

**AN EVALUATION OF BIOCHEMICAL OXYGEN DEMAND OF ORGANIC  
WASTES BY MEASUREMENT OF CARBON DIOXIDE EVOLUTION FROM  
AERATED ACTIVATED SLUDGE**

by

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## I. INTRODUCTION

For some time, prior to legislation of the National Water Pollution Control Act, by Congress in 1948, investigators across the country had been trying to improve current techniques and devise new methods of measuring pollution in waters destined for human consumption. Figures from the Act showed that over 8,000 industries were in need of either new waste treatment plants, or replacements, and additions of new units to existing facilities. Furthermore, there were over 4,000 municipalities serving 40 million people where data on pollution was not complete or no data was on record.

The effectiveness of measures taken to mitigate the severity of this pollution problem depends in part on the analytical techniques employed. Most methods available for the investigation and determination of pollution and its effects are time consuming, complex and expensive.

One of the most popular methods currently employed for measuring the degree of stream pollution is the Biochemical Oxygen Demand (B. O. D.) determination. This determination has several shortcomings, the greatest of which is a required 5 day incubation period. Many efforts have been made to replace the B. O. D. determination with

one which is less time consuming. To date, however, such efforts have enjoyed little success.

In the activated sludge treatment of sewage, the incoming sewage, is mixed with previously formed sludge and the subsequent mixture is aerated. Several investigations have been performed on this type of treatment with the view in mind of utilizing the carbon dioxide ( $\text{CO}_2$ ), released during the process as a measure of the oxidizing ability or activity of an aerated sludge, such sludge being procured from a domestic or industrial waste treatment plant, or from a sludge formed in the laboratory for the purpose of carrying out an investigation.

For this investigation, a sludge was formed and maintained in the laboratory and used together with synthetic sewage as a medium to simulate an activated sludge treatment process. The carbon dioxide evolved during aeration of the mixture was measured, and an attempt was made to correlate the  $\text{CO}_2$  values with the results of 5 day B. O. D. tests run concurrently on the aerating mixture.

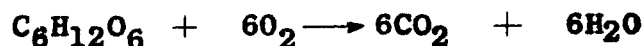
The process used in determining carbon dioxide evolution is not new or novel, but its application as a tool for measuring the B. O.D. of an industrial or domestic waste or any polluted water is a recent field of investigation.

## II. REVIEW OF LITERATURE

Probably the most extensive and recent work on the utilization of  $\text{CO}_2$  evolved from an aerating activated sludge-sewage mixture was performed by Nandor Porges et al (9). Their investigations of dairy wastes showed that a correlation existed between the  $\text{CO}_2$  evolved and the 5 day B. O. D. as measured by the Warburg apparatus. Solutions of skim milk (1,000 p.p.m.) lactose (500 p.p.m.) and casein (350 p.p.m.) were used, and each was found to have its own rate and extent of oxidation when acted upon by the sludge organisms. All activity was essentially completed in 6 hours. Analyses showed that 60 - 68% of the substrate was converted to cell material, and the remainder was oxidized.

Determinations of the Respiratory Quotient (R. Q.) for each of these systems demonstrated that the volume of  $\text{CO}_2$  evolved was almost exactly equal to the oxygen ( $\text{O}_2$ ) consumed. That is to say  $\text{R. Q.} = \text{CO}_2/\text{O}_2 = 1$

A typical oxidation equation is as follows:



(Glucose + oxygen  $\longrightarrow$  carbon dioxide + water)

$$(\text{R. Q.} = \frac{6\text{CO}_2}{6\text{O}_2} = \frac{6}{6} = 1)$$

Thus it appears that under a highly aerobic environment, the  $\text{CO}_2$  evolved may serve as a measure of sludge activity.

Earlier investigators, Wooldrige and Standfast (13) of England, found that the ratio of  $\text{CO}_2/\text{O}_2$  was much less than unity for a domestic sludge and sewage mixture, but that the ratio increased as oxidation proceeds.

Salle (3) made investigation upon the respiratory quotient of various organisms each in a carbohydrate, fat and protein medium. His investigations showed an agreement with Nandor Porges et al (9) in that a carbohydrate or lactose medium gave an R.Q. of unity, but that for both fats and proteins, the R.Q. was considerably less. The ratio varied; 0.8 being given as an approximate average, although in some cases the value was even greater than unity.

Wooldrige and Standfast (13) showed that the R.Q. for sewage sludge mixtures other than carbohydrates was closer to unity in an acid medium (i.e. one whose  $\text{pH} < 7$ ). Their explanation for this condition was that in a solution which is quite acid ( $\text{pH} < 5$ ), there is greater inhibition of non-carbonaceous oxidation e.g. ammonia ( $\text{NH}_3$ ) oxidation.

Nandor Porges et al (9) were able to support this in their most recent investigation. They discovered that acidification of an aerating mixture of a proteinaceous nature was necessary to release all the  $\text{CO}_2$  evolved. They

explained this phenomenon by stating that the alkaline substances produced by the oxidation of the proteins combined with some of the  $\text{CO}_2$  thus preventing its removal from the aerating sludge. They suggested that, in some cases acidification of the aerating mixture may be replaced by the use of buffers.

Porter (4) reported similar low values of R.Q. for several bacteria and protozoa in jar experiments and stated that the fundamental reason for these low values was the failure of nearly all techniques to determine the amount of  $\text{CO}_2$  dissolved free or chemically combined in the medium. Moreover, due to secondary changes in the medium a longer aeration period may result in an increased  $\text{CO}_2$  production. Porter also found that an R.Q. greater than the theoretical value is due to the decarboxylation of amino acids and other organic acids resulting in a further release of  $\text{CO}_2$  without a corresponding  $\text{O}_2$  intake.

Hoover et al (10) found that the volume of air admitted to the aerating mixture did not affect the  $\text{CO}_2$  evolution. From original air quantities of 1 volume of air per minute per 1 volume of aerating mixture, reductions in air volume to 1/2, 1/4 and 1/8 per volume of mixture resulted in no change in  $\text{CO}_2$  evolution.



### III. THE INVESTIGATION

#### Preliminary Investigation and Apparatus

Although the device used for the measurement of CO<sub>2</sub> evolution is not complex, considerable time was spent in its construction. The apparatus used is shown in Plates 1 and 2. As shown in Plate 2 not less than four units were used at one time.

The apparatus functioned very efficiently throughout the investigation, although a proper technique in manipulation was essential in achieving consistent results.

The group of investigators at the Eastern Regional Research Laboratory in Philadelphia, Pennsylvania concentrated their efforts chiefly on the oxidation of dairy wastes and made a comparison of results with the Warburg apparatus. The investigation contained herein was made with the idea of reducing the number of variables to a minimum, and a comparison of results was made with those obtained by using the standard 5 day B. O. D. bottle dilution test.

