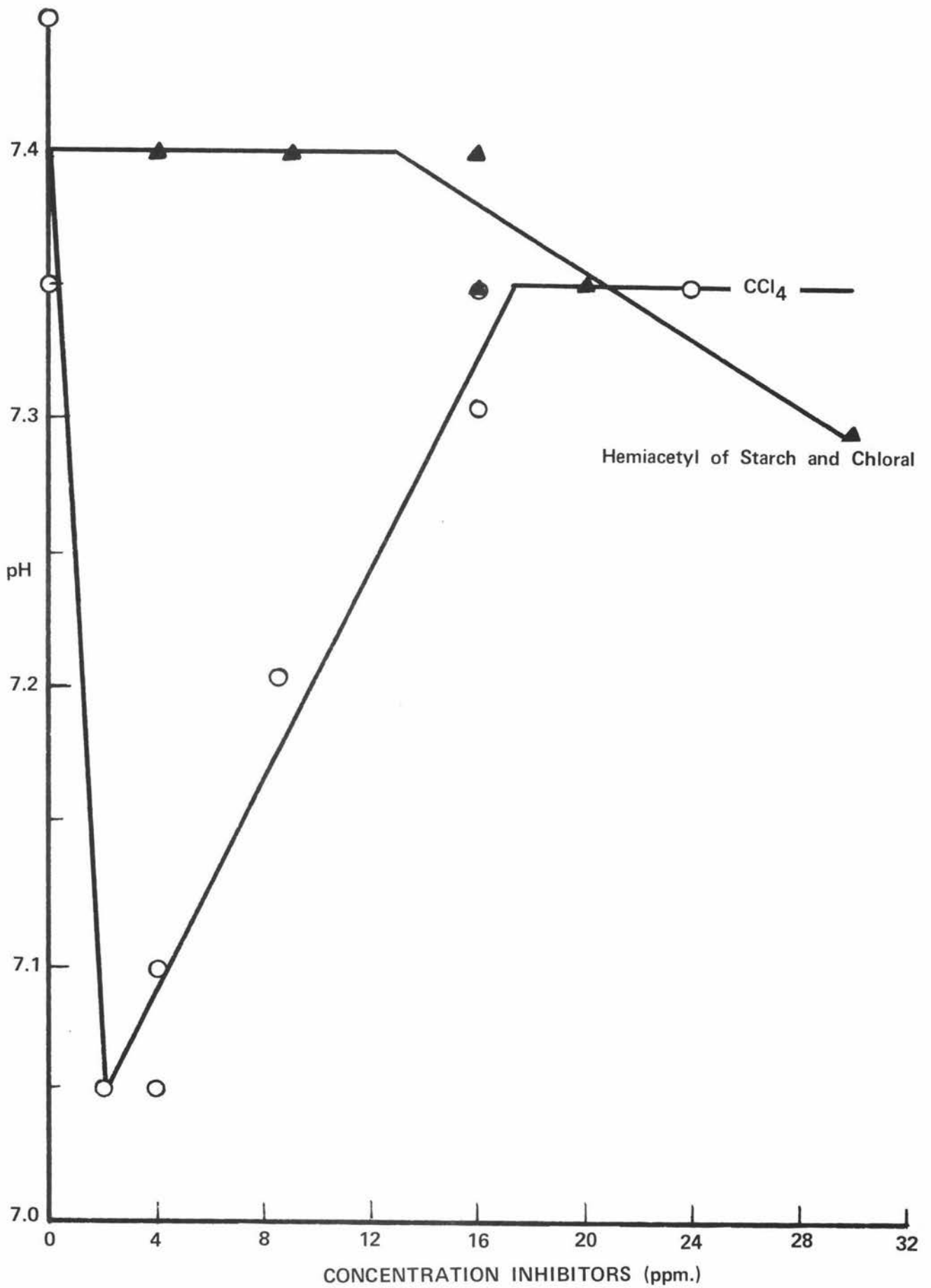


FIGURE 36. CHANGES OF pH WITH THE CONCENTRATION OF CHEMICAL INHIBITORS.

