

THE EFFECT OF AGITATION ON THE BIOCHEMICAL  
OXYGEN DEMAND OF SEWAGE

by

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## I. INTRODUCTION

The treatment of sewage and industrial wastes is accomplished by three basic processes: the separation of solids from liquids; the biological decomposition of liquids under aerobic conditions; and the biological decomposition of solids under anaerobic conditions. The end result of the latter two processes is the reduction of organic matter to a more stable form.

Today the standard method for measuring the reduction of organic matter is the 5-day, 20°C., biochemical oxygen demand (B.O.D.) test. The biochemical oxygen demand is determined by diluting the waste with a water whose own B.O.D. has been satisfied, holding the samples at a constant temperature of 20°C. for a period of 5 days, and measuring the difference between dissolved oxygen content at the beginning and the end of the test. A glass-stoppered bottle is filled with the diluted sample and placed in a constant temperature apparatus to incubate under quiescent conditions. This method is used to determine the amount of oxygen required to stabilize organic matter in sewage, industrial wastes, and polluted streams.

The 5-day B.O.D. test has many shortcomings including possibly the quiescent conditions under which the sample is maintained. If agitation has an effect upon B.O.D., the B.O.D. test as a measure of actual conditions in the stream would be in error.

Work done at the Institute of Engineering Research, Berkeley, California during the past few years has indicated that the B.O.D.

of sewage can be measured by the manometric technique employing the Warburg apparatus.

In the manometric technique, a volume of sample is placed in a relatively large flask that is closed to the outside atmosphere. The entire system is continuously agitated and oxygen is supplied to the sample by absorption from the flask atmosphere.

In recent years, many investigators have employed the manometric technique to measure the oxygen uptake of sewage and activated sludge. In the majority of cases, B.O.D. values of sewage and activated sludge were found to be higher when the samples were being agitated than when they were subjected to quiescent conditions. In general, investigators have attached no significance to agitation. Two researchers, however, have taken exception to this point of view. One believes that agitation increases the rate of gas transfer between the gas and liquid phase and in that manner prevents the oxygen supply from becoming a limiting factor, while the other believes that the conditions of oxygen saturation and gentle motion caused by agitation of the sample undoubtedly favors higher reaction rates.

Although the Warburg apparatus and the standard bottle test utilize different principles in the measurement of B.O.D., it is believed that the effect of agitation may be found by a comparison of the two methods if all other variables are held constant.

## II REVIEW OF LITERATURE

The biochemical oxygen demand (B.O.D.) may be defined as the milligrams of oxygen per liter water (parts per million) required for the stabilization of organic matter in water by aerobic bacterial action.<sup>(1)</sup> This demand is determined entirely by the availability of material as a biological food and the amount of oxygen utilized by the microorganisms during oxidation.<sup>(2)</sup>

The biochemical oxygen demand bottle test is undoubtedly the most valuable criterion available in the sanitary field for measuring the concentration of organic material in water.<sup>(3)</sup> Essentially the test consists of the determination of the dissolved oxygen concentration present in each of two duplicate samples, the first determination being made at the beginning and the second at the end of the prescribed incubation period. The difference in the dissolved oxygen concentration of these two samples is the biochemical oxygen demand for the temperature and incubation period employed.<sup>(4)</sup>

The B.O.D. bottle test has been widely used in the determination of allowable organic waste loadings on streams.<sup>(5)</sup> There has been considerable doubt, however, as to the validity of applying data obtained from a test in which the oxygen consumption takes place under quiescent conditions to the solution of such loading problems for turbulent streams.

The Warburg instrument is enjoying current popularity in the sewage and industrial waste field as a manometric technique for determining the biochemical oxygen demand.<sup>(6) (7) (8) (9) (10)</sup> The fundamental principle

involved in the operation of the instrument is that at constant temperature and constant gas volume any changes in the amount of a gas can be measured by the changes in its pressure.<sup>(11)</sup>

Several investigators have attempted to explain, through research, the effect of agitation on oxygen uptake.

Gellman and Heukelekian<sup>(9)</sup>, using the Warburg instrument, found that the difference in the oxygen demand of shaken and quiescent sewage was increased where strong sewage samples were used. When a weak sewage was used, agitation caused no increase in the measured oxidation rate. Their conclusions were that agitation itself did not increase the rate of metabolism, but only the rate of gas transfer between the gas and liquid phase and in this manner prevented the oxygen supply from becoming a limiting factor.

Dawson and Jenkins<sup>(10)</sup>, employing the Warburg instrument in their experimental work on activated sludge, showed that oxygen content, within broad limits, was not a limiting factor. The investigators used 4 ml of activated sludge in each of six Warburg flasks and .4 ml of potassium hydroxide was used as an absorbent. Three of the flasks were filled with oxygen and three with air. All six flasks were shaken. The average of three determinations of oxygen uptake in 5 hours at 28°C. was as follows:

<u>Atmosphere</u>	<u>Manometers Reading</u>
Pure oxygen	33.6
Air	32.4.

Since the concentration of a gas is proportional to its partial pressure, the amount of oxygen absorbed by the sample from the pure

oxygen atmosphere would be much greater than the amount of oxygen absorbed from the atmosphere of air; however, respiration rates of the organisms in both types of atmosphere were almost the same as shown by the manometer readings.

Further evidence has been given that oxygen content is not a limiting factor in the respiration rate of organisms. Rich<sup>(11)</sup> found by a series of tests on the respiration rates of Zoogloea ramigera that the rate is probably independent of the oxygen tension of their suspension for oxygen concentrations down to 1.5 ppm and possibly even lower. Zobell<sup>(12)</sup> (13) reported the same conclusion for oxygen consumption in sea water and in working with lake water bacteria found that the oxygen uptake was independent of the oxygen tension and although the oxygen tension might influence growth, it did not influence the respiration rate. Schalyer<sup>(14)</sup> states that experiments carried by O. Warburg, et al, show that with the exception of Bacterium azobacter, the respiration rates of most bacteria are largely independent of the oxygen content of the medium.

Caldwell and Langelier<sup>(6)</sup> in their investigation of manometric measurement of the B.O.D. of sewage found that the 3-day, 25°C. values of B.O.D. were in general greater than the 5-day values at 20°C. obtained by the standard dilution method. From this work, the investigators stated that apparently the velocity of reaction as it occurred in the manometric method was more than double the standard rate, even allowing for temperature differences. In this work, the researchers used a higher concentration in the Warburg flasks than they did in the bottle

tests. When comparable dilutions were used in both methods the results agreed reasonably well. The investigators in their discussion of the velocity of carbonaceous oxidation stated that it seemed reasonable to suppose factors tending to promote optimum conditions for effective collisions between active surfaces should favor higher reaction rates. Concentration was one such factor and in the manometric method the conditions of continuous saturation and gentle motion were undoubtedly also favorable to higher reaction rates.

### III. OBJECT OF THE INVESTIGATION

The purpose of this investigation was to determine the effect of agitation upon the biochemical oxygen demand of sewage. The effect was to be measured by making a statistical analysis of experimental data obtained by the B.O.D. test, a test in which quiescent conditions prevail, and by the Warburg test in which the sewage is agitated continuously.

#### IV. THE INVESTIGATION

The investigation was divided into four general parts: (a) preliminary work; (b) determination of the biochemical oxygen demand by the B.O.D. bottle test; (c) determination of the biochemical oxygen demand employing a manometric technique; and (d) comparison of the two methods by the application of statistics.

##### Preliminary Work

In order that an evaluation of the effect of agitation upon the B.O.D. of sewage could be made, it was necessary to control certain variables. These variables were temperature, composition of the sewage, dilutions, and type and number of microorganisms present for the stabilization of organic matter. Temperature variation proved to be no problem since the temperature of the water bath of the Warburg apparatus and the temperature of the incubator for the bottle test could be easily controlled at 25°C. Although 20°C. is generally used, the investigator found it too difficult to keep the temperature of the Warburg instrument as low as 20°C. Average domestic sewage is not constant enough in its composition for experimental work and, from the start, was ruled out as a test sewage. It was decided that a synthetic sewage of the type used by Butterfield and Wattie<sup>(12)</sup> should be tried. The results of several experimental runs showed that the synthetic sewage had a 5-day B.O.D. of approximately 250 parts per million. (See Appendix A for composition). Since previous work had shown that high concentrations of sewage could be tested in the Warburg

apparatus, the bottle test controlled the dilution factor. A trial run indicated that a  $2\frac{1}{2}\%$  sewage was the strongest concentration that could be used. All tests were run on two dilutions, a 2% and a  $2\frac{1}{2}\%$  concentration of sewage.

Because the synthetic sewage was sterilized, it was necessary to introduce microorganisms in order that a B.O.D. could be exerted. Lipman<sup>(13)</sup> has shown that the micro-flora of soil include nearly all forms of bacteria necessary to stabilize organic matter. A known amount of dried, pulverized garden soil was suspended in a given amount of water for twenty-four hours prior to use and the supernatant was drawn off for use as a seed source. It was thought that this type of seed was relatively constant in both number and type of microorganisms present. (Refer to Appendix B for composition).