Previous investigators made no mention of the condition of sludge used in the aerating mixture. It was found in the present investigation that sludge condition has a very decided effect on the oxidation potentiality of the

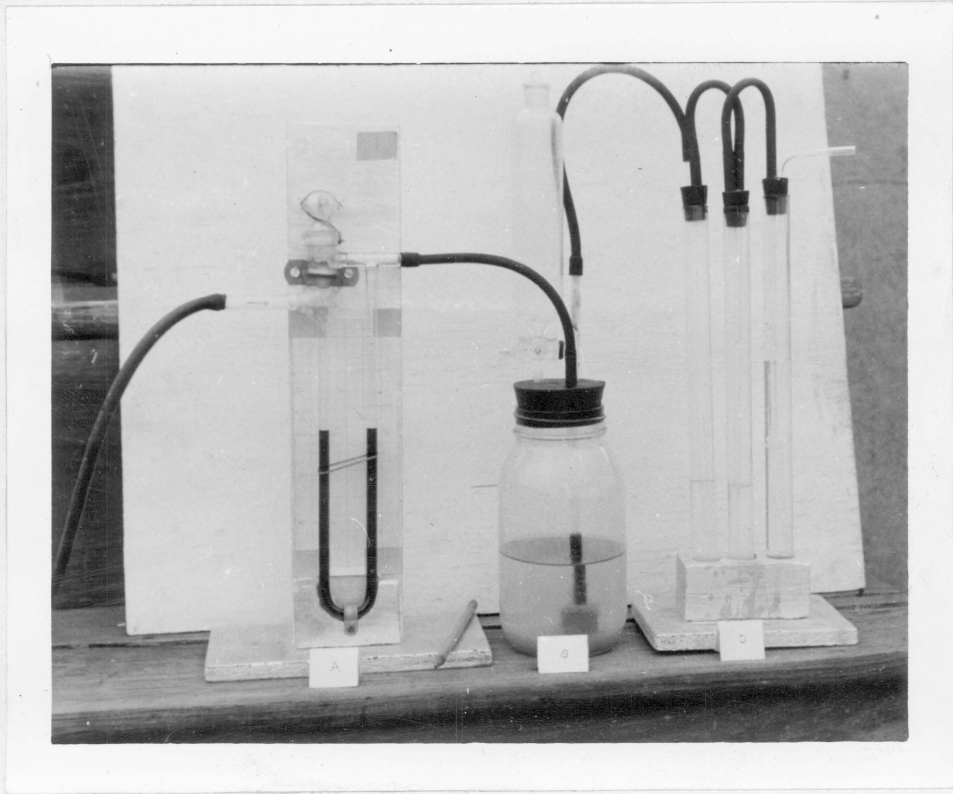


Plate 1

Assembly of One Aeration Unit

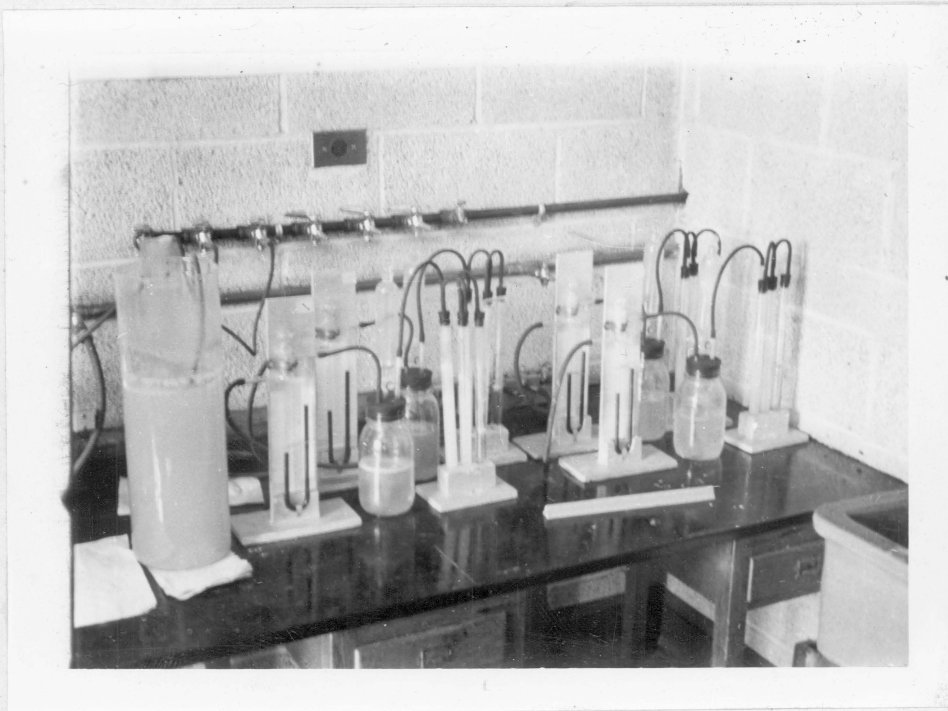


Plate 2

View Showing Four Aeration Units  
Operating Simulanteously

sludge when the sludge is mixed with synthetic sewage. Extensive trials were made with varying values of settleable solids, suspended solids and sludge volume index (S.V.I.). A graph of suspended solids versus CO<sub>2</sub> evolution may be seen on page 24, Figure 2.

#### IV. MAJOR INVESTIGATION

The previous investigators, using principally dairy wastes, could make a direct estimation of the oxygen demand from the CO<sub>2</sub> evolution. There the respiratory quotient (R.Q.) equaled unity viz:  $CO_2/O_2 = 1$ . No alkaline condition was produced and the respiratory quotient was unity. However it was found that a proteinaceous waste such as was used in the present investigation has a respiratory quotient of approximately 0.8 (A. J. Salle (3) and Wooldrige & Standfast (13)). The alkaline substances produced combined with some of the CO<sub>2</sub> preventing its removal from the aerating sludge. The Philadelphia investigators found that an addition of dilute H<sub>2</sub>SO<sub>4</sub> in sufficient amount to reduce the pH to 3 or less was sufficient to cause a greater amount of CO<sub>2</sub> evolved to be removed from the aerating mixture. Use of a buffer was not attempted in place of acid, but may be useful in further investigations.

Referring to Plate 1 and Figure 1:

The rubber tube leading to the manometer from the left fed oxygen-free air from the soda-lime scrubber. The flow was controlled by a needle valve at the manifold near the scrubber. The flow rate used was one volume of air per minute per volume of sewage sludge mixture (500 ml. air / min.

per 500 ml. mixture). The air was dispersed in the 500 ml. of sludge-substrate mixture contained in the quart jar (B). The glass tube through the jar stopper was fitted with a diffuser stone to serve as the sparger. The spent air carrying evolved  $\text{CO}_2$  left the jar through the upright tube containing glass wool and entered the three tubes (C) at the right (the glass wool prevented passage of liquid droplets). The first two tubes each contained 25 ml. of 0.1N  $\text{Ba}(\text{OH})_2$ , while the third contained a dilute solution of phenolphthalein. Thorough removal of the  $\text{CO}_2$  by the  $\text{Ba}(\text{OH})_2$  was secured by running glass tubes to within 1/16 inch of the bottom of the tubes, forcing the  $\text{CO}_2$  laden air to move upwards through the column of liquid. The third tube contained a glass tube with a Rose bulb at the end; that is, the end of the glass tube was blown to form a small bulb and perforated with four or five small holes. If any  $\text{Ba}(\text{OH})_2$  solution from the first two tubes were accidentally forced over into the tube containing the Rose bulb in the phenolphthalein solution, this would cause the solution to turn pink. Several times during the trials, a nessler tube containing  $\text{Ba}(\text{OH})_2$  solution was connected to the exit tube containing the phenolphthalein. Titration of the  $\text{Ba}(\text{OH})_2$  solution in this tube after several hours during a trial showed no presence of any  $\text{Ba CO}_3$  formation due to a loss of  $\text{CO}_2$ .

Thus it was assumed all the  $\text{CO}_2$  evolved from the aerating mixture was absorbed in the two tubes containing the  $\text{Ba}(\text{OH})_2$  solution.

#### Preliminary Preparation

Since a source of fresh sludge was not available, such had to be built up and nurtured in the laboratory. The seed used was domestic sewage from the outfall sewer of the city of Blacksburg. The nutrient material was synthetic sewage made up as per Butterfield and Wattie (15).

The formation of sludge was carried out in the laboratory. An 8 liter glass cylindrical container was used for this purpose. A domestic sewage volume of about 5 liters was used at the start and to this was added 50 ml. of asbestos fiber emulsion (17) which formed a nucleus for the microorganisms and thus aided considerably in the sludge formation. From time to time as sludge was used up, more asbestos emulsion was added. The sewage was then aerated by the use of two difuser stones fitted to a glass "Y" and connected to the air line leading to the compressor. The flow was controlled by a needle valve at the outlet. After a settling period of 10 minutes, sixty percent of the supernatant was replaced by fresh domestic sewage. However, as a sludge began to form the domestic sewage was replaced by synthetic sewage (12) and it was found that the sludge could be readily built up by two or three feedings per day with concentrated synthetic sewage.

