APPLICATION OF THE MANOMETRIC TECHNIQUE TO A STUDY OF THE BIOCHEMICAL OXYGEN DEMAND OF A NEUTRALIZED ACID WASTE

by

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I. INTRODUCTION

The Biochemical Oxygen Demand (B.O.D.) Test is widely used by sanitary engineers to estimate the amount of oxygen necessary to stabilize the decomposing organic matter in a body of water. Daily B.O.D. values extending over a period of several days are used to trace the course of a biological oxidation reaction under different conditions and to study the rate of change in oxygen demand over varying periods of time. Although the standard dilution test (bottle method) is most often used to get a single B.O.D. reading, it is cumbersome and time consuming when used to obtain a series of daily B.O.D. values from one sample.

A manometric method has been developed which entails the use of the Warburg apparatus to obtain B.O.D. values for any period of time and which allows the oxidation rate to be charted for any time interval. Although this method will give higher B.O.D. values for the first few days of the reaction than will the standard dilution method, it has the advantage of giving values for as many time intervals as are desired simply by reading the manometer at any time.

The investigation herein described consisted of a manometric study of the effect of several variables on the B.O.D. of a neutralized acid industrial waste. Such information was desired so that the waste could be disposed of in a practical and efficient manner.

II. THE REVIEW OF LITERATURE

General

"The biochemical oxygen demand (frequently referred to as B.O.D.) of sewage, sewage effluents, polluted waters or industrial wastes is the oxygen (in parts per million by weight) required during stabilization of the decomposable organic matter by aerobic bacterial action." (1).

Although widely employed, the standard dilution method (1) for determining the B.O.D. of sewage and industrial wastes has several shortcomings, foremost among which is the necessity of dealing with high dilutions of the original waste. Certain "direct methods," however, have been developed which do not entail such high dilutions.

Development of Direct Methods for Biochemical Oxygen Demand Determinations

Adeney (3) in 1908 probably made the first attempt to apply the direct method (direct oxygen absorption technique) to determine the oxygen requirements of a stabilizing waste.

Sierp (19) in 1928 developed an apparatus wherein sewage was maintained in contact with pure oxygen, the absorption of which was measured in a calibrated eudiometer tube. Symons and Buswell (20) used the Sierp procedure while Wooldridge and Standfast (22) used the Barcroft differential manometer for measurement of the B.O.D. of sewage. In 1935, Burtle and Buswell (2) (4) used a Nordell oxygen demand apparatus (later called the odeeometer) to measure the B.O.D. of sewage.

Several other modifications of the Sierp apparatus have been made and the direct method has been used for other than B.O.D. determinations of sewage and industrial waste (9) (14).

Manometric methods for estimating exchange of gases in biological and chemical reactions have been used for many years. Dixon (8) divided manometric apparatus into three groups: (a) the Haldane gas analysis type, (b) the Barcroft and Haldane type, commonly called the Warburg apparatus, and (c) the Barcroft or differential type. The Warburg type is now being used most widely for studies of biochemical oxidation of sewage and industrial wastes. This apparatus enables the enclosed reaction system to be kept at a constant volume and temperature, and from the change in the pressure reading on the manometer the gas evolved or absorbed is calculated.

Recently, studies have been made by Caldwell and Langelier (5) on the B.O.D. of sewage, by Dawson and Jenkins (6) on the oxygen requirements of activated sludge and by Dawson and Jenkins (7) on the effect of the addition of chemicals on the oxygen uptake of activated sludge. Using manometric means, Gellman and Heukelekian (10) (12) studied the problem for formaldehyde oxidation and the effect of certain environmental factors on biochemical oxidation of wastes. Gellman and Heukelekian (11) also formulated a proposed standard method for direct oxygen measurements of sewage and industrial wastes.

Effect of Certain Variables on Manometric Biochemical Oxygen Demand

Hydrogen Ion Concentration

Dawson and Jenkins (6) found that activated sludge took up oxygen over a hydrogen ion concentration (pH) range of 4 to 13, but that the uptake below 5 and above 12 was negligible. In addition, they found the optimum pH range for oxygen uptake to be between 7 and 8 with the maximum at pH 7.4. They concluded that the oxygen uptake was less sensitive to increase in pH than to a decrease and that a drop from pH 6 to pH 5 reduced the respiratory activity of the sludge about 75 per cent.

From studies with industrial wastes, Gellman and Heukelekian (12) reported that adjustment of the pH values between 6 and 8 prevented the initial pH from affecting the oxidation process and that the optimum pH range is considerable, but is greater for some wastes than for others. Also, for some wastes the optimum pH is more on the alkaline side while for others it is on the acid side of neutrality. They noted the effect of pH was more pronounced and the range more restricted during shortened periods of incubation. Furthermore, they report that in poorly buffered wastes the tendency of the pH was to gravitate toward the acid side of the neutral point, especially when acid intermediate products were produced in the oxidation process.

Type of Seed

Gellman and Heukelekian (12) found that the origin of domestic sewage used to seed a dextrose peptone substrate had a minor influence on the rate of biochemical oxidation of the substrate. They reported also that industrial wastes containing large numbers of bacteria are not affected by variations in sewage seed origin, but that with wastes containing relatively few bacteria little oxidation is obtained without seeding.

Age of Seed

No direct reference to the effect of seed age on the B.O.D. of an industrial waste was found in the literature.

Lipman (18) showed that the bacterial content of dry soil was large and varied for a long period and that to produce active stages of bacteria the soil suspensions should be prepared at least 24 hours prior to use.

Adapted Seed

Gellman and Heukelekian (12) found that by adaptation a seed material could be produced which readily oxidized phenol concentrations as high as 2,000 ppm. With unadapted seed prolonged initial lag periods in oxidation occurred which increased with the concentration of phenol. They noted further that the initial rate of oxidation of certain industrial wastes was increased by an increase in adapted seed volume. This effect was minimized with increased periods of incubation.

Dilution Water

In their Procedure for Proposed Direct Method of Determining B.O.D., Gellman and Heukelekian (11) recommended a B.O.D., nitrogen, phosphorus ratio of 60 to 3 to 1 in order to develop properly the B.O.D. of industrial waste. When Caldwell and Langelier (5) measured the B.O.D. of an industrial waste manometrically, they added 1 ml of Normal sodium dibasic phosphate solution per liter of waste and sewage seed solution.

Dilution

It is reported that oxygen demand values (12) obtained by the direct method for sewage and wastes are somewhat higher than for the standard dilution method, the increase apparently being caused by the higher concentration of organic matter present in the flask when the direct method is used. The minimum manometric B.O.D. values of sewage occurred at a dilution of 10% and were approximately equal to the values obtained by the standard dilution method. Spent sulfite liquor gave no difference in the 21 hour B.O.D. values for dilutions from 5% to 50% when seeded with adapted seed.

Heukelekian (23) reported that the oxygen demand was relatively higher in the diluted samples than in the original waste. He concluded from this that there was an inhibiting agent in the waste which at some concentration was sufficiently dilute to permit the oxygen demand to be fully exerted. He stated further that the dilution at which the inhibiting action was counteracted depended upon the concentration and potency of the inhibiting agent. In an earlier report, Heukelekian (13) had concluded that many organic substances are oxidizable at low concentrations and toxic at high concentrations.

III. OBJECT OF INVESTIGATION

The object of this investigation was to use the manometric method to determine the effect of the following variables on the B.O.D. values of a neutralized acid industrial waste: pH, type of seed, age of seed, adapted seed, dilution water, and dilution.

IV. THE INVESTIGATION

General

The industrial waste for this study came from a large commercial plant and had the following general characteristics: (a) a low B.O.D., usually less than 100 ppm, (b) a pH of about 5, (c) continuous and almost constant flow, and (d) comprised mainly of neutralized nitric and sulfuric acids. The organic constituents of the waste and the presence or absence of materials toxic to the B.O.D. reaction were not known.

It was desired to determine the effect of different environmental conditions on the B.O.D. of the waste. This was accomplished by varying one of the reaction conditions and observing the effect of the change on the B.O.D. values while keeping the other factors of the reaction constant.

As the optimum condition for each variable was determined, it was selected as a reaction constant and the next variable investigated until the list of different conditions was completed.

The constants for the manometric B.O.D. test in the experiments were temperature and agitation. The reaction flasks were maintained at a temperature of 25° C., and were agitated at the rate of 80 oscillations per minute (opm).

The variables of the B.O.D. reaction in this investigation were pH, type of seed, age of seed, adapted seed, dilution water, and dilution.

The pH values used in the tests ranged from 4.1 to 7.1. The pH of 4.1 was used because the waste treatment process sometimes left the waste with this pH. A pH of 7.1 was selected because this was in the optimum pH range for the B.O.D. reaction as reported in the literature.

To allow the waste to exert a normal B.O.D., it was necessary to add some type of bacterial seed and to mix the waste with a diluting water which contained mineral nutrients for the bacteria. Seeds selected were river water (collected below the outfall of the waste being studied), fresh sewage, settled sewage, and garden soil.

Dilution waters employed included river water (collected below the waste outfall), demineralized tap water, and dilution water prepared by adding the nutrients recommended in Standard Methods for the Examination of Water and Sewage (1) to double distilled tap water (standard dilution water).

Seeds with ages from 6 hours to 73 days were investigated for the effect of age of seed on the B.O.D. of the waste.

The seeds used in the study of effect of adaptation of seeds on B.O.D. were river water (collected below waste outfall), activated sludge, garden soil, and adapted garden soil.

Dilutions of 10% and 50% were used to determine the effect of dilution on the B.O.D. of the waste.