#### Determination of B.O.D. by the Bottle Test

##### General

The biochemical oxygen demand was determined by measuring the oxygen depletion during the stabilization of organic matter. This was accomplished by determining the dissolved oxygen content of five diluted samples without incubation and five series of five bottles each after incubation periods at 25°C. of one, two, three, four, and five days. The procedure was employed on both 2% and  $2\frac{1}{2}\%$  samples. Duplicate sets of five bottles each of diluting water were made up and dissolved oxygen determinations made initially and after five days to determine how much B.O.D. was exerted by the diluting water.

### Apparatus

The apparatus necessary for the B.O.D. determination was, for the main part, standard laboratory equipment. This included pipettes, a burette, various sizes of graduated cylinders, seventy standard 300 ml B.O.D. bottles, two 3000 ml Fernbach flasks, cylindrical battery jars, and rubber tubing. Erlenmeyer flasks with 250 ml volumes were used to hold the samples for titrations. The diluting water was contained in carboys of eighteen liter capacity and a siphon was used for removing the water. An air compressor with a connecting line and diffuser stones was used for saturating the diluting water with oxygen and for aerating the distilled water needed in the standardization procedure of the sodium-thiosulfate. (See Appendix H). An incubator with a controlled temperature of 25°C. was used for the incubation of the samples.

### Preparation and Dilution of Samples

The synthetic sewage was previously prepared in a concentrated form. Five ml of concentrated sewage were diluted to a liter with diluting water. (Refer to Appendix H for composition of standard diluting water). The resulting solution gave a sewage comparable to that of average domestic sewage and exerted a 5-day, 25°C. B.O.D. of approximately 250 ppm. All dilutions were made up in 2500 ml quantities in Fernbach flasks and transferred by siphoning into a large cylindrical battery jar. The 2% dilution required 50 ml of sewage, diluted to 2500 ml with diluting water. The sewage was then seeded on the basis of 1 ml of soil seed suspension per 100 ml of diluted sewage sample.

The  $2\frac{1}{2}\%$  dilution required 62.5 ml of sewage diluted to 2500 ml and was seeded in the same manner as the 2% dilution.

When ten liters of each of the dilutions had been made, they were transferred to clean, dry standard 300 ml B.O.D. bottles. After filling, by siphoning, thirty bottles for each of the two dilutions, they were stoppered making sure that no air was entrained and that each one had a water seal. Ten bottles were filled with the diluting water in the same manner.

#### Oxygen Depletion

Dissolved oxygen content (D.O.) was determined immediately on 5 bottles of the 2% sample, 5 bottles of the  $2\frac{1}{2}\%$  sample, and 5 bottles of the diluting water. The remaining 65 bottles were placed in the incubator. Five bottles of the 2% and 5 bottles of the  $2\frac{1}{2}\%$  samples were removed each day for the following five days and their dissolved oxygen content determined by employing the Alsterberg modification of the Winkler method. (See Appendix C). At the end of the fifth day the D.O. contents of the remaining 5 bottles of diluting water were found in a like manner. Corrections were made for the loss of dissolved oxygen due to the diluting water and from the corrected D.O. contents obtained, the B.O.D.'s of the diluted samples for all five days were calculated. (See Appendix D for sample data sheet of B.O.D. calculations.).

## Determination of B.O.D. by the Manometric Technique

### Description of Apparatus

The basic parts of a Warburg test unit are the reaction flask and corrected manometer. These are mounted on a supporting mechanism. The flasks are immersed in a constant temperature water bath and their contents agitated continuously.

The reaction flasks are of the Warburg-type with a volume of approximately 20 milliliters. The manometer is essentially an U-tube, 30 cm. in height, with a 1 mm. bore, and an adjustable fluid reservoir. The male ground glass joint attached to one leg is fashioned to fit the female joint of the reaction flask. This leg is closed to the atmosphere during tests. The other leg of the manometer is always open to the atmosphere. (Figure 1).

The supporting mechanism consists of a detachable manometer support, a shaking mechanism and a constant water bath. The detachable supports serve as a rigid fixture for the manometers, a linkage between shaking mechanism and manometer and has a screw adjustment for control of fluid reservoir. A shaking mechanism furnishes the agitation at a constant frequency; the frequency in this case being 80 oscillations per minute. The constant temperature water bath consists of a reservoir of water equipped with a stirring mechanism, thermoregulator, and a heating element controlled by the thermoregulator.

### Preparation of the Warburg Instrument

All manometers must be free of leaks and thoroughly cleaned prior to introduction of the manometer fluid. There are many methods used to

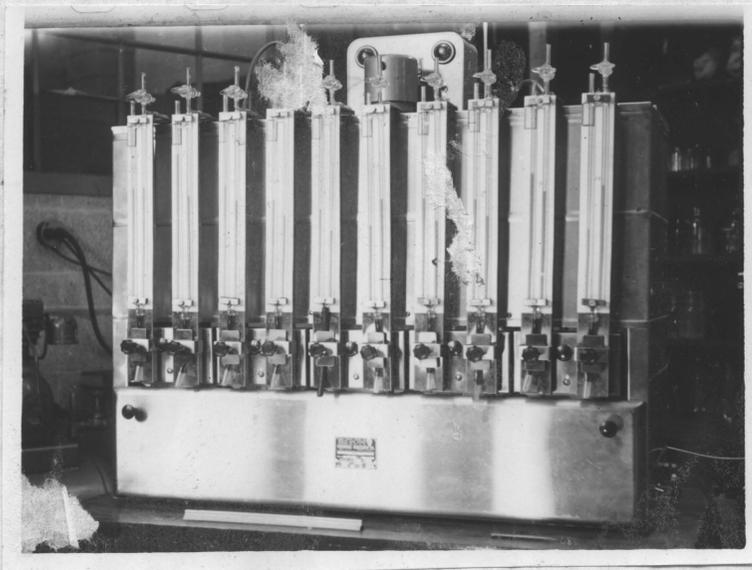


Plate 1

Warburg Apparatus for Manometric  
Measurement of B.O.D.

clean the manometers and this investigator found that boiling the manometers in distilled water with one of the better commercial detergents followed by rinsing with distilled water seven or eight times, and drying with ether and dry air was most successful. In any case, the manometer must be clean and free of moisture before the fluid is introduced.

The manometer fluid must contain a wetting agent, must be coherent to prevent stranding and must be resistant to decomposition. It should be colored so that it may be easily seen. The specific gravity of the fluid must be known. The fluid used in this work was "Brodies" solution. (See Appendix E).

To introduce manometer fluid, the fluid reservoir was filled and attached to the reservoir connection of the manometer. With the reservoir in place the manometer was mounted on the supporting mechanism. The fluid was then brought up into the barrels. This was done carefully in order to avoid entrapment of air at the junction of the reservoir and the reservoir connection. When the fluid was well up in each barrel, it was forced up to the 300 mm mark in both legs. The reservoir was released quickly and the "Brodies" solution allowed to fall to the index mark. If any manometer fluid remained stranded in the barrel, the manometers were not adequately cleaned.

Preventing leakage in the manometer stopcocks and all other glass to glass connections was essential. All joints were thoroughly cleaned with xylol or ether and wiped dry with a piece of lens paper or linen cloth. Caution was taken to ensure that no lint remained on the joint.

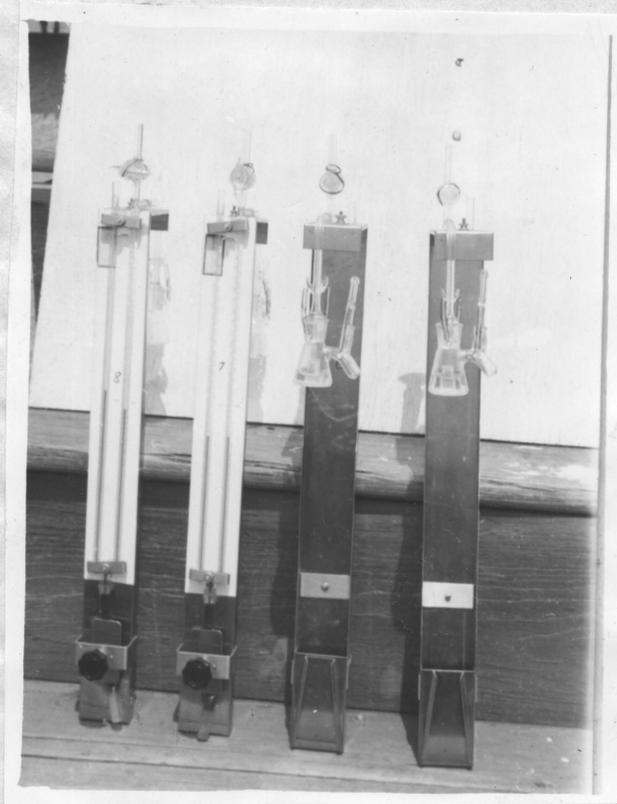


Plate 2

Warburg-Type Manometer  
with Attached Reaction  
Flask

Thin streaks of a good grade stopcock grease were applied to the stopcock, and the stopcock inserted in the female joint and a slight rotational twist exerted; all of the area was covered by the lubricant. In the case of the reaction flasks and all other joints, vaseline was used instead of stopcock grease.

The reaction flasks were cleaned in the same manner as the manometers.

#### Calibration of Reaction Flasks

The procedure used for the calibration of the reaction flasks and manometers was developed by Lazarow<sup>(14)</sup>. The method is described below.