The fluid used in the manometer tubes was Brodie's Solution (16) which was found to have excellent non-wetting properties.

A 1N oxalic acid solution was made and standardized (14). Also a 0.1N solution of barium hydroxide was made up (15) and standardized against the oxalic acid solution.

Dilution water was made up in concentrated form (1).

#### Minor Investigation and Laboratory Procedure

Several trial runs were made to ascertain the effect of the condition of sludge used. A typical procedure in which four units were used simultaneously was as follows:

The air diffusion assembly was removed from the liquid and allowed to settle (10 minutes). Very nearly all of the supernatant was decanted, and a 1 liter graduate was filled to the mark with the remaining sludge. Upon settling of the sludge in the graduate, the supernatant was decanted and dilution water added as an elutriant. This "washing" was repeated twice more, then the final mixture of sludge and dilution water was agitated and a 100 ml. volume drawn off and placed in Jar #1. Further agitation followed and again 100 ml. were drawn off and placed in Jar #2. The latter jar was used to measure the endogenous rate of oxidation. Similarly



Jars #3 and #4 were each filled with 120 ml. sludge. To each of these sludge volumes was to be added sufficient sewage and dilution water to make a 500 ml. volume in each jar. The arrangement of the system was now:

Jar #1 -- 100 ml. sludge and 400 ml. of synthetic sewage (12) of double strength.

Jar #2 -- 100 ml. sludge and 400 ml. of dilution water. Jar #3 -- 120 ml. sludge and 380 ml. synthetic sewage. Jar #4 -- 120 ml. sludge and 380 ml. dilution water. Double strength synthetic sewage was used throughout.

The amount of sludge used is in accordance with the usual volume in percentage pumped to aerating activated sludge tanks in practice i.e. 20 - 25%.

#### General Procedure

After the sludge had been placed in the jar, a three hole stopper with the assembly was fitted into the mouth of the jar. The sparger tube was connected with the flowmeter. Carbon dioxide (CO<sub>2</sub>) free air was then passed through the apparatus for 15 minutes to sweep out residual CO<sub>2</sub> in the jar and tubes. The air flow was now stopped and synthetic sewage added to the jar by way of the separatory funnel to give a final volume of 500 ml. in the jar. The

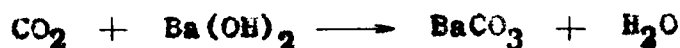
glass cock on the separatory funnel was closed to the atmosphere and the upright air tube containing the glass wool was connected to the tubes containing the  $\text{Ba}(\text{OH})_2$  solution. Air flow was resumed and adjusted to 500 ml. per minute. Silicone anti-foam mixture was added in very small amount to the aerating mixture and to the tubes containing the  $\text{Ba}(\text{OH})_2$ . Aeration was carried out for exactly one hour and the tubes containing the  $\text{Ba}(\text{OH})_2$  solution were replaced with another set also containing 25 ml. of  $\text{Ba}(\text{OH})_2$  solution. Air was permitted to flow during the inter-change of the solution to avoid back pressure. The contents of the tubes containing the  $\text{Ba}(\text{OH})_2$  solution were transferred to a 500 ml. Erlenmeyer flask with a minimum amount of exposure to the atmosphere in order to avoid further absorption of  $\text{CO}_2$ . The contents of the Erlenmeyer flask were then titrated to the colorless end point, phenolphthalein being used as an indicator. The tubes containing the  $\text{Ba}(\text{OH})_2$  solution were replaced at hourly intervals and, as was mentioned previously, all data was recorded and plotted.

As stated in the preliminary investigation, acidulation of the aerating mixture near the end of the 6 hour period was necessary to release most of the  $\text{CO}_2$  produced. It was found that a volume of 25 ml. of 0.02N  $\text{H}_2\text{SO}_4$  solution would reduce the pH of the aerating mixture to about

pH 3. This acidulation was carried out near the end of the aeration period and the mixture allowed to aerate for another 30 minutes.

### Calculations

The spent air from the aerator was relieved of  $\text{CO}_2$  by the  $\text{Ba}(\text{OH})_2$  solution as per the following equation:



The  $\text{BaCO}_3$  was precipitated as an insoluble salt. The excess  $\text{Ba}(\text{OH})_2$  was titrated by the oxalic acid, which does not react with the  $\text{BaCO}_3$ . The difference in value between the  $\text{Ba}(\text{OH})_2$  present at the beginning in the 25 ml. of standard solution, and that found by the oxalic acid gives the amount of  $\text{Ba}(\text{OH})_2$  combined with  $\text{CO}_2$ .

#### A Sample Calculation:

At start 50 ml.  $\text{Ba}(\text{OH})_2$  0.102N = 51 ml. 0.1N  
 titration 3.1 ml.  $(\text{COOH})_2$  1.06N = 32.8 ml. 0.1N  
 $\text{Ba}(\text{OH})_2$  carbonated = 18.2 ml. 0.1N  
 1 ml. of 0.1N  $\text{Ba}(\text{OH})_2$  = 1 ml. 0.1N  $\text{CO}_2$  = 2.2 mg.  $\text{CO}_2$   
 $\text{CO}_2$  produced = 18.2 x 2.2 = 40 mg. per 500 ml.  
 $\text{CO}_2$  produced, mg. per liter = 80 p.p.m.

The results obtained during a typical 6 hour aeration period were tabulated and calculated as follows:

Jar #1 - 100 ml. sludge 400 ml. sewage  
 Jar #2 - 100 ml. sludge - 400 ml. dilution H<sub>2</sub>O  
 Jar #3 - 120 ml. sludge 380 ml. sewage  
 Jar #4 - 120 ml. sludge - 380 ml. dilution H<sub>2</sub>O  
 Ba(OH)<sub>2</sub> solution = 0.102N  
 (COOH)<sub>2</sub> solution = 1.06N

	Ml. of Oxalic Acid					
	12:15	1:15	2:15	3:15	4:15	5:15
Jar #1	3.1	3.5	3.0	3.55	3.6	2.8
Jar #2	3.9	4.4	4.1	4.23	4.25	4.3
Jar #3	2.8	3.1	2.9	3.70	3.35	2.9
Jar #4	4.0	4.25	4.15	4.35	4.1	4.1

	P. p. m. Carbon Dioxide					
Jar #1	80.0	60.8	84.4	58.4	56.4	94.0
Jar #2	42.6	19.4	33.0	26.4	26.4	23.8
Jar #3	94.0	80.0	89.2	78.4	68.2	89.2
Jar #4	37.8	26.4	30.8	21.6	33.0	33.0

	Cumulative Results					
Jar #1	37.4	78.8	130.2	162.4	192.4	262.6
Jar #3	56.2	109.8	168.2	255.0	260.0	316.2

Applying the respiratory quotient correction:

$$262.6 \times \frac{1}{0.8} = 328 \text{ p.p.m.}$$

$$316.2 \times \frac{1}{0.8} = 385 \text{ p.p.m.}$$

In each titration, the blank value or the endogenous value in p.p.m. is subtracted from the sewage oxidation value.

$$\text{viz: Jar \#1} = 80 \text{ p.p.m.}$$

$$\text{Jar \#2} = \underline{42.6} \text{ p.p.m.}$$

$$\text{Net value} = 37.4 \text{ p.p.m.}$$

The net values are cumulative through the 6 hour period.