Description of Apparatus

The Warburg Apparatus used in this investigation was rectangular in shape and of a size adequate to hold 10 Warburg manometers. (Plate 1). The apparatus was equipped with a shaking mechanism adjustable from 50 to 150 opm and had a shaking stroke amplitude of 0 to 4 cm. The temperature was regulated by a thermoregulator which maintained any desired temperature in

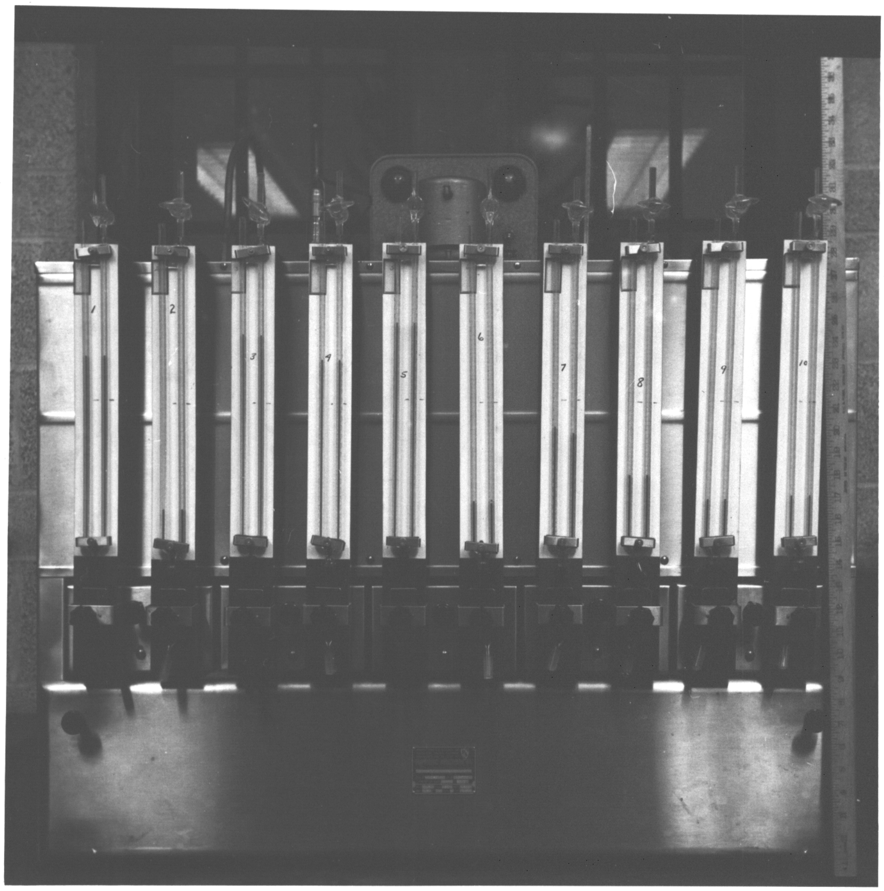

Plate 1

Warburg Apparatus

the 15 gallon water bath from room temperature to 60° C. within two-hundreths of a degree, plus or minus. The water in the water bath was kept in continuous circulation by means of a motor-driven stirrer, and raised to the desired temperature by a heating tube immersed in the water.

The shaking apparatus of the manometer had 10 dovetail sockets to hold the manometer supports. These supports had white plastic reflectors to facilitate reading, and had a built-in guard to prevent breakage of manometers while in operation. (Plate 1).

A Warburg manometer was mounted on each manometer support. The manometer was a graduated U-tube with one leg open to the atmosphere and the other leg connected to a reaction flask. The bottom of the U-tube was connected to a flexible plastic well which held the excess manometer fluid; a compression device, held in place by a clamp and a spring, could be adjusted to raise or lower the liquid level in the manometer. A three-way stopcock on the closed leg of the manometer controlled the outlet to the atmosphere. (Plate 2).

The reaction flask was connected to the closed leg of the manometer by ground glass fittings and held together by two rust-proof steel springs attached to glass hooks on the manometer and flask. The reaction flasks had a volume of approximately 20 ml. and contained a cylindrical glass well that was open at the top and fused to the bottom of the reaction flask. The flask also had a side-arm tube with an opening to the air, controlled by a ground glass insert and secured by steel springs as was the other ground glass connection. (Figure 1).

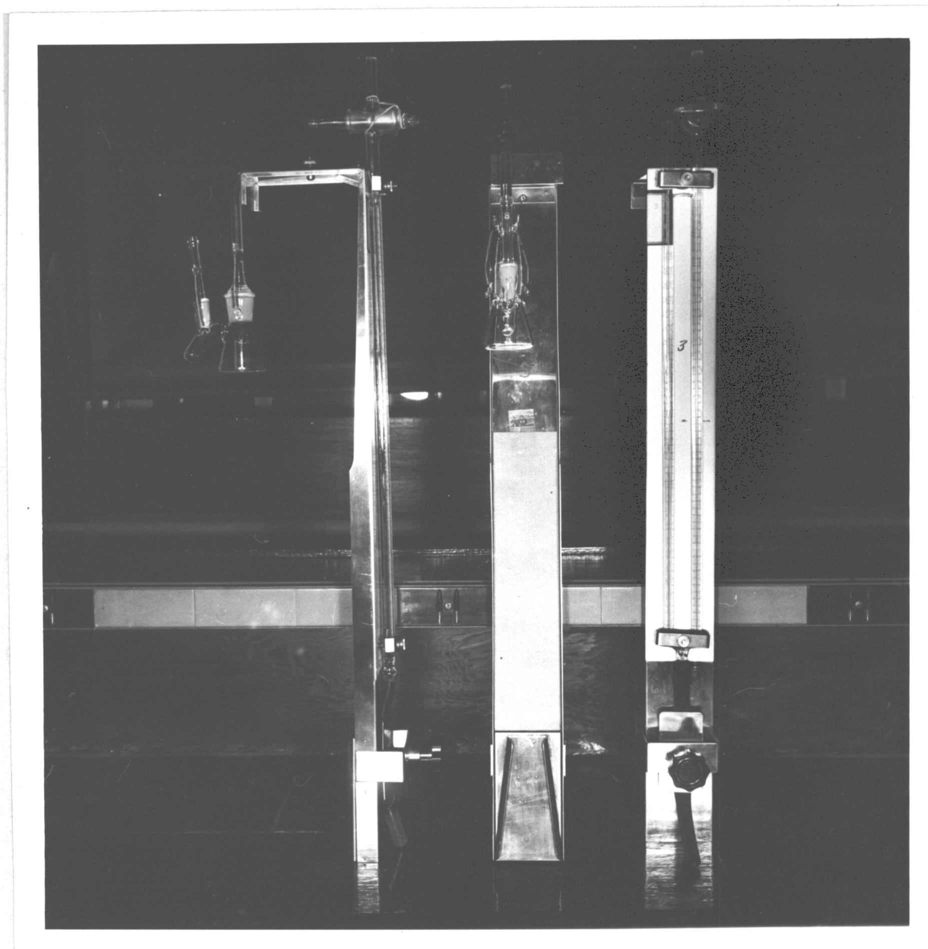

Plate 2

Warburg Manometers and Reaction Flasks

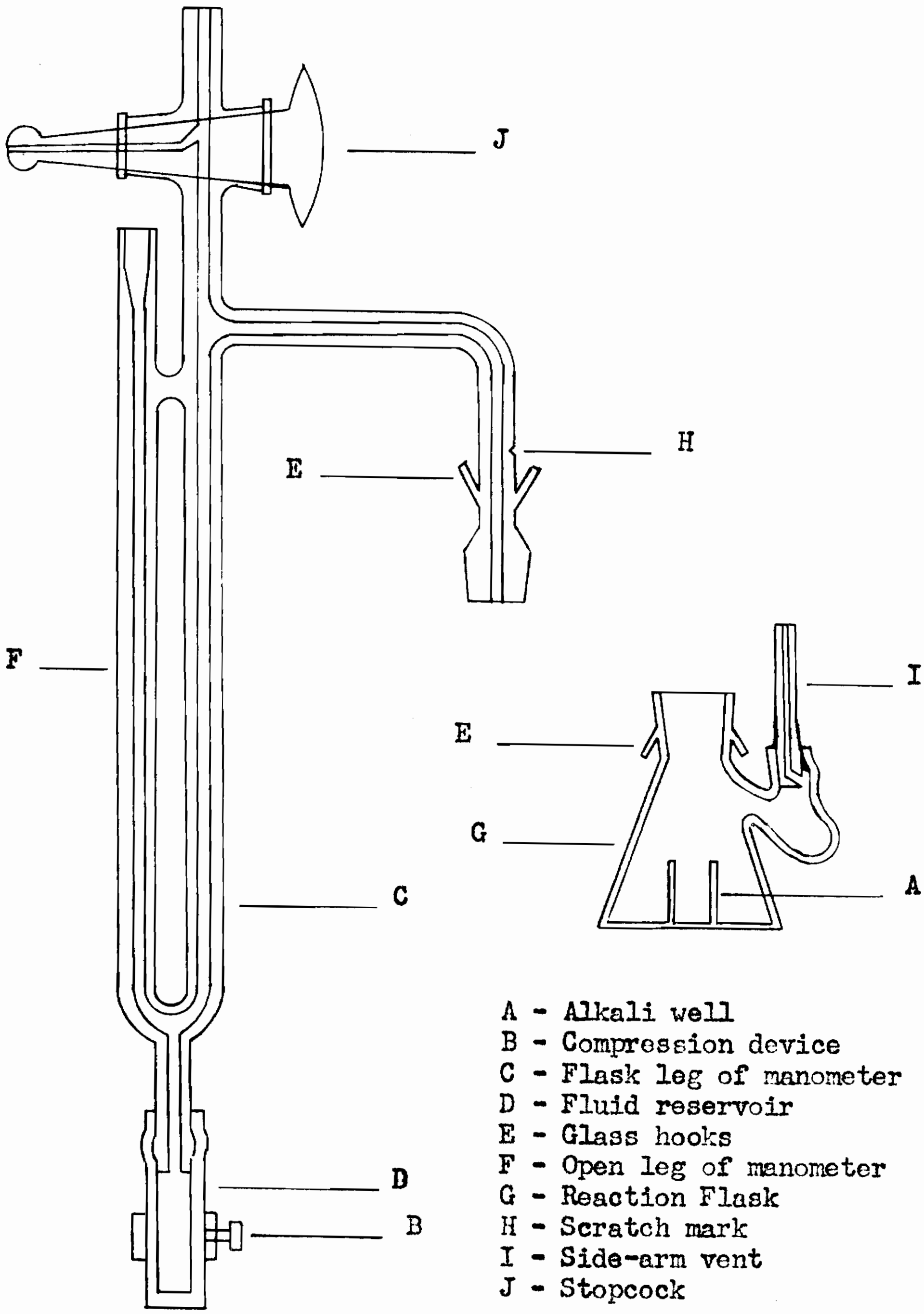

Figure 1

Warburg Manometer and Reaction Flask

Determination of Flask and Manometer Volumes

Several basic methods and variations of these methods for determining flask and manometric volumes are given in the literature. Three methods deserve special mention: (a) The method of Vogler or Krebs as described by Ludwig, et al., (17). (b) The use of a calibrator as described by Lazarow (15). (c) A variation of the mercury calibration method as described by Loomis (16).