The Warburg vessel, W, (Figure 1) was attached to the manometer. The vent, V, of the side arm was opened, and the vessel was placed in the water bath at 25°C. A short period was allowed for temperature equilibration. The calibrator, A, made by the Micro-Metric Instrument Company, Cleveland, Ohio, was filled with water and a glass adapter, B, with a male tapered joint, was inserted into the calibrator and held in place by means of the spring clips, D. The calibrator was placed in a vertical position, adapter end up, and all the air removed from the calibrator chamber by advancing the plunger, P. A small segment of amber gum rubber tubing, G, was placed over the end of the adapter. The vertical part, H, above the manometer stopcock was moistened with water and attached to the adapter by inserting it into the rubber tube, G. The calibrator was clamped to the manometer.

The plunger, P, of the calibrator was advanced until the capillary tube of H and the stopcock, S, were just filled with water. This was

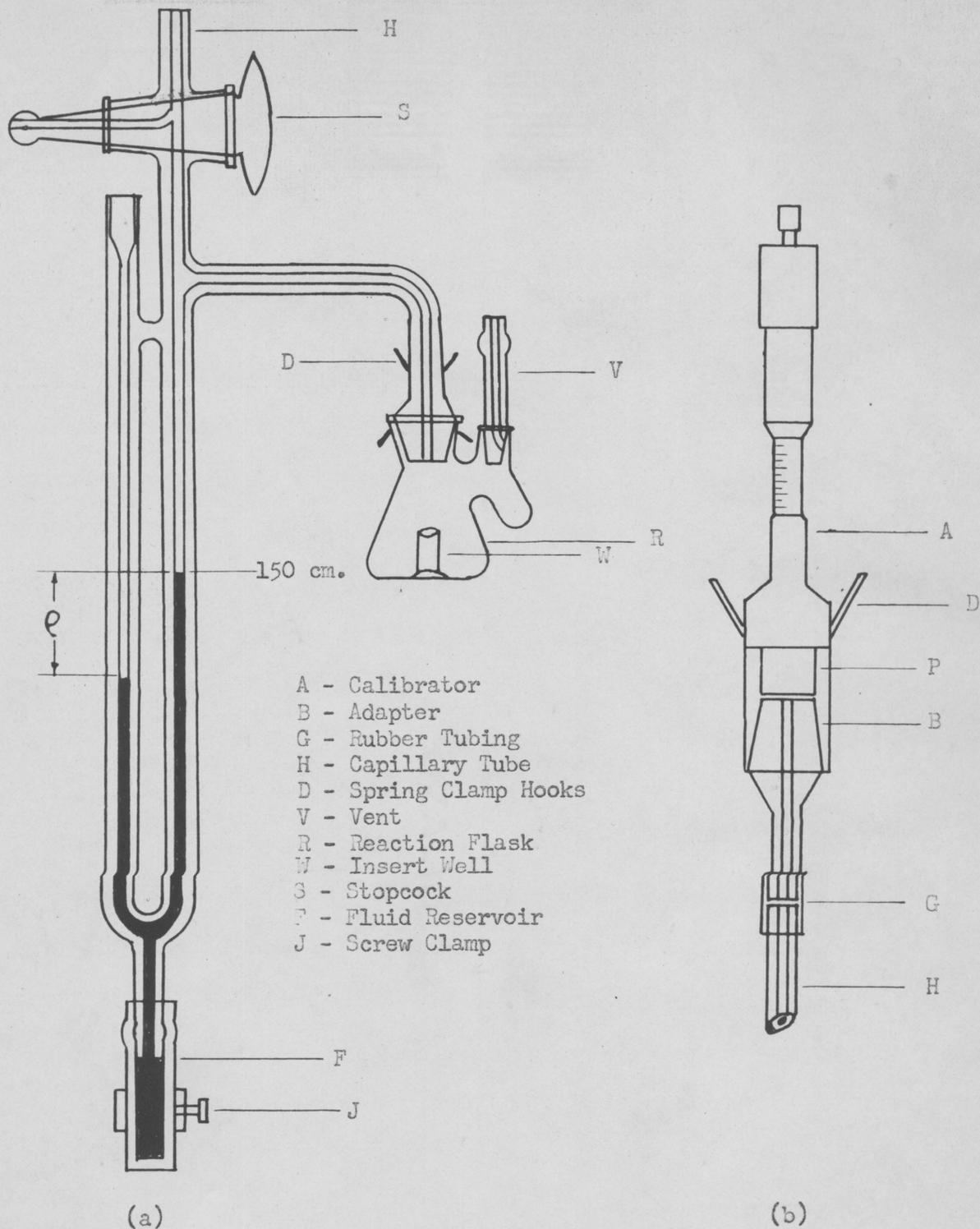


Figure 1. Warburg Manometer, Reaction Flask, and Calibrator

dead space and was not part of the volume used in the calculation. The "Brodies" solution in the manometer was adjusted to the 150 mm mark by adjusting the screw clamp on the fluid reservoir at the bottom of the manometer and the manometer vent, V, closed. Both arms should then read 150 millimeters. If the level of liquid was not exactly equal on both sides, it was adjusted by means of the calibrator. After the initial reading of the calibrator was recorded, sufficient gas was removed to make the difference in pressure between the right and the left limbs of the manometer equal to the value of  $p$  as calculated from the equation  $p = .129 (P_B - P_W)$ .  $P_B$  was the barometric pressure in millimeters of mercury with the temperature correction for the barometric pressure subtracted.  $P_W$  was the vapor pressure of water in millimeters of mercury at the temperature used. When the level of "Brodies" solution in the right limb of the manometer was maintained at the 150 mm mark and the level in the left limb reads 150 minus  $p$ , the final reading of the calibrator was recorded.

Since the diameter of the calibrator plunger has a cross-sectional area of 1 square centimeter, each millimeter advancement of the plunger corresponds to a volume displacement of 0.1 ml. Because the volume of the vessel plus manometer is 100 times the gas volume removed, each millimeter advancement of the plunger corresponds to 10 milliliters.

#### Performing the B.O.D. Test

The samples for this test were made up in the same manner as for the bottle test. The 2% sample contained 2 ml of sewage, the 2½% sample,

2½ ml of sewage. Both samples were diluted to 100 ml with diluting water and each was seeded with 1 ml of seed.

After thoroughly mixing the sample, a 4 ml volume was withdrawn and placed in the reaction flask. The pipette was wiped with Kleenex during the transfer, so that drops adhering to the outside of the pipette would not fall into the insert. The same precaution was followed in the transfer of the gas absorbent.

Before connecting the flasks, the manometers were checked for air bubbles in the liquid, poor seating of the stopcock, and cleanliness of the joint. The stopcock was open and the manometer fluid brought up to the index mark. This investigator used the 150 mm as the index.

Thin streaks of vaseline were placed at three points about the manometer joint and the mouth of the reaction flask brought up over the joint. A few rotating motions of the flask spread the vaseline evenly about the connection and made it air tight. After joining, rust-proof spring clamps were stretched between the specially provided tips and held the flask firmly in place. Each flask was attached to the manometer with which it was calibrated.

Since one leg of the manometer is open to the atmosphere, the entire system is affected by changes in atmospheric pressure, and, therefore, two of the flask-manometers were employed as barometric controls or thermobarometers.

Both thermobarometers were set up in identical fashion with the previously described test set-up, except that 4 ml of seeded sample were replaced by 4 ml of the standard diluting water.

Next the water bath was filled to a point 1 inch from the top, marked, and maintained at this level throughout the test.

The manometer and flasks were attached to the shaking device and the flasks suspended in the water bath. The stopcock was left open and a period of from 45 to 60 minutes allowed to obtain thermal equilibrium.

Following the equilibration period, the manometer fluid was adjusted to the index point and the stopcock closed. A rubber band stretched about the stopcock eliminated any chance of its opening during the shaking. At the time of closing a reading was taken of the open leg.

A range of B.O.D. values corresponding to the 5-day bottle test was made by recording the open leg readings at 0, 24, 48, 72, 96, and 120 hours. (See Appendix F for typical arrangement of B.O.D. data). The manometer fluid in the flask leg of the manometer was always brought to the index at the instant the open leg reading was made.

After the final reading was taken, all manometer stopcocks were opened prior to removing the flasks from the water bath, to prevent loss of the manometer fluid.

#### Calculation of B.O.D.

The calculation of B.O.D. required finding the difference between the open leg readings of the sample manometers and the open leg readings of the thermobarometers by subtraction and multiplying the result by the flask constant. (Refer to Appendix G for calculation of the flask constant).

### Analysis by Statistical Methods

The B.O.D. values obtained by both methods for the fourth and fifth days were analyzed statistically comparing the same dilutions of the Warburg test with the bottle test. The overlapping test as described by Velz<sup>(16)</sup> was used. (See Appendix I for procedure). Confidence limits of 99.73% were established. Since the simple overlapping test was unduly severe, a less severe test for statistical significance between the two means was used employing 68% confidence limits. (See Appendix I).

In order to evaluate the effect of dilution, the 2% and the 2½% dilutions of both the bottle and the Warburg were compared using the simple overlapping test.

