

CORRELATION OF BIOCHEMICAL OXYGEN DEMAND
WITH
OXIDATION-REDUCTION POTENTIAL OF
SETTLED SEWAGE

by

Henry Ray Thacker

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in candidacy for the degree of
MASTER OF SCIENCE
in
Sanitary Engineering

APPROVED:

Lewis A. Purdue
Director of Graduate Studies

APPROVED:

Russell C. Brinker
Head of Department

Walter Pittman
Dean of Engineering

Lewis J. Rich
Major Professor

May, 1953

Blacksburg, Virginia

LD
5655
V855
1953
T522
C.2

TABLE OF CONTENTS

	Page
I. Introduction	8
II. Review of Literature	9
III. Object of the Investigation	12
IV. The Investigation	13
The Standard B.O.D. Determinations	15
Apparatus	15
Collection of the Samples	18
Preparation of the Samples	18
Determination	19
The O-R Potential Determinations	19
Apparatus	20
Maintenance and Operation of the pH Meter	20
The Collection of the Sample	22
Performance of the Test	22
Procedure	24
Collection of the Samples	24
Initial Laboratory Work	26
The Final Laboratory Work	28
Statistical Correlation of the Data	29
Data and Results	29
V. Discussion of Results	50
VI. Conclusions	52

	Page
VII. Summary	53
VIII. Acknowledgments	54
IX. Bibliography	55
X. Vita	57
XI. Appendices	58
Appendix A. Apparatus and Chemicals	
Employed	59
Appendix B. Preparation of Reagents	61
Appendix C. Instructions for the Use of	
the Beckman Model N-2	
pH Meter	65
Appendix D. Correction Factor for the	
Standardization of 0.025N	
Sodium Thiosulfate Solution	
With Saturated Solution of	
Dissolved Oxygen	70
Appendix E. Dissolved Oxygen Determination	
by the Alsterberg Modification	
of the Winkler Method	73
Appendix F. Biochemical Oxygen Demand	
Sample Calculations	74
Appendix G. Statistical Correlation of	
Data	76

LIST OF PLATES

	Page
Plate 1. The Site of the Investigation Was the V.P.I. Sewage Treatment Plant	14
Plate 2. The Apparatus Used in the B.O.D. Determina- tion.	16
Plate 3. The Apparatus Used in the O-R Potential Determination.	21
Plate 4. The Samples were Collected from the Effluent of the Primary Clarifier at the V.P.I. Sewage Treatment Plant	25
Plate 5. All Laboratory Work was Performed at the V.P.I. Sewage Treatment Plant Laboratory . . .	27

LIST OF TABLES

	Page
Table 1. Results of O-R Potential and B.O.D. Determinations for Tests 1 Through 12. . . .	31
Table 2. Results of O-R Potential and B.O.D. Determinations for Tests 13 Through 24 . . .	32
Table 3. Results of O-R Potential and B.O.D. Determinations for Tests 25 Through 36 . . .	33
Table 4. Results of O-R Potential and B.O.D. Determinations for Tests 37 Through 48 . . .	34
Table 5. Results of O-R Potential and B.O.D. Determinations for Tests 49 Through 60 . . .	35

LIST OF FIGURES

	Page
Figure 1. Relationship Between B.O.D. and Field Determined O-R Potential	36
Figure 2. Relationship Between B.O.D. and Laboratory Determined O-R Potential.	37
Figure 3. Relationship Between Field and Laboratory Determined O-R Potential.	38
Figure 4. Distribution of Field Determined O-R Potentials	39
Figure 5. Distribution of Laboratory Determined O-R Potentials.	40
Figure 6. Distribution of B.O.D. Determinations	41
Figure 7. The Relation of B.O.D. to Field Determined O-R Potential for Tests 1 Through 20.	42
Figure 8. The Relation of B.O.D. to Field Determined O-R Potential for Tests 21 Through 40	43
Figure 9. The Relation of B.O.D. to Field Determined O-R Potential for Tests 41 Through 60	44
Figure 10. The Relation of B.O.D. to Laboratory Determined O-R Potential for Tests 1 1 Through 20.	45
Figure 11. The Relation of B.O.D. to Laboratory Determined O-R Potential for Tests 21 Through 40	46
Figure 12. The Relation of B.O.D. to Laboratory Determined O-R Potential for Tests 41 Through 60	47

	Page
Figure 13. The Relation of B.O.D. to Field Determined O-R Potential Showing Curve of Best Fit	48
Figure 14. The Relationship of B.O.D. to Laboratory Determined O-R Potential Showing Curve of Best Fit.	49

I. INTRODUCTION

The ultimate stabilization of the decomposable organic material found in domestic sewage and certain industrial wastes is attainable only through complex reactions of a biochemical nature. Chemically these reactions are of the oxidation-reduction type in which both free and combined oxygen play an essential part. Investigators have long experienced difficulty in developing satisfactory control tests for sewage treatment processes due to the fact that in sewage mixtures many separate oxidation-reduction reactions are taking place simultaneously.

Control tests have been developed progressively from the crude putrescibility test to the relative stability determination and more recently to the chemical oxygen demand (C.O.D.) and the biochemical oxygen demand (B.O.D.). The B.O.D. test has been accepted as the standard method for determining the amount of decomposable organic matter present in sewage. Since the B.O.D. test measures the amount of oxygen utilized in the stabilization of the sewage for a 5-day, 20° centigrade incubation period, immediate results cannot be obtained. Investigators have attempted without success to develop a more rapid type of measurement than the standard B.O.D. test.

II. REVIEW OF LITERATURE

Clark⁽¹⁾ found that an electrical potential exists within an oxidation-reduction system and that as long as certain other conditions remain unchanged, the potential is quantitatively dependent upon the ratio of oxidant to reductant present. The oxidation-reduction (O-R) potential concept was further investigated by Hewitt⁽²⁾ who applied the fundamental theories to biological systems. He expressed the O-R potential in terms of electron migration in which the loss and gain of electrons result in oxidation and reduction respectively. In an oxidation-reduction system, when one substance is oxidized, another is simultaneously reduced.

Rohlich⁽³⁾ was the first to apply the O-R potential to the study of sewage mixtures. He concerned himself principally with a highly aerobic treatment process (activated sludge) in which dissolved oxygen (D.O.) concentration plays an essential part. It was shown that the oxidation-reduction potential of activated sludge-sewage mixtures becomes more positive as the system becomes more oxidized. Moreover, good correlation was found to exist between the stage of oxidation, as measured by the rate of oxygen utilization, and the O-R potential. The O-R potential increased positively throughout the aerobic units of the plant when the overall treatment system appeared to be functioning properly.

Hood⁽⁴⁾ prompted by Rohlich's⁽³⁾ work with activated sludge, endeavored to apply O-R potential principles to the efficient operation of sewage treatment processes in general. He found that under normal operating conditions, one may expect to find positively ascending values for the O-R potential as the hydraulic descent is made through the plant. Any unit or units which does not increase proportionally the potential, under normal conditions, may be accepted as not functioning properly. Hood's findings suggested that it would be a simple matter to detect offending units within a system merely by noting those units which do not increase the O-R potential of the sewage mixture.

Hood and Rohlich⁽⁵⁾ in an attempt to establish quantitative values for O-R potential existing within the treatment plant, found that the potentials occurring in normal oxidation processes varied from +200 to +600 millivolts while those in anaerobic processes varied from -100 to -200 millivolts. This investigation indicated that early detection of conditions detrimental to proper operation of the plant can be made by utilization of an O-R potential survey.

Porter⁽⁶⁾ expressed the characteristics of the O-R potential as an "intensity factor" rather than a "capacity factor". In this respect, O-R potential may be considered similar to temperature, as temperature gives one no information as to

the heat capacity, just as the O-R potential gives one no indication of "poising effect", the capacity term used in O-R systems. Faison⁽⁷⁾, however, explored the possibility that normal domestic sewage is sufficiently uniform in composition to allow a correlation to be made between the intensity factor, O-R potential, and the capacity factor, B.O.D. Working with unsettled sewage, Faison was unsuccessful in establishing such a correlation.

The present investigator, knowing that settled sewage exhibits greater uniformity, felt that Faison's investigation should be continued on settled sewage.

III. OBJECT OF THE INVESTIGATION

The object of this thesis research was to determine whether or not a correlation exists between values obtained from performing the standard biochemical oxygen demand determination and the oxidation-reduction potential determination on settled sewage. The samples were collected from the effluent of the primary clarifier at the V.P.I. Sewage Treatment Plant. The results obtained from these two tests were to be statistically compared and the degree of correlation was to be ascertained.

IV. THE INVESTIGATION

The research investigation consisted essentially of performing two different determinations, B.O.D. and O-R potential, on similar samples of settled sewage. The samples were obtained from the effluent of the primary settling tank of the V.P.I. Sewage Treatment Plant. The inherent characteristics of the two determinations together with the existing local conditions prompted the division of the investigation procedure into three distinct phases: (1) the collection of the samples; (2) the initial laboratory work; and (3) the final laboratory work. The investigator attempted to minimize procedural errors by making every effort to maintain a consistent technique throughout the investigation, paying particular attention to the time element to insure the elapse of the same increment of time between successive phases of the determinations.

The initial phase of the investigation consisted of collecting the necessary samples for the O-R potential, and performing the B.O.D. and the D.O. tests. The D.O. samples were fixed immediately after collection to insure a more representative value. The samples were carried at once to the laboratory where the second phase of the investigation could be initiated.

The initial laboratory work was divided into three parts: (1) the determination of the D.O. of the undiluted samples; (2) the preliminary work on the B.O.D. test; and (3) the

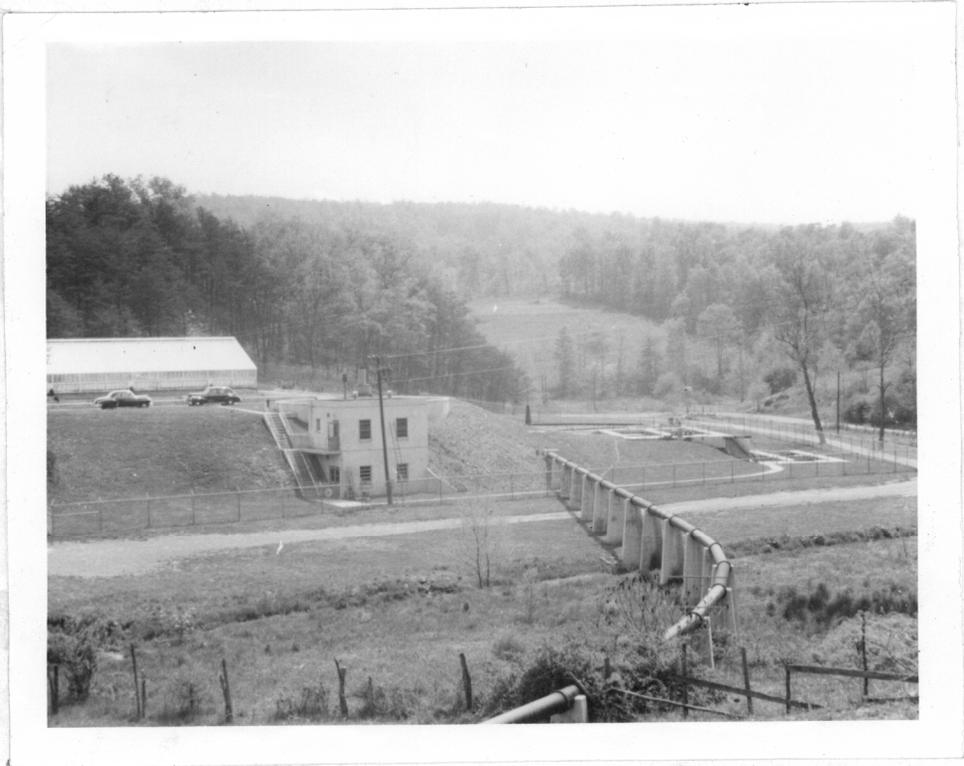


Plate 1.

The Site of the Investigation Was
The V.P.I. Sewage Treatment Plant

O-R potential determinations. The initial work on the B.O.D. test consisted of preparing a duplicate range of dilutions from the raw sewage, incubating one set of samples and performing a test for dissolved oxygen on each sample of the second set of dilutions.

The third and final phase of the investigation consisted of performing tests on the incubated B.O.D. samples to determine the dissolved oxygen content. Calculations of the 5-day, 20°C B.O.D., of the sewage, were then made on the basis of the initial and final dissolved oxygen content of the diluted B.O.D. samples. These values were tabulated and filed for analysis following the completion of the research.

The Standard Biochemical Oxygen Demand Determination

The biochemical oxygen demand is the quantity of oxygen utilized by bacteria in the stabilization of decomposable organic material. The B.O.D. values were determined by the dilution method using the Alsterberg Modification of the Winkler Method⁽⁸⁾ for determining the dissolved oxygen content of the samples. The 5-day, 20°C B.O.D.'s were determined with the results expressed in parts per million (p.p.m.).

Apparatus:

The sample was collected in a two (2) liter bottle, using rubber gloves. The dilution water was prepared, aerated,



Plate 2.

The Apparatus Used in the
B.O.D. Determination

and stored in carboys of approximately twenty (20) liter capacity. Just prior to making the dilutions, the dilution water was seeded with sewage by using a pipette while the water was contained in a four (4) liter aspirator. The aspirator was equipped with a two (2) foot rubber hose, a stopcock, and an eighteen (18) inch glass tube to facilitate the filling of the graduated cylinders. Four-one (1) liter graduated cylinders, a one hundred (100) milliliter pipette, a fifty (50) milliliter pipette, a twenty-five (25) milliliter pipette, and a siphon were employed in the dilution and the transference of the samples. Several three hundred (300) milliliter B.O.D. bottles were used for holding the diluted samples both for the initial and final dissolved oxygen determinations. Dropping bottles were employed to hold the reagents and to facilitate their addition to the B.O.D. bottles. After being fixed, the samples were transferred from the B.O.D. bottles to four-two hundred and fifty (250) milliliter Erlenmeyer flasks by means of a two hundred (200) milliliter pipette and a four (4) milliliter pipette. The titrations were made with a burette. The samples were titrated in Erlenmeyer flasks. A 20° B.O.D. incubator furnished the proper conditions for the incubation of the duplicate samples. Other miscellaneous standard laboratory equipment were used in preparation of the reagents and performance of the B.O.D. determination itself.