While the first two calibration methods named above are well established and can be performed easily, it is believed that a calibration with mercury is most reliable. The calibration with mercury gives the volume for the flask and manometer separately while the other methods mentioned give only the total volume for flask and manometer. It is convenient to have separate volumes for flasks and manometers as it is often necessary to interchange the manometers and flasks because of breakage or for other reasons.

The total volume (V) of the flask-manometer system was measured in three separate steps. A scratch mark was placed on the neck of the manometer about one inch above the top of the manometer-flask joint. The volume below the scratch mark when the flask was attached was designated as the volume of the flask (Vc) while the volume above the scratch mark was designated as the manometer volume (Vm).

To Determine Vc

The empty flask was weighed on an analytical balance and then was filled with mercury. The ground glass manometer joint was firmly seated in the flask and the amount of mercury in the flask was adjusted by means of a medicine dropper until the mercury rose to the scratch mark on the manometer. The flask and mercury were then weighed and the weight of the mercury found by difference. The weight of the mercury divided by the density of mercury for its specific temperature gave the flask volume (Vc).

<u>To Determine Vm</u>

(a) The volume from the scratch mark to the 25 centimeter mark on the manometer was measured as described below:

A funnel supported by a ring stand was connected to the stopcock controlled manometer outlet by a piece of rubber tubing. A clamp was placed on the rubber tubing and the funnel was filled with about 100 ml. of clean mercury. The inverted manometer was held above the mercury level, the clamp on the rubber tube was removed, and the manometer was slowly lowered with the stopcock open until the mercury rose in one side of the manometer leg to the scratch mark and in the other leg to the 25 cm mark. The manometer stopcock was then closed one-eighth of a turn with the marking dot up. At this point, the clamp was put back on the rubber tube and the stopcock was rotated another one-eighth of a turn (in the same direction with the marking dot up and the stopcock horizontal). When the last one-eighth turn was made on the stopcock, the mercury contained in the manometer from the scratch mark to the 25 centimeter graduation point was caught in a tared container as it came from the air (side) outlet of the stopcock. The volume corresponding to this quantity of mercury was computed as before .

(b) The volume between the 25 centimeter and the 15 centimeter marks was not measured directly; however, a value was calculated for a 10 centimeter length of the manometer tube. Mercury was introduced into the closed leg of the manometer to cover a distance of about 25 centimeters on the upper part of the graduated scale. The column was read and the mercury was weighed as before. This operation was performed twice for each manometer and the volume calculated for a 10 centimeter graduated portion of the manometer.

The sum of the volume of parts (a) and (b) gave the total volume from the scratch mark to the 15 centimeter graduation of the manometer (Vm).

To Determine V

The total flask-manometer volume was found by adding the flask volume to the manometer volume. V = Vc + Vm.

Tables 1, 2, 3, and 4 give the volumes of the flask and manometer systems.

Table 1

Warburg Flask Volumes

Flask No.	Temp. C.	Density Mercury	Wt. Mercury gms	Vol. Flask ml
1	27.0	13.5291	230.8086	17.06
2	29.0	13.5242	216.4824	16.01
3	28.0	13.5266	260.0222	19.22
4	27.5	13.5279	227.1896	16.79
5	31.0	13.5193	240.6859	17.80
6	27.0	13.5291	229.9323	17.00
7	28.8	13.5247	253.2703	18.73
8	31.0	13.5193	269.6769	19.95
9	31.0	13.5193	262.6857	19.43
10	30.6	13.5203	272.4557	20.15
11	23.0	13.5389	231.1934	17.08
12	24.0	13.5364	216.5466	16.00
22	28.0	13.5266	263.7710	19.50
23	28.0	13.5266	214.7107	15.87

Table 2

Manometer Volume from Scratch Mark
to 25 cm Mark on Flask Leg

No.	Temp. C.	Density Mercury	Weight Mercury 1st Trial gms	Weight Mercury 2nd Trial gms	Weight Mercury Avg. gms	Vol. to 25 cm Mark ml
17	27.0	13.5291	6.9587	6.9502	6.9544	0.51
72	26.5	13.5303	7.7100	7.7096	7.7098	0.57
61	27.0	13.5291	8.6626	8.6630	8.6628	0.64
44	27.0	13.5291	8.0811	8.0812	8.0812	0.60
20	28.0	13.5266	8.3962	8.3964	8.3963	0.62
83	26.8	13.5297	7.8618	7.8612	7.8615	0.58
7	26.5	13.5303	7.8599	7.8598	7.8599	0.58
27	24.0	13.5364	7.8541	7.8546	7.8544	0.58
58	24.0	13.5864	8.1710	8.1704	8.1707	0.60
23	22.8	13.5395	8.4839	8.4843	8.4841	0.63
88	28.0	13.5266	7.3142	7.3143	7.3142	0.54
86	22.0	13.5413	7.5318	7.3292	7.2305	0.53
81	23.0	13.5389	6.8285	6.8321	6.8303	0.51

Table 3

Manometer Tube Volume for 10 cm Length
on Graduated Flask Leg

Man. No.	Temp. C.	Density Mercury	Range Measured cm	Length of Column cm	Weight Mercury gms	Vol. ml
17	27.0	13.5291	6.1-30.0	64.9	19.4435	0.22
72	27.0	13.5291	1.8-30.0	51.5	13.7012	0.20
61	27.0	13.5291	1.3-30.0	52.3	15.1302	0.21
44	27.0	13.5291	0.8-29.9	55.0	14.7063	0.20
20	28.0	13.5266	2.7-29.9	52.9	14.6314	0.20
83	26.8	13.5297	1.4-30.0	51.5	14.5919	0.21
7	26.5	13.5303	0.6-29.3	56.5	14.8977	0.20
27	26.5	13.5303	0.6-30.0	57.1	16.4270	0.21
58	24.0	13.5364	1.0-29.2	54.4	16.0122	0.22
23	24.0	13.5364	1.0-30.0	57.2	16.1378	0.21
86	23.0	13.5389	3.8-29.5	54.8	9.9396	0.14
81	23.0	13.5389	3.9-30.0	26.1	6.8799	0.20
88	28.0	13.5266	6.0-29.3	23.3	6.3475	0.20

Table 4

Flask and Manometer Volumes in ml

Flask No.	Man. No.	Flask Vol. to Scratch Mark on Man.	Man. Vol. - Scratch Mark to 25cm Grad.	Man. Vol. for 10cm Length	Flask Vol. plus Man. Vol. to 15cm Grad.
1	17	17.06	0.51	0.22	17.79
2	72	16.01	0.57	0.20	16.78
3	61	19.22	0.64	0.21	20.07
4	44	16.79	0.60	0.20	17.59
5	20	17.80	0.62	0.20	18.62
6	83	17.00	0.58	0.21	17.79
7	7	18.73	0.58	0.21	19.52
8	27	19.95	0.58	0.21	20.74
22	58	19.50	0.60	0.22	20.32
9	58	19.43	0.60	0.22	20.25
10	23	20.15	0.63	0.21	20.99
23	88	15.87	0.54	0.20	16.61
11	86	17.08	0.53	0.14	17.75
12	81	16.00	0.51	0.20	16.71

Preparation of Equipment

Warburg Manometers

The manometers were thoroughly cleaned by passing the following series of fluids in the order listed through all parts of the manometer tube: A detergent solution prepared by dissolving a household detergent (Tide) in hot distilled water, distilled water, 95% ethyl alcohol, ethyl ether, and dry air.

The manometers were then mounted on the manometer supports. The plastic tube that held the manometer fluid was completely filled with Brodie's Solution (Appendix A). This plastic tube was forced on the glass connection at the bottom of the U-tube, in such a manner that no air was introduced into the plastic reservoir.

The manometer stopcock and manometer were thoroughly cleaned with xylol, and dried with lens paper. A medium weight burette stopcock grease was used to make three longitudinal streaks of grease on the ground glass portion of the stopcock. The stopcock plug was firmly inserted into the stopcock barrel by a slight rotational twist. The portions of grease came together over the entire matching area without entrapping air; otherwise, the entire process was repeated.

Warburg Apparatus

The water bath of the instrument was filled with distilled water to within 1 inch of the top and the water level marked. The thermoregulator was set at 25° C., the shaking apparatus was set for 80 opm, and the Warburg instrument lubricated according to the instructions outlined in the operating manual.

Reaction Flasks

The reaction flasks were cleaned by boiling in a detergent solution for 20 minutes, and rinsed thoroughly by dipping the flasks into three successive beakers of boiling distilled water. The flasks were placed in a dry-heat oven at 103° C. for 8 hours, allowed to cool, and then wrapped in a clean lint-free towel until used.

Pipettes

The volumetric pipettes used were cleaned by washing thoroughly in a strong detergent solution and rinsing three times in distilled water. The pipettes were placed in an open pipette container and dried in the dry-heat oven at 103° C. for 8 hours.