### Data and Results

The data obtained from the investigation of the B.O.D. values of the synthetic sewage were tabulated and are presented in Tables 1 through 6. The simple overlapping test was applied to the four and five-day B.O.D. values to determine whether or not a significant difference existed between the means of the values obtained by the Warburg and bottle tests. The results of the statistical test are illustrated graphically in Figures 2 through 5. The overlapping test was also applied to the five-day B.O.D. values of the two different dilutions of the same test, one for the bottle test and one for the Warburg. These results are presented graphically in Figures 6 and 7. The less severe test of statistical significance between the two means was applied to the four and five-day B.O.D. values and the results are presented in Table 7.

Figure 2 shows the 5-day B.O.D. values of the 2% dilution of both the Warburg and bottle tests. Using 99% confidence limits, the spread each side of the means produces an overlap and indicates no significant difference between the values found in the two types of tests. When the test was applied to the 5-day, 2½% dilution values, (Figure 3) the 4-day, 2% dilution values, (Figure 4) and the 4-day, 2½% dilution values, (Figure 5), no overlap occurred indicating there was a significant difference between the two sets of values.

Figures 6 and 7 indicate by the overlapping test that there is no significant difference between the two dilutions in either of the tests.

Table 7 shows the results of a less severe test than those shown in Figures 2, 3, 4, and 5. The confidence limits in this test were lowered to 68% and in all cases a significant difference developed between the two sets of values.

Table 1

Results of B.O.D. Determinations  
Expressed in ppm for Test 1

Hrs.	Bottle Test		Warburg Test	
	2% Dilution	2½% Dilution	2% Dilution	2½% Dilution
24	112 108 114 110 95	114 103 108 107 116	129 97 110 75	89 101 91 100
48	148 148 165 173 156	171 164 150 157 170	142 136 131 133	183 165 140 141
72	189 186 180 183 180	197 192 202 200 196	199 171 198 180	224 214 186 182
96	203 198 198 183 199	204 207 223 207 221	236 220 233 217	234 234 221 217
120	236 238 230 230 240	234 241 251 227 239	258 256 264 257	254 265 256 253

Table 2

Results of B.O.D. Determinations  
Expressed in ppm for Test 2

Hrs.	Bottle Test		Warburg Test	
	2% Dilution	2½% Dilution	2% Dilution	2½% Dilution
24	110 93 100 91 99	92 109 98 118 106	84 80 81 80	94 122 90
48	136 167 154 157 157	143 147 140 137 143	161 139 152 146	130 157 129 141
72	181 185 185 187 185	184 198 189 192 194	182 183 176 181	180 207 183 174
96	208 203 208 213 210	206 202 206 205 208	212 220 217 212	222 220 223 225
120	251 246 248 248 254	252 259 253 251 253	248 241 253 241	264 255 258 266

Table 3

Results of B.O.D. Determinations  
Expressed in ppm for Test 3

Hrs.	Bottle Test		Warburg Test	
	2% Dilution	2½% Dilution	2% Dilution	2½% Dilution
24	103 94 108 102 98	114 108 101 116 99	73 98	98 99
48	160 149 171 153 153	153 153 148 151 157	155 129 122 117	138 120 112 124
72	189 183 190 187 183	201 193 196 201 200	183 181 173 180	180 200 194 186
96	219 213 202 204 215	238 214 224 223 237	217 221 212 219	220 223 216 237
120	248 259 259 282 265	251 235 233 269 251	250 258 254 252	266 250 248 256

Table 4

Results of B.O.D. Determinations  
Expressed in ppm for Test 4

Hrs.	Bottle Test		Warburg Test	
	2% Dilution	2½% Dilution	2% Dilution	2½% Dilution
24	92 97 97 101 117	115 105 93 95 93	117 83	112
48	158 158 170 158 139	154 154 142 142 130	155 129 122 117	138 120 112 124
72	168 198 178 178 183	182 214 186 195 208	183 181 173 180	180 200 194 186
96	219 213 202 204 215	238 214 224 223 237	228 220 218 230	234 227 221 225
120	242 260 242 230 256	259 268 242 230 237	242 263 251 246	259 253 248 257

Table 5

Results of B.O.D. Determinations  
Expressed in ppm for Test 5

Hrs.	Bottle Test		Warburg Test	
	2% Dilution	2½% Dilution	2% Dilution	2½% Dilution
24	125 111 111 106 118	110 104 104 130 127	71 89	88 50
48	141 143 165 144 156	143 162 147 173 162	169 133 140 187	186 154 172 156
72	215 212 208 198 185	201 186 199 210 186	201 176 189 192	217 197 183 206
96	222 220 226 220 220	194 207 216 205 230	222 219 225 229	238 219 224 230
120	260 245 242 262 241	252 242 259 244 240	260 253 248 266	259 246 588 267

Table 6

Results of B.O.D. Determinations  
Expressed in ppm for Test 6

Hrs.	Bottle Test		Warburg Test	
	2% Dilution	2½% Dilution	2% Dilution	2½% Dilution
24	100 106 99 99 108	92 102 116 114 106	55	122 81 88
48	136 153 140 139 135	162 150 150 164 172	142 122 128 122	120 125 111 119
72	206 194 200 210 210	203 189 192 207 196	183 219 169 179	211 218 185 217
96	198 211 216 210 216	220 215 205 212 222	238 213 207 221	240 224 227 202
120	256 260 252 252 252	260 241 237 242 243	269 250 264 243	259 263 242 271

Time and Dilution Factor	Type of Test	Mean $\bar{x}$ ppm	Std. Dev.	Std. Error S.E. Mean	No. of obs. n	Confidence Level	Std. Error S.E. Diff.	Diff. of Means	Test Results
	Warburg	254	7.95	1.62	24				
5-Day B.O.D. Values 2½% Dilution	Bottle	246	10.60	1.93	30	68%	2.38	11	11-(1x2.38)=+8.62 Significant Diff.
	Warburg	257	6.84	1.39	24				
4-Day B.O.D. Values 2% Dilution	Bottle	221	7.75	1.41	30	68%	2.94	13	13-(1x2.94)=+10.06 Significant Diff.
	Warburg	208	9.80	1.99	24				
4-Day B.O.D. Values 2½% Dilution	Bottle	212	12.23	2.23	30	68%	2.79	13	13-(1x2.79)=+10.21 Significant Diff.
	Warburg	225	8.24	1.68	24				

Table 7

Significance of Difference Between Bottle and Warburg  
B.O.D. Values as Determined by the Less Severe  
Overlapping Test