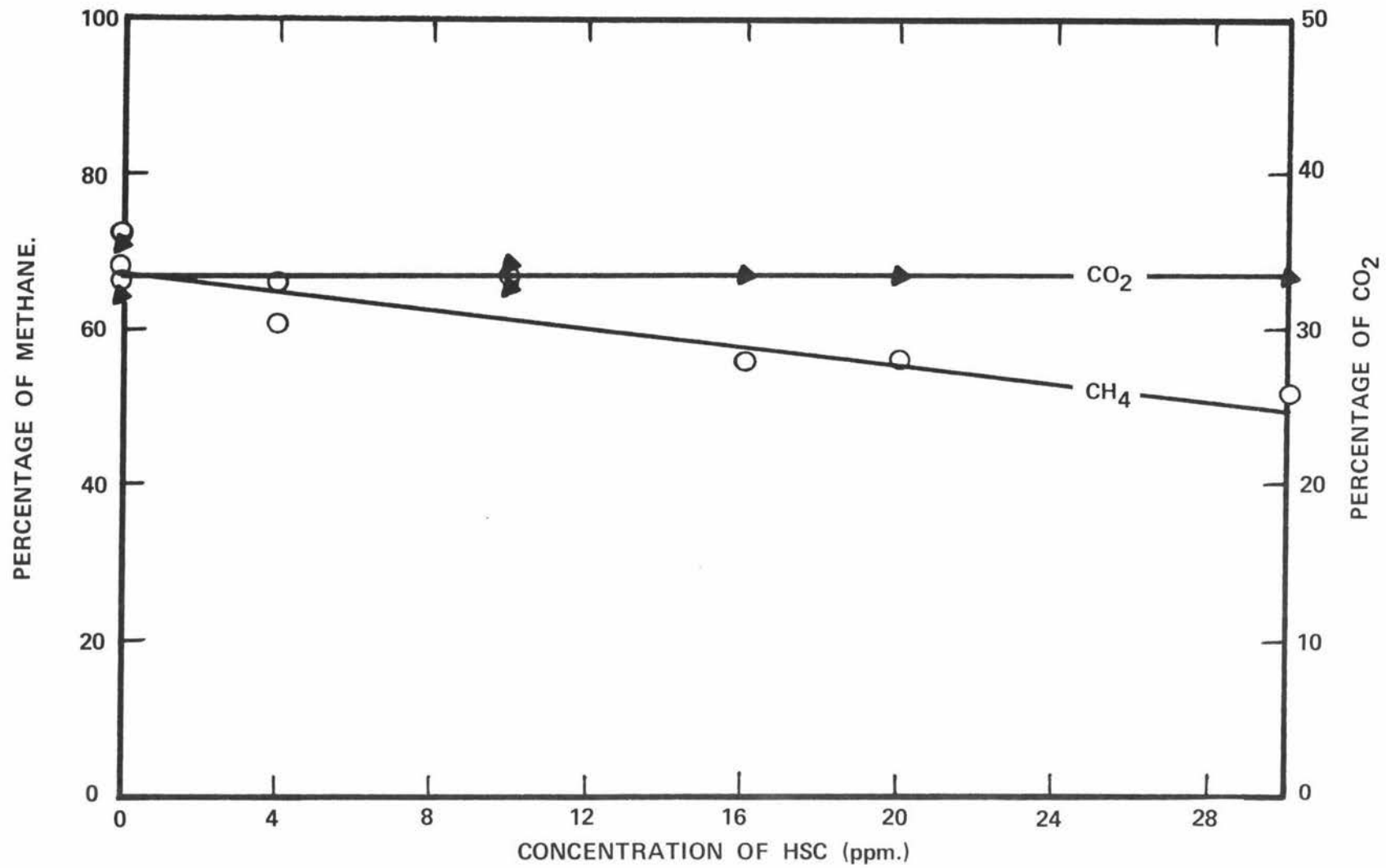


FIGURE 37. GAS PRODUCTION WITH HSC INHIBITION.