Collection of the Samples:

The sewage required for the B.O.D. determination was collected immediately after the collection of the sample for the O-R potential. The "grab method" of sampling was used since a representative sample was not required in these correlation studies. The sample was collected in a clearly marked, clean, wide-mouthed stoppered bottle. The time of collection was carefully noted to act as a check on consistent laboratory procedures. The bottle was filled by carefully holding it horizontal with the mouth pointed in the direction of flow, being careful to avoid undue turbulence. The sample thus collected was stoppered and immediately carried to the laboratory for the second phase of the investigation.

Preparation of the Samples:

After collection of the sample, it was immediately brought to the field laboratory where a duplicate range of dilutions were made. These dilutions were made by placing the necessary quantities of the sample, depending on the dilution desired, in each of the four-one (1) liter graduated cylinders. Being very careful to avoid agitation, the seeded diluting water was then added from the aspirator until the solution reached the liter mark. In order to act as a control on the amount of oxygen utilized by the diluting water alone,

one cylinder contained only the diluting water. Two-three hundred (300) milliliter B.O.D. bottles were then carefully filled by using a siphon from each of the cylinders. Prior to performing the final test, one complete range of samples was placed in a 20°C incubator to allow for a 5-day depletion of dissolved oxygen. The other duplicate range was set up to facilitate the determination of the initial dissolved oxygen content of the sample.

Determination:

The Alsterberg Modification of the Winkler Method was used in determining the dissolved oxygen concentration on all the B.O.D. samples. The oxygen depletion in the samples was indicative of the amount of decomposable organic matter present to be stabilized aerobically by the bacteria. Therefore, a quantitative determination could be made of the "concentration" of the sewage. For an example of B.O.D. calculations refer to Appendix F.

Oxidation-Reduction Potential Determination

The Oxidation-Reduction Potential was determined by measuring the electrical potential existing in the sample by means of a pH meter and the proper electrodes. The O-R potential in a liquid is due to the respective concentrations of oxidant and reductant present.

Apparatus:

The apparatus required for determination of the Oxidation-Reduction Potential in the sewage samples consisted essentially of a Beckman Model N-2 pH meter with the proper electrodes. Three (3) platinum electrodes were used to serve as a check for any variations which might occur due to operational difficulties, and the average of the readings recorded as the O-R potential value. A calomel electrode was employed as the reference electrode. A thermometer was used for adjusting the temperature compensator on the pH meter. A stand was used for holding the three platinum electrodes an equal distance from the reference electrode in the actual determinations. A three-way switch was utilized with the three platinum electrodes, thereby eliminating the necessity of a great deal of rewiring of the electrodes to the main meter unit. The solution was contained in a fifteen hundred (1500) milliliter beaker during the test. Other apparatus employed were a wash bottle, a small beaker for standardization, and several pin jack connectors.

Maintenance and Operation of the pH meter:

Since the measurement of the Oxidation-Reduction Potential depended upon the proper care of the meter and electrodes, great care was taken to carefully follow the manufacturers' instructions. In general this consisted of carefully washing



Plate 3.
The Apparatus Used in the
O-R Potential Determination

and maintaining the electrodes, frequent checks for any drift in the meter, routine checks for standardization variation, and utmost care in handling the meter and electrodes. For additional information, refer to Appendix C.

The Collection of the Sample:

The sample of sewage was collected in a clean, fifteen hundred (1500) milliliter beaker by placing it under the surface of the sewage in an inverted position and then turning it back to its normal position allowing it to be filled with the sewage. A mark was etched into the beaker marking that volume which was consistently collected. The time of collection was carefully noted in order to allow the same increment of time to elapse between the various readings. Rubber gloves were used since the beaker was immersed in the sewage. The sample, upon collection, was carried immediately to the field laboratory where it was allowed to settle before the Oxidation-Reduction potential determinations were made.

Performance of the Test:

The temperature of the sewage sample was determined at once, and recorded as the field temperature. Inasmuch as pH exerts an influence upon the values obtained for the O-R potential, the pH of the sewage was determined also. The pH was determined with the Beckman pH meter with the exception

that a glass electrode was used in lieu of the platinum electrode. For more detailed instructions on the use of the pH meter, see Appendix C.

Upon collection of the sample, the filled beaker was placed in its proper position and allowed to settle for exactly thirty (30) minutes. After the proper adjustment of the meter readings were then made to determine the electrical potential measured with each electrode. The values were not allowed to deviate more than five (5) percent from the average of all three electrodes. This tendency to deviate was eliminated in most cases by carefully cleaning the electrodes. The average value given by the electrodes was used in calculating the millivolts for the correlation of the research data.

A similar reading was made at the end of another thirty (30) minutes time interval on a sample obtained from the B.O.D. sample bottle. It was constantly necessary to clean the electrodes and vigilance was required to eliminate false readings due to operational difficulties. The temperature of the sample was determined and recorded as the laboratory temperature.

For a more detailed discussion of the procedure, refer to Appendix C.

Procedure

At the beginning of each series of determinations the necessary apparatus was assembled in the laboratory to allow the tests to be made with a minimum elapse of time. The same arrangement of apparatus was used in each test to promote similar conditions and increase the accuracy of technique. The pH meter was standardized and the electrodes were carefully cleaned as described in Appendix C. The necessary containers used in collecting the samples were then carried to the collection point and the first phase of the testing procedure was initiated.

Collection of the Samples:

Four separate samples were collected for the initial laboratory phase of the investigation. The first was collected in a 1500 milliliter beaker for the initial O-R potential determination; the second was collected in a four liter bottle to be used for the B.O.D. test and the second O-R potential reading; and the last two samples were collected in 300 milliliter B.O.D. bottles to determine the dissolved oxygen in the undiluted sample. The samples were collected from the effluent of the primary settling tank at the V.P.I. Sewage Treatment Plant. The time of collection of the first sample was carefully noted and recorded to insure the same



Plate 4.

The Samples were Collected from the Effluent
of the Primary Clarifier at the
V.P.I. Sewage Treatment Plant

elapse of time between the various phases of the determinations, particularly the O-R potential determinations.

Initial Laboratory Work:

Upon collection, the samples were carried immediately to the field laboratory where the second phase of the procedure was conducted. The two samples used for the dissolved oxygen determination on the undiluted sample were immediately "fixed" by the addition of the appropriate reagents for the Alsterberg Modification of the Winkler Method. While the D.O. samples were being prepared, the O-R potential sample was set aside for the pH determination and at the same time the temperature was checked and recorded as the "field" temperature. After recording the pH reading, the O-R sample was arranged in order to obtain the initial reading after a proper "settling" time was reached. In this particular investigation, a thirty-minute period seemed to be most favorable. The B.O.D. sample was then prepared by adding the proper amount of sample sewage to the dilution water in order to obtain a dilution range adequate to determine the B.O.D. of the sample. After the duplicate range had been prepared, one set was placed in the 20°C incubator for five days, and the second range was prepared immediately for the initial dissolved oxygen determination. This phase of the initial laboratory work was generally accomplished prior to the

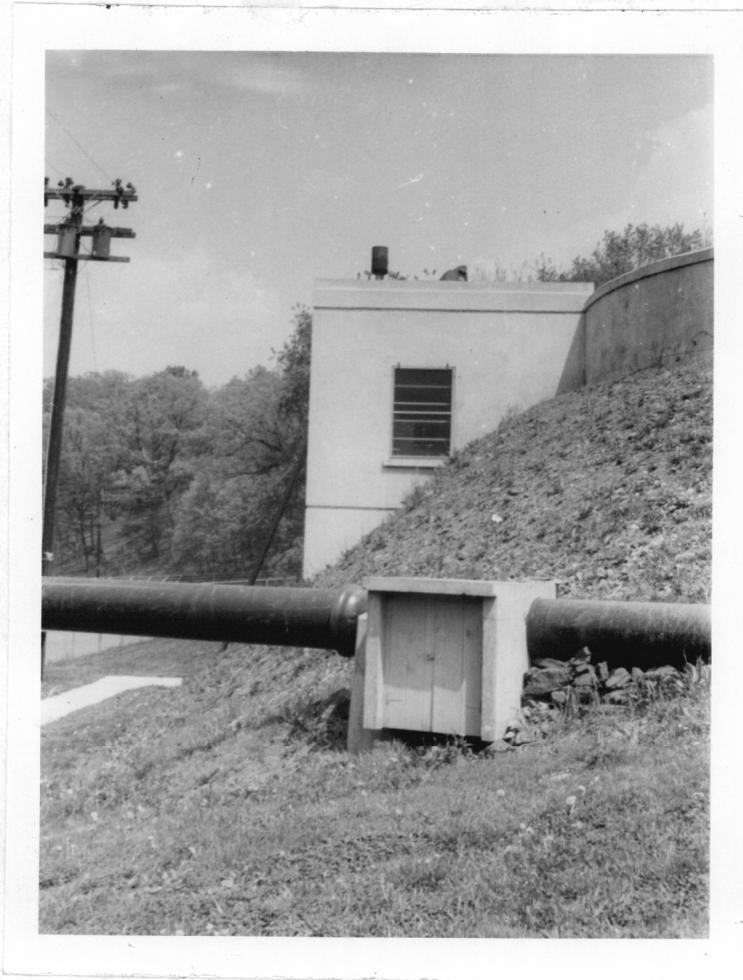


Plate 5.

All Laboratory Work was Performed at the
V.P.I. Sewage Treatment Plant Laboratory

initial O-R potential reading. After the first O-R potential reading was made and recorded, the second sample was prepared by refilling the sampling beaker with a portion of the remaining sewage from the B.O.D. sampling bottle. The necessary titrations were then made for the dissolved oxygen determinations on both the diluted B.O.D. samples and the undiluted D.O. samples. These values were recorded and, upon completion of the final phase of the test, those of the B.O.D. samples were arranged to facilitate calculation of the 5-day, 20°C B.O.D. of the sample. After an elapse of sixty minutes, from the time of the collection of the sample, the second O-R potential reading was made and recorded. The temperature of the sewage was determined at this time and was recorded as the laboratory temperature.

The Final Laboratory Work:

The final phase of the research consisted of performing the final dissolved oxygen determinations on the duplicated range of the B.O.D. samples which had been incubated for a five-day period at 20°C. The same procedure was followed for the final D.O. determination as was used for the initial D.O. determination. The results were recorded and the 5-day, 20°C B.O.D. of each set of samples was recorded.

Statistical Correlation of the Data:

The results obtained from the laboratory phase of the research were tabulated and then statistically and graphically analyzed to determine if correlation exists between the O-R potential and B.O.D.

Data and Results

The data and results obtained from the investigation have been tabulated in Tables 1 through 5. Figures 1 to 3 illustrate the graphical relationship existing between the O-R potentials and the B.O.D. The results of the statistical analysis of the data have been presented in Figures 4 through 14.

The comparisons between the B.O.D. and the O-R potentials have been shown in Figures 1 and 2. Figure 3 illustrates the variations which occurred between the field and laboratory determined O-R potential. In order to determine whether the data follow a normal probability curve, the field O-R potential, the laboratory O-R potential, and the B.O.D. values were plotted on arithmetic probability paper in Figures 4, 5, and 6.

The field determined O-R potential was plotted in increments of twenty tests each against the B.O.D. in Figures 7, 8, and 9. The laboratory determined O-R potential and the B.O.D.

were presented in Figures 10, 11, and 12 as in the figures devoted to the field determined O-R potentials. The correlation between the B.O.D. and the total field O-R potentials is shown in Figure 13, while the correlation between the B.O.D. and the total laboratory O-R potentials is presented in Figure 14. Figures 7 through 14 were statistically analyzed and the results were added to each figure. For the detailed statistical treatment, see Appendix G.

TABLE 1.
RESULTS OF O-R POTENTIAL AND B.O.D. DETERMINATIONS
FOR TESTS 1 THROUGH 12

Test No	Date 1953	Collection Time, Hours	D.O.D. (5-day, 20°C) p.p.m.	O-R Potential (mv)		D.O. p.p.m.	pH	Temperature °C	
				field	lab.			field	lab.
1	21 March	1610	71	138	140	2.40	7.50	13.0	16.0
2	23 March	1230	65	260	189	2.73	7.42	15.0	17.0
3	23 March	1430	55	84	132	2.95	7.48	16.0	19.0
4	24 March	1300	42	241	221	4.91	7.32	14.0	18.0
5	24 March	1410	62	208	206	4.15	7.21	14.0	18.0
6	24 March	1515	62	208	179	4.72	7.25	15.0	18.0
7	24 March	1700	49	231	216	4.22	7.37	15.0	18.0
8	30 March	0905	73	269	265	4.57	7.20	14.0	16.0
9	30 March	1010	53	252	236	3.60	7.38	13.0	16.0
10	30 March	1345	102	188	171	1.13	7.32	15.0	17.5
11	30 March	1445	142	188	175	1.51	7.28	15.0	17.5
12	30 March	1545	93	181	128	1.47	7.40	15.5	17.5

TABLE 2.
RESULTS OF O-R POTENTIAL AND B.O.D. DETERMINATIONS
FOR TESTS 13 THROUGH 24

Test No	Date 1953	Collection Time, Hours	B.O.D. (5-day, 20°C) p.p.m.	O-R Potential (mv)		D.O. p.p.m.	pH	Temperature °C	
				field	lab.			field	lab.
13	1 April	0900	105	172	240	3.50	7.18	16.5	18.5
14	1 April	1000	84	238	208	3.30	7.20	16.0	18.0
15	1 April	1305	95	166	150	0.60	7.20	17.0	19.0
16	1 April	1415	104	166	157	1.60	7.15	17.5	19.0
17	1 April	1500	200	173	150	.90	7.32	18.5	19.5
18	1 April	1600	182	150	144	.80	7.28	18.0	19.0
19	3 April	0915	60	266	209	2.80	7.42	15.0	17.0
20	3 April	1015	80	190	176	1.70	7.30	15.0	18.5
21	3 April	1300	156	173	155	.80	7.32	16.5	19.0
22	3 April	1400	92	166	160	1.10	7.30	16.5	18.0
23	3 April	1500	80	149	127	1.50	7.35	16.5	18.0
24	3 April	1600	84	180	168	.30	7.10	16.5	18.0