Preparation of Waste Samples for Test

The waste samples used for the B.O.D. test were adjusted to a pH of 7, except where noted, by the addition of powdered sodium carbonate.

It was necessary to add a dilution water and bacterial seed to prepare the waste for the B.O.D. test. A 50% dilution of waste was prepared by pouring 500 ml. of neutralized waste into a graduated cylinder. Dilution water was added to the waste to bring the total volume to approximately 950 ml. One ml. of fresh sewage was added to the diluted waste and the total volume was made up to 1,000 ml. by the further addition of dilution water. Different concentrations of waste samples with various seeds were prepared in the same manner as the above samples. An exception was made when river water was used as dilution water which made the addition of bacterial seed unnecessary.

Performing the B.O.D. Test

The water level of the water bath was checked and a correction for the water volume was made, if necessary. The water bath thermoregulator was set at 25° C. and the electric switch, which controlled the thermoregulator, the stirring mechanism, and the heater in the water bath, was turned on. While the water was coming to the control temperature of 25° C., other preparations for the test were made.

The inside top edge of the alkali container in the reaction flask was coated with a layer of petroleum jelly (Vaseline) by means of a wooden splint. A calibrated medicine dropper was used to introduce 0.5 ml. of 10% potassium hydroxide solution into the alkali well, and then 4 ml. of the prepared waste sample were introduced into the reaction flask with a volumetric pipette. It was essential that the potassium hydroxide be put into the alkali well and the sample of waste be pipetted into the reaction flask without any spilling or intermingling of the liquids.

At the midpoint on the ground-glass surface of the male manometer-flask joint, three drops of sealing lubricant were deposited. (This lubricant was prepared by mixing thoroughly equal portions by volume of anhydrous lanolin and Vaseline.) The manometer joint was then forced into the ground-glass flask entrance and the flask rotated slightly until the drops of lubricant ran together. If air bubbles remained in the joint, the flask was removed from the manometer, the joint was cleaned with lens paper, and the above process repeated until correct joint connections was obtained. Two rust-proof steel springs were then attached to the glass hooks on each side of the manometer tube and the flask. The procedure used for the manometer-flask joint was also used for seating, sealing, and securing the glass inserts of the side-arm tube on the reaction flasks.

Any changes in the atmospheric pressure after the B.O.D. test was started produced a change in the manometer reading. This change of pressure was determined by using a control reaction flask or thermobarometer flask which contained only diluting water. Two thermobarometers were used for each test run.

The manometer supports which held the assembled manometers and reaction flasks were placed on the dovetail sockets of the Warburg shaking apparatus. The shaking apparatus was started, with the manometer stopcock open to the atmosphere, and allowed to run for 1 hour.

At the end of the equilibration period, the shaking apparatus was stopped and the entire manometer-reaction flask system inspected for leaks or other defects. When the inspection and adjustments were completed, the manometer stopcocks were closed and the manometer fluid was brought to the index point of 20 cm. by varying the manometer fluid reservoir adjustment. The readings on the open legs of the manometers were recorded as were the manometer numbers and the time of the readings. When the readings were completed and recorded, the shaking mechanism of the Warburg apparatus was started and allowed to run.

The B.O.D. values were determined after any elapsed time for each sample flask by stopping the shaking apparatus and adjusting the manometer fluid to the index point on the closed manometer leg. The readings on the open legs of the sample flasks and control barometers were taken and recorded

Table 5.--Virginia's civilian labor force by industry

Industry	Number of employees (Thousands)		
	1940	1950	1962
Manufacturing	187.4	225.1	291.3
Wholesale and retail trade	115.8	188.6	224.1
Government	66.0	173.5	207.4
Agriculture	223.0	167.5	117.0
Total[1/]	905.4	1,150.2	1,340.8

[1/] Total row is not a summation of four other rows

Sources: Federal Reserve Bank of Richmond, 1963.
U.S. Bureau of the Census, 1961.

Table 5

Effect of pH on B.O.D.

Warburg Test 1			
Hrs.	Types of Seed+		
	River Water	Fresh Sewage	Settled Sewage
24	2.4	0.0	0.0
48	2.4	0.0	0.0
72	2.3	0.0	0.0
96	2.2	0.0	0.0
120	3.5	0.0	0.0

Warburg Test 1			
Hrs.	Types of Seed++		
	River Water	Fresh Sewage	Settled Sewage
24	0.0	0.0	0.0
48	0.0	0.0	0.0
72	0.0	8.4	9.8
96	5.0	12.8	9.0
120	4.2	12.7	5.2

All samples had initial pH of 4.5.
+Samples were run at 50% dilution.
++Samples were run at 25% dilution.

Table 6

Effect of pH on B.O.D.

Warburg Test 2				
Hrs.	Initial pH Values			
	7.1	7.1	4.1	4.1
24	0.0	2.7	0.0	0.0
48	15.5	19.5	0.0	0.0
72	28.5	21.0	0.0	0.0
96	32.0	20.5	9.6	1.0
120	33.0	17.9	15.0	7.5

Bottle Test				
Hrs.	Initial pH Values			
	7.1	---	---	---
120	42	---	---	---

River water used as seed and as dilution water.
All samples run at 50% dilution.

Table 7

Effect of Different Seeds on B.O.D.

Warburg Test 3				
Hrs.	Type of Seed			
	River Water	Fresh Sewage	Settled Sewage	Garden Soil
24	25.6	17.8	24.8	11.9
48	86.0	68.7	65.5	65.3
72	95.0	79.2	75.6	79.5
96	101.2	83.8	80.6	87.1
120	107.8	89.3	85.0	92.7

Bottle Test				
Hrs.	Type of Seed			
	River Water	Fresh Sewage	Settled Sewage	Garden Soil
120	97	90	110	----

All sample dilutions were 50%.

Table 8

Effect of Different Seeds on B.O.D.

Warburg Test 4				
Hrs.	Type of Seed			
	River Water	Fresh Sewage	Settled Sewage	Garden+ Soil
24	33.9	30.0	31.7	31.5
24				28.3
48	48.7	37.2	41.5	43.7
48				38.3
72	56.9	43.9	46.2	49.0
72				46.9
96	61.5	45.8	48.8	51.7
96				49.5
120	63.0	47.5	49.1	54.2
120				51.1

Bottle Test				
Hrs.	Type of Seed			
	River Water	Fresh Sewage	Settled Sewage	Garden Soil
120	56	47	45	----

All sample dilutions were 50%.

+ Duplicate samples were run using garden soil seed.

Table 9

Effect of Seed Age on B.O.D.

Warburg Test 5					
Hrs.	Age of River Water Seed in Days+				
	67	36	7	1/4	1++
24	4.6	5.1	2.7	2.7	2.1
24	4.6	2.3	---	1.6	3.2
48	9.7	5.0	6.3	27.8	6.0
48	9.5	7.0	---	20.5	7.4
72	23.7	10.1	11.6	36.1	16.5
72	22.0	28.1	----	34.6	20.5
96	38.3	29.6	25.0	42.1	25.6
96	43.7	41.6	----	40.5	27.8
120	50.7	43.3	32.2	45.0	27.8
120	50.4	48.5	----	43.9	41.2

Bottle Test		
Hrs.	Age of River Water in Days	
	67	1/4
120	74	58

All sample dilutions were 50%.

+Duplicate samples were analyzed for each seed.
++Garden soil seed in river water and aged 1 day.

Table 10a

Effect of Seed Age on B.O.D.

Warburg Test 6						
Hrs.	Age of River Water Seed in Days					
	73	42	13	6	1/4	1+
24	10.5	5.7	7.1	4.2	8.7	11.5
47	59.6	54.9	55.4	55.4	54.2	55.7
72	68.5	62.4	62.7	64.0	62.0	62.6
96	73.8	68.2	66.5	68.8	67.4	66.7
120	79.6	73.4	70.2	73.0	71.0	73.4

Bottle Test						
Hrs.	Age of River Water in Days					
	--	--	--	--	1/4	--
120	--	--	--	--	55	--

All sample dilutions were 50%.
+Garden soil seed in Standard Method dilution water.

Table 10b

Effect of Seed Age on B.O.D.

Warburg Test 6			
Hrs.	Age of River Water in Days		
	6	1/4	1+
24	6.3	42.7	11.8
47	62.9	68.9	59.5
72	78.7	92.6	63.8
96	81.9	96.2	88.0
120	86.6	96.2	87.4

Bottle Test			
Hrs.	Age of River Water in Days		
	--	1/4	--
120	--	55	--

All sample dilutions were 10%.
+Garden soil seed in Standard Methods dilution water.

Table 11

Effect of Adapted Seed on B.O.D.

Warburg Test 7				
Hrs.	Types of Seed+			
	River Water	Garden Soil	Adapted Garden Soil	Activated Sludge
24	4.3	0.0	0.0	12.7
24	0.0	0.0	0.0	10.3
48	44.5	9.5	10.2	37.5
48	30.4	4.9	25.3	34.0
72	64.6	38.0	28.1	56.5
72	45.1	39.1	45.5	52.0
96	107.5	40.6	32.3	73.4
96	50.6	54.2	51.9	65.3
120	210.6	52.2	43.8	97.2
120	66.8	73.3	63.4	81.3

Bottle Test				
Hrs.	Types of Seed			
	River Water	Garden Soil	Adapted Garden Soil	Activated Sludge
120	65	--	--	40

All sample dilutions were 50%.

+Duplicate samples were analyzed for each type of seed.

Table 12

Effect of Adapted Seed on B.O.D.