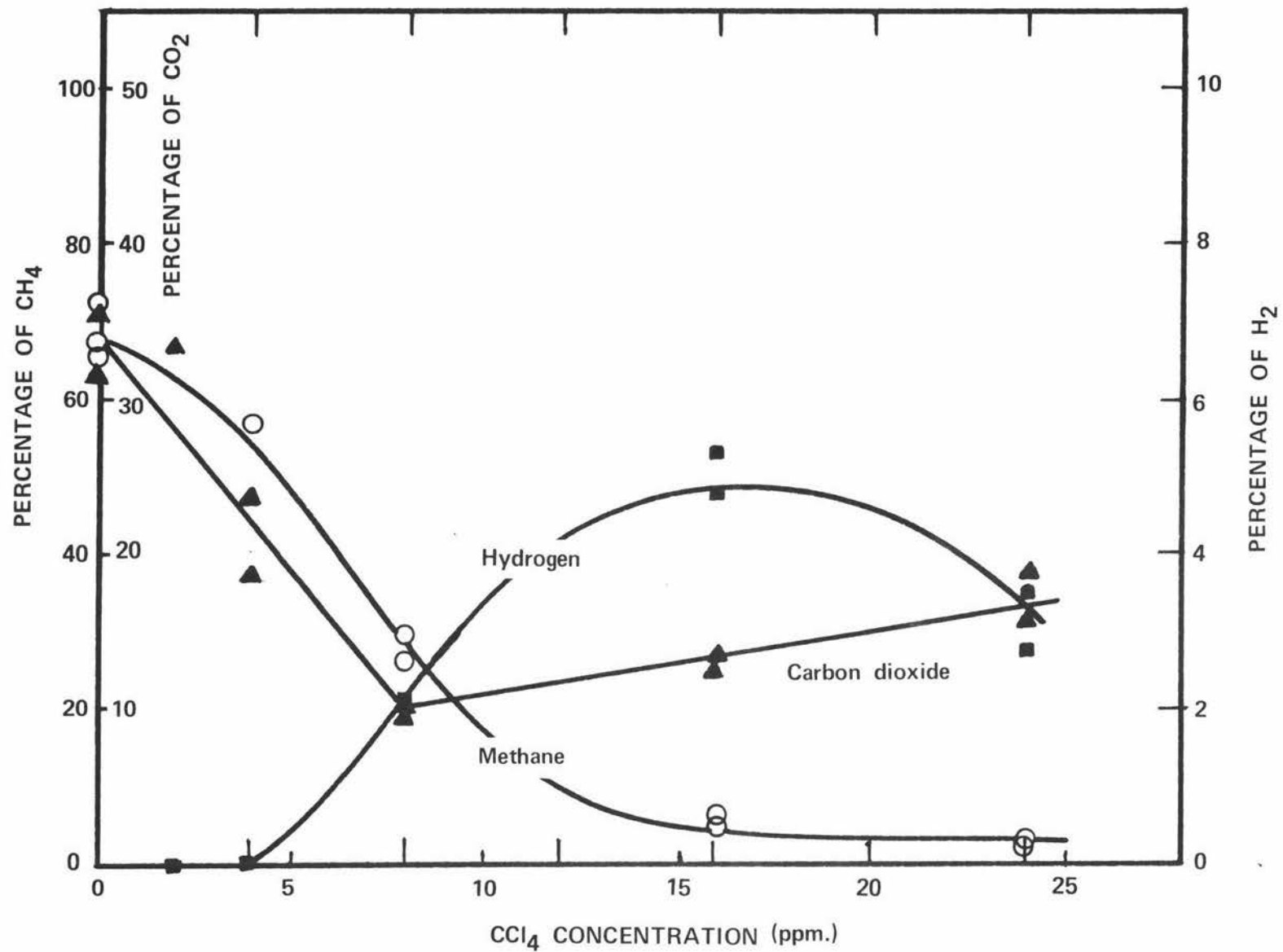


FIGURE 38. GAS PRODUCTION WITH CCl₄ INHIBITION.

digestion, sharply decreased from 70 to 30 per cent when the cultures were dosed with 8 ppm CCl_4 , and only 2 per cent of methane accumulated with the presence of 24 ppm of CCl_4 in the mixed liquor. Accumulation of hydrogen resulted from the inhibition of methane formation, hydrogen being detected when the sludge was dosed with more than 4 ppm CCl_4 . The proportion of hydrogen gas increased as CCl_4 became more concentrated. The maximum concentration of hydrogen (5 per cent) was detected when the culture was dosed with 16 ppm CCl_4 .

Carbon dioxide was also reduced when the sludge mixed liquor was dosed with CCl_4 . Only 10 per cent of CO_2 was found to accumulate when methane formation was inhibited with 8 ppm CCl_4 . When the dose of CCl_4 was increased to 24 ppm the concentration of CO_2 increased to 17.5 per cent. The general pattern of inhibition of CO_2 is in agreement with that reported by Sykes and Kirsch (30).

The total volatile fatty acid (VFA) data are summarized in Figure 39. A higher concentration of VFA accumulated when the culture was inhibited with CCl_4 rather than HSC; 680 ppm VFA was analysed when the mixture was dosed with 4 ppm CCl_4 , and only 320 ppm VFA was found to accumulate with the presence of 30 ppm HSC in the sludge liquor.

The VFA found in the mixed liquor inhibited with HSC were mainly acetic and propionic acids (Figure 40). Acetic and propionic acids increased sharply when more than 10 ppm of HSC were added into the sludge liquor. Butyric acid started to accumulate when the dose of HSC was increased to 20 to 30 ppm. With the presence of 30 ppm of HSC in the sludge the concentration of acetate, propionate and butyrate were found to be 12.4, 6.8 and 2.7 ppm respectively. No other acids were found to accumulate in the sludge inhibited with HSC. Figure 41 summarizes the data of volatile acids accumulated when methane formation was inhibited with CCl_4 . Once the cultures were dosed with CCl_4 , large quantities of acetic acids and lesser amounts of propionic, butyric, iso-valeric and valeric acids were found to accumulate. The proportions of each acid varied with CCl_4 except valerate. The valerate concentration was quite constant at the concentration of about 4ppm. Maximum concentration of butyric acid was observed when the sludge