TABLE 3.
RESULTS OF O-R POTENTIAL AND B.O.D. DETERMINATIONS
FOR TESTS 25 THROUGH 36

Test No	Date 1953	Collection Time, Hours	B.O.D. (5-day, 20°C) p.p.m.	O-R Potential (mv)		D.O. p.p.m.	pH	Temperature °C	
				field	lab.			field	lab.
25	8 April	1245	180	69	41	0.00	7.35	17.5	18.0
26	8 April	1330	112	24	0	0.00	7.42	18.0	18.0
27	8 April	1430	132	54	2	0.00	7.28	17.5	18.0
28	8 April	1515	144	1	46	0.00	7.25	17.5	18.0
29	10 April	0945	62	170	163	2.16	7.50	16.5	18.0
30	10 April	1300	90	153	137	1.51	7.15	17.5	18.5
31	10 April	1400	152	122	106	1.65	7.23	18.0	19.5
32	10 April	1500	156	119	108	1.79	7.40	17.5	19.0
33	10 April	1600	148	126	83	0.71	7.25	18.0	19.5
34	15 April	0845	38	244	214	3.72	7.30	14.5	16.5
35	15 April	0945	48	184	172	2.92	7.43	15.0	17.0
36	17 April	0945	34	154	160	2.97	7.51	14.5	17.0

TABLE 4.
RESULTS OF O-R POTENTIAL AND B.O.D. DETERMINATIONS
FOR TESTS 37 THROUGH 48

Test No	Date 1953	Collection Time, Hours	B.O.D. (5-day, 20°C) p.p.m.	O-R Potential (mv)		D.O. p.p.m.	pH	Temperature °C	
				field	lab.			field	lab.
37	17 April	1445	96	133	104	0.42	7.29	16.0	19.0
38	17 April	1545	84	79	103	0.00	7.10	17.0	19.0
39	18 April	1300	113	104	112	0.00	7.29	16.0	18.5
40	18 April	1400	145	109	94	0.52	7.32	17.0	18.0
41	18 April	1500	102	97	104	0.24	7.42	17.0	18.0
42	18 April	1600	145	94	87	0.00	7.30	16.5	18.0
43	18 April	1700	93	103	106	0.75	7.22	16.5	18.0
44	18 April	1800	182	71	79	0.00	7.29	16.5	17.5
45	18 April	2045	152	106	104	0.00	7.40	16.5	17.5
46	18 April	2145	172	114	100	0.00	7.33	16.5	18.5
47	18 April	2245	140	112	92	0.00	7.25	16.0	18.0
48	18 April	2345	176	112	109	0.00	7.25	16.0	18.2

TABLE 5
RESULTS OF O-R POTENTIAL AND B.O.D. DETERMINATIONS
FOR TESTS 49 THROUGH 60

Test No	Date 1953	Collection Time, Hours	B.O.D. (5-day, 20°C) p.p.m.	O-R Potential (mv)		D.O. p.p.m.	pH	Temperature °C	
				field	lab.			field	lab.
49	19 April	0045	90	123	118	1.23	7.38	15.5	18.0
50	19 April	0145	92	126	127	1.08	7.40	15.5	18.0
51	19 April	0245	100	143	138	1.27	7.30	15.0	17.5
52	19 April	0345	78	147	145	1.93	7.27	15.0	17.5
53	19 April	0445	40	166	156	2.97	7.23	15.0	17.0
54	19 April	0545	36	158	140	3.35	7.22	14.0	17.0
55	19 April	0845	34	170	161	4.28	7.28	15.0	17.0
56	19 April	0945	26	157	153	4.48	7.10	14.5	17.5
57	19 April	1045	55	153	148	3.20	7.30	14.0	17.0
58	19 April	1145	106	126	126	3.44	7.27	15.0	17.0
59	19 April	1245	88	118	109	2.12	7.40	15.0	17.5
60	19 April	1345	102	109	107	1.79	7.22	15.0	17.5

35

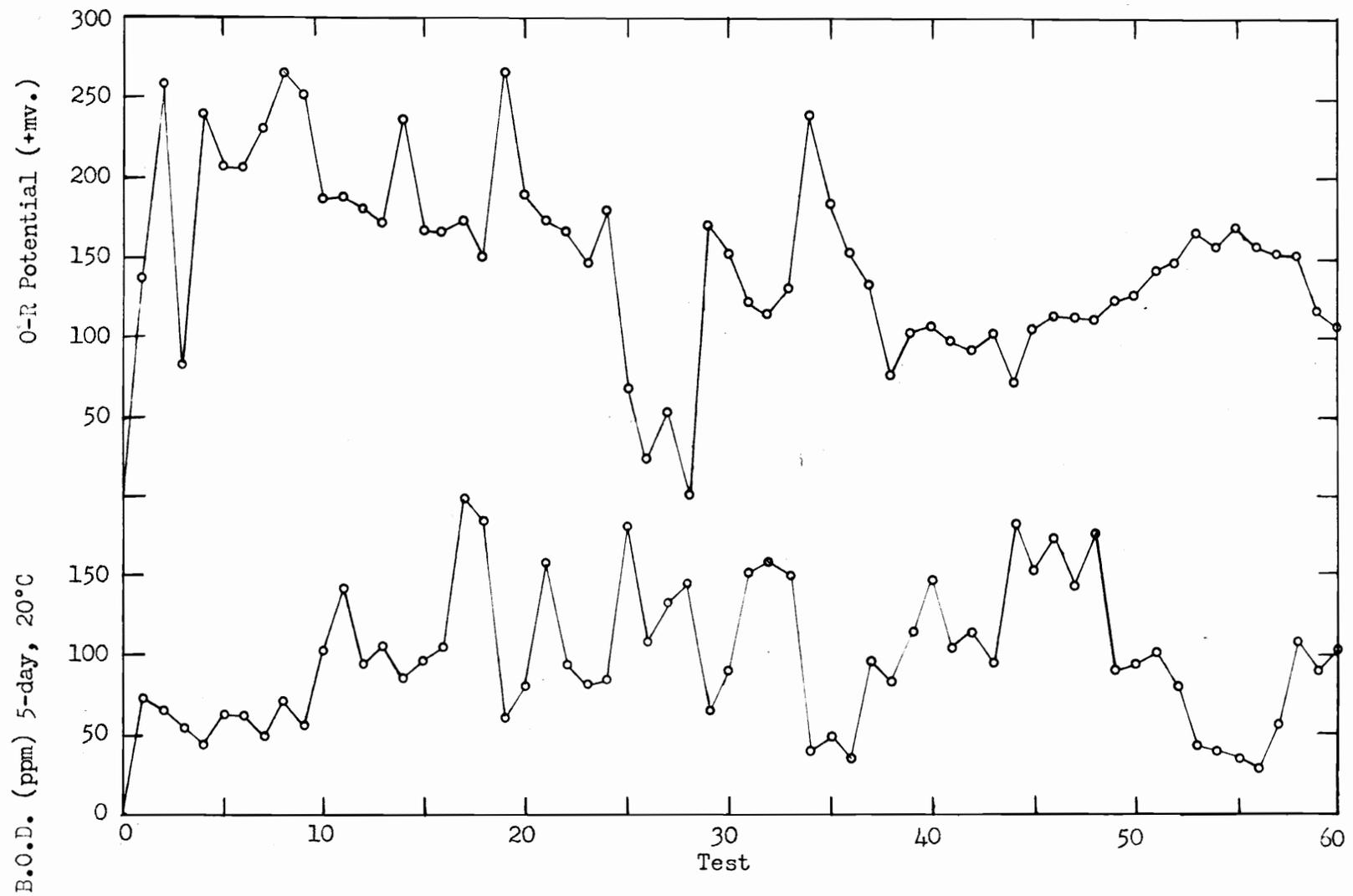


FIGURE 1.

RELATIONSHIP BETWEEN B.O.D. AND FIELD DETERMINED O-R POTENTIAL

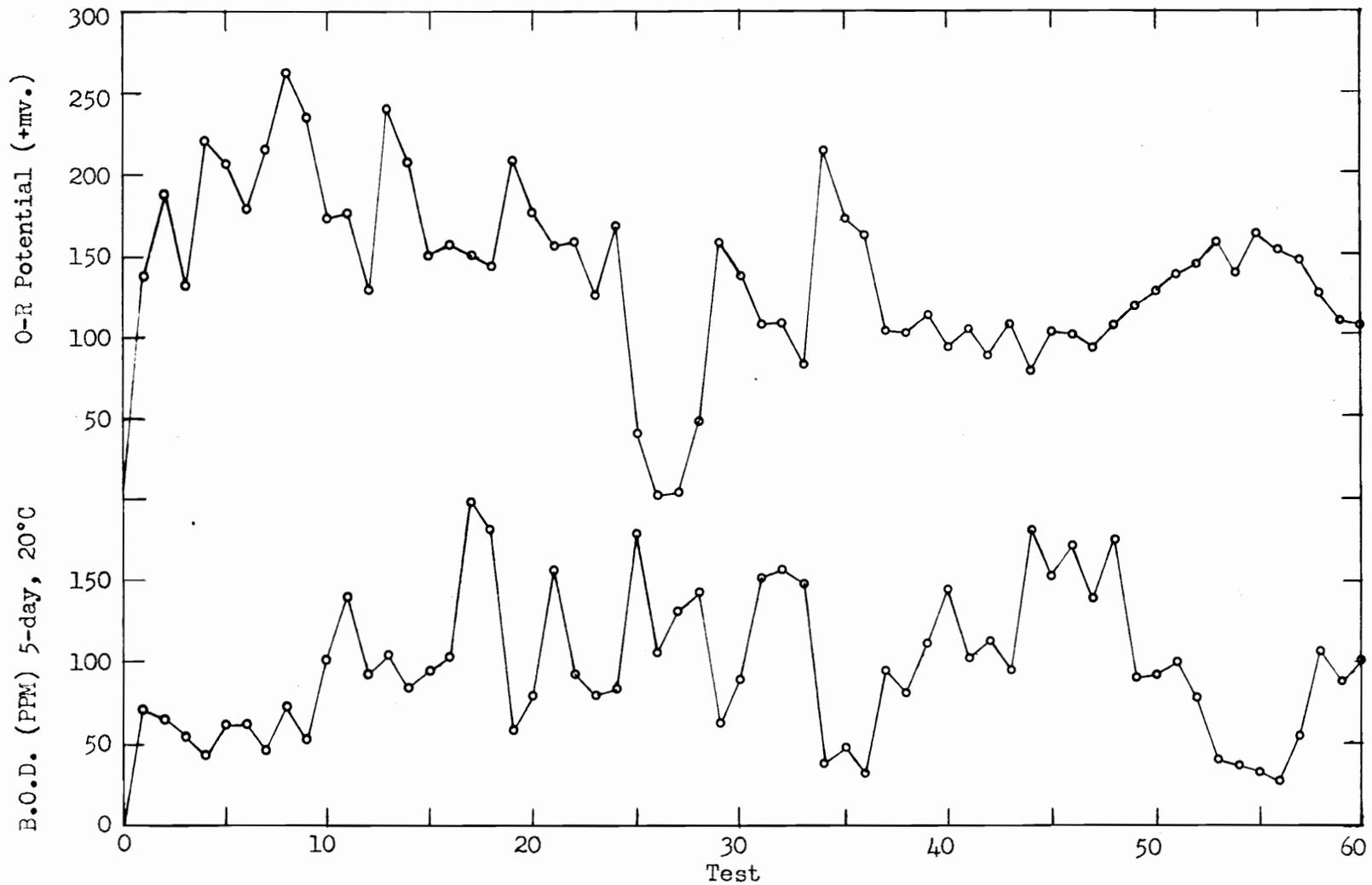


FIGURE 2.

RELATIONSHIP BETWEEN B.O.D. AND LABORATORY DETERMINED O-R POTENTIAL

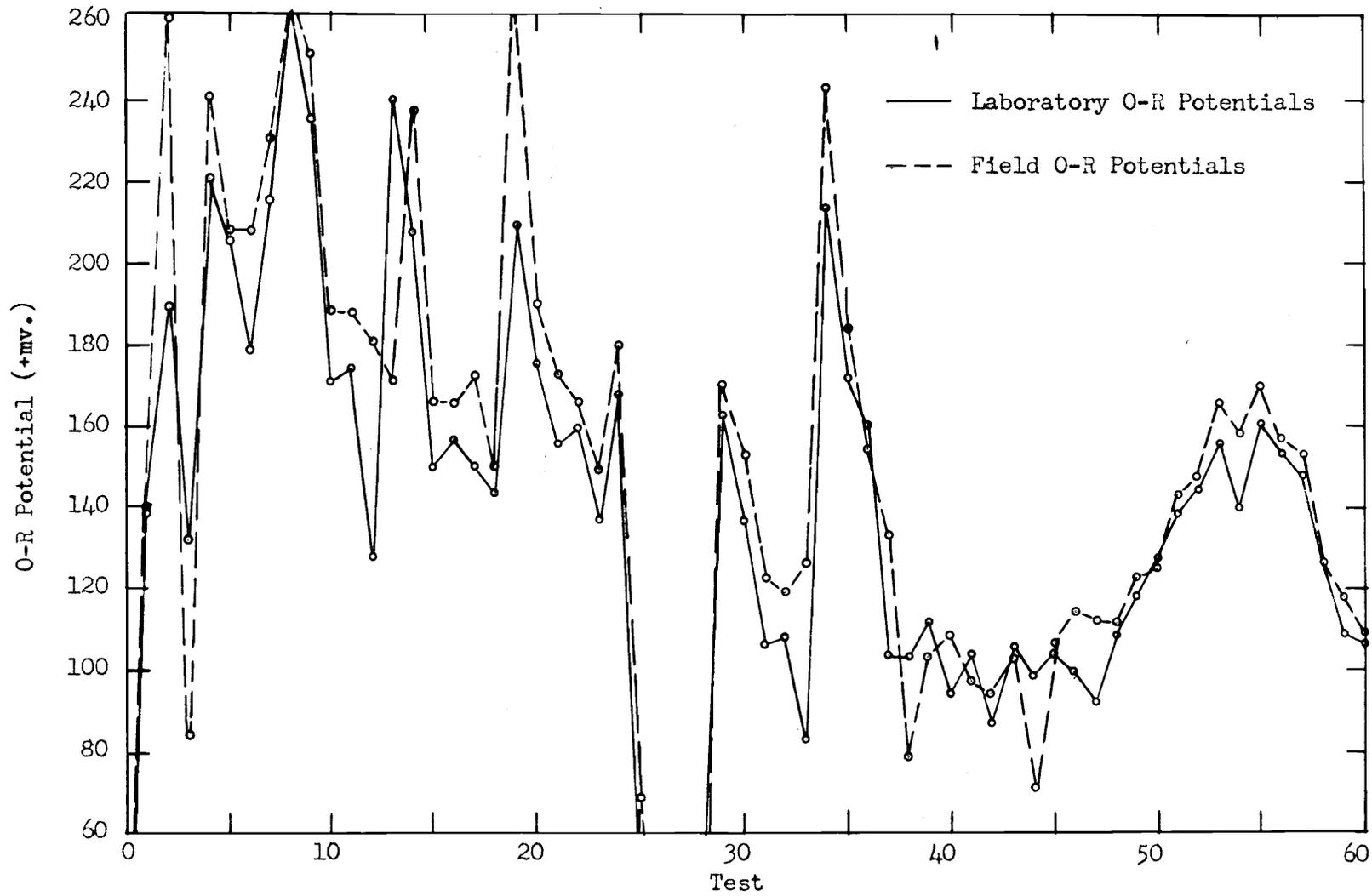


FIGURE 3.