Warburg Test 8				
Hrs.	Types of Seed+			
	River Water	Garden Soil	Adapted Garden Soil	Activated Sludge
24	41.5	0.0	40.2	46.6
24	73.1	0.0	45.1	47.4
48	64.3	2.2	84.0	81.6
48	119.5	28.2	85.3	72.8
72	71.4	20.3	118.3	107.5
72	166.3	51.6	116.9	102.1
96	89.6	71.2	164.8	133.4
96	224.7	70.2	142.7	115.7
120	110.9	102.3	213.4	155.8
120	272.0	93.6	181.3	142.1

Bottle Test				
Hrs.	Types of Seed			
	River Water	Garden Soil	Adapted Garden Soil	Activated Sludge
120	55	--	--	46

All sample dilutions were 10%.

+Duplicate samples were analyzed for each type of seed.

Table 13

Effect of Dilution Waters on B.O.D.

Warburg Test 9			
Hrs.	Types of Dilution Water+		
	Standard Methods	River Water	Demineralized Tap Water
24	0.0	9.9	47.0
24	0.0	13.9	27.9
48	0.0	27.2	53.9
48	0.0	51.6	14.5
72	0.0	33.7	55.5
72	0.0	71.4	----
96	0.0	36.7	54.9
96	0.0	91.8	----
120	0.0	41.9	54.4
120	0.0	110.3	----

Bottle Test			
Hrs.	Types of Dilution Water		
	Standard Methods	River Water	Demineralized Tap Water
120	50	--	--

All dilutions were 10%.

+Duplicate samples were analyzed for each type of dilution water and seeded with river water.

Table 14

Effect of Dilution Waters on B.O.D.

Warburg Test 10			
Hrs.	Types of Dilution Water+		
	Standard Methods	River Water	Demineralized Tap Water
24	59.6	12.1	20.1
24	28.3	20.2	21.0
48	116.1	40.6	30.6
48	47.9	58.2	42.9
72	155.4	61.0	41.7
72	60.1	86.7	58.5
96	189.1	88.7	41.7
96	57.6	104.2	53.1
120	227.0	113.8	42.8
120	64.0	125.2	49.4

All dilutions were 10%.

+Duplicate samples were analyzed for each type of dilution water and seeded with river water.

Table 15

Effect of Dilution on B.O.D.

Warburg Test 11				
Hours	10% Dil.+	Ave. 10% Dil. Value	50% Dil.++	Ave. 50% Dil. Value
24	12.5	19.4	2.5	2.4
24	26.3		2.0	
24			2.5	
24			2.6	
48	47.6	56.0	20.0	19.0
48	64.4		17.4	
48			19.1	
48			19.3	
72	47.4	56.2	23.7	22.4
72	65.0		19.9	
72			22.5	
72			23.3	
96	45.6	53.7	24.3	22.4
96	61.8		18.4	
96			23.5	
96			23.4	
120	44.8	53.4	25.3	24.3
120	62.0		20.0	
120			23.7	
120			24.0	

All samples were seeded with river water.

+Duplicate samples were analyzed for 10% dilution.

++Quadruplicate samples were analyzed for 50% dilution.

Table 16

Effect of Dilution on B.O.D.

Warburg Test 12				
Hours	10% Dil.+	Ave. 10% Dil. Value	50% Dil.+	Ave. 50% Dil. Value
24	11.7	7.8	0.0	0.8
24	13.9		0.0	
24	0.0		1.0	
24	5.5		2.0	
52	105.3	101.7	24.8	34.8
52	122.6		39.6	
52	79.5		40.4	
52	99.3		34.2	
73	128.2	128.5	47.2	52.5
73	166.2		49.9	
73	90.6		52.2	
73	129.1		60.5	
100	177.8	168.7	55.9	63.7
100	227.7		59.0	
100	114.4		66.0	
100	154.9		73.9	
121	208.0	191.7	63.2	71.0
121	266.1		65.3	
121	119.5		72.3	
121	173.2		83.1	
314	471.3	434.6	86.0	113.0
314	677.8		107.2	
314	251.1		114.6	
314	338.2		144.2	
484	697.9	629.0	115.8	156.8
484	960.3		149.4	
484	384.0		150.1	
484	473.7		222.0	

All samples were seeded with river water.

+Quadruplicate samples were analyzed for each dilution.

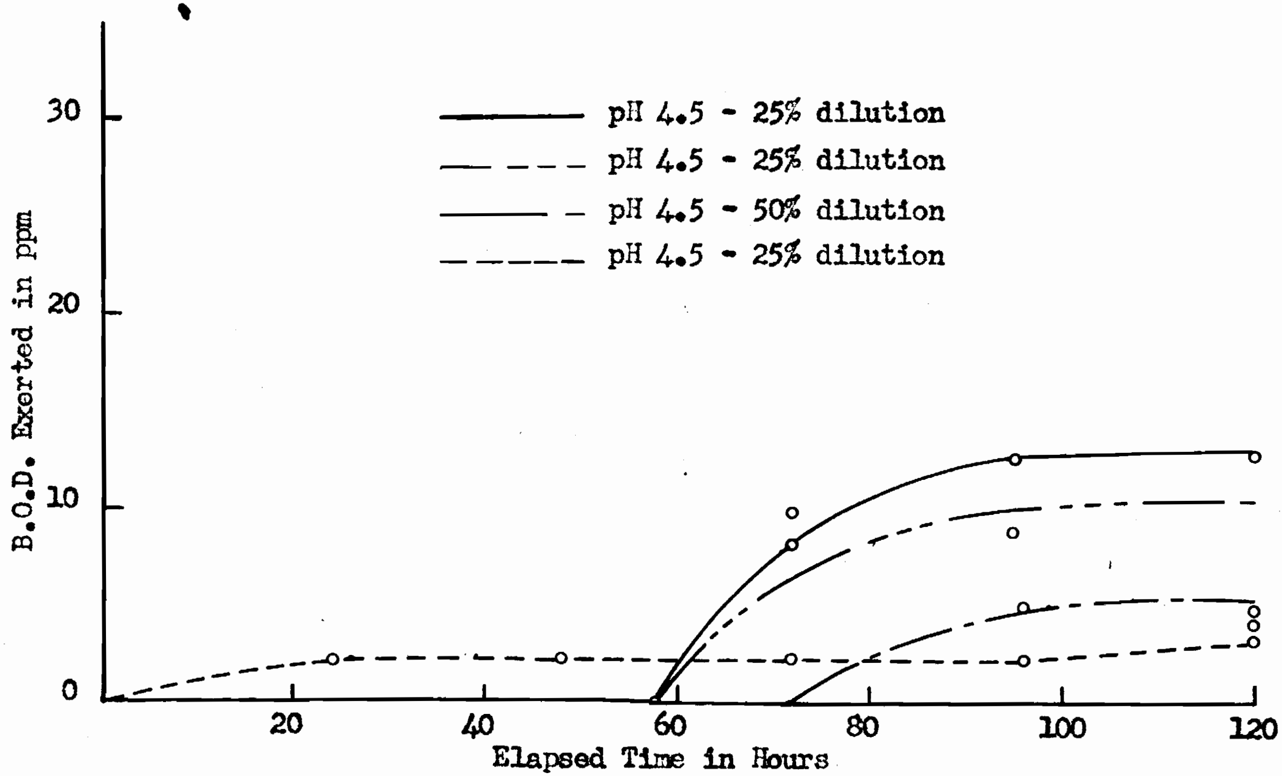

Figure 2

Effect of pH on B.O.D.

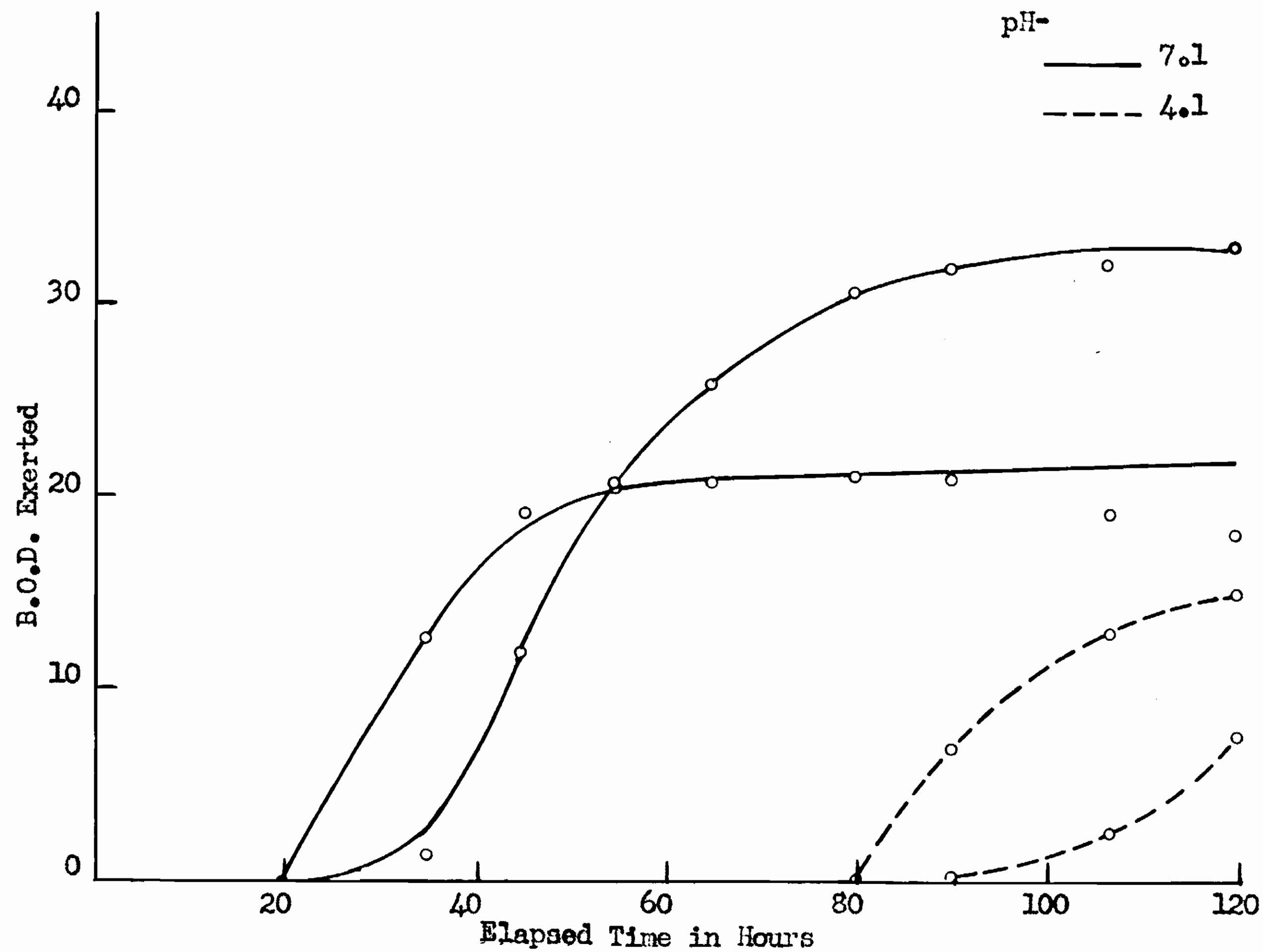

Figure 3

Effect of pH on B.O.D.