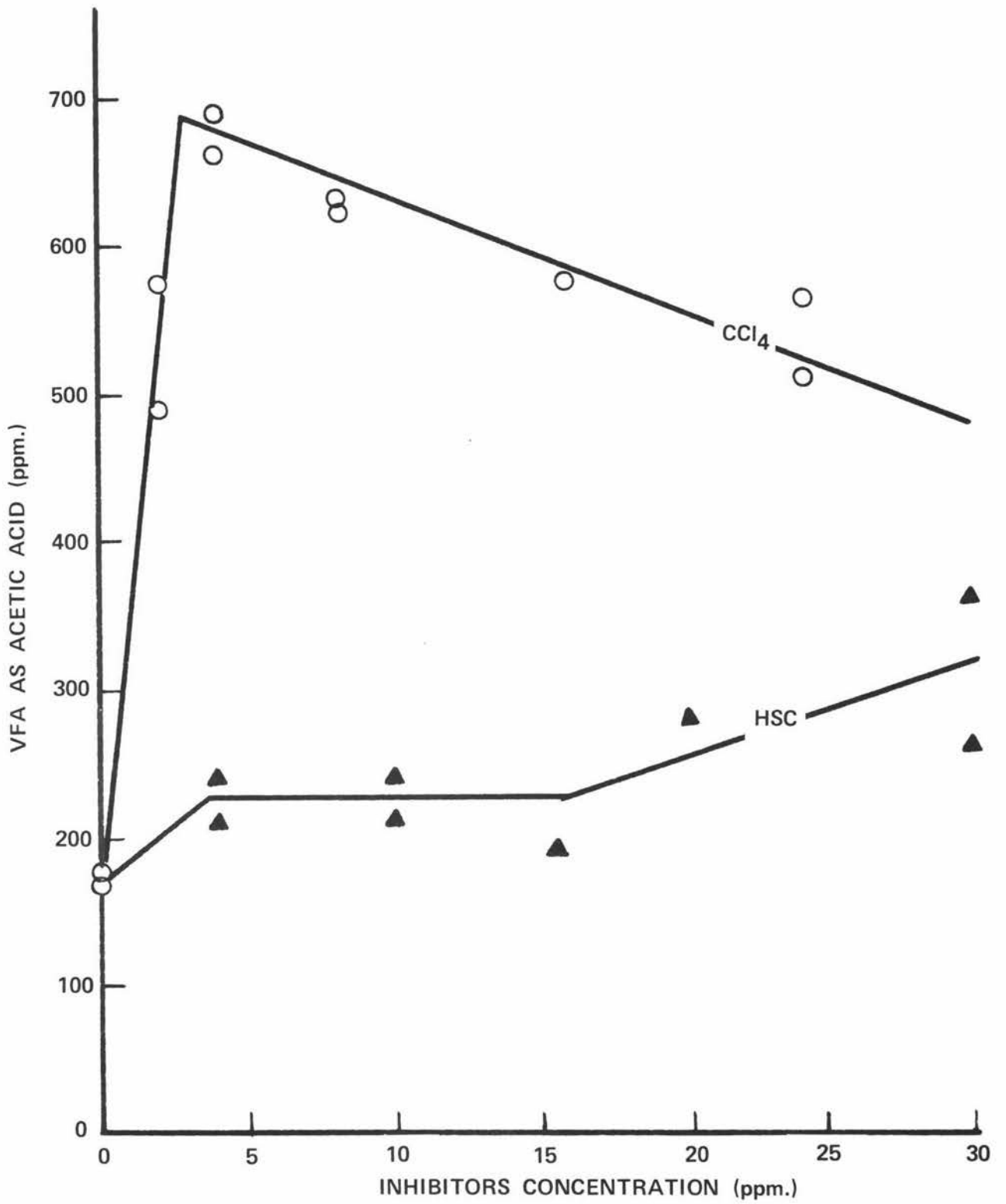


FIGURE 39. VOLATILE FATTY ACID ACCUMULATION WITH INHIBITION OF METHANE PRODUCTION.