RELATIONSHIP BETWEEN FIELD AND LABORATORY DETERMINED O-R POTENTIAL

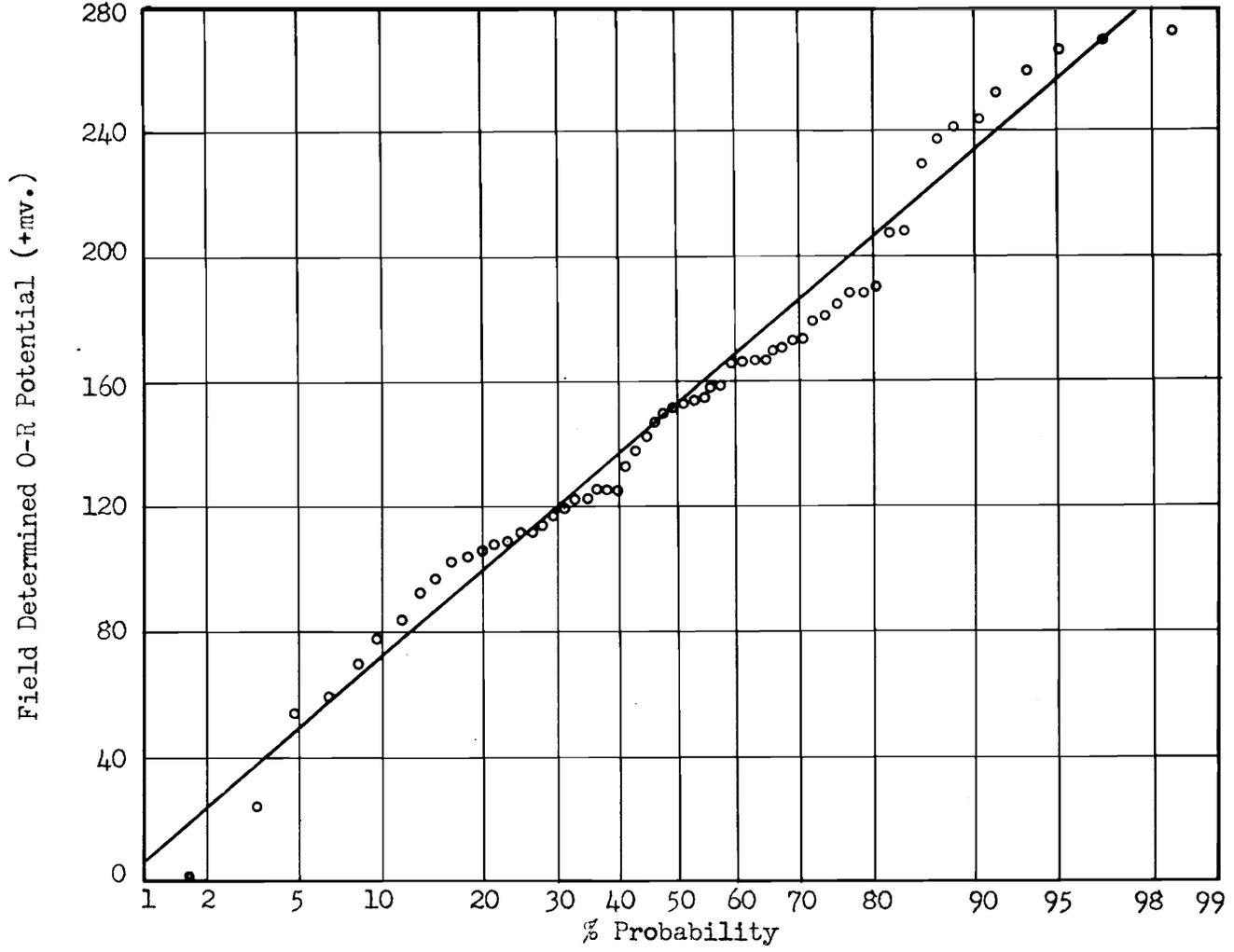


FIGURE 4.

DISTRIBUTION OF FIELD DETERMINED O-R POTENTIALS

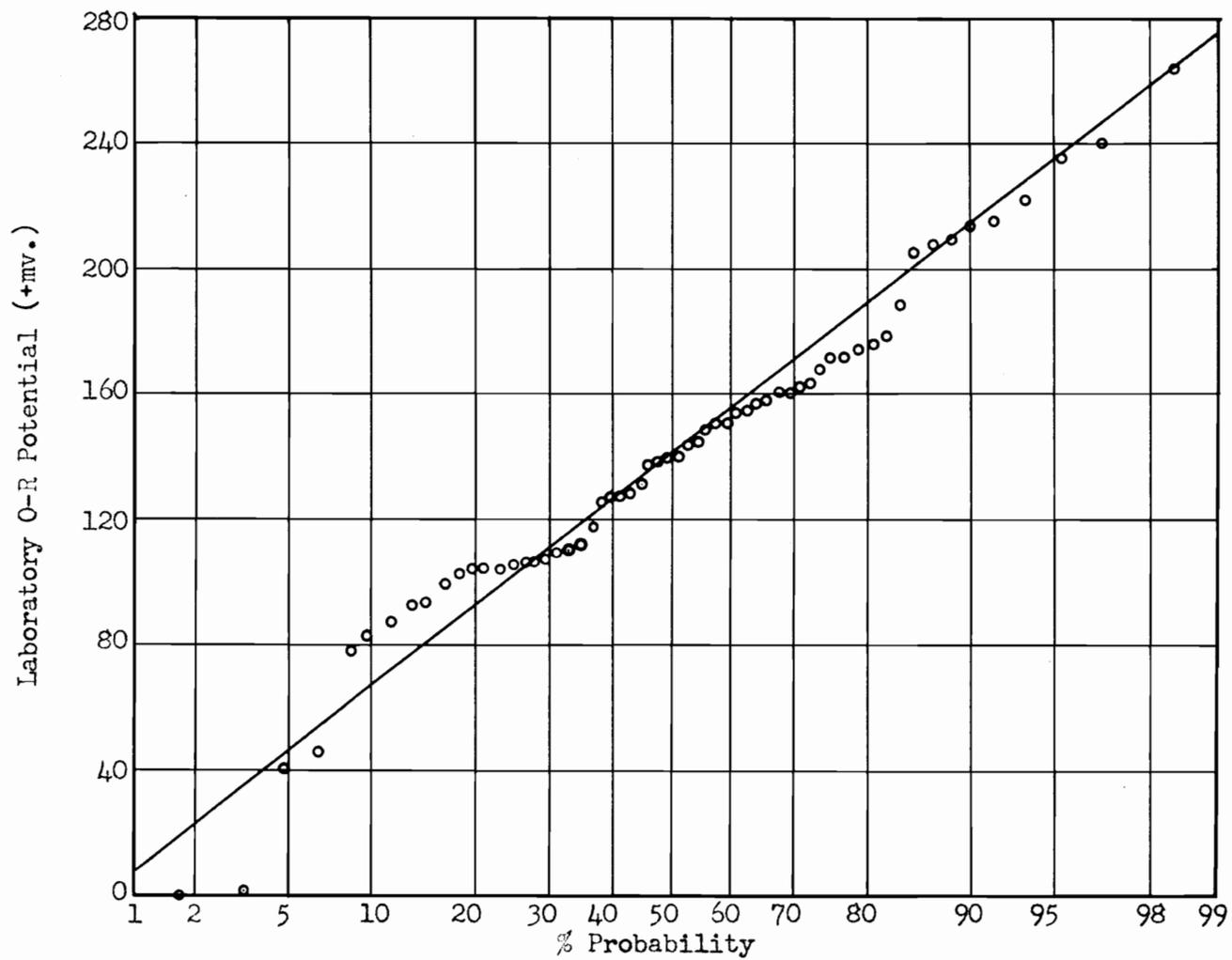


FIGURE 5.

DISTRIBUTION OF LABORATORY DETERMINED O-R POTENTIALS

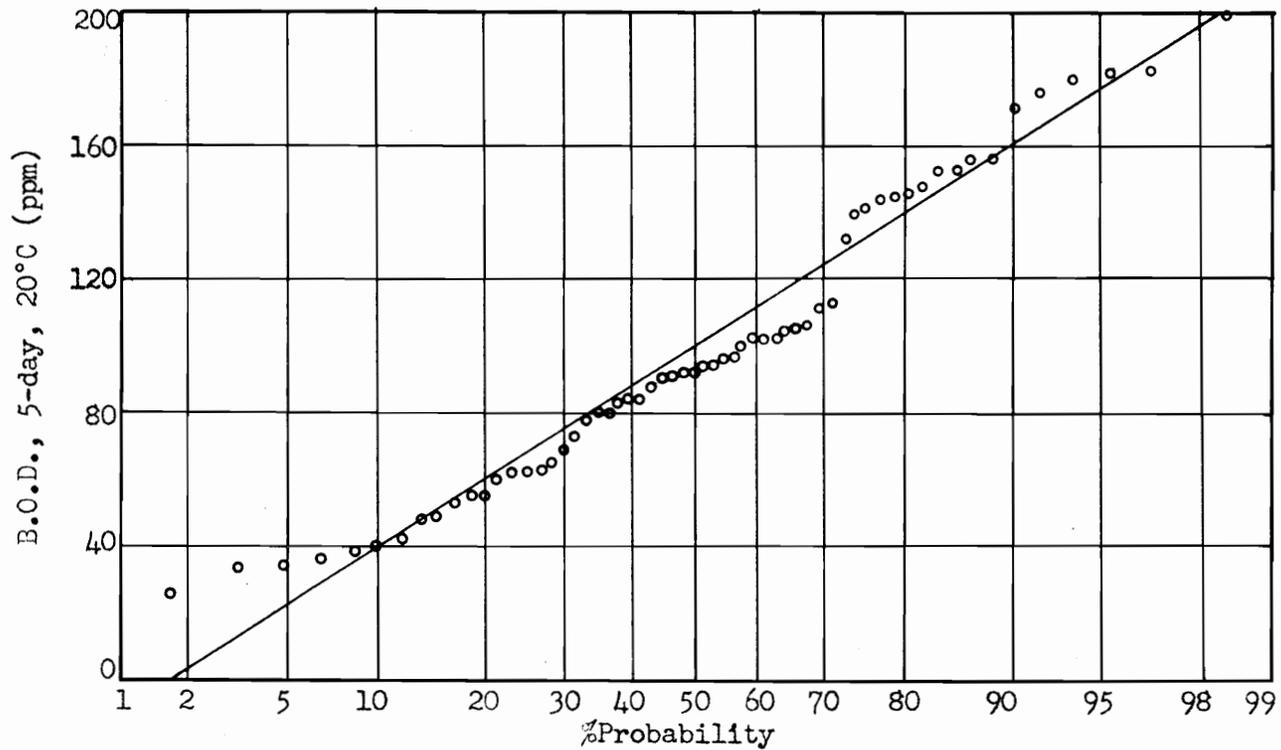


FIGURE 6.

DISTRIBUTION OF B.O.D. DETERMINATIONS

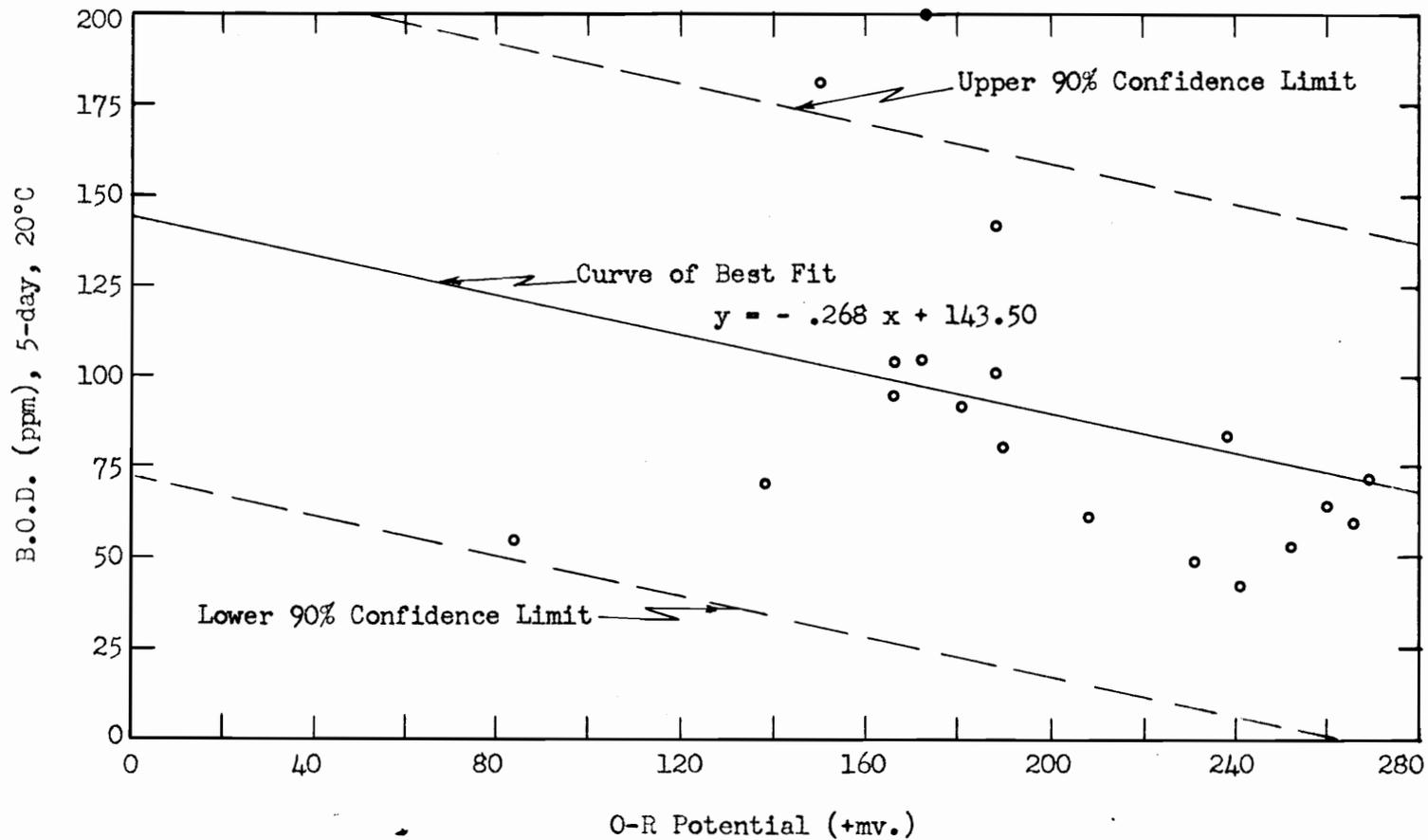


FIGURE 7.