#### Final Investigation

Following the study of the relationship between sludge condition, such as suspended solids, sludge volume index and carbon dioxide evolved, the results were tabulated and plotted as shown on Table 1 and Figure 2. Having determined the above relationship, several 6 hour runs were subsequently made using sludge in a very active condition. At the same time, bottle 5 day B.O.D. determinations were made on the sludge-sewage supernatant liquor after a settling period of 5 minutes. B.O.D. incubations were made at the start and end of each run. The

Schematic Diagram

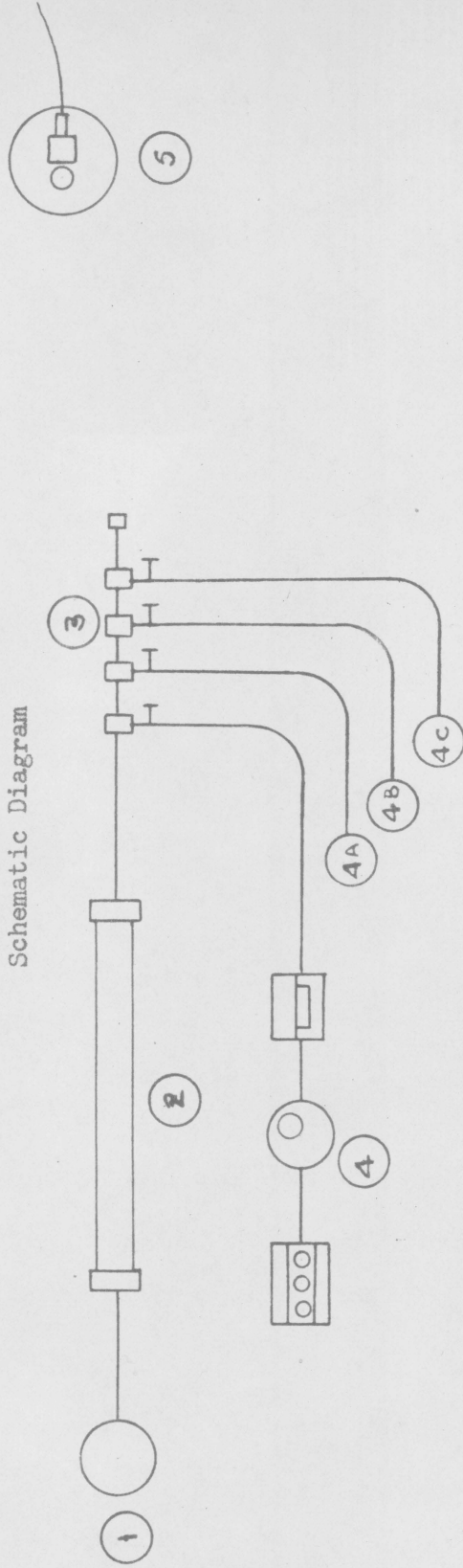


Figure 1

Plan of Apparatus Assembly

- (1) Ingersol-Rand air compressor Type 30 fitted with Fisher Scientific pressure regulating valve.
- (2) Air scrubber of 2" dia. galvanized iron pipe 36" long 2 lbs. soda-lime 4-8 mesh in pipe. Glass wool packing at ends to ensure retention of particles in pipe.
- (3) Manifold assembly of 1/4" dia. pipe with 1/4" Fisher brass needle valve at each take-off for individual control.
- (4)A,B,C. Four complete outfits as shown on Plate 1.
- (5) Fisher Scientific Air Flowmeter for manometer calibration.

Table 1  
Comparison of Suspended Solids With Carbon  
Dioxide Evolution

<u>Suspended Solids</u> (p.p.m.)	<u>Carbon Dioxide</u> (p.p.m.)
170	92
270	58
310	92
310	130
380	119
480	181
500	115
550	138
610	196
670	192
730	134
790	212
820	189
900	202
1000	238
1020	253
1110	242
1110	247
1200	253
1310	240

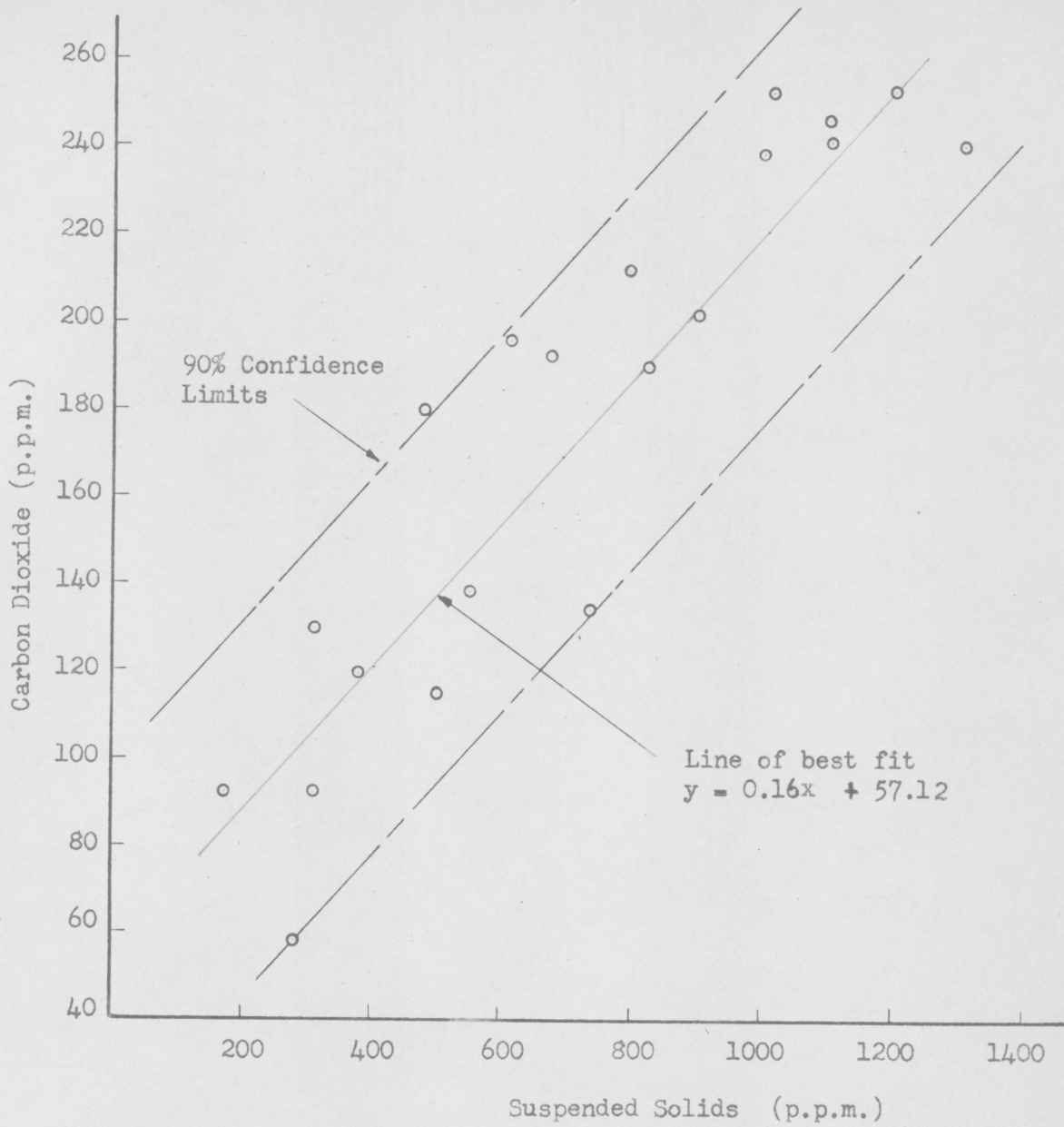


Figure 2

Correlation Graph of Suspended Solids  
and Carbon Dioxide Evolved



results of these runs were compared with the evolved  $\text{CO}_2$  values and are shown on Table 2 and Figure 3. In each case, the bottle B.O.D. values were considerably in excess of the carbon dioxide values, although the final results of the  $\text{CO}_2$  values had been increased by the approximate Respiratory Quotient of 0.8.