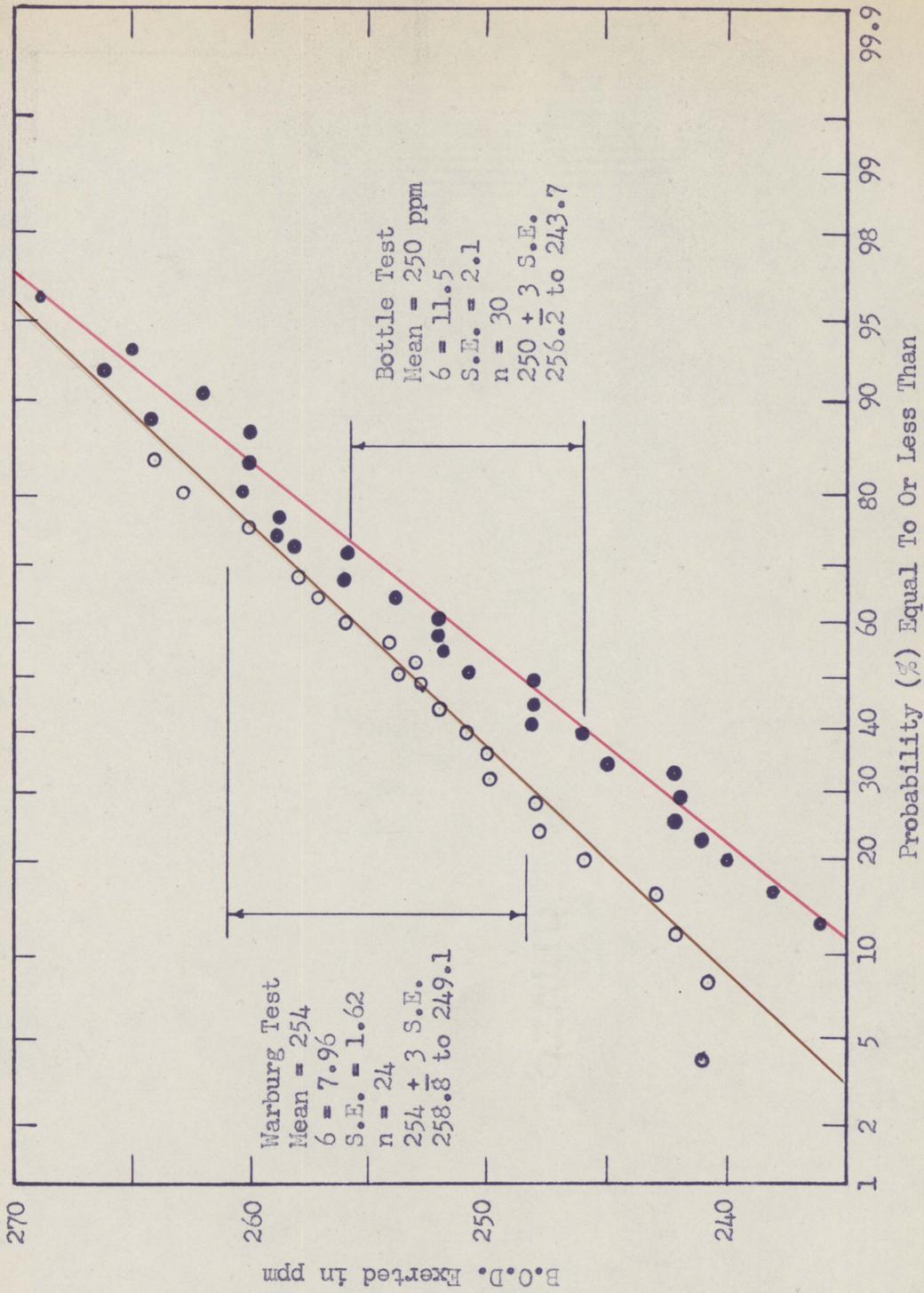


Figure 2

Probability Distribution of 5-Day B.O.D. Values Employing 2% Dilution

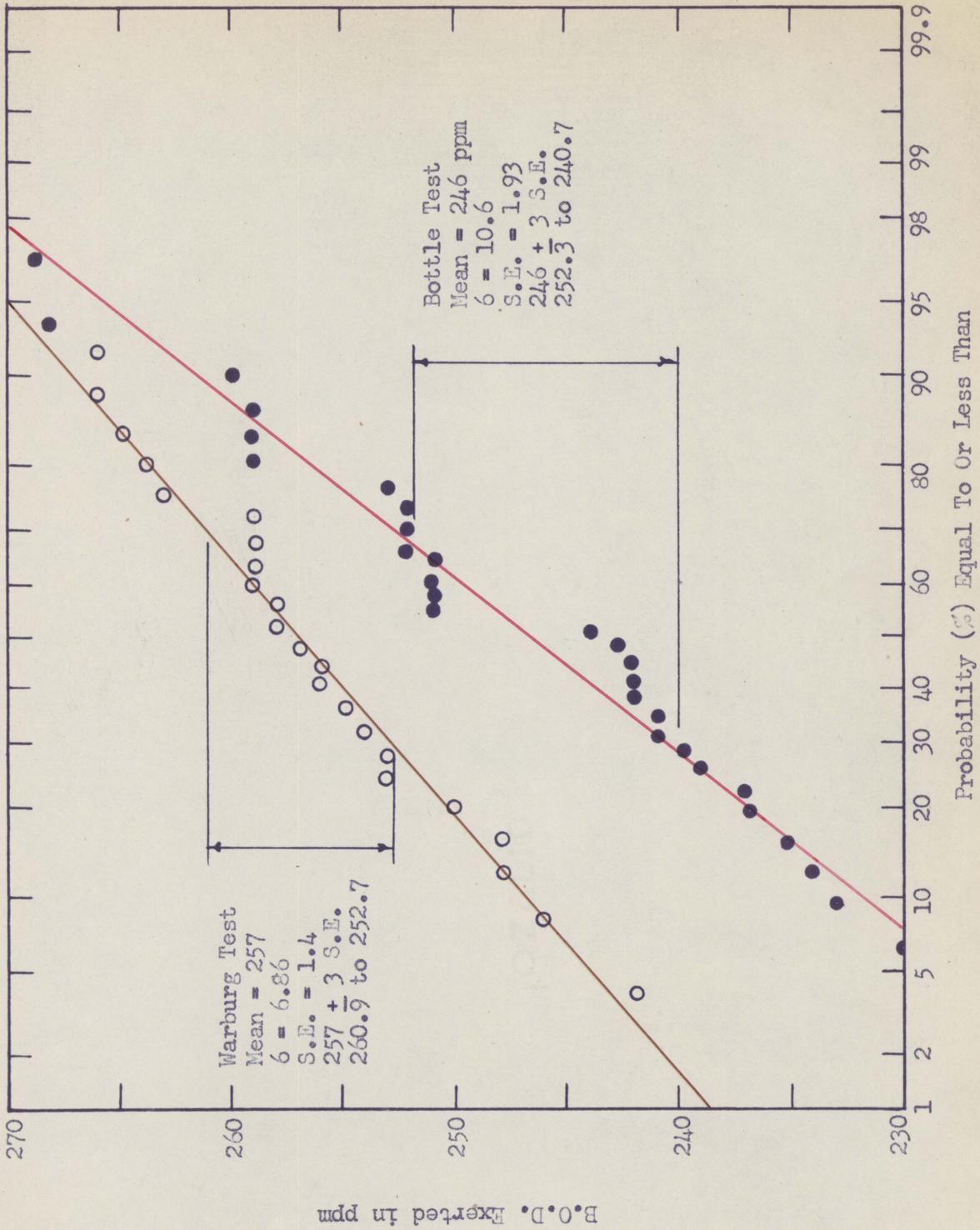


Figure 3

Probability Distribution of 5-Day B.O.D. Values Employing  $2\frac{1}{2}\%$  Dilution