THE RELATION OF B.O.D. TO FIELD DETERMINED
O-R POTENTIAL FOR TESTS 1 THROUGH 20

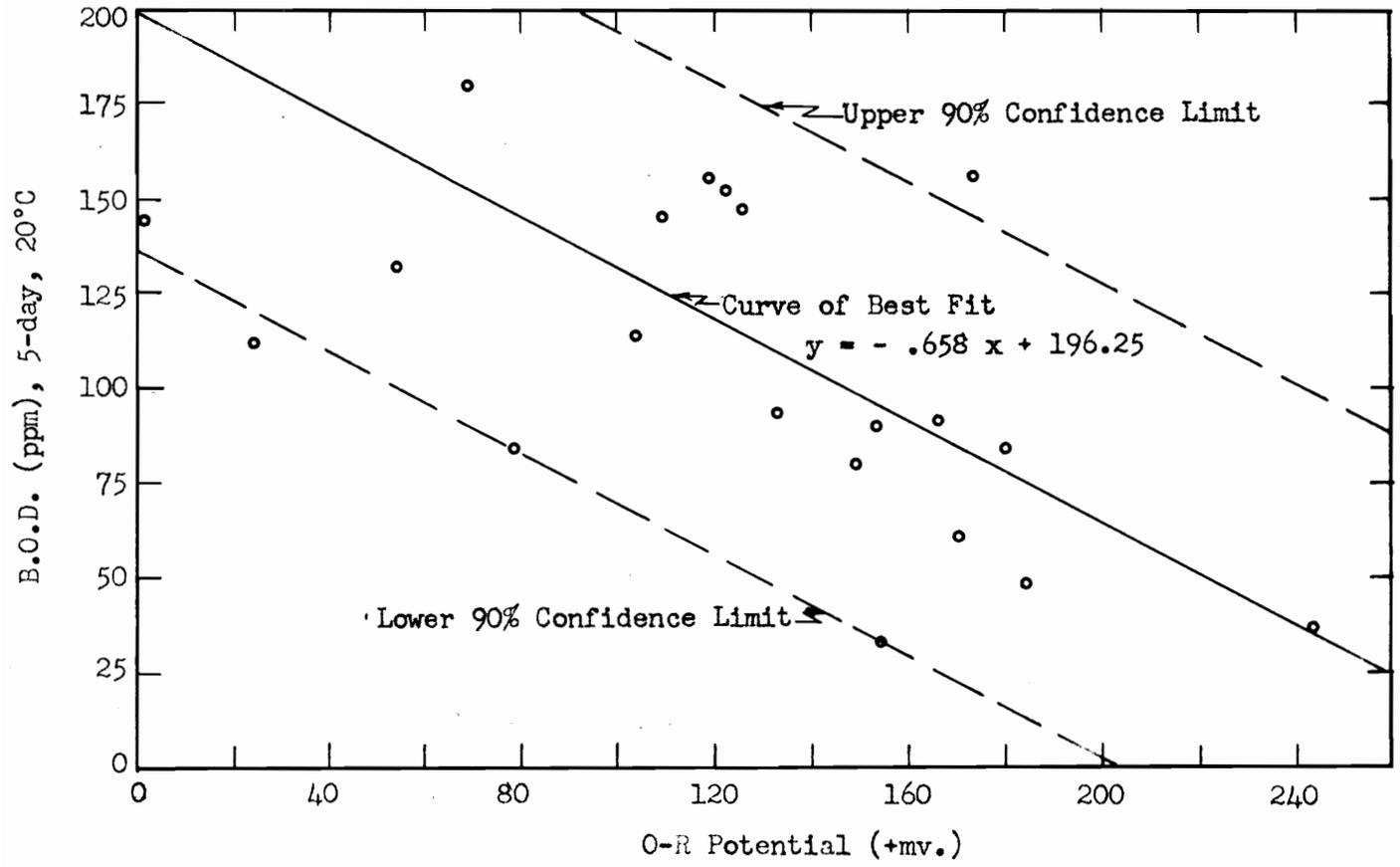


FIGURE 8.
 THE RELATION OF B.O.D. TO FIELD DETERMINED
 O-R POTENTIAL FOR TESTS 21 THROUGH 40

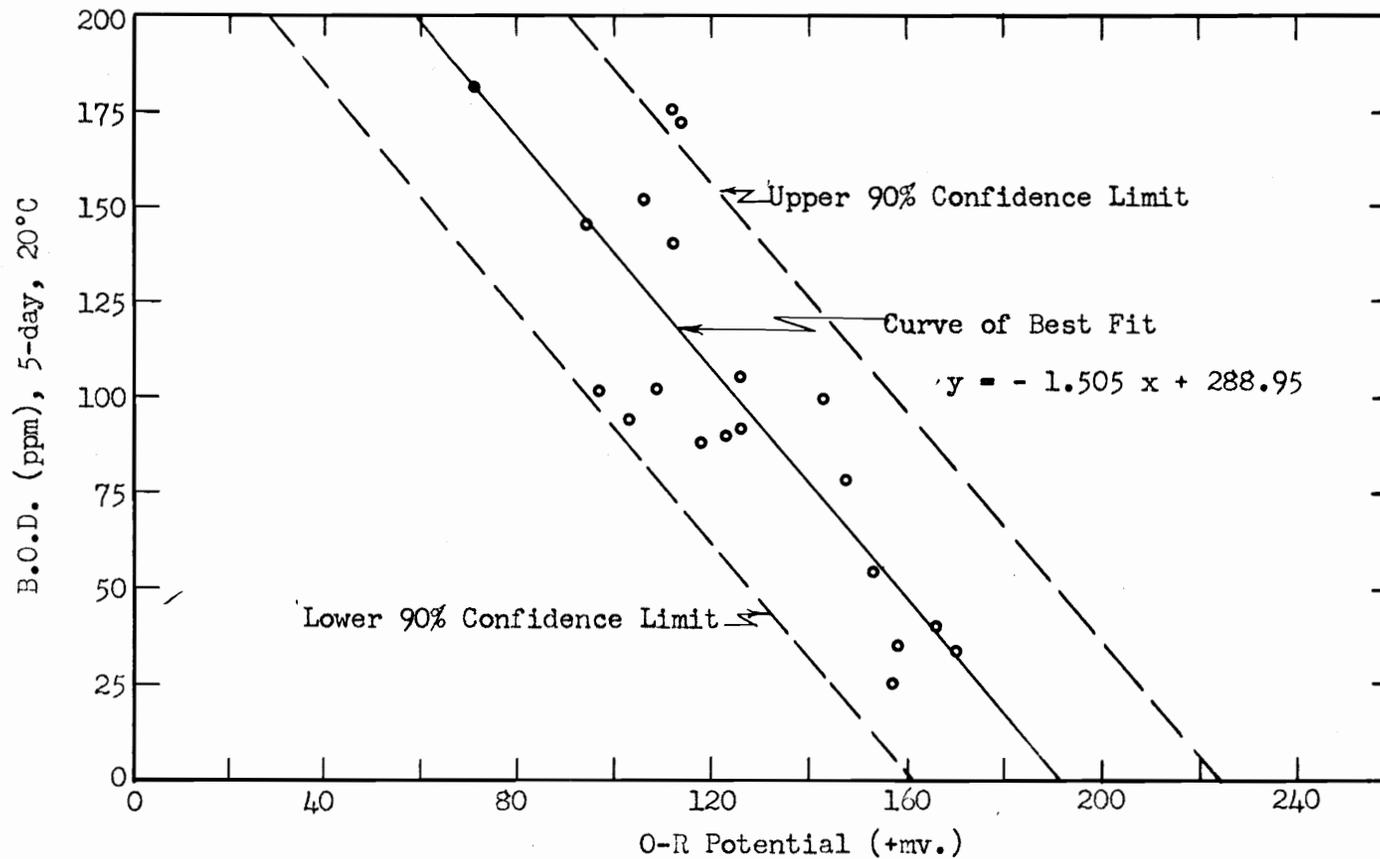


FIGURE 9.

THE RELATION OF B.O.D. TO FIELD DETERMINED
O-R POTENTIAL FOR TESTS 41 THROUGH 60

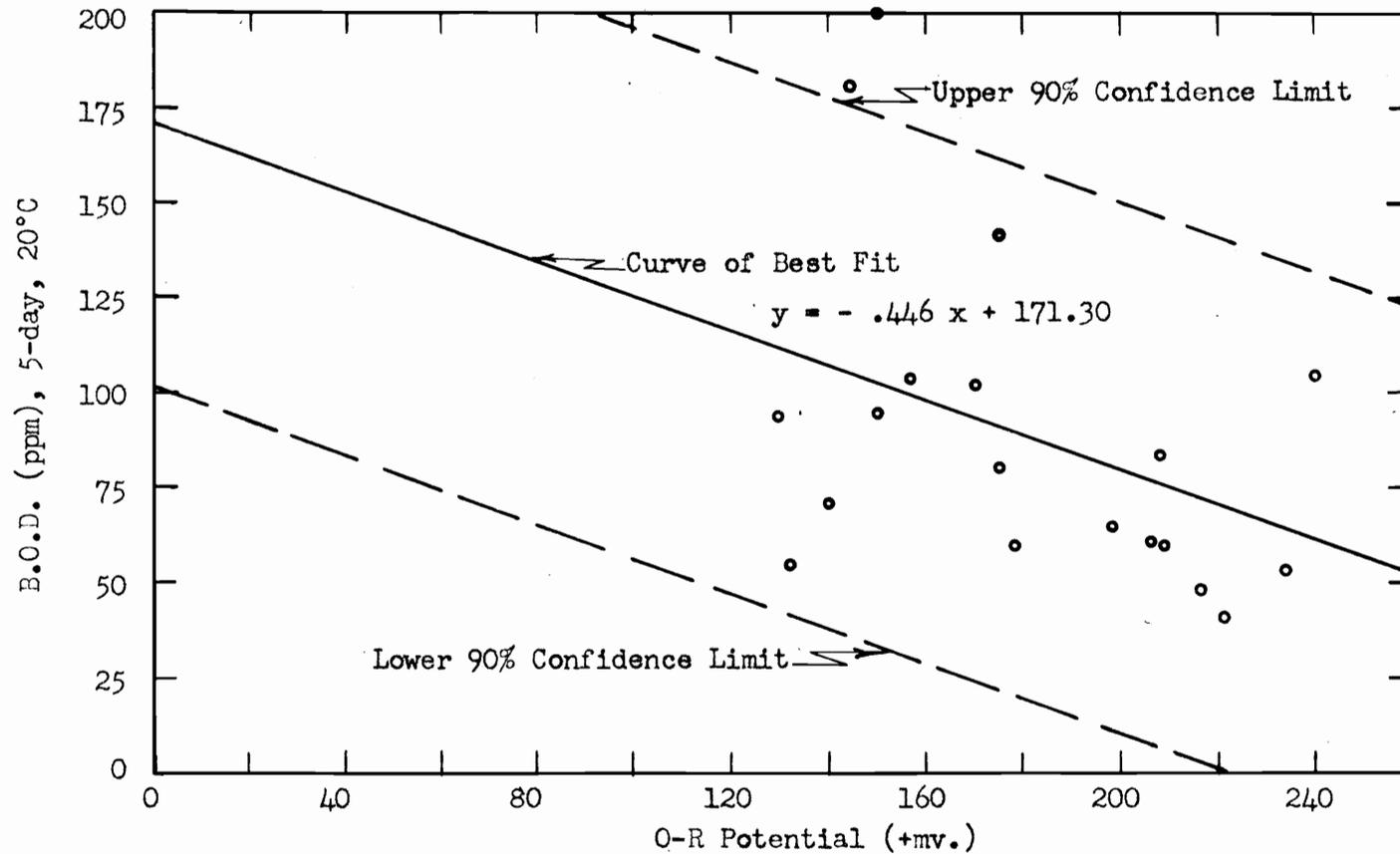


FIGURE 10.