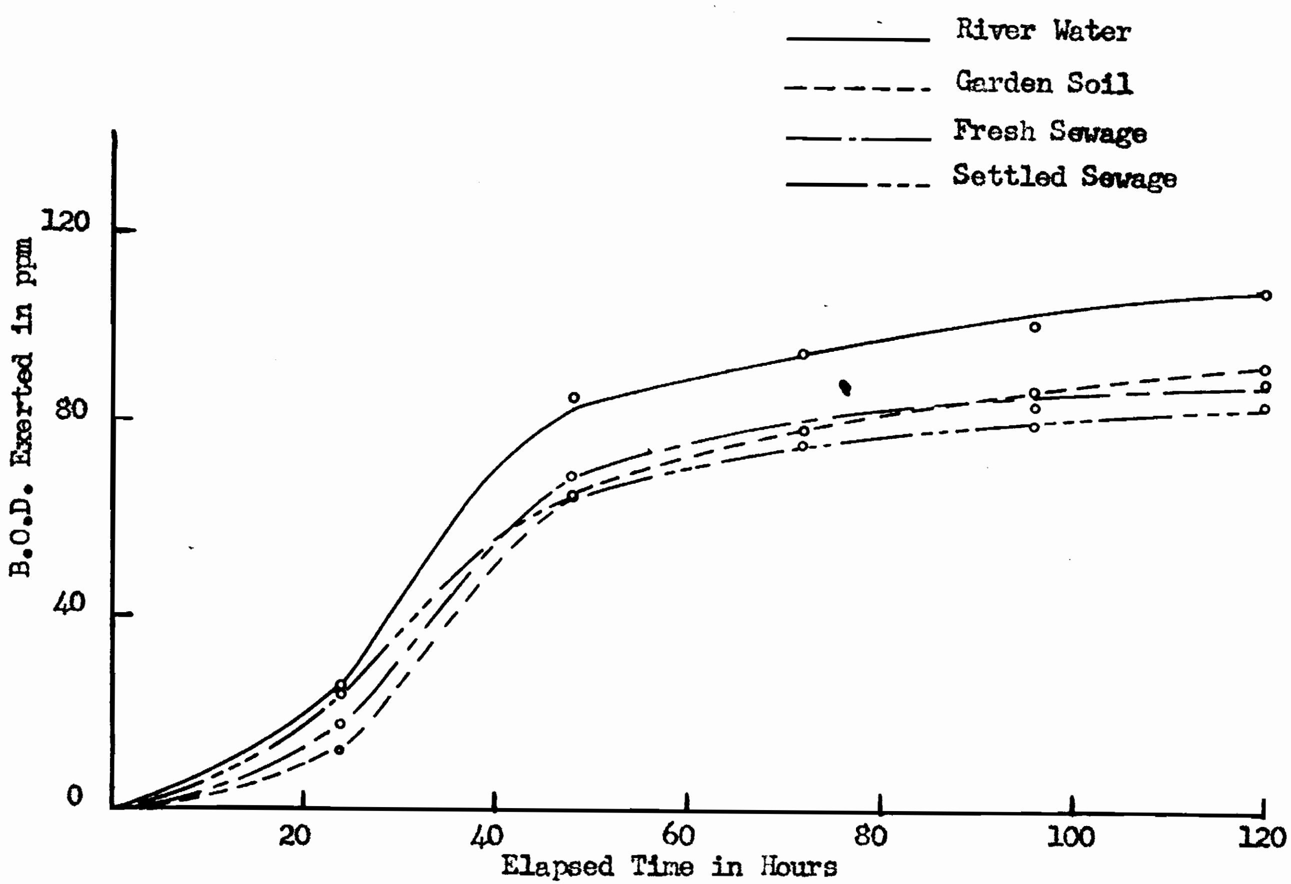

Figure 4

Effect of Different Seeds on B.O.D.

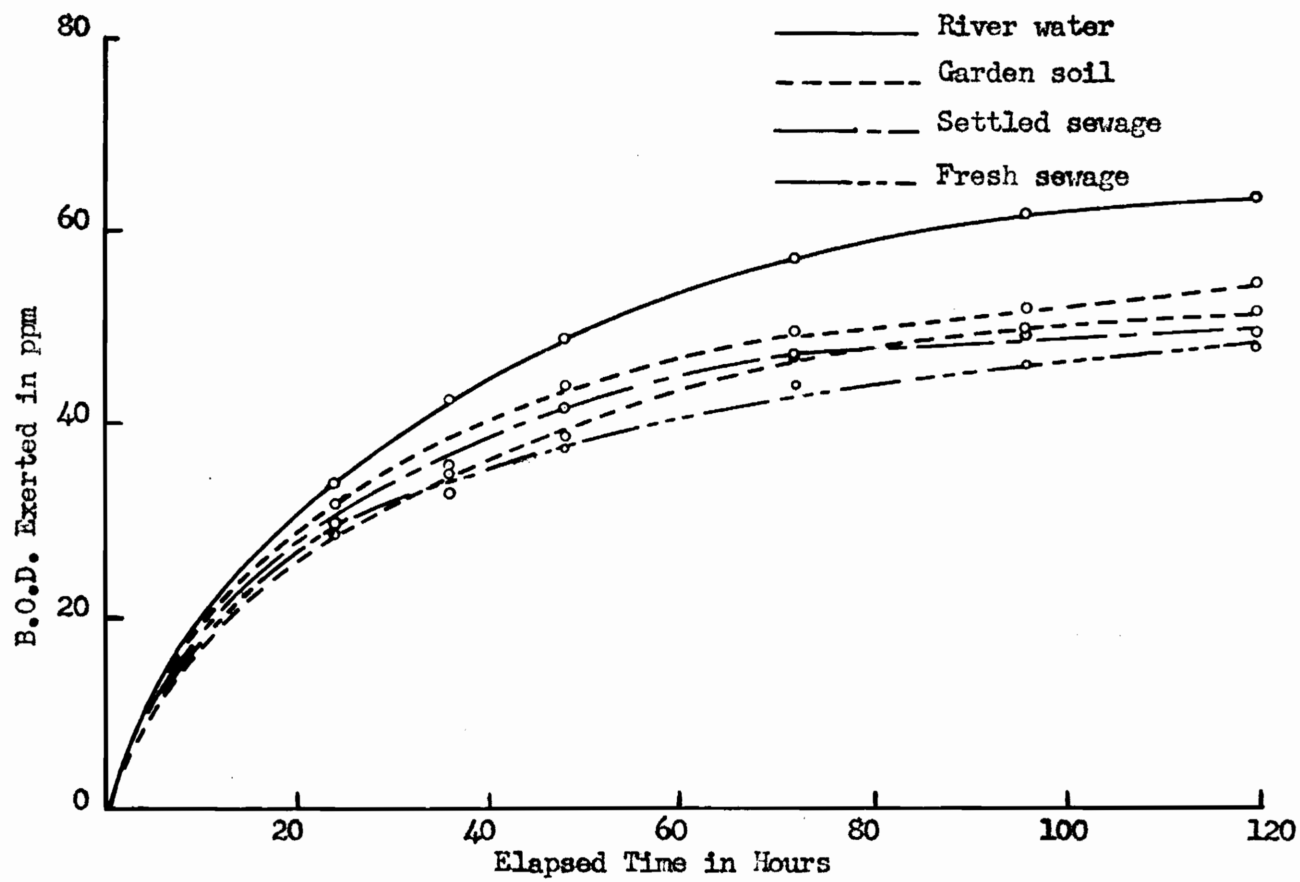

Figure 5

Effect of Different Seeds on B.O.D.