The 5 day B.O.D. bottle dilution showed considerable variance with varying volumes of liquor inoculum. The incubations of 1 ml. and 2 ml. volumes showed no relationship to the 3 ml. volume of inoculum when the results of the former two were appropriately increased to compare with the 3 ml. results. Since the greater dilutions resulted in consistently smaller B.O.D. values, it was decided to assume results of the lower (3 ml.) dilution as a more correct value. This decision was in accord with Langelier's investigations (11).

Earlier trials made during the preliminary investigations showed a greatly increased carbon dioxide evolution from the aerating mixture by the addition of acid. Consequently, in light of these findings, it was decided to add acid towards the termination of a 6 hour aeration period then allowing further aeration for about 30 minutes. Once more bottle B.O.D. determinations were made at the start and also at every two hour period during the run. Since the action of the acid renders the

**Table 2**  
**Comparison of Carbon Dioxide Evolution With**  
**5 Day B.O.D., With and Without Acidulation**

<u>Trial</u>	<u>Without Acid</u>		<u>With Acid</u>	
	<u>B.O.D.</u> <u>(p.p.m.)</u>	<u>CO<sub>2</sub></u> <u>(p.p.m.)</u>	<u>B.O.D.</u> <u>(p.p.m.)</u>	<u>CO<sub>2</sub></u> <u>(p.p.m.)</u>
1	303	343	251	266
2	319	320	289	300
3	334	282	300	354
4	339	298	325	384
5	352	306	328	400
6	354	302	329	375
7	364	314	338	280
8	375	349	344	368
9	386	376	363	360
10	389	325	364	352
11			366	385
12			400	328

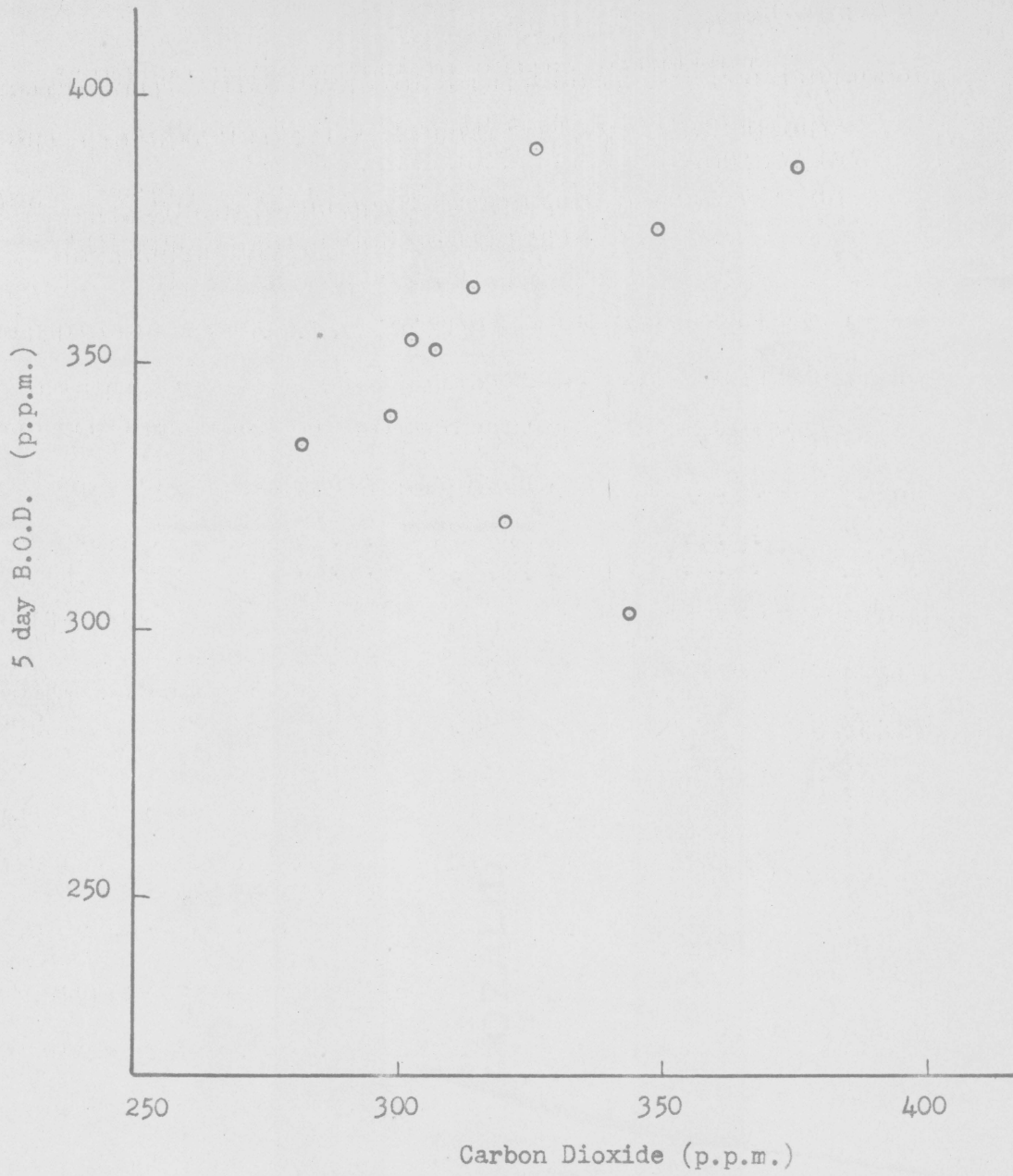
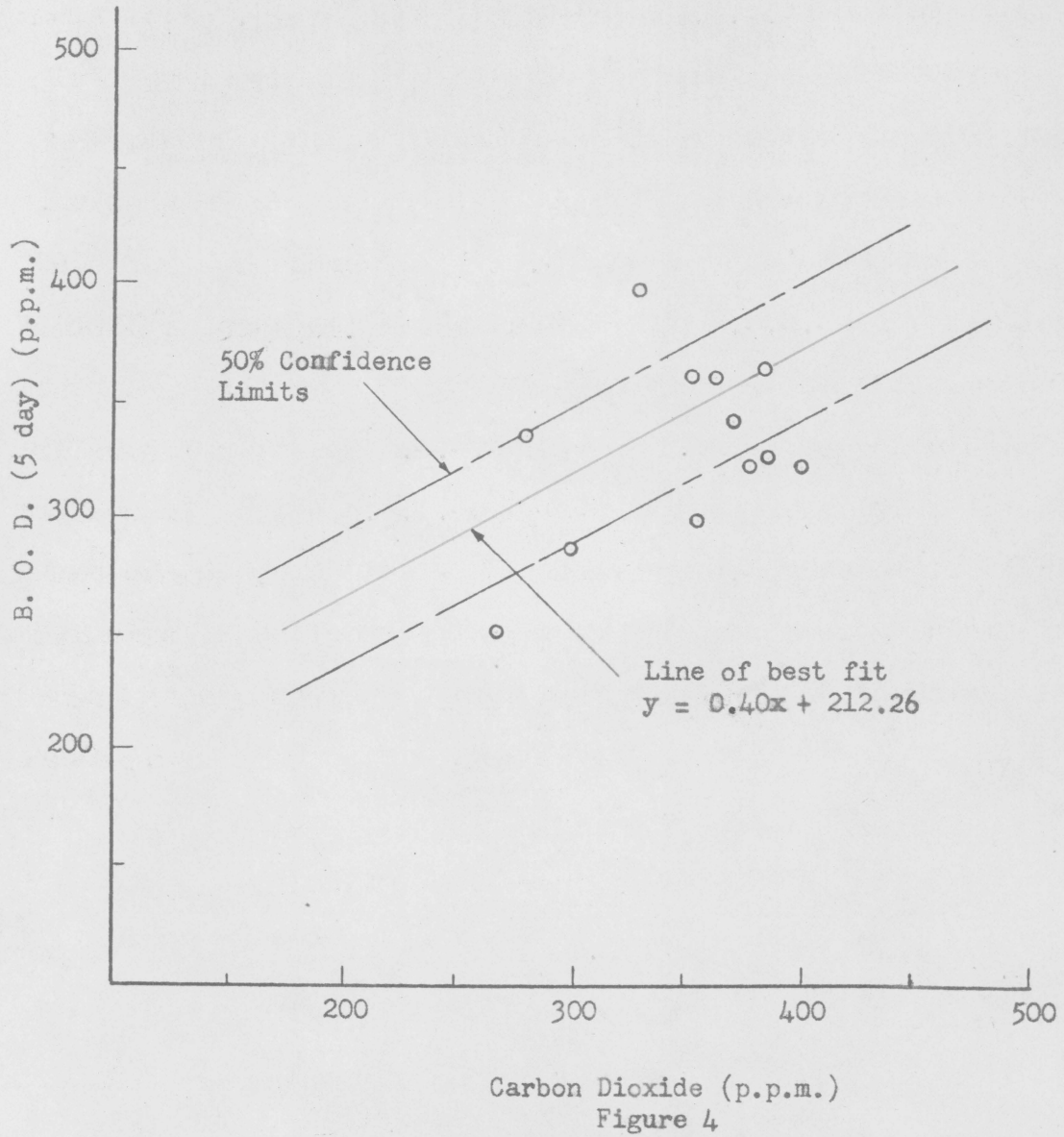