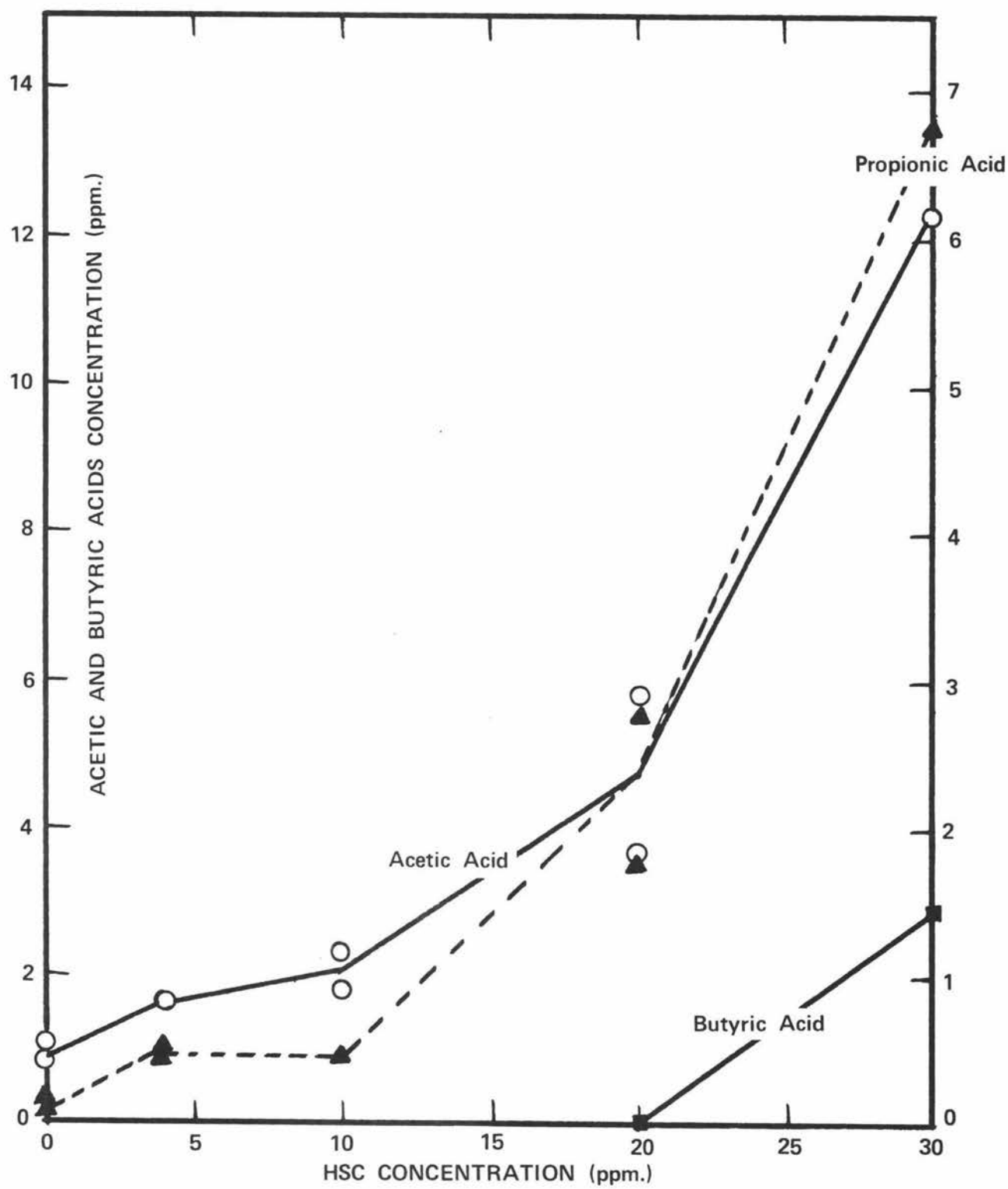


FIGURE 40. VOLATILE FATTY ACID ACCUMULATION WITH HSC INHIBITION.

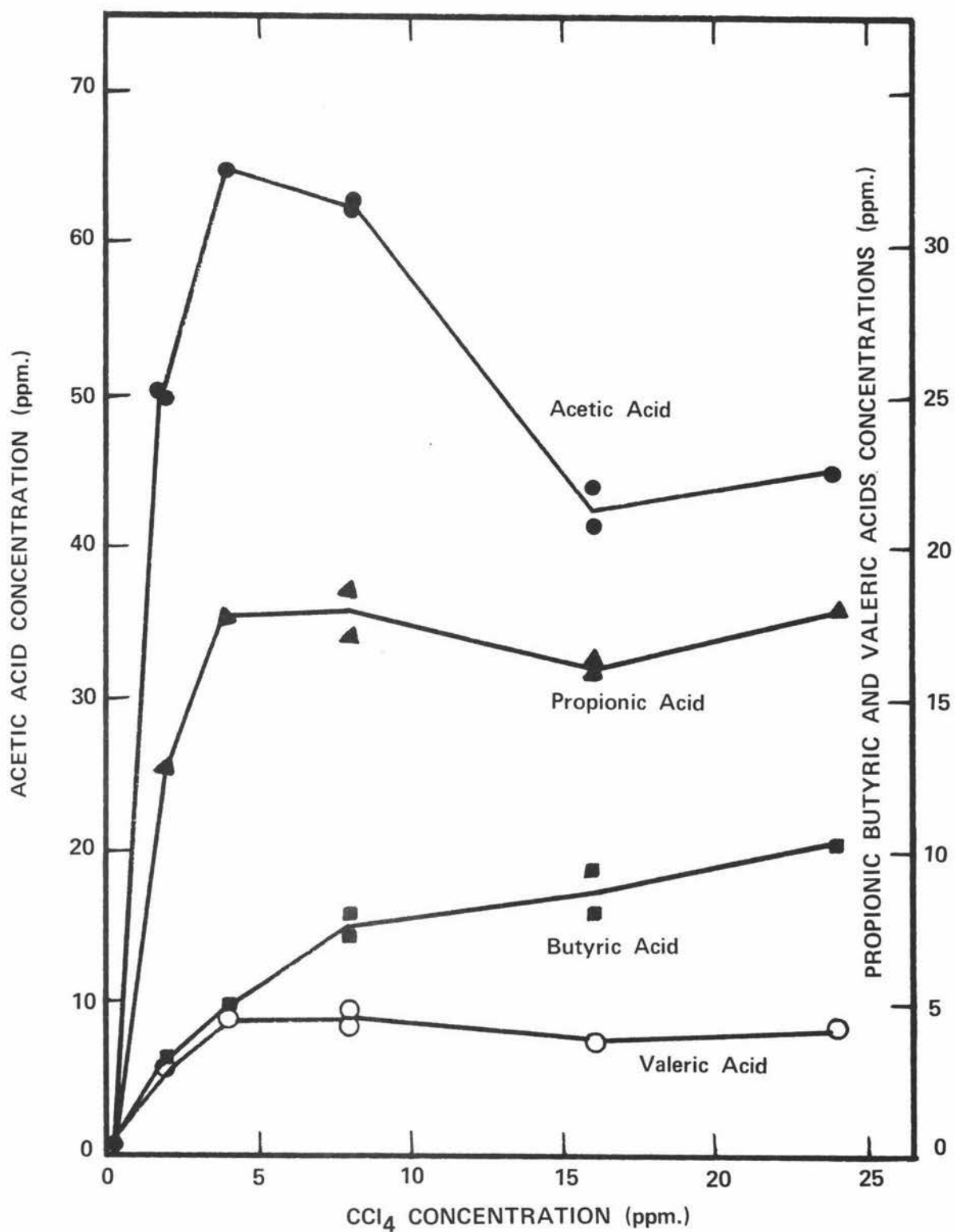


FIGURE 41. VOLATILE FATTY ACIDS ACCUMULATION WITH CCl_4 CONCENTRATION.

was dosed with 24 ppm CCl_4 . Acetic acid constituted for about 50 per cent of the total volatile fatty acid. The maximum concentrations of acetic and propionic acids of 62.5 to 64.5 ppm, and 17.5 ppm were reported when the anaerobes were dosed with 4-8 ppm CCl_4 . Caproic acid and its isomers were also found to accumulate in very small quantities.