THE RELATION OF B.O.D. TO LABORATORY DETERMINED
O-R POTENTIAL FOR TESTS 1 THROUGH 20

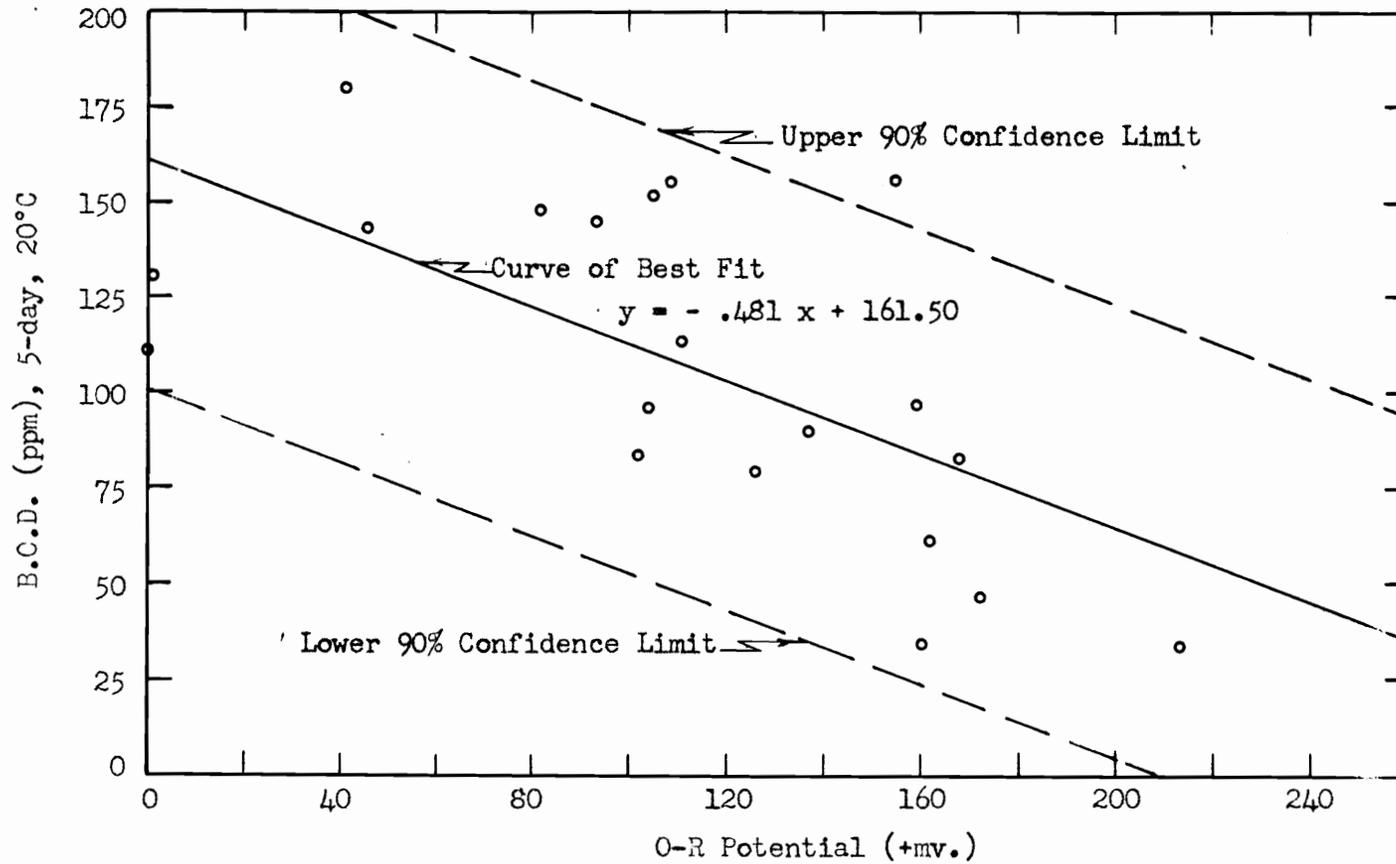


FIGURE 11.

THE RELATION OF B.O.D. TO LABORATORY DETERMINED
O-R POTENTIAL FOR TESTS 21 THROUGH 40

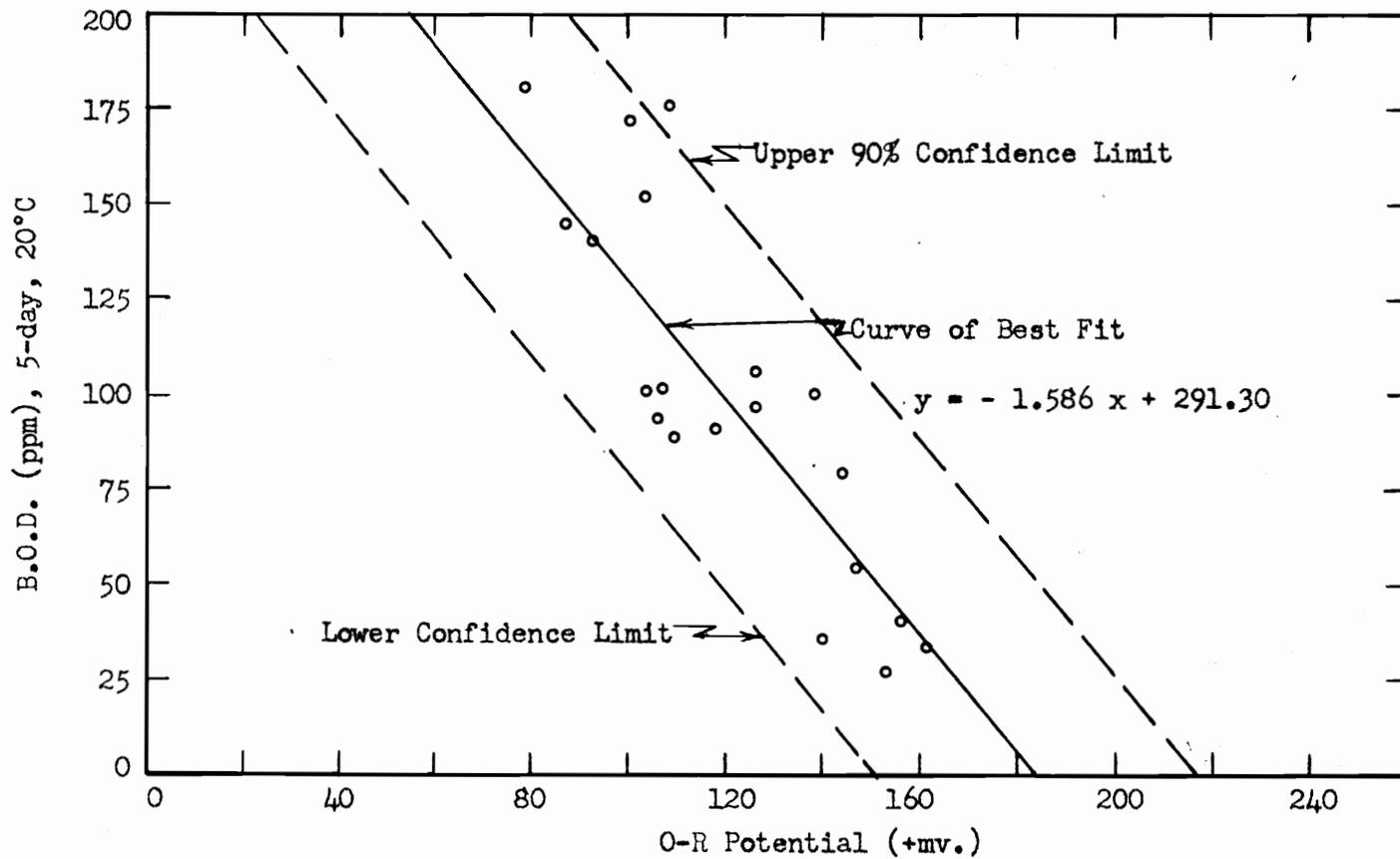


FIGURE 12.

THE RELATION OF B.O.D. TO LABORATORY DETERMINED
O-R POTENTIAL FOR TESTS 41 THROUGH 60

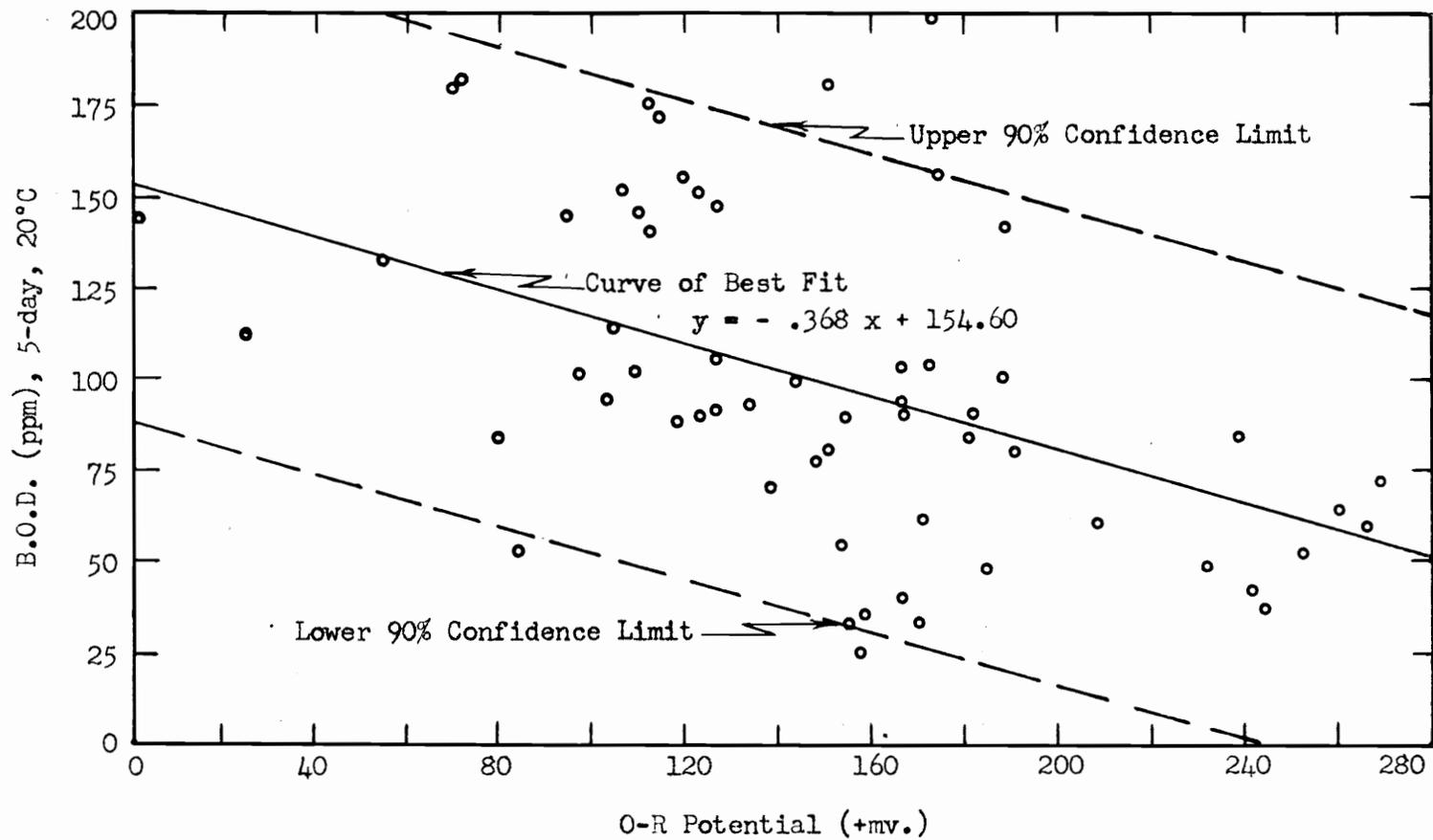


FIGURE 13.

THE RELATION OF B.O.D. TO FIELD DETERMINED
 O-R POTENTIAL SHOWING CURVE OF BEST FIT

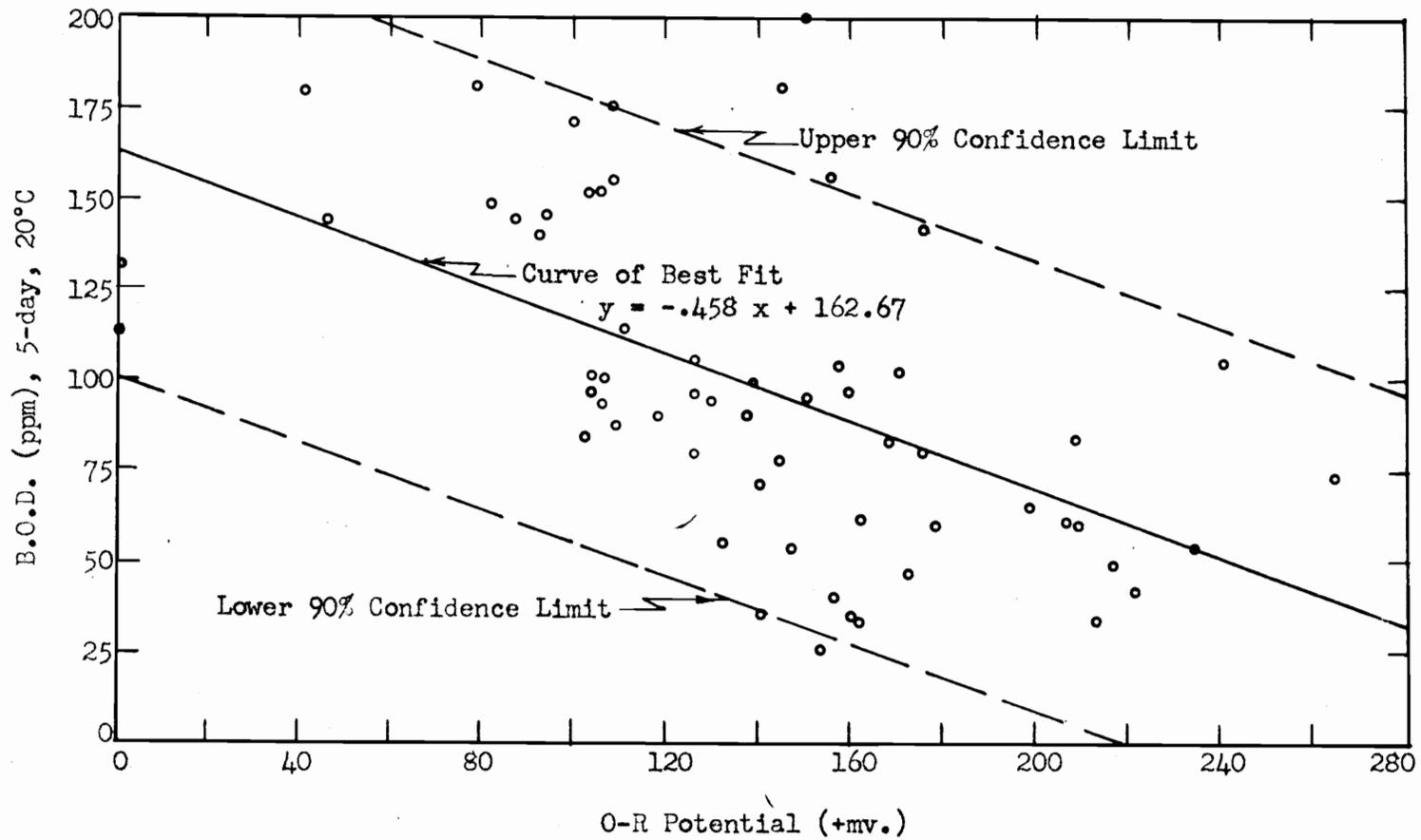


FIGURE 14.