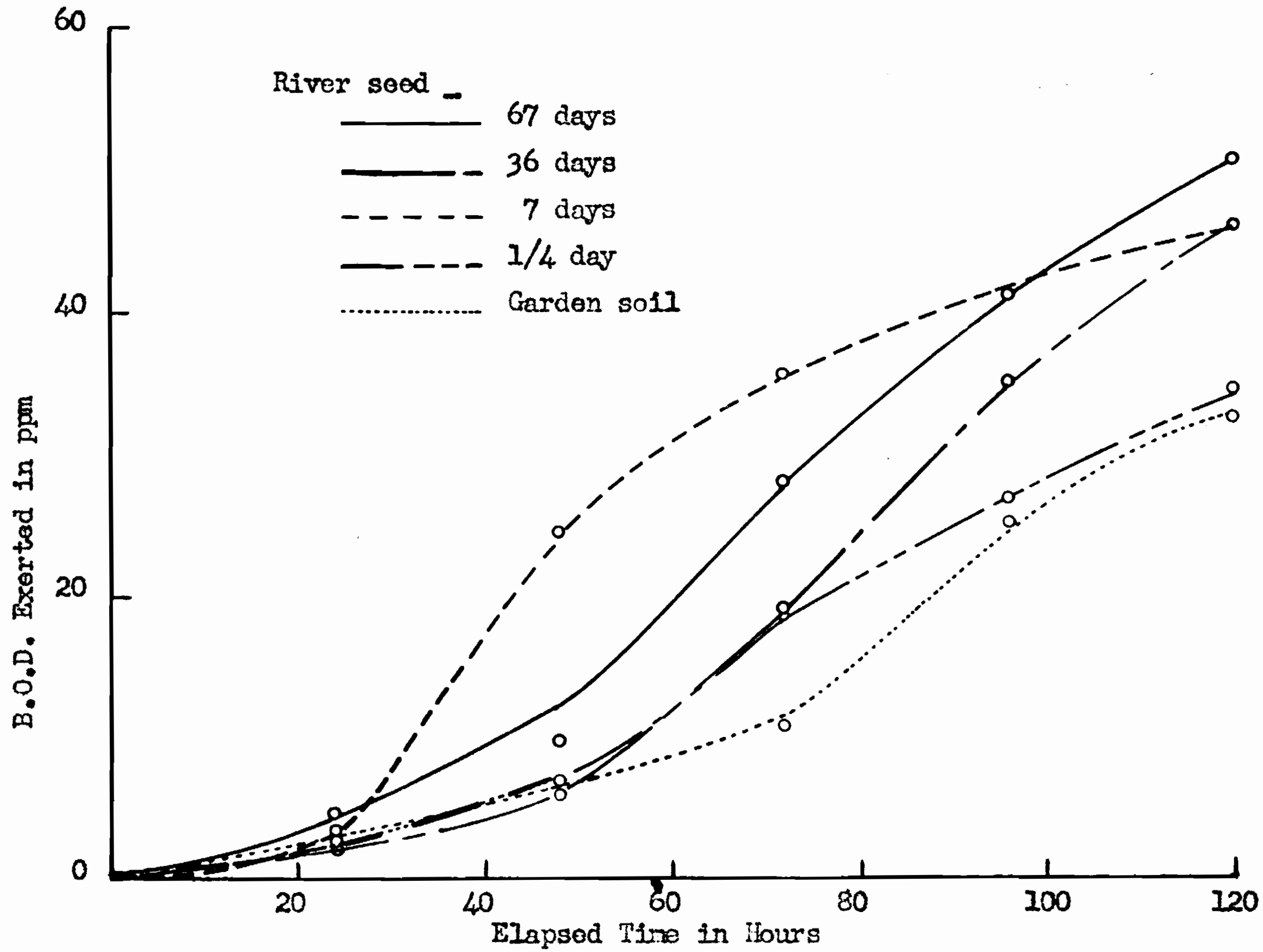

Figure 6

Effect of Seed Age on B.O.D.

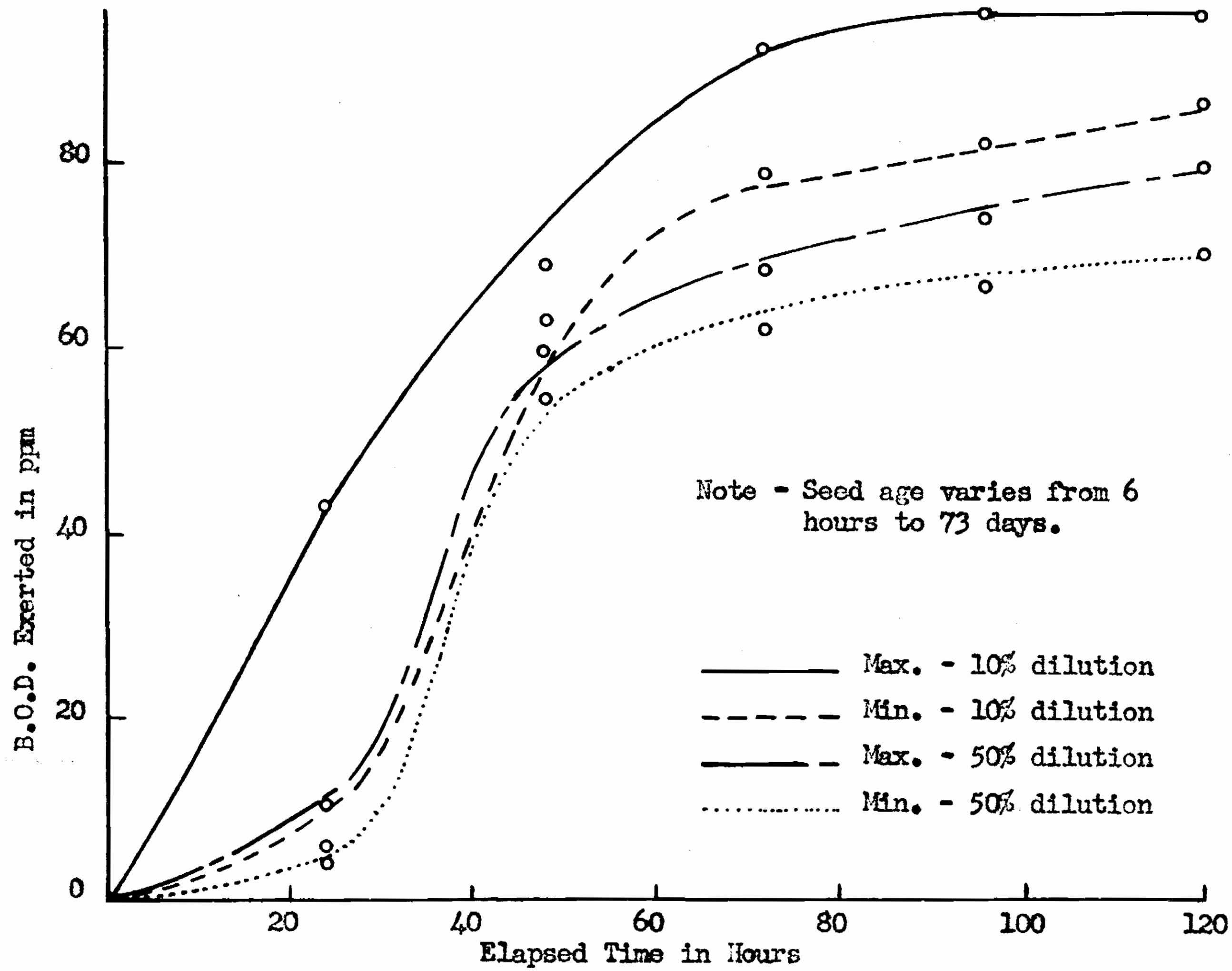

Figure 7

Effect of Seed Age on B.O.D.

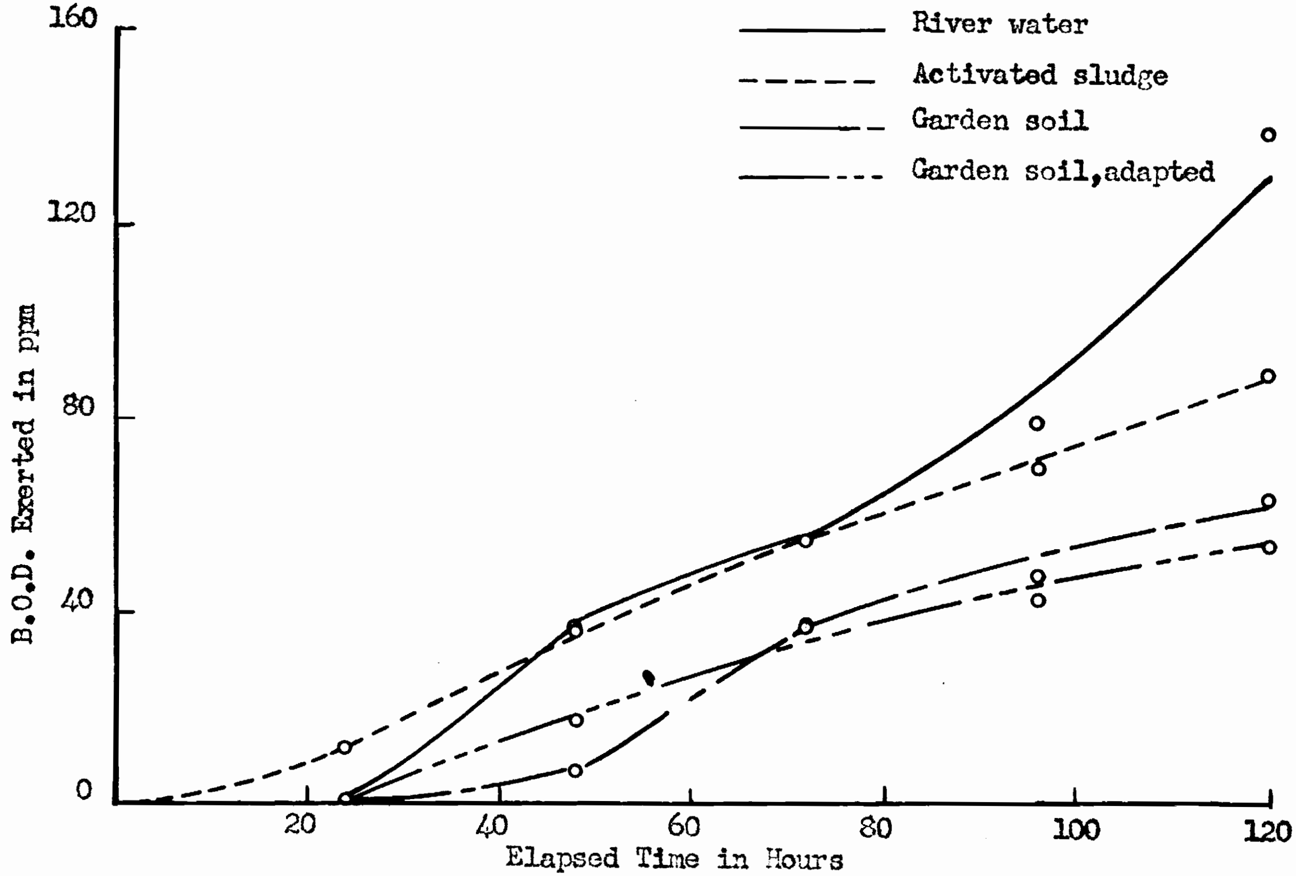

Figure 8

Effect of Adapted Seed on B.O.D.