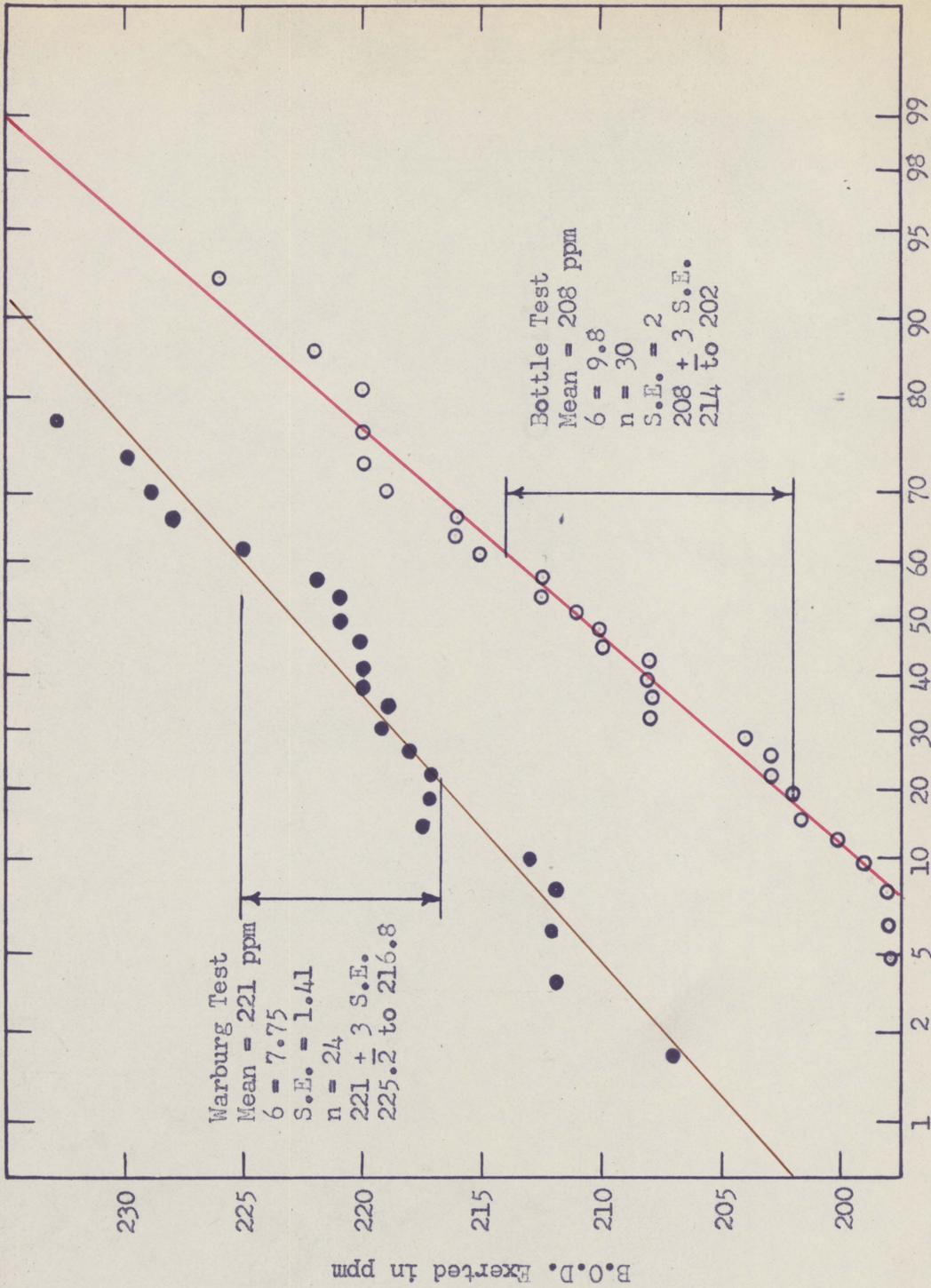


Figure 4

Probability Distribution of 4-Day B.O.D. Values Employing 2% Dilution

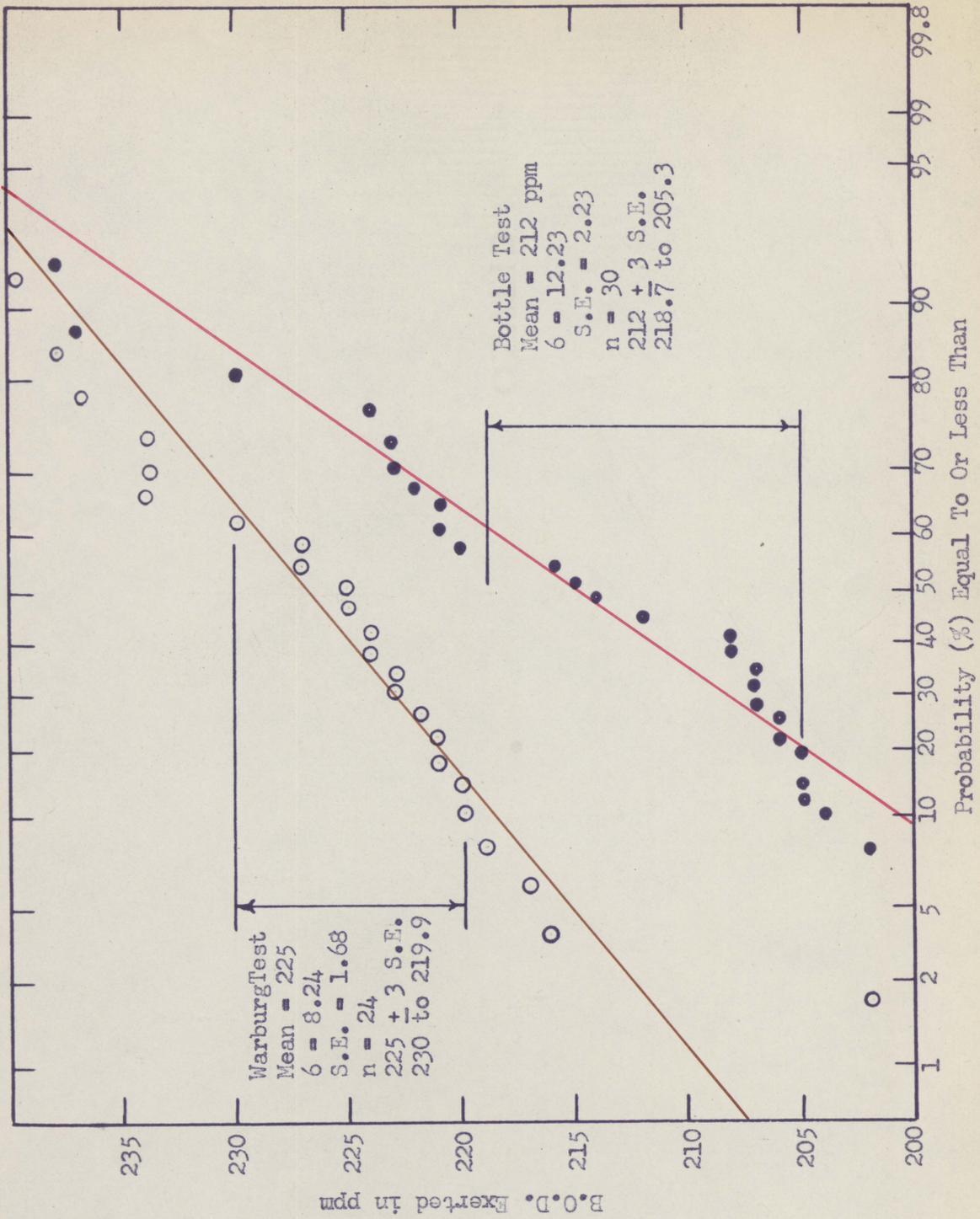


Figure 5

Probability Distribution of 4-Day B.O.D. Values Employing 2 1/2% Dilution

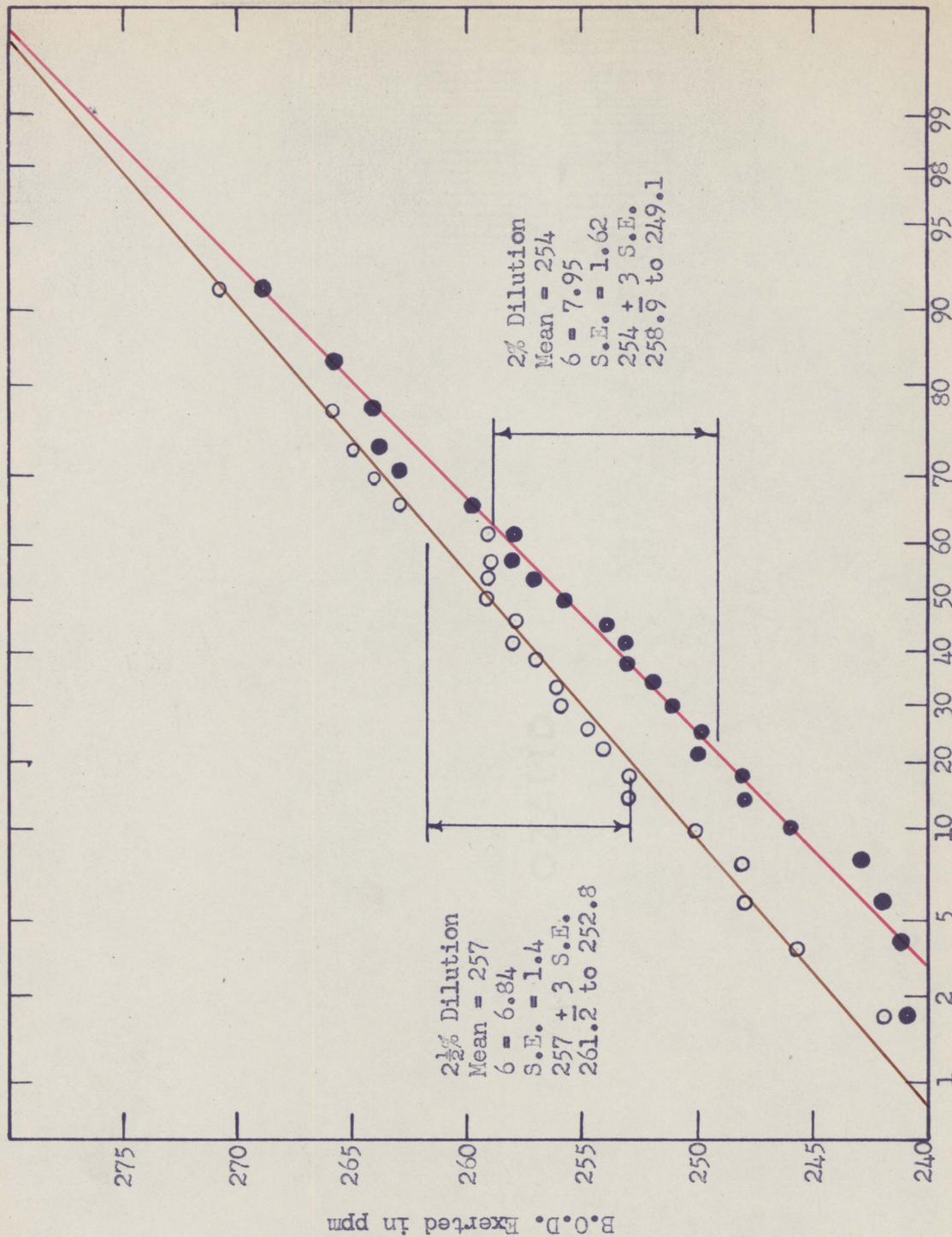


Figure 6  
 Probability Distribution of 5-Day B.O.D. Values Employing The Warburg Test