THE RELATIONSHIP OF B.O.D. TO LABORATORY DETERMINED
O-R POTENTIAL SHOWING CURVE OF BEST FIT

V. DISCUSSION OF RESULTS

The results obtained from the statistical analyses of the investigation data revealed that poor correlation exists between the B.O.D. and the O-R potential of settled sewage. The field and laboratory determined O-R potentials when compared with the corresponding B.O.D. values yielded a variation of ± 65.46 p.p.m. and ± 63.04 p.p.m., respectively (Figures 13 and 14). These values were established by a 90% confidence limit.

There appeared to be a general tendency for the O-R potential to increase as the B.O.D. decreased. (Figures 1 and 2.). It was also observed that correlation between the O-R potential and B.O.D. increased with each successive series of tests. (Figures 7 through 12). Better laboratory technique was no doubt responsible for this trend toward closer correlation. The best degree of correlation (± 47.34) obtained was between the field O-R potential and B.O.D. for tests 41 through 60. (Figure 9). Generally, however, the laboratory determined O-R potentials yielded a slightly better degree of correlation than corresponding field O-R potential values. This variation can be attributed to the longer "settling" time provided by the laboratory determined O-R potential which allowed the system to become better poised. The investigation also revealed that after

all of the measurable dissolved oxygen was depleted from the sample, the O-R potential remained at a highly oxidized level for some time before it began to decrease.

It is absolutely essential to meticulously clean the electrodes prior to performing any O-R potential determination. (See Appendix C). Figure 3 graphically illustrates the manner in which variations between the field and laboratory determined O-R potentials decreased as the investigation proceeded. Both the field and laboratory O-R potentials followed a normal probability curve when plotted on normal arithmetic probability paper. (Figures 4 and 5).

The dilution water used in the B.O.D. determinations caused some difficulty when it was aerated and aged for at least a week. The B.O.D. values when plotted on arithmetic probability paper followed a normal probability curve (Figure 6).

VI. CONCLUSIONS

Based on the results of the statistical and graphical treatment of the research data obtained on settled sewage, the following conclusions are drawn:

1. Insufficient correlation exists between O-R potential and B.O.D. for the former test to replace the latter as a measure of the concentration of organic material present in sewage.

2. The O-R potential offers possibilities as a measure of the state of oxidation even when the dissolved oxygen concentration is low.

3. Results of the investigation followed closely the principles of O-R potential established by Rohlich and Hood(5).

VII. SUMMARY

The purpose of this investigation was to determine whether correlation exists between the O-R potential and the biochemical oxygen demand of settled sewage. The source of the samples was the effluent end of the primary clarifier at the V.P.I. Sewage Disposal Plant.

The investigation consisted essentially of performing the standard biochemical oxygen demand determination and the oxidation-reduction potential determination on similar sewage samples. The data obtained were statistically analyzed and graphically presented. The standard deviation was ascertained and a ninety percent confidence limit was established.

The final results based on statistical methods of analysis show that no valid correlation exists between O-R potential and B.O.D. of settled sewage.

VIII. ACKNOWLEDGMENTS

The author would like to take this opportunity to express his deepest appreciation to his thesis advisor, Dr. L. G. Rich, for constant encouragement, prudent guidance, and constructive criticism; to Mrs. Elma LaVecchia for her excellent and untiring typing assistance; to the V.P.I. Sewage Treatment Plant operators for the use of their laboratory and some equipment; and to my family whose confidence, faith, and assistance have made this thesis possible.

IX. BIBLIOGRAPHY

- (1) Clark, W. M., The Determination of Hydrogen Ions, 3rd Edition, 367-403, The Williams and Wilkins Company, Baltimore, 1928.
- (2) Hewitt, L. F., Oxidation-Reduction Potentials in Bacteriology and Biochemistry, 6th Edition, E. and S. Livingstone, Ltd., Edinburgh, 1953.
- (3) Rohlich, G. A., Sarles, W. B., and Kessler, L. H., "Oxidation-Reduction Potentials in Activated Sludge". A Symposium on Hydrobiology, University of Wisconsin Press, 288-302.
- (4) Hood, J. W., "Measurement and Control of Sewage Treatment Process Efficiency By Oxidation-Reduction Potential", Sewage Works Journal.
- (5) Hood, J. W., and Rohlich, G. A., "Oxidation-Reduction Potentials in Sewage Treatment", Water and Sewage Works, 96, 352-354, 1949.
- (6) Porter, J. R., "Bacterial Chemistry and Physiology", 2nd Printing, 51, John Wiley and Sons, Inc., 1947.
- (7) Faison, F. A., "Correlation of Biochemical Oxygen Demand with Oxidation-Reduction Potential", Master's Thesis, V.P.I., 1952, Sanitary Engineering Division Library.

- (8) Standard Methods for the Examination of Water and Sewage, American Public Health Association, 9th Edition, 1946.
- (9) Instructions for the Beckman Model N pH Meter, Beckman Instruments, Inc., South Pasadena, California.
- (10) Moggio, W. A., and Burland, P. D., "Standardization of N/40 Sodium Thiosulfate Solution with Saturated Solutions of Dissolved Oxygen", Sewage and Industrial Wastes, 23, 1243, 1951.
- (11) Imhoff, K., and Fair, G. M., Sewage Treatment, 5th Printing, John Wiley and Sons, New York, 1949.
- (12) Gainey, P. L., and Lord, T. H., Microbiology of Water and Sewage, Prentice-Hall, Inc., New York, 1952.

X. VITA

The author was born in Petersburg, Virginia on May 29, 1928. He graduated from Petersburg High School in June 1945 and began his undergraduate studies in Civil Engineering at the Virginia Polytechnic Institute in the fall of that year. He graduated from the Institute in June 1949 with a Bachelor of Science degree in Civil Engineering. Upon graduation, he began work on a Master of Science degree in Sanitary Engineering and continued this study until the summer of 1950 when he resigned to enter service. He entered service in the Corps of Engineers, U. S. Army and completed two years of active duty. Upon termination of the tour, he reenrolled at the Institute in order to complete the requirements for the Master of Science degree in Sanitary Engineering.

Henry B. Thacker

XI. APPENDICES

Appendix A

Apparatus and Chemicals Employed

I. Apparatus Employed:

a. In the Main Laboratory:

1 Balance, analytical
 1 Balance, triple beam
 1 Barometer, mercurial, precision, 50 ml
 Beakers, Griffin low form, 2500 ml
 Bottles, storage, miscellaneous size
 4 Bottles, dropping
 1 Bottle, washing
 1 Burette, 50 ml
 1 Burner, Fisher, high temperature
 1 Cylinder, graduated, 1 liter
 1 Cylinder, graduated, 250 ml
 1 Cylinder, graduated, 50 ml
 Filter paper
 1 Funnel, 8 inch
 4 Flasks, Erlenmeyer, 250 ml
 1 Flask, Erlenmeyer, 1000 ml
 1 Flask, Erlenmeyer, 4000 ml
 Glass tubing
 pH, comparator, Hellinge, Model
 Incubator, B.O.D.
 1 Mortar, Pestle, 100 mm dia.
 Pipettes, from 1 ml to 200 ml
 Rubber stoppers
 Rubber tubing
 1 Spatula, 6-inch blade
 1 Support, burette
 1 Thermometer, centigrade
 1 Tripod
 Miscellaneous other normal laboratory apparatus
 Aeration apparatus

b. In the "Field and Field Laboratory":

1 Beaker, Griffin low form, 1000 ml
 1 Beaker, Griffin low form, 50 ml
 3 Bottles, storage, 20 liters
 1 Bottle, sampling, 3 liters
 1 Bottle, aspirator, 4 liters
 4 Bottles, dropping, 125 ml
 1 Bottle, washing
 Approx. 100 Bottles, B.O.D., 300 ml
 1 Burette, automatic, 32 oz-bottle

- 4 Cylinders, graduated, 1 liter
- 4 Flasks, Erlenmeyer, 250 ml
- Glass tubing
- 3 Pipettes, 2 ml
- 1 Pipette, 4 ml
- 1 Pipette, 10 ml
- 1 Pipette, 25 ml
- 1 Pipette, 50 ml
- 1 Pipette, 100 ml
- 1 Pipette, 200 ml
- 1 Pr. Rubber gloves
- Rubber tubing
- 1 Thermometer (°C)
- 1 pH meter, Beckman, Model N-2
- 2 Glass electrodes
- 2 Calomel electrodes
- 3 Platinum electrodes
- 1 Electrode stand
- 1 Three-way switch
- Cleansing tissue
- 1 Beckman 700 terminal connector

II. Chemicals Employed:

a. In the Main Laboratory:

- Manganous sulfate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$)
- Potassium hydroxide (KOH)
- Potassium iodide (KI)
- Sodium azide (NaN_3)
- Soluble starch
- Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$)
- Chloroform
- Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)
- Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)
- Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)
- Potassium acid phosphate (KH_2PO_4)
- Sodium hydroxide
- Ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$)
- Concentrated sulfuric acid (H_2SO_4)
- Nitric acid
- Distilled water
- Manganous sulfate solution
- Alkaline-iodide-sodium azide solution
- Starch solution
- Standard sodium thiosulfate

b. In the Field and Field Laboratory:

Manganous sulfate solution
 Alkaline-iodide-sodium azide solution
 Concentrated sulfuric acid
 Starch solution
 Standard sodium thiosulfate
 Nitric acid
 Saturate KCl solution
 Distilled water
 Standard dilution water
 Beckman pH 7.00 buffer solution

Appendix B

Preparation of Reagents (8)

(1) Nitric acid solution (10%):

Used in cleaning the platinum electrodes in the O-R Potential determination.

Ten (10) milliliters of concentrated nitric acid were added to ninety (90) milliliters of distilled water.

(2) Ferric Chloride solution:

Used in the preparation of the standard diluting water for the B.O.D. determinations.

Twenty-five hundredths (0.25) grams of ferric chloride ($\text{Fe Cl}_2 \cdot 6\text{H}_2\text{O}$) were dissolved in one (1) liter of distilled water.

(3) Calcium chloride solution:

Used in the preparation of the standard diluting water for the B.O.D. determinations.

Twelve and seventy-nine hundredths (12.79) grams of $\text{Ca Cl}_2 \cdot 2\text{H}_2\text{O}$ were dissolved in one liter of distilled water.

(4) Magnesium sulfate solution:

Used in the preparation of the standard diluting water for the B.O.D. determinations.

Ten (10.0) grams of $Mg SO_4 \cdot 7H_2O$ were dissolved in one liter of distilled water.

(5) Phosphate buffer stock solution:

Used in the preparation of the standard diluting water for the B.O.D. determinations.

Thirty-four (34) grams of potassium acid phosphate (KH_2PO_4) were added to five hundred (500) milliliters of distilled water.

Approximately one hundred and seventy-five (175) milliliters of one normal sodium hydroxide (1N NaOH), more or less, was added until a pH of 7.2 was reached.

One and five tenths (1.5) grams of ammonium sulfate ($(NH_4)_2 SO_4$) was added and the entire solution was diluted to one liter with distilled water.

(6) Standard Dilution water:

Used in the preparation of the samples for the B.O.D. determinations.

To each carboy of distilled water, containing approximately twenty (20) liters, the following reagents were added in the stated quantities: ten (10) milliliters of the ferric chloride solution; fifty (50) milliliters of the calcium chloride solution; and twenty-five (25) milliliters of the phosphate buffer starch solution.

The water was then aerated for twenty-four (24) hours and aged for several days before using. Two (2) milliliters of sewage were added to each liter of the standard dilution water as each separate B.O.D. determination was performed.

(7) Manganous sulfate solution:

Used in the Dissolved Oxygen determinations.

Three hundred and sixty-four (364) grams of $MnSO_4 \cdot H_2O$ were dissolved in distilled water, filtered and then made up to one liter.

(8) Alkaline-iodide-sodium azide solution:

Used in the Dissolved Oxygen determinations.

Seven hundred (700) grams of potassium hydroxide (KOH), one hundred and fifty (150) grams of potassium iodide (KI), and ten (10) grams of sodium azide (NaN_3) were dissolved separately in small quantities of distilled water. The solutions of potassium hydroxide and iodide were mixed, diluted to nine hundred and fifty (950) milliliters and were cooled to room temperature. Then ten (10) milliliters of sodium azide were mixed with forty (40) milliliters of distilled water and then it was added slowly, with constant stirring to avoid local heating, and the entire solution made up to one (1) liter.

(9) Concentrated sulfuric acid:

Used in the Dissolved Oxygen determinations.

Approximately two hundred (200) milliliters of concentrated sulfuric acid, with a specific gravity of 1.83 to 1.84, were placed in a reagent bottle in order to facilitate its use in the field.

(10) Starch solution:

Used in the Dissolved Oxygen determinations.

A starch solution of approximately 0.5% was prepared by adding five (5) grams of soluble starch to a small amount of distilled water in a mortar and then it was ground to a thin paste. This was poured into one (1) liter of boiling distilled water, stirred and allowed to cool. The resulting solution was allowed to settle overnight and the clear supernatant was drawn off into a reagent bottle. The solution was preserved by adding one and twenty-five hundredths (1.25) grams of salicylic acid to the liter of starch solution prepared.

(11) Stock sodium thiosulfate solution:

Used in the preparation of the standard sodium thiosulfate solution.

Twenty-four and eighty-two hundredths (24.82) grams of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ were dissolved in one (1) liter of distilled water; this resulted in a solution of 0.1N sodium thiosulfate. Five (5) milliliters of chloroform were added to each liter in order to preserve the solution.

(12) Standard sodium thiosulfate solution:

Used in the Dissolved Oxygen determination.

Two hundred and fifty (250) milliliters of 0.1N stock sodium thiosulfate solution were diluted to one (1) liter by adding freshly boiled distilled water.

Appendix C

Instructions for the Use of the Beckman Model N-2 pH Meter⁽⁹⁾

Both the pH and the Oxidation-Reduction potential of the sewage was measured with a Beckman Model N-2 pH Meter using, of course, the proper electrodes. It is the purpose of this appendix to briefly list the procedure followed together with a brief discussion of electrode maintenance as they affected the thesis research determinations.