3. Discussion

Hemiacetyl of starch and chloral (HSC) was found not to be as active a methane inhibitor as CCl_4 . Methane inhibition amounting to a 97.2 per cent reduction was obtained with the presence of 24 ppm of CCl_4 . On the other hand, 30 ppm of HSC in the sludge liquor could only inhibit 26.5 per cent of the methane formation after 24 hours digestion.

Trio (42) claimed that only 12 ppm of HSC was needed for approximately 90 per cent of the otherwise expected methane inhibition in rumen liquor, this inhibition of methane formation resulting in the accumulation of hydrogen gas. In this study no hydrogen accumulated, and only 26.5 per cent of methane formation was inhibited even when the concentration of the inhibitor used was two and a half times that reported by Trio (42). This implies that the sludge in anaerobic digesters is less sensitive to HSC than the rumen bacteria.

The study on inhibition of methane formation by CCl_4 revealed an interesting point on the relationships of the digester performance and the concentration of CCl_4 . Figures 36 and 39 illustrate that the activity of the bacterial sludge reached the maximum level in terms of VFA production in the presence of 2.5 ppm CCl_4 . Consequently, the pH of the mixed liquor dropped to 7.05. At a higher concentration of CCl_4 VFA gradually decreased (Figure 39), while the pH increased (Figure 36). The pH of the mixed liquor returned to normal (about 7.35) when the concentration of CCl_4 was increased to 16 ppm. The pH was still at 7.35 when CCl_4 was increased to 24 ppm. The reason presumably is that the overall activity of the sludge was inhibited by the presence of a high concentration of CCl_4 . In addition, the activity of the flora was also inhibited by the fermented gas, especially hydrogen. The sensitivity of the flora to the inhibitor will vary from sludge to sludge (Thiel (29)).

Inhibition of methane formation resulted in the accumulation of hydrogen gas, and the maximum quantity of hydrogen was obtained when CCl_4 was increased to 16 ppm. At a higher concentration, accumulated hydrogen decreased. It was suggested by Thiel (29) that either the hydrogen producing bacteria were inhibited by hydrogen, or the hydrogen flow was directed towards the production of lactic acid. Sykes and Kirsch (30) demonstrated that accumulated hydrogen, from CCl_4 inhibition, inhibited hydrogen production by hydrogen producing bacteria.

The inhibiting effect of oxygen (Section B) on the performance of anaerobic digestion was quite different from that of methane analogue compounds especially CCl_4 . The compositions of the gas produced from the sludge subjected to oxygen toxicity were quite constant. The percentages of methane and CO_2 were 60 to 65 and 40 to 35 respectively. No hydrogen was found to accumulate. The role of oxygen in aeration was quite different from that in the continuous aeration reported by Fields and Agardy (44). They demonstrated that additions of oxygen cause a dramatic decrease in CO_2 production and total gas production with the methane, on the other hand, remaining fairly constant.

The utilization of oxygen by obligate anaerobes was claimed to be through the formation of peroxide, and the peroxide formed acted as an inhibitor (44). Oxygen probably killed the anaerobes, depending upon their sensitivity. On the other hand, CCl_4 selectively inhibited methane formation and caused accumulation of the precursor, hydrogen.

The presence of oxygen in a digester was reported by Fields and Agardy (44) to result in a rapid increase in volatile acid levels. Slyter and Wolin (38) demonstrated inhibition by CuSO_4 and O_2 , and this was accompanied by an increase in propionate formation. Aeration prior to sedimentation, similarly, resulted in the accumulation of VFA. In the presence of 1460 mg/litre of oxygen in the sludge mixed liquor a 46 per cent increase in VFA resulted. Acetic acid also accumulated in the presence of oxygen. When oxygen was increased to 1460 mg/litre, propionic acid increased rapidly. No other acids were found.

Accumulation of volatile acids in the presence of oxygen was similar to that when the sludge mixed liquor was dosed with

HSC. Acetic and propionic acids constituted most of the acids. As the concentration of HSC was increased the proportions of those acids were quite constant, until the HSC was at about 30 ppm, when propionate accumulated slightly faster and butyrate also started to accumulate. Other volatile acids were present in very small quantities.

It was reported by Rufener and Wolin (41) in the inhibition study of rumen liquors that VFA were unaffected by CCl_4 concentrations which inhibited methane formation except for the changes in the proportion of the produced VFA, while Sykes and Kirsch (30) demonstrated a decrease in the production of propionate. The results from both groups were quite different from those of this investigation. In the presence of CCl_4 there was a sharp increase in VFA. Acetate, propionate, butyrate, isovalerate and valerate accumulated in large quantities. Only a small quantity of caprate was present.

VI SUGGESTIONS FOR FUTURE WORKS

As a result of the findings in this study the following suggestions are made for further research.