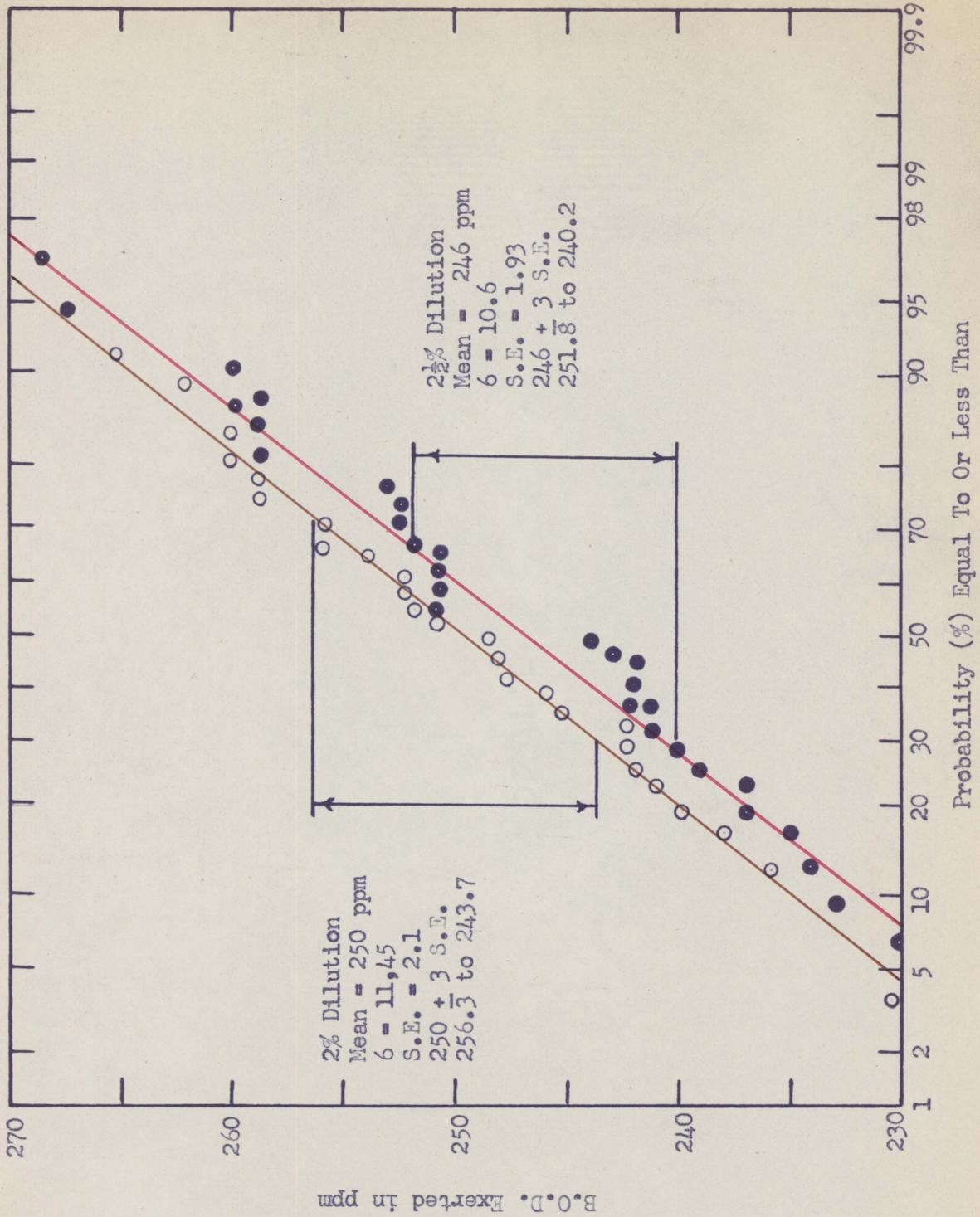


Figure 7

Probability Distribution of 5-Day B.O.D.  
Employing the Bottle Test

## V DISCUSSION OF RESULTS

The results of the analysis of data indicated in three of the four sets of values tested that there was a statistically significant difference between the B.O.D. values obtained by the bottle and Warburg tests. Using 99.73% confidence limits, no significant difference was found to exist between the 5-day, 2% dilution values obtained by the two tests. It was thought that perhaps the dilution factor may have had an effect upon the results, but an analysis using the overlapping test indicated no significant difference between the B.O.D. values of the two dilutions.

A less severe test was applied to the aforementioned set of values and at a 68% confidence level a statistically significant difference resulted in all four cases.

Since much evidence is available to show that oxygen content, within broad limits, did not affect the rate at which the microorganisms respired, the significance of the higher B.O.D. values as determined by the manometric technique would seem to indicate that agitation did increase the metabolism of the bacteria. The agitation may have accomplished this in one or two ways. Agitation may have caused the envelope of waste surrounding the bacteria to diffuse into the medium at a faster rate and consequently reduced the possibility of the waste inhibiting the bacterial respiration. The second possibility was that agitation caused the bacteria to be in continual contact with the food supply. This continuous food supply may have resulted in an increased oxygen uptake by the organisms present.

For two reasons, only the four and five-day B.O.D. values were analyzed: first, most routine sewage examinations report their B.O.D. values on a 5-day basis; and second, the one, two, and three-day values were somewhat erratic, especially those determined on the Warburg instrument. This was probably due to the small amount of original sample being tested. In the case of the Warburg instrument, it was 0.08 ml and 0.1 ml for the 2% and 2½% dilutions, respectively. It was also possible that the varying B.O.D. values may have been caused by one or any combination of the following: poor technique in titrating the samples for the bottle test and in reading the manometers for the Warburg test; failure to produce a sewage of constant strength; failure to get an unvarying soil seed; and improper aerating and aging of diluting water.

## VI CONCLUSIONS

The mean 4-day and 5-day B.O.D. values of the Warburg instrument were 4 to 13 ppm higher than those determined by the bottle test. The analysis showed that a statistically significant difference between the values determined by the two types of tests could be expected at least 68% of the time. On the basis of this evidence, the investigator felt that agitation has a tendency to affect the B.O.D. values of sewage.

Although the resulting B.O.D. values of the test were not consistent, the author believed the cause was faulty technique on his part rather than a weakness in the procedure followed during the investigation.

The ease with which B.O.D. values were found by the manometric method as compared to the bottle test was offset somewhat by the care and skill involved in cleaning and calibrating the flasks, the preliminary work involved, and in implementing the test.

The results of this work are by no means conclusive. Much experimentation along this line remains to be done. This investigator believed more accurate results could be obtained if higher concentrations of sewage could be used in the bottle test or under similar quiescent conditions.

VII SUMMARY

The purpose of this investigation was to determine what effect agitation had upon the biochemical oxygen demand of sewage. In order to determine the effect of agitation, the composition of the sewage, the seed source, the temperature and the dilutions were held constant.

The investigation consisted essentially of determining the biochemical oxygen demand of synthetic sewage by the standard bottle test method and by the manometric technique employing the Warburg instrument. The first of these methods gave B.O.D.'s exerted under quiescent conditions while the Warburg instrument yielded B.O.D. values of a sewage that had been continuously agitated while being incubated.

The data obtained were statistically analyzed by the overlapping test to determine if there was any statistically significant difference. The test was made for both ninety-nine and sixty-eight percent confidence levels.

The final results based on statistical methods of analysis showed that for ninety-nine percent confidence limits there was a significant difference in some cases. For sixty-eight percent confidence limits there was always a significant difference. These results indicate that agitation has some effect upon the biochemical oxygen demand of sewage.

VIII ACKNOWLEDGEMENTS

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XI APPENDICES

Appendix APreparation of Synthetic Sewage Developed by  
Butterfield and Wattie(12)

The composition is as follows:

Peptone, Difco, Bacto grade .....	.3 grams
Meat Extract, Liebigs .....	.2 grams
Urea, C.P. grade .....	.05 grams
Disodium hydrogen phosphate, C.P. ....	.05 grams
Sodium Chloride, C.P. ....	.015 grams
Potassium Chloride, C.P. ....	.007 grams
Calcium Chloride, C.P. ....	.007 grams
Magnesium Chloride, C.P. ....	.005 grams
Water, distilled .....	.20 ml.

The above named compounds were dissolved in the 20 ml of distilled water, poured into a test tube, and sterilized in an autoclave for 20 minutes and at 15 pounds pressure. The tube was then removed, sealed, and placed in the refrigerator until ready for use.

Before using this concentrated synthetic sewage, it was necessary to dilute to above quantity to 2000 ml with distilled water. The resulting sewage has a 5-day, 25°C. B.O.D. of approximately 250 ppm.

Appendix BPreparation of Soil Seed

Two or three hundred grams of fresh moist garden soil was collected, rolled into a ball and allowed to dry at room temperature. When the clump was thoroughly dry, it was pulverized in a mortar and pestle and sieved through a 200-mesh screen. The soil powder was placed in a beaker and put in a dessicator for further drying.

One hundred mg of soil was added to 100 ml of diluting water to make a soil seed solution. The solution was thoroughly shaken and allowed to stand twenty-four hours before using. The supernatant was then drawn off and used as the seed source.

Appendix CDissolved Oxygen by the Alsterberg Modification  
of the Winkler Method

To a full sample in a 300 ml bottle, the following was added from a pipette with the tip below the surface of the water: 2 ml of manganese sulfate solution, and 2 ml of alkaline-iodide solution containing sodium azide. The sample was then thoroughly shaken, allowed to settle halfway, then shaken again. With the tip of the pipette above the surface of the sample, 2 ml of concentrated  $H_2SO_4$  were added and the solution shaken thoroughly.

A corrected volume of 204 ml of this sample was transferred to a 250 ml Erlenmeyer flask.

The sample was titrated with the standard sodium thiosulfate solution using the starch indicator. The milliliters of thiosulfate used were recorded and the parts per million of dissolved oxygen present calculated.

Sample calculation for dissolved oxygen:

$$\begin{aligned} \text{D.O. (ppm)} &= (\text{ml thio. used}) (.025/N \text{ of thiosulfate}) \\ \text{D.O.} &= (6.32) (.025/.0245) \\ \text{D.O.} &= 6.19 \text{ ppm.} \end{aligned}$$

Appendix DTypical Arrangement of Data for B.O.D. Calculations

Date: 10 January 1953  
 Starting Time: 3:00 pm  
 Percentage Dilution: 2%

Seed Source: Soil Seed 1 ml.100 ml  
 Initial D.O. of Diluting Water: 7.60  
 Final D.O. of Diluting Water: 7.45

(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	7.55	7.50	---	---	---	---
0	7.60	7.56	---	---	---	---
120	2.55	2.50	5.00	.15	4.85	243
120	2.60	2.56	5.00	.15	4.85	243

Column (1) = hours the sample had been incubated

Column (2) = mls of thiosulfate used

Column (3) = dissolved oxygen in ppm

Column (4) = depletion in D.O.

Column (5) = depletion of D.O. corrected for the depletion attributed to diluting water

Column (6) = B.O.D. of the diluted sample

Column (7) = B.O.D. of the original sewage sample before dilution.