Figure 3

Plot of B. O. D. (5 day) vs Carbon Dioxide  
(No acidulation)

biological organisms inert in the aerating mixture, the final B.O.D. samples were taken just prior to the acidulation. In each instance of B.O.D. sampling the air flow to the jar containing the aerating mixture was shut down and a five minute period of settling was allowed. The sample for inoculation was then drawn by a pipette from the supernatant liquid. No endogenous rate of oxidation correction was made in the B.O.D. determination but the 5 day incubation value of the dilution water was allowed for in the B.O.D. calculations. Corresponding values of bottle S.O.D. and  $\text{CO}_2$  evolved from these latter runs are shown on Table 2 and Figure 4. One of the principal functions of the acid upon the medium was to break down the bicarbonates formed during the aeration period.



Carbon Dioxide (p.p.m.)  
Figure 4  
Graph of B. O. D. (5 day) vs Carbon Dioxide  
(With acidulation)

**Table 3**  
**DATA COLLECTED DURING A TYPICAL 6 HOUR AERATION**  
**PERIOD SHOWING EFFECT OF ACIDULATION**

	ml. Oxalic Acid					
	12:15	1:15	2:15	3:15	4:15	5:15
	acid at 4:45					
Jar #1	3.1	3.5	3.0	3.55	3.6	2.8
Jar #2	3.9	4.4	4.1	4.25	4.25	4.3
Jar #3	2.9	3.1	2.9	3.70	3.35	2.9
Jar #4	4.0	4.25	4.15	4.35	4.1	4.1

	p.p.m. Carbon Dioxide					
Jar #1	30.0	60.8	84.4	58.6	56.4	94.0
Jar #2	42.6	19.4	33.0	26.4	26.4	23.8
Jar #3	94.0	80.0	89.2	78.4	68.2	89.2
Jar #4	37.8	26.4	30.0	21.6	33.0	33.0

	Cumulative Results					
	37.4	78.8	130.2	162.4	192.4	262.6
	56.2	109.8	168.2	225.0	260.0	316.2

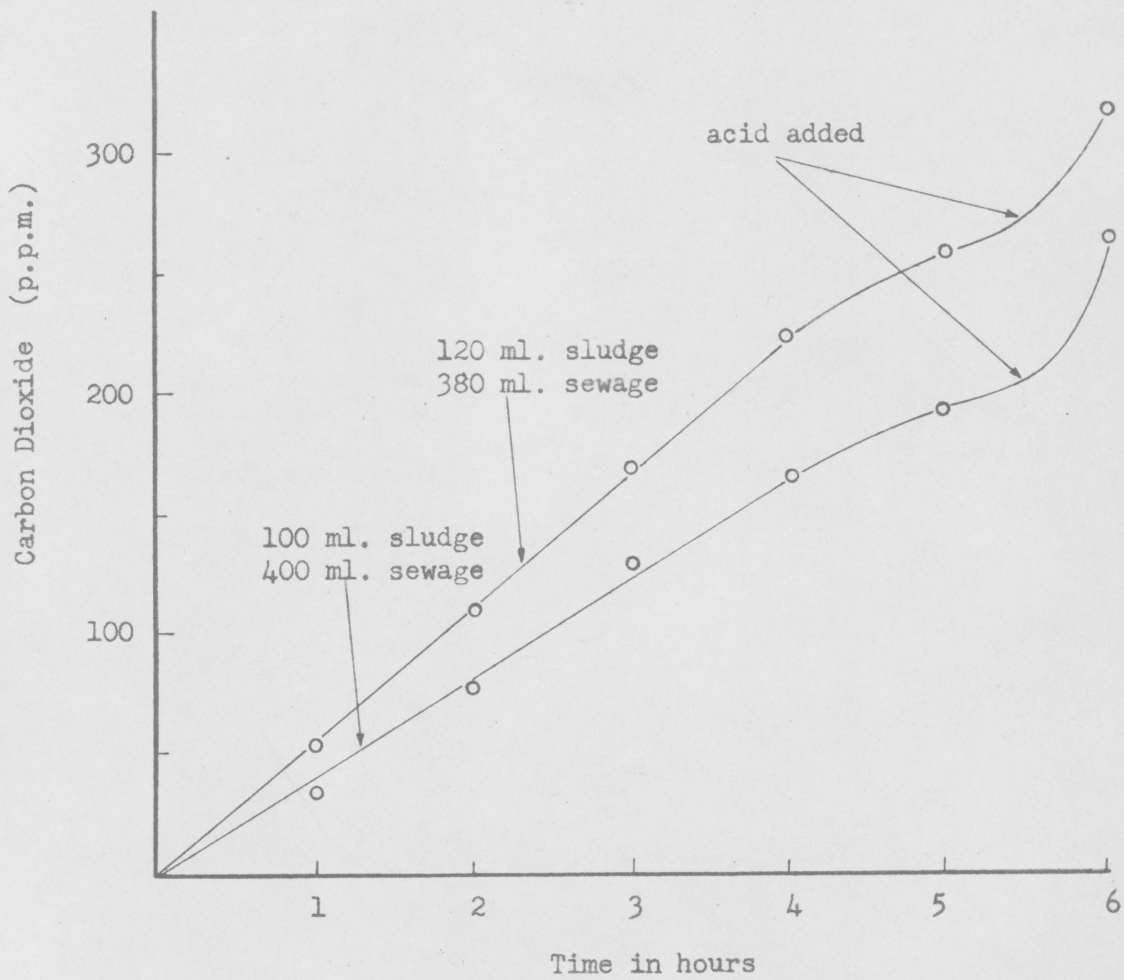


Figure 5

Graph of CO<sub>2</sub> Evolved vs Time in Hours

Showing Effect of Acidulation

## V. DISCUSSION OF RESULTS

As was surmised, the  $\text{CO}_2$  evolution varied with the sludge condition. Although the limits of confidence values appear far removed from the curve of best fit, they are in accordance with the measured values. It was found that the values of the sludge volume index (S.V.I.) varied between 80-120 which is within the range suggested by Imhoff and Fair (7).