1. Extension of the data obtained in this study to the continuous anaerobic contact process with continuous aeration-degasification.
2. Study of the design parameters of the aeration vessels, including the location and geometry of air diffusers and the shape of the aeration vessels.
3. Study of the effects of aeration degasification on the performance of anaerobic digesters with different loading rates.
4. Study of the effects of the sizes of air bubbles and the intensity of turbulence on the continuous flocculation of anaerobic sludge suspension.
5. The effects of aeration on settling characteristics of sludge.
6. Study of the effects of the other gases such as nitrogen, carbon dioxide, pure oxygen and possibly the gas produced from the digestion process itself, on the performance of the digesters.

APPENDIX 1.

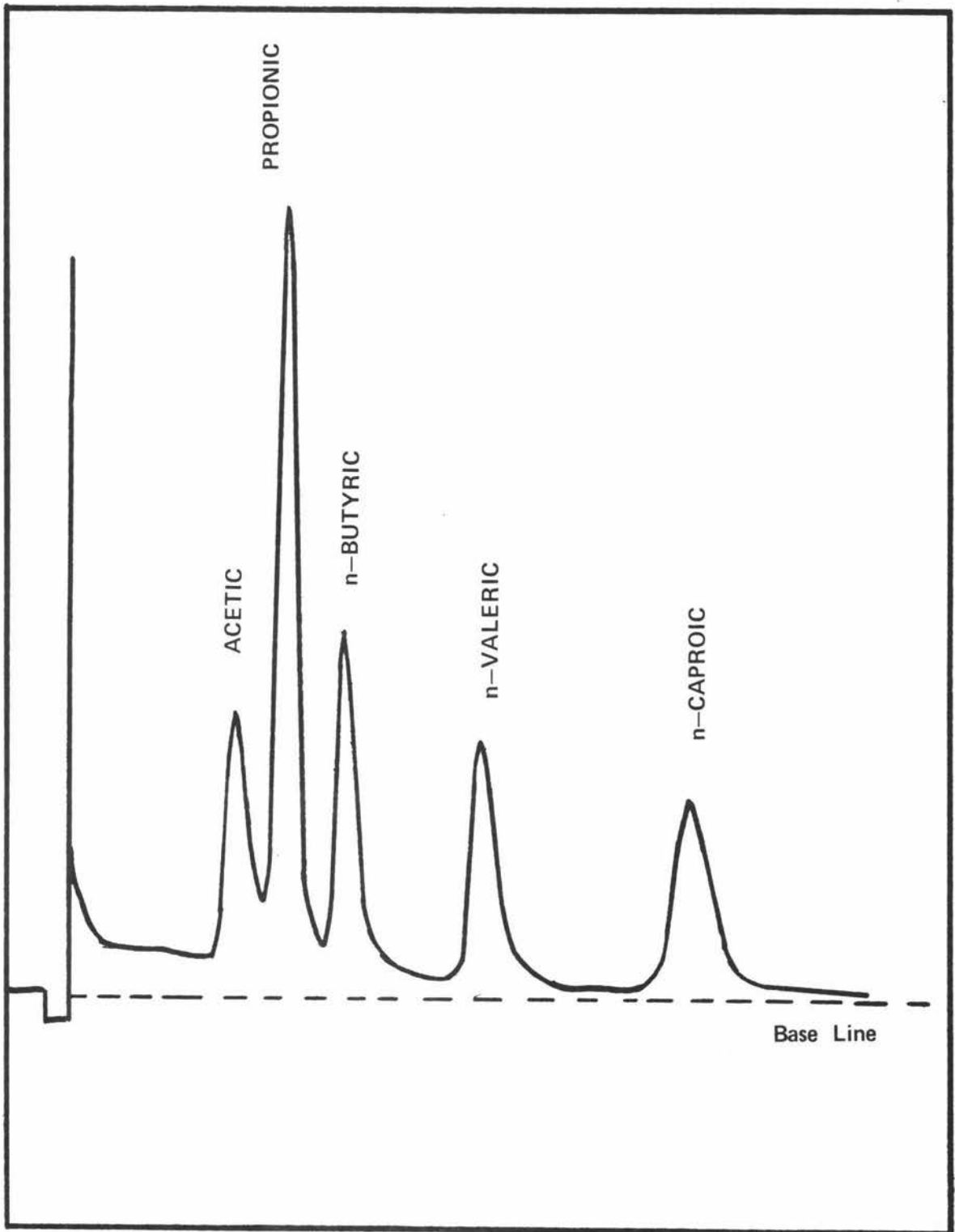


FIGURE 42. TYPICAL CHROMATOGRAM SHOWING SEPARATION OF ACETIC, PROPIONIC, n-BUTYRIC, n-VALERIC AND n-CAPROIC ACIDS.

APPENDIX 2.