Appendix EComposition of "Brodies" Solution for Manometers

The composition of "Brodies" solution is as follows:

Sodium Chloride, C.P. ....	23 grams
Sodium Choleate .....	5 grams
Evan Blue .....	0.1 grams
Thymol .....	trace
Distilled Water .....	500 ml

The sodium choleate and the Evan blue were dissolved in a few ml of water and added to 400 ml of water containing the sodium chloride. This was mixed thoroughly, filtered and made up to 500 ml with water. A crystal of thymol was added to prevent mold growths. The specific gravity of the solution was determined and it was kept in a cool place, tightly stoppered to prevent evaporation.

Appendix FTypical Arrangement of B.O.D. Data for the Warburg Instrument

Test Number: 1  
 Date: 20 January 1953  
 Time of Start: 3:00 pm  
 Equilibration Period: 1 hr.  
 Shaking Rate: 80 rpm  
 Index: 150

Test Temperature: 25°C.  
 Gas Absorbent: .5 ml - 10% KOH  
 Seed Source: Soil 1 ml/100 ml  
 $V_f$ : 4.5 ml  
 $P_0$ : 1000

					Open leg manometric readings at "t" hrs					
(1)	(2)	(3)	(4)	(5)	0	24	48	72	96	120
2%	.08	18.95	14.45	200.10	15.00	15.40	17.90	15.25	13.80	11.95
Cntrl.	-	19.55	15.05	213.30	15.00	15.80	18.50	16.00	14.90	13.20
2½%	.1	20.03	15.53	221.05	15.00	15.25	18.10	15.15	13.85	11.99

Column (1) = Name of sample, whether 2% dilution, 2½% dilution, or barometric Control

Column (2) =  $V_s$ , volume of original sample being tested (ml)

Column (3) =  $V$ , total volume of gas before sample, seed, and gas absorbent were added

Column (4) =  $V_q$ , total volume of gas in flask, connection, and manometer to index

Column (5) =  $K$ , temperature-fluid (flask) constant.

Appendix GCalculation of Flask Constants(11)

The formula for calculating B.O.D. from the manometer deflections was derived by Langelier and Caldwell:

$$(a) w = Kh$$

where

- w = oxygen uptake in ppm
- h = observed manometric deflection after correction
- K = temperature-fluid constant.

The value of K may be taken as

$$(b) K = \frac{1430}{P_o V_s} \left[ \frac{273 V_g}{T} + V_f \alpha \right]$$

$V_g$  = total volume of gas in flask, correction, and manometer to the index point (in ml).

$V_s$  = volume of original sample being tested (in ml).

$V_f$  = total volume of fluid (sample plus added diluents and seed) undergoing testing in flask.

$\alpha$  = solubility of oxygen.

= .028 ml/ml at 25°C.

$P_o$  = normal pressure of manometer fluid (in cm).

=  $76 \times 13.6/\rho$  where  $\rho$  = specific gravity of manometer fluid.

T = temperature (in degrees absolute).

Employing "Brodies" solution for manometer fluid ( $\rho = 1.033$ ), and substituting value of K given by formula (b) in formula (a)

$$(c) w = h \left[ (1.31 V_g/V_s) + (.0403 V_f/V_s) \right]$$

Appendix HPreparation of Reagents

## (1) Manganous sulfate solution:

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

## (2) Alkaline-iodide sodium azide:

Seven hundred grams of KOH, 150 grams KI, and 10 grams of  $\text{NaN}_3$  were used per liter. These compounds were dissolved separately in small portions of distilled water. The solutions of potassium hydroxide and iodide were mixed, diluted 950 ml, and cooled to room temperature. The sodium azide solution was added slowly with constant stirring to avoid local heating. The resulting solution was then diluted to a liter.

## (3) Starch indicator:

To five grams of soluble starch in a mortar, a little distilled water was added and the starch ground to a paste. This was poured in a liter of boiling distilled water, stirred and allowed to cool. The solution was preserved with 1.25 grams of salicylic acid.

## (4) Sodium thiosulfate solution:(15)

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 one liter of distilled water; this corresponded to a 0.1N solution. The solution was preserved with .4 grams NaOH. Two hundred fifty ml of this solution were made up to one liter. The normality of this solution was found in the following manner:

One and one-half liters of distilled water were aerated vigorously for 30 minutes to assure saturation with dissolved oxygen. After 30 minutes the temperature of the water and the barometric pressure were noted and recorded. Three standard B.O.D. bottles were filled with aerated water immediately by siphoning, and the bottles stoppered. The standard dissolved oxygen determination using the Alsterberg modification of the Winkler method was then made.

The normality of the thiosulfate was calculated from the following formula:

$$N = .025 \times \frac{O_s}{v} \times \frac{P_o}{P_s} \times \frac{V}{200}$$

where

$O_s$  = D.O. Saturation in ppm at the observed temperature in °C and 760 mm pressure

$v$  = volume of thiosulfate used for titration

$P_o$  = observed barometric pressure

$P_s$  = 760 mm

$V$  = volume of original sample titrated in ml

$N$  = resulting normality of thiosulfate being tested.

(5) Ferric chloride solution:

Twenty-five hundredths grams of  $FeCl_3 \cdot 6H_2O$  were dissolved in one liter of distilled water.

(6) Calcium chloride solution:

Twelve and seventy-nine hundredths grams of  $CaCl_2 \cdot 2H_2O$  were dissolved in one liter of distilled water.

(7) 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  $\text{N}/44$  NaOH were added until a pH of 7.2 was reached. Then 1.5 grams of  $(\text{NH}_4)_2\text{SO}_4$  were added and the solution diluted to one liter.

(8) Magnesium sulfate solution:

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

(9) Standard diluting water:

To each carboy of distilled water (approximately 18 liters) the following were added: 0.5 ml of the ferric chloride solution; 2.5 ml of the calcium chloride solution; 2.5 ml of the magnesium sulfate solution; and 1.25 ml of the phosphate buffer solution. The diluting water was aerated continuously for 24 hours and aged for at least four days before using.

(10) Potassium hydroxide solution (10%):

Twenty-five grams of KOH were dissolved in distilled water and diluted to 250 ml.

Appendix IThe Overlapping Test for Statistical Significance  
Between Two Series of Data(16)

Where:

- S.E. = the standard error of the mean  
 $\sigma$  = the standard deviation  
 n = number of measurement in the series  
 $\bar{X}$  = mean value  
 $\sum X$  = sum of the individual measurements

$$\text{Mean value} = \sum X / n$$

$$\text{Standard deviation} = \sqrt{\frac{\sum (X - \bar{X})^2}{n}}$$

$$\text{Standard error S.E.} = \sigma / \sqrt{n}$$

(1) Simple Overlapping Test:

$$\text{Confidence Limit} - 99:73\% = \bar{X} \pm 3 \times \text{S.E.}$$

To test for statistical significant difference, find the upper and lower confidence limits for both series. If the lower limit of the higher mean value is less than the higher limit of the lower mean value, then overlapping occurs and there is no significant difference; if the reverse is true, then there is a significant difference.

Sample Calculation:

"A" Series

$$\begin{aligned} \bar{X} &= 250 \\ n &= 30 \\ &= 11.45 \\ \text{S.E.} &= 2.1 \end{aligned}$$

"B" Series

$$\begin{aligned} \bar{X} &= 254 \\ n &= 24 \\ &= 7.95 \\ \text{S.E.} &= 1.62 \end{aligned}$$

Confidence Limit = 99.73% =  $\bar{X} \pm 3 \times \text{S.E.}$

"A" Series

$$250 \pm 3 \times 2.1 \\ 256.2 \text{ to } 243.7$$

"B" Series

$$254 \pm 3 \times 1.62 \\ 258.9 \text{ to } 249.1$$

"B" Series has the higher mean and its lower limit is 249.1.

"A" Series has the lower mean and its higher limit is 256.2.

Since the lower limit (249.1) of the higher mean (254) is less than the higher limit (256.2) of the lower mean (250), overlapping occurs and there is no significant difference.

(2) Less Severe Overlapping Test:

Confidence Limit - 68% =  $\bar{X} \pm 1 \times \text{S.E.}$

To test for statistical significance, find the mean ( $\bar{X}$ ) and the standard deviation ( $\sigma$ ) of both series. Compute the Standard Error (S.E.) of each mean. Compute the S.E. of the difference between the two means by the following formula

$$\text{S.E. diff.} = \sqrt{\frac{(\text{S.E.})^2}{\text{mean}_1} + \frac{(\text{S.E.})^2}{\text{mean}_2}}$$

Obtain the difference between the two means. Use 68% confidence limits. Subtract the S.E. diff from the difference of the two means.

If the resulting value is negative, the difference is not statistically significant. If there is a statistical significance, then the answer is positive.

Sample Calculation:

Series A

$$\text{Mean} = 135 \\ \text{S.E.} = 6.2$$

Series B

$$\text{Mean} = 142 \\ \text{S.E.} = 7.5$$

$$\text{Difference of Means} = 142 - 135 = 7$$

$$\text{S.E.}_{\text{diff}} = \sqrt{(6.2)^2 + (7.5)^2} = 9.7$$

Testing at 68% certainty:

$$7 - (1 \times 9.7) = -2.7$$

There is not a statistically significant difference.