The comparison of values of B.O.D. and  $\text{CO}_2$  from runs which were not acidulated showed no correlation. It was evident that all of the  $\text{CO}_2$  formed was not released so that it could combine with the  $\text{Ba}(\text{OH})_2$  solution. Although the Respiratory Quotient ratio of 0.8 (commonly accepted for proteins) was applied to the  $\text{CO}_2$  values, they were still less than the B.O.D. values by approximately 30%.

The series of runs in which acid was added to the aerating mixture showed a sharp increase in the  $\text{CO}_2$  evolved with apparently some correlation, but a calculation of statistical correlation failed to show a significant relationship. Apparently this is due to an inability of the technique to determine the amount of  $\text{CO}_2$  dissolved free, or chemically combined in the medium. The generally accepted value of 0.8 for the Respiratory



Quotient is conceded by other investigators to be only approximate and will vary from one medium to another. It would seem that the sludge condition is an influencing factor, and the subsequent effect of this condition upon the sewage in the mixture could not be accurately anticipated. Although the suspended solids and settleable solids may be in accord from one medium to another, the age of the sludge had some influence on the end result of an aeration period as far as CO<sub>2</sub> liberation was concerned.

## VI. CONCLUSIONS

It is evident from the results obtained that for a proteinaceous waste no significant correlation can be established between carbon dioxide evolution and bottle B.O.D. As other investigators have determined, the Respiratory Quotient varies from one medium to another. Furthermore, the true value of  $\text{CO}_2$  liberated and that which combines with the barium hydroxide  $\text{Ba}(\text{OH})_2$  in the tubes varies in quantity from one hour to the next to some extent, and certainly varies considerably for different mixtures.

The bicarbonates formed and other alkaline substances produced during the oxidation process absorb varying quantities of carbon dioxide and, although the addition of acid to the mixture releases some of the combined  $\text{CO}_2$ , varying amounts remain in combination and these quantities cannot be determined.

Towards the end of some of the runs it was evident that considerably more carbon dioxide would have been liberated with an extension of the time period. It is possible that some of these runs requiring further time before no further liberation of  $\text{CO}_2$  could be measured would have shown a correspondingly greater B.O.D. However, as the greater majority of runs showed an approach

to exhaustion at the end of the 6 hour period with subsequent poor correlation, it is not likely that the isolated runs requiring further time to reach depletion would have shown a closer correlation with the corresponding B.O.D. values.

The writer is of the opinion that the method of investigation employed is suitable for carbohydrate wastes where, under aerobic conditions, the oxidation products are only carbon dioxide and water as borne out conclusively by the Philadelphia group of investigators. However with proteins and fats, the intermediate products formed during the oxidation process, unite in varying proportions with the evolving carbon dioxide and thus prevent a true measure of the  $\text{CO}_2$  to be made by the method used in this investigation.

## VII. SUMMARY

The object of this research investigation was to establish whether or not a correlation exists between bottle B.O.D. and CO<sub>2</sub> liberated from a highly aerobic sewage-sludge mixture. The first part of the project consisted of reducing the number of variables in the operation to a minimum. To this end, considerable time and effort was spent finding the relationship existing between the condition of the sludge and its oxidizing potentiality. A definite strong statistical correlation was found to exist between the CO<sub>2</sub> liberated and the suspended solids or organic matter in the sludge.

The apparatus functioned very well as could be readily seen from the consistent results obtained with the four units operating simultaneously.

Before acidulation of the aerating mixtures, the relationship between bottle B.O.D. and CO<sub>2</sub> was practically non-existent. The mixtures which were acidulated served in the majority of cases to demonstrate some proportional relationship with bottle P.O.D. values, but failed to do so under statistical analysis.

Possibly the sampling of the liquor supernatant at the start for the initial B.O.D. determination may not

give an accurate indication of the subsequent oxidation potential of the sludge in the medium.

It was felt by the writer that as much thought and effort was applied to the project as conditions permitted. The principle and apparatus used were entirely satisfactory, but this method of analysis evidently is not applicable to the type of waste investigated.

### VIII. ACKNOWLEDGEMENTS

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## XI. APPENDICES

### APPENDIX A

#### Correction Factor for Standardization of 0.025N Sodium Thiosulfate Solution With Saturated Solutions of Dissolved Oxygen (8).

One and one-half liters of distilled water were aerated vigorously for a minimum of 30 minutes to assure saturation with dissolved oxygen. The exact temperature of the aerated water was recorded together with the barometric pressure. Immediately, three B.O.D. bottles (300 ml. each) were filled with the aerated water by siphoning and the bottle stopper replaced with no air bubbles entrained. The standard dissolved oxygen determination was then made on each bottle using the Alsterberg modification of the Winkler method.

Sample calculation for correction factor "f":

$$N = 0.025 \times \frac{O_s}{O} \times \frac{P_o}{P_s} \times \frac{V}{204}$$

Where:

$O_s$  = D. O. Saturation (p.p.m) at the observed temperature (°C) and 760 mm.

$O$  = ml. of thiosulfate used in the titration

$P_o$  = observed barometric pressure (mm.)

$P_s$  = 760 mm.

$N$  = Normality of sodium thio. solution

$V$  = corrected volume of sample titrated (ml.)

$$\text{Sample N} = 0.025 \times \frac{8.68}{7.98} \times \frac{712.3}{730} \times \frac{204}{204}$$

$$N = 0.0255$$

$$\text{Therefore "f"} = \frac{0.025}{0.0255} = 0.980$$

#### APPENDIX B

##### Dissolved Oxygen by the Alsterburg Modification of the Winkler Method:

To a full sample in a 300 ml. B.O.D. bottle, the following was added from a pipette with the tip below the surface of the water: 2 ml. of manganous sulfate solution and 2 ml. of alkaline-iodide solution containing sodium oxide. The sample was then shaken thoroughly by inverting several times, allowed to settle half way to the bottom and then shaken again. With tip of the pipette just above the surface of the solution, 2 ml. of concentrated  $\text{H}_2\text{SO}_4$  were added and the solution thoroughly shaken until clear.

Then 204 ml. (corrected volume for chemicals added) of this sample was transferred to a 500 ml. Erlenmeyer flask by pipettes.

$$\text{Volume correction: } \frac{200 \times 300}{300-6} = 204 \text{ ml.}$$

The sample (204 ml.) was then titrated with the standard sodium thiosulfate solution using the starch

iodide indicator solution. The milliliters of thio-sulfate used in the titration were recorded and the parts per million (p.p.m.) of dissolved oxygen present were calculated.

$$\text{Dissolved oxygen (p.p.m.)} = \frac{(204)}{204} \times (\text{ml. thio. used}) \times ("f")$$

#### APPENDIX C

##### Typical Arrangement of Data for B.O.D. Calculations:

Innoculum Sample	Original D.O.	Final D.O.	Final Blank	Corr. Diff.	Conc. Factor	B.O.D. Final
3 ml.	7.95	3.24	7.63	4.41	100	441
3 ml.	7.90	3.27	7.68	4.38	100	438

#### APPENDIX D

##### Preparation of B.O.D. Reagents

###### (1) Manganous sulfate solution:

Three hundred and sixty-four grams of  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  were dissolved in distilled water, filtered, and the filtrate made up to one liter.

###### (2) Alkaline-iodide sodium oxide:

Seven hundred grams of KOH, 150 grams KI and 10 grams of  $\text{Na}_2\text{S}_2\text{O}_3$  were used per liter. These compounds were dissolved separately in small portions of distilled water. The solutions of potassium hydroxide (KOH) and potassium iodide (KI,) were mixed, diluted to 950 ml. and cooled to

room temperature. Then the sodium azide (10 gm. in 40 ml. of water) was added slowly with constant stirring to avoid local heating. The combined volume was made up to 1 liter.