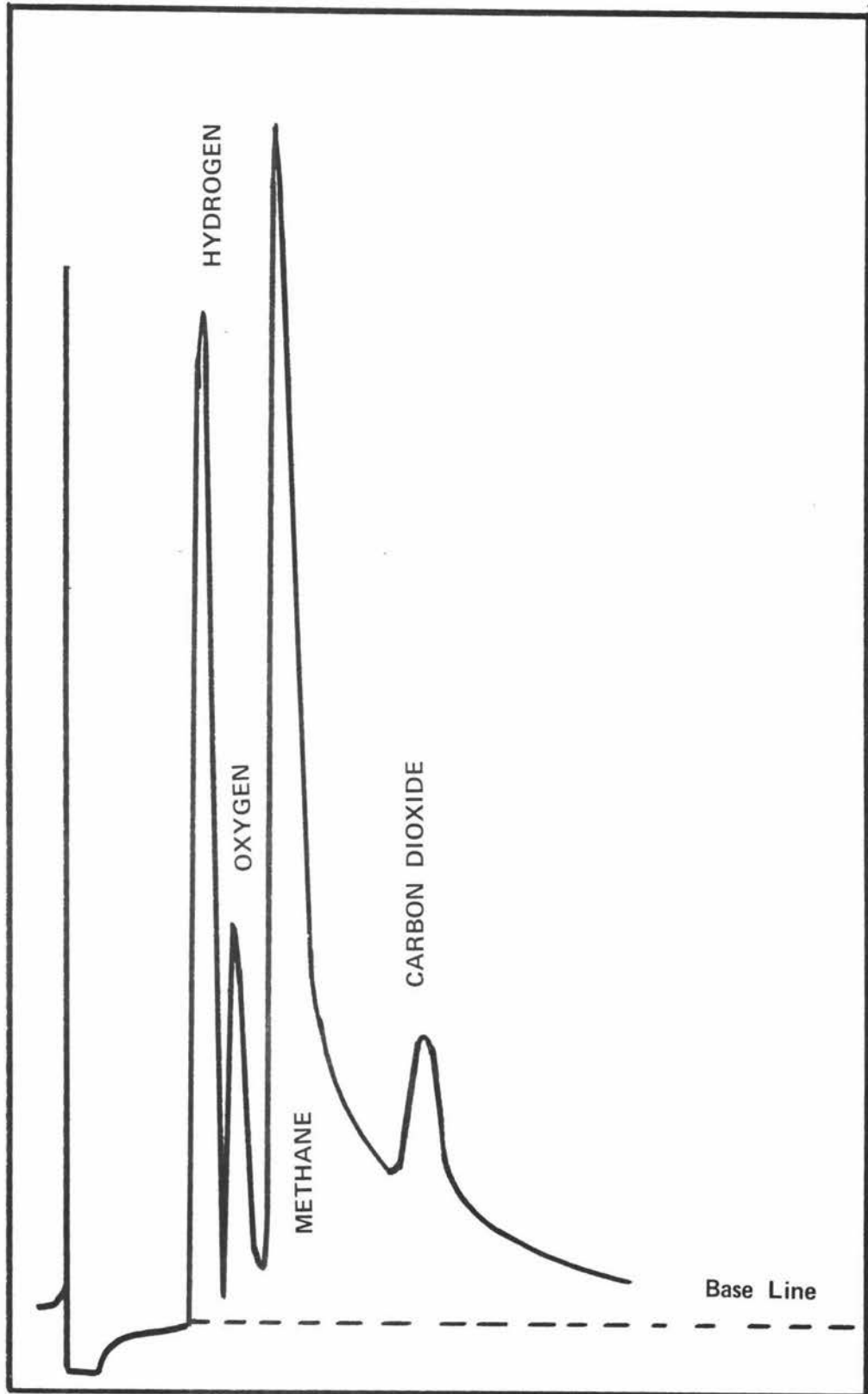


FIGURE 43. TYPICAL CHROMATOGRAM SHOWING SEPARATION OF GASES HYDROGEN, OXYGEN, METHANE AND CARBON DIOXIDE.

APPENDIX 3

DAILY GAS PRODUCED (LITRES)		DIFFERENCE (D)	(D ²)
FERMENTER NO. I	FERMENTER NO. II		
6.01	5.87	-0.14	0.0196
6.29	6.29	-	-
7.16	7.05	-0.11	0.0121
7.05	7.05	-	-
6.56	6.73	0.17	0.0289
6.82	6.86	0.04	0.0016
6.25	6.56	0.31	0.0961
		0.27	0.1583

$$t = \frac{\bar{D}}{s^2}$$

The means of the difference between the two sets of samples (\bar{X})

$$= \frac{0.27}{7} = 0.0386$$

$$s^2 = \frac{\sum D^2 - \frac{(\sum D)^2}{N}}{N - 1}$$

$$= \frac{0.1583 - \frac{0.0729}{7}}{6}$$

$$= \frac{0.1583 - 0.0104}{6}$$

$$= \frac{0.1479}{6} = 0.0247$$

$$t_6 = \frac{0.0386}{0.0248} = 1.56$$

Not significant

APPENDIX 4

DAILY GAS PRODUCED (LITRES)		DIFFERENCE (D)	(D ²)
FERMENTER NO. I	FERMENTER NO. II		
6.54	6.58	0.04	0.0016
6.65	6.55	-0.10	0.01
6.45	6.90	0.45	0.2025
6.71	6.92	0.21	0.0441
6.33	6.59	0.26	0.0676
6.87	6.64	-0.23	0.0529
6.65	6.72	0.07	0.0049
7.11	7.01	-0.10	0.01
		0.70	0.3936

$$t = \frac{\bar{X}}{s^2}$$

$$\bar{X} = \frac{0.70}{8} = 0.0875$$

$$s^2 = \frac{0.3936 - \frac{0.49}{8}}{7}$$

$$= \frac{0.3936 - 0.0613}{7}$$

$$= 0.0475$$

$$t_7 = \frac{0.0875}{0.0475} = 1.84$$

Not significant

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