A. Buffer Standardization.

Using the General Purpose Glass Electrode and the Fiber type Reference Electrode the following procedure was for standardization:

1. The Range Switch was turned to the Check position.
2. The electrodes were rinsed with distilled water and immersed in a beaker of buffer solution of known pH.
3. The Range Switch was turned to the 0-R or 6-14 position depending on the pH of the buffer.
4. The temperature of the buffer solution was determined and the Temperature Compensator was set at this value.

5. The Standardization Central (STD) was turned until the meter needle indicated the pH of the buffer solution at the temperature of measurement.

6. The Range Switch was then turned to the Check position and the Check Control (CHK) turned until the Check pointer coincided with the meter needle. This allowed checks to be made routinely without buffer standardization since it was necessary to merely bring the meter needle to coincide with the check points if any slight drift was noticed. If the drift was appreciable, the buffer standardization was repeated.

B. pH Measurements:

Using the General Purpose Glass Electrode and the Fiber Type Reference Electrode, the following procedure was used for pH determinations:

1. The electrodes were rinsed with distilled water and wiped dry with absorbent tissue. They were then immersed in the sample placed in a beaker.

2. The temperature of the sample was determined and the Temperature Compensator was set at that value.

3. The Range Switch was then turned to the proper pH range, i.e., 0-8 or 6-14, and the pH of the sample was read and recorded.

C. O-R Potential Measurements:

Using the Fiber Type Reference Electrode and a metallic electrode, the following procedure outline was used in the oxidation-reduction potential measurements:

1. The Temperature Compensator was set at 29°C regardless of the temperature of the sample. The Range Switch was then turned to the Check Position and the meter needle was made to coincide with the 7.00 pH value used by the Standardization Control.

2. The electrodes were then rinsed with distilled water and wiped dry with absorbent tissue. The electrodes were next immersed in the sample. The Range Switch was turned to the proper range and the value on the pH scale was recorded. A reading of 7.00 pH indicated 0 mv; readings above 7.00 pH indicated negative voltage; and readings below 7.00 indicated positive voltage.

3. The following formula was used to convert pH scale reading to millivolts:

$$(7.00 - \text{pH scale reading}) = \text{MV}$$

For example:

$$\begin{aligned} &\text{Assume a pH scale reading of 10.0} \\ &(7.0 \text{ pH scale reading}) \quad 6.00 = \text{mv} \\ &(7.0 - 10.0) \quad 60.0 = (-3) \quad 60.0 = -180 \text{ mv.} \end{aligned}$$

D. Care of the Electrodes and Operating Precautions:

In general extreme care was taken in using each type of electrode and the specific instructions pertaining to each electrode were closely followed.

1. The Beckman Fiber Type Reference Electrode was prepared for service in the following manner: the rubber tip and rubber plug were removed from the electrode allowing

the atmosphere to reach into the electrode. The electrode was then washed with distilled water and wiped dry with absorbent tissue. If the KCl solution needed replenishing, additional amounts were added with a dropper. For temporary storage the electrode was immersed in a KCl solution in a small beaker, and for longer periods the electrode was removed from solution, cleaned and the rubber tip replaced closing the fiber end from the air. After each measurement the electrode was cleaned as above and at no time was the sample allowed to dry on the glass.

2. The General Purpose Glass Electrode:

The new electrode was soaked in a buffer solution for eight hours prior to use and during the first two days frequent checks were made to note any lack of stability. The electrode was stored for short periods of time immersed in a beaker of buffer solution, and for longer periods it was cleaned, dried, and returned to its box. The glass electrode was cleaned with distilled water and wiped dry with absorbent tissue. Here also, as in the care of the reference electrode, the glass electrode was carefully cleaned after each determination and never was any sample left to dry on the exterior of the electrode body. Precautions were taken to be careful also never to hit the delicate glass membrane at the base of the electrode particularly against the bottom of the sampling beaker.

3. The Platinum Electrode:

Prior to use each day the platinum electrodes were rinsed in a ten (10) percent solution of nitric acid and carefully washed with distilled water, then dried with absorbent tissues. The same care was shown the use of this electrode as was shown the reference electrode in that the platinum electrode was washed and dried both prior to each determination and immediately after each. If erratic results became apparent during the day, the electrodes were scrubbed with a mild, abrasive cleaner, Bon-Ami. Since three different platinum electrodes were used on each determination the drift of any one of the three could be immediately noted and corrected by one of the methods listed above. The electrodes were rinsed with distilled water, dried and were left in the electrode rack exposed to the air for any storage period since they are by their very nature inert metallic electrode.

The following rules were used to prevent injury to the electrodes in their use with the pH meter:

1. The electrodes were never removed from solution until the Range Switch was turned to either the Check or Off position.

2. The electrodes were carefully cleaned prior to immersion in the sample and immediately after the reading was noted.

3. The instrument and electrodes were checked periodically to insure proper functioning and if any deviations were noted they were promptly corrected either by standardization, cleaning, or replacement.

4. The instrument was always turned off to conserve the batteries if a delay period longer than three (3) minutes was expected.

Appendix D

Correction Factor for the Standardization of 0.025N Sodium Thiosulfate Solution With Saturated Solution of Dissolved Oxygen (10)

The method outlined herein is based upon the forced saturation with oxygen of distilled water under given atmospheric pressure, temperature, and time of aeration as a standardization medium for the standard sodium thiosulfate solutions used in the Winkler method dissolved oxygen determination. In brief, it is a method based upon the reverse application of the Winkler method where the amount of dissolved oxygen in the water is known and the concentration of the sodium thiosulfate is unknown. Due to the relatively small solubility of oxygen in water, the method is limited to concentrations of N/10 or less.

One and one-half liters of distilled water were aerated vigorously for at least thirty (30) minutes to assure saturation with dissolved oxygen. The temperature of the aerated

distilled water and barometric pressure was observed and recorded at the end of the aeration period. Upon completion of the aeration period, three standard B.O.D. bottles were immediately filled by siphoning with the aerated water. The stopper was inserted in the bottles after filling and upon being certain that no bubbles of air were entrained in the water. The sodium azide modification of the Winkler method was then used to determine the dissolved oxygen present as determined with the given sodium thiosulfate reagent. All precautions used in the normal Winkler method were employed in the above determination. The correction factor for the sodium thiosulfate was determined by the formula below using the data in the included table, and that observed in the above procedure.

$$N = 0.025 \times \frac{P_s}{V} \times \frac{P_o}{P_s} \times \frac{V}{200}$$

Where:

O_s = D.O. saturation (ppm) at the observed temperature (PC) and 760 mm pressure. From the table on D.O. values.

P_s = 760 mm

P_o = Observed barometric pressure (mm)

N = Normality of sodium thiosulfate solution

V = Corrected volume of original sample titrated (ml)

v = Volume of thiosulfate solution used for titration

f = Correction factor.

SATURATION VALUES OF DISSOLVED OXYGEN IN
 PARTS PER MILLION, FOR TEMPERATURES FROM
 15° TO 30° C. (FROM TABLE BY STREETER (2))
 UNDER NORMAL ATMOSPHERE AT 760 MM. PRESSURE

Temp. °C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
15	10.15	10.13	10.11	10.09	10.07	10.05	10.03	10.01	9.99	9.97
16	9.95	9.93	9.91	9.89	9.87	9.85	9.82	9.80	9.78	9.76
17	9.74	9.72	9.70	9.68	9.66	9.64	9.62	9.60	9.58	9.56
18	9.54	9.52	9.50	9.48	9.46	9.44	9.43	9.41	9.39	9.37
19	9.35	9.33	9.31	9.30	9.28	9.26	9.24	9.22	9.21	9.19
20	9.17	9.15	9.13	9.12	9.10	9.08	9.06	9.04	9.03	9.01
21	8.99	8.98	8.96	8.94	8.93	8.91	8.89	8.88	8.86	8.85
22	8.83	8.81	8.80	8.78	8.77	8.75	8.74	8.72	8.71	8.69
23	8.68	8.66	8.65	8.63	8.62	8.60	8.59	8.57	8.56	8.54
24	8.53	8.51	8.50	8.48	8.47	8.45	8.44	8.42	8.41	8.39
25	8.38	8.36	8.35	8.33	8.32	8.30	8.28	8.27	8.25	8.24
26	8.22	8.20	8.19	8.17	8.16	8.14	8.13	8.11	8.10	8.08
27	8.07	8.05	8.04	8.02	8.01	7.99	7.98	7.96	7.95	7.93
28	7.92	7.90	7.89	7.87	7.86	7.84	7.83	7.81	7.80	7.78
29	7.77	7.75	7.74	7.73	7.71	7.70	7.69	7.67	7.66	7.64
30	7.63	-	-	-	-	-	-	-	-	-

Sample calculations:

$$N = 0.025 \times \frac{7.29}{7.03} \times \frac{705.7}{760.} \times \frac{204}{200} = .02455$$

$$N = .0246$$

Therefore:

$$"f" = \frac{.0246}{.0250} = .984$$

Appendix E

Dissolved Oxygen Determination by the Alsterberg Modification of the Winkler Method (8)

To a full sample in a three hundred (300) milliliter B.O.D. bottle, the following reagents were added in the order given and in the quantities as shown by use of pipettes: two (2) milliliters of manganous sulfate solution (introduced just under the liquid surface), and two (2) milliliters of alkaline-iodide-sodium azide solution (also introduced just under the liquid surface). The sample was then inverted and shaken thoroughly; the precipitate was then allowed to settle half-way of bottle. The sample was shaken again and allowed to settle. Two (2) milliliters of concentrated sulfuric acid (H₂SO₄) were added to the sample above the surface by means of a pipette and the solution was again shaken thoroughly.

Two hundred and four (204) milliliters (corrected volume) of this sample were transferred to a two hundred and fifty (250) milliliter Erlenmeyer flask by means of a two hundred

(200) milliliter and a four (4) milliliter pipette. The corrected volume was found as given below:

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

The sample was then titrated to colorless with the standard sodium thiosulfate solution using the starch solution as an indicator. The total number of milliliters of thiosulfate used was recorded and the quantity of dissolved oxygen present was calculated in parts per million (p.p.m.).

Sample calculation for dissolved oxygen:

$$\begin{aligned} \text{D.O. (ppm)} &= \frac{200}{204} \times (\text{ml. thio. used}) ("f") \\ &= \frac{200}{204} \times 2.04 \times .984 \end{aligned}$$

$$\text{D.O.} = 1.97 \text{ ppm.}$$

Appendix F

Biochemical Oxygen Demand Sample Calculations (8)

The data relative to B.O.D. determinations were arranged in the following manner and calculations of the 5-day, 20°C B.O.D. made as illustrated:

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
0	7.62	7.22	.40	-			
$2\frac{1}{2}$	7.40	5.84	1.56	1.16	21%		
5	7.28	3.17	4.11	3.71	51%	20	74.2

Where:

Column (1) = percent sewage in the diluted sample

Column (2) = initial Dissolved Oxygen in ppm

- Column (3) = final Dissolved Oxygen in ppm
- Column (4) = Dissolved Oxygen depletion
- Column (5) = depletion of D.O. corrected by depletion attributed to the diluting water
- Column (6) = percent oxygen depleted
- Column (7) = concentration factor of the diluted sample
- Column (8) = 5-day, 20°C, B.O.D. of the sample in ppm.

Appendix G

Statistical Correlation of Data

B.O.D. vs. Field O-R

Test No.	B.O.D. Y	O-R X	x ²	XY	y ²
1	71	138			
2	65	260			
3	55	84			
4	42	241			
5	62	208			
6	62	208			
7	49	231			
8	73	269			
9	53	252			
10	102	188			
11	142	188			
12	93	181			
13	105	272			
14	84	238			
15	95	166			
16	104	166			
17	200	173			
18	182	150			
19	60	266			
20	80	190			
n = 20	Σ 1779	Σ 4069	Σ 876,009	Σ 348,895	Σ 192,545

I. Method of Least Squares for Determination of the Equation of Best Fit:

where:

- Y = observed B.O.D.
- X = field determined O-R potential
- n = number of determinations
- m = slope
- b = "Y" intercept

The Normal Equation:

1. $m \sum X + Nb - \sum Y = 0$
2. $m \sum X^2 + b \sum X - \sum XY = 0$

Substituting the proper value from the table in the normal equation:

1. $4069m + 20b - 1779 = 0$
2. $876,009m + 4069b - 348,895 = 0$

Solving the equations simultaneously:

1. $827,634.5m + 4069b - 361,848.6 = 0$
2. $876,009.0m + 4069b - 348,895.0 = 0$

$$m = - \frac{12953.6}{48374.5} = \underline{\underline{-.268}}$$

Substituting the value of the slope (m) in equation 1

$$4069 (-.268) + 20b - 1779 = 0$$

$$b = 143.5$$

Curve of Best Fit

$$\underline{\underline{Y = -.268 X + 143.5}}$$

II. Pearson's Coefficient of Correlation

where:

- r = Pearson's coefficient
- Y = observed B.O.D.
- X = field determined O-R potential
- n = number of determinations

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{n}}{\sqrt{\left[\sum X^2 - \frac{(\sum X)^2}{n} \right] \left[\sum Y^2 - \frac{(\sum Y)^2}{n} \right]}}$$

$$r = \frac{348,895 - \frac{(1779)(4069)}{20}}{\sqrt{\left[876,009 - \frac{(4069)^2}{20} \right] \left[192,545 - \frac{(1779)^2}{20} \right]}}$$

$$r = - \underline{\underline{.32085}}$$

III. Confidence Limits:

$$(\sigma_{r^2}) = \left[1 - r^2 \right] \left[\sum Y^2 - \frac{(\sum Y)^2}{n} \right] \frac{1}{n-2}$$

$$(\sigma_r)^2 = \left[1 - (-.23085)^2 \right] \left[192,545 - \frac{(1779)^2}{20} \right] \frac{1}{18}$$

$$(\sigma_r)^2 = 1709.54$$

$$\sigma_r = \pm 41.3$$

Degrees of freedom = 20 - 2 = 18

For 90% confidence at 18° of freedom

"t" value = 1.734 (obtained from tables)

$$\sigma_r \text{ for 90\% confidence} = \pm 41.3 \times 1.734 = \pm 71.61$$

The Equations of Confidence Limits:

$$\text{Upper Y} = - .268 X + 215.11$$

$$\text{Lower Y} = - .268 X + 71.89$$