**(3) Starch Iodide Indicator:**

To five grams of soluble starch placed in a mortar, a little cold water was added, and the mixture ground to a thin paste. This was poured into one liter of boiling distilled water, stirred, and allowed to cool. The solution was preserved with salicylic acid (1.25 gm. per liter).

**(4) Stock Sodium Thiosulfate Solution:**

Twenty-four and eighty-two hundredths grams of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  were dissolved in 1 liter of distilled water: this corresponded to a 0.1 N solution. The solution was preserved with NaOH (0.40 gm per liter) after making up to the volume mark.

**(5) Standard Sodium Thiosulfate Solution:**

Two hundred and fifty milliliters of stock thiosulfate solution were diluted to 1 liter with freshly boiled distilled water. This was then standardized according to Appendix A.

(6) Ferric Chloride Solution and others:

Ferric Chloride Solution:

Twenty-five hundredths grams of  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$  were dissolved in 1 liter of distilled water.

(7) Calcium Chloride Solution:

Twelve and seventy-nine hundredths grams of  $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$  were dissolved in 1 liter of distilled water.

(8) Magnesium Sulfate Solution:

Ten grams of  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$  were dissolved in 1 liter of distilled water.

(9) Phosphate Buffer Solution:

Thirty-four grams of potassium acid phosphate ( $\text{KH}_2\text{PO}_4$ ) were dissolved in 500 ml. of distilled water. Approximately 390 ml. of  $\text{N}/44$   $\text{NaOH}$  (plus or minus) were added until a pH of 7.2 was reached. Then 1.5 grams  $(\text{NH}_4)_2 \text{SO}_4$  were added and the whole diluted to 1 liter.

(10) Standard Diluting Water:

To each carboy of distilled water (1 carboy approximately 20 liters) were added in the following:

- 10 ml. of ferric chloride solution
- 50 ml. of calcium chloride solution
- 50 ml. of magnesium sulfate solution
- 25 ml. of phosphate buffer stock solution

The diluting water was then seeded with about 50 ml. of supernatant fluid from the aerating sludge. The seeded dilution water was aerated with small air bubbles from a diffuser stone for 24 hours and aged for several days before using.

#### APPENDIX E

(12) Preparation of Standard Synthetic Sewage as per U. S. Public Health Reports as a Nutrient Medium for Activated Sludge.

Peptone, Difco, Bacto grade	gram	0.3
Beef extract, Difco, Bacto grade	"	0.2
Urea C. P. grade	"	0.05
Disodium hydrogen phosphate C. P.	"	0.05
Sodium chloride C. P.	"	0.015
Potassium chloride C. P.	"	0.007
Calcium chloride C. P.	"	0.007
Magnesium chloride C. P.	"	0.005
Water, distilled	milliliters	1000

As considerable quantity of this sewage was required to maintain an active sludge at all times, two feedings of fresh synthetic sewage per day were a minimum. As a convenience in handling the sewage, it was made up in concentrated form of sufficient volume to make 100 liters.



The handling was further facilitated by measuring out 20 ml. volumes of concentrated sewage into 25 ml. screw-cap bottles which were then sterilized in the autoclave for 20 minutes at 15 pounds pressure. Upon sterilization, the sewage was placed in the refrigerator, and kept there until used.

#### APPENDIX F

##### (13) Preparation of Dilution Water for Elutriation of Sludge and for Endogenous Respiration:

Disodium hydrogen phosphate	C. P.	gram	0.05
Sodium chloride	C. P.	"	0.015
Potassium chloride	C. P.	"	0.007
Calcium chloride	C. P.	"	0.007
Magnesium chloride	C. P.	"	0.005
Water distilled		milliliters	1000

It was found convenient to make up a concentrated solution of dilution water in such strength that a twenty milliliter volume placed in a screw cap bottle could be diluted to make a two liter volume of dilution water for elutriation purpose and as a medium for endogenous rate.

## APPENDIX G

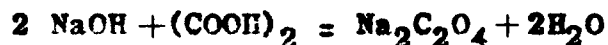
Preparation of Reagents Used in Making Carbon  
Dioxide Determinations.

(14) Oxalic Acid Solution: 1 N

Sixty-three grams of hydrous oxalic acid  $(\text{COOH})_2$  were dissolved in distilled water and made up to one liter.

Oxalic Acid Standardization:

The reaction involved in the titration of oxalic acid is:



Twenty milliliters of the above oxalic acid solution were diluted to fifty milliliters with distilled water and placed in an evaporating dish. Two drops of phenolphthalein indicator were added. The mixture was stirred and standard 1N NaOH solution was run in from a burette until the colorless solution turned slightly pink. The burette readings were recorded. The pink color in the solution was discharged with standard HCl and a few drops added in excess. The solution was boiled for three minutes, but no color reappeared. Another similar sample was carefully titrated with the NaOH solution until a faint pink color appeared. The volume of NaOH

used was recorded and calculation of oxalic acid normality was made as per:

$$\text{Volume} \times \text{Normality} = \text{Volume} \times \text{Normality}$$

(15) Barium Hydroxide Solution: 0.1 N

Eight and fifty-seven hundredths grams of anhydrous barium hydroxide were dissolved in distilled water and made up to a liter. This was standardized against the above oxalic acid solution.

#### APPENDIX H

##### Preparation of Manometer Fluid and Asbestos Emulsion:

(16) Brodie's Manometer Solution:

Twenty-three grams of NaCl

Five grams sodium choleate (MERCK)

The above were dissolved in 500 ml. of distilled water

Evan Blue (100 mg/500 ml.) was the dye used.

(17) Asbestos Emulsion for Sludge Nucleus:

5 grams of well separated asbestos fibres in a liter of distilled water.

## APPENDIX I

Equation for the Curve of Best Fit by the Method of Least Squares (14)

Where:

X = Observed suspended solids in p.p.m.

Y = Observed CO<sub>2</sub> in p.p.m.

n = Number of observations

m = Slope of line

b = The "y" intercept of the line

$$\text{First Normal Equation: } \sum Y = m \sum X + nb \quad (1)$$

$$\text{Second Normal Equation: } \sum XY = m \sum X^2 + b \sum X \quad (2)$$

by substituting values:

$$3,523 = 14,240m + 20 b \quad (1)$$

$$2,872,750 = 12,342,400m + 14,240 b \quad (2)$$

Solving simultaneously:

$$155m = 25.9$$

$$m = 0.16$$

$$\text{Whence } b = 57.12$$

∴ Equation of Curve of Best Fit

$$Y = 0.16X + 57.12$$

## APPENDIX J

Calculation of Coefficient of Statistical Correlation  
Between 5 Day B.O.D. and Carbon Dioxide (Pearson)

Where:

$r$  = Coefficient of correlation

$x$  = Observed B.O.D. in p.p.m.

$Y$  = Observed  $CO_2$  in p.p.m.

$n$  = Number of observations

$$r = \frac{\sum xY - \sum x \sum Y/n}{\sqrt{\left(\sum x^2 - \frac{(\sum x)^2}{n}\right) \left(\sum Y^2 - \frac{(\sum Y)^2}{n}\right)}}$$

$$r = \frac{364,374}{397,600} = 0.918$$

Since by definition, this value is greater than 0.7 there exists a strong Statistical Correlation.

## APPENDIX K

Statistical Limits of Confidence

$(\sigma_r)^2$  = Standard deviation

r = Coefficient of correlation

$$(\sigma_r)^2 = \left(1 - r^2\right) \left(\frac{\sum (Y)^2}{n} - \frac{(\sum Y)^2/n}{2}\right)$$

$$= (1 - .842) \times 3985$$

$$\sigma_r = \sqrt{626}$$

$$= 25$$

For 90° confidence and 18° freedom

"t" (statistical tables) = 1.734

∴ Confidence limit = 25 x 1.734

= 43.4 p.p.m.