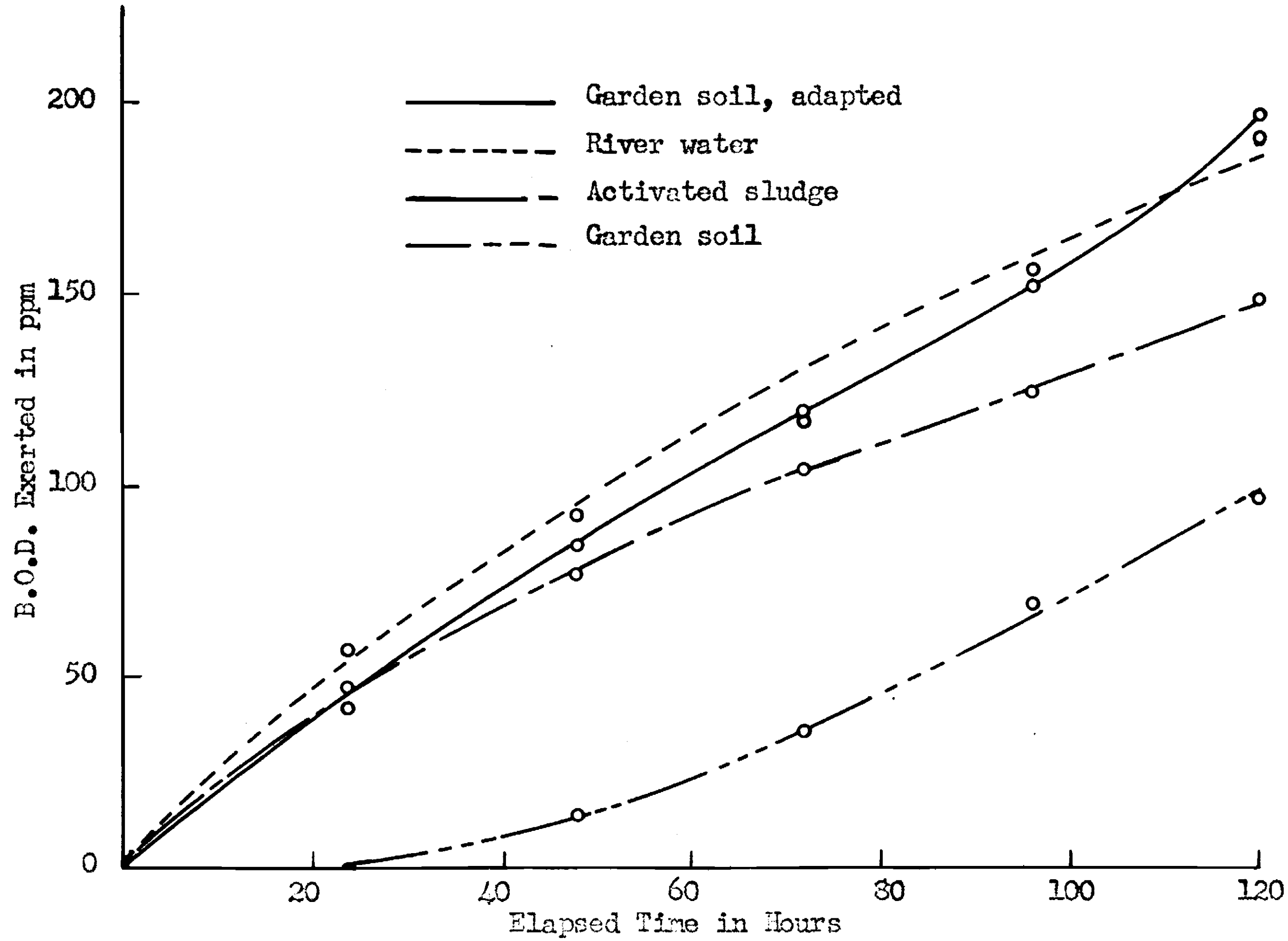

Figure 9

Effect of Adapted Seed on B.O.D.

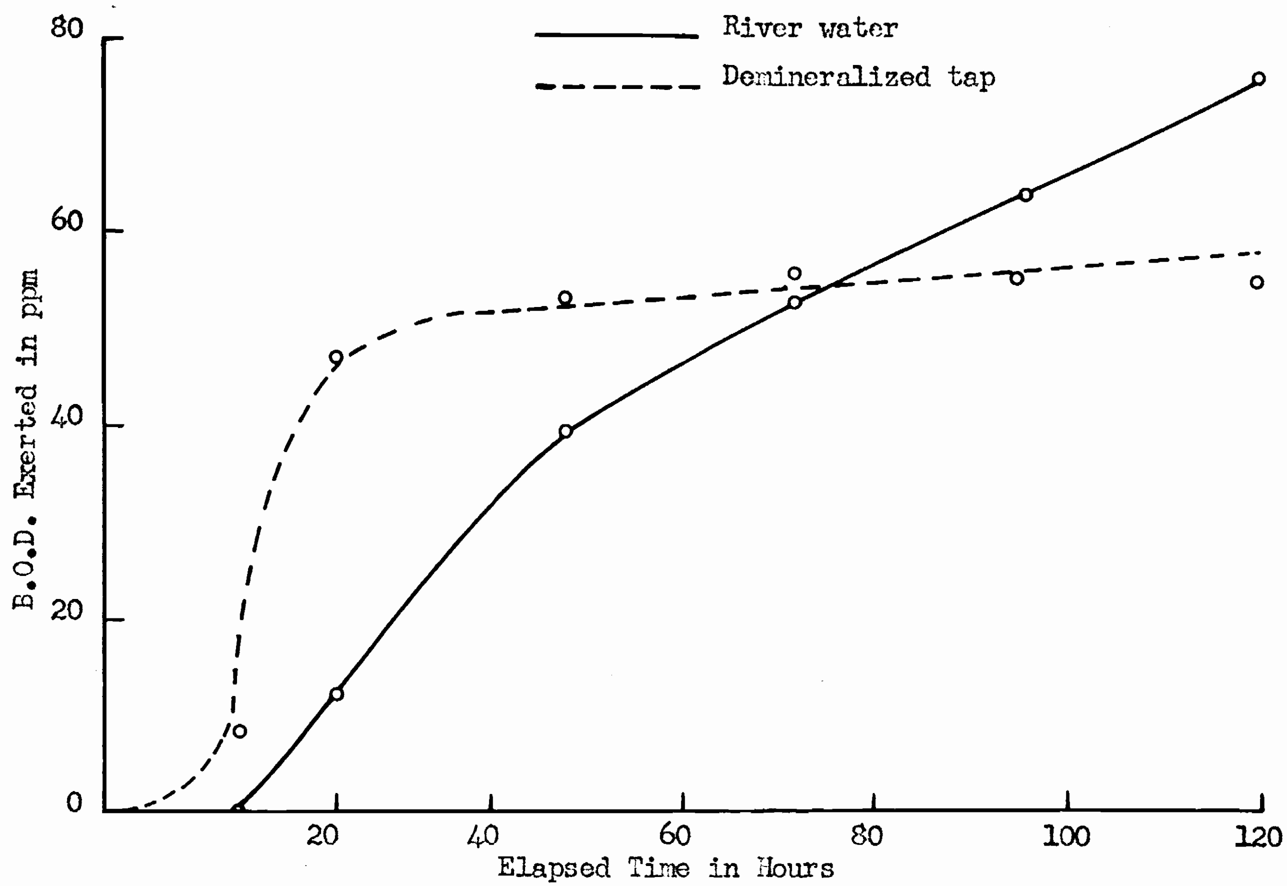

Figure 10

Effect of Dilution Waters on B.O.D.

Table 8.--Lumber used in furniture manufacturing by quality class and species groups, 1960[1/]

Species and quality	Furniture class		
	Wood household (not upholstered)	Wood household (upholstered)	Wood office
Softwood			
High	26	5	2
Medium	37	62	80
Low	37	33	18
Hardwood			
High	24	11	21
Medium	68	80	74
Low	8	9	5
Sweetgum			
High	26	6	9
Medium	67	80	88
Low	7	14	3
Hard Maple			
High	35	44	33
Medium	58	55	63
Low	7	1	4
Red Oak			
High	25	2	20
Medium	52	92	76
Low	23	6	4
Yellow Poplar			
High	12	8	6
Medium	74	77	85
Low	14	5	9

[1/] National Figures

Source: Gill, 1965:54

which is critical to those Virginia forest industries, such as sawmills and veneer and furniture plants, which must have good size sawlogs. Trees available for sawlogs are rapidly becoming smaller in diameter, even to the point of disappearing as sawlog material."

If wood is to continue to be used for furniture, then quality wood supplies must be available. Due to technological advances in lamenating, the gluing of shorter pieces of quality wood to form larger pieces, and the combining of wood with other materials to improve properties, it has been possible to decrease the quantity of quality wood per unit of furniture. The increased use of plywood, particleboard and hardboard in furniture can decrease the need of quality wood since these prefabricated products can be used for core stock and other less visible areas.

Quantity Requirements of Lumber

Virginia produces large quantities of lumber. "The Virginia cut has exceeded one billion feet each year since 1939, except in 1945 when it was just under one billion (General Assembly of Virginia, 1955:19)." Since 1956, more than one-half of the hardwood produced in the South has been cut in five states. In order of hardwood production, 1957-1961, the leading states are: Virginia, Tennessee, North Carolina, Arkansas and Mississippi (Siegel, 1963:n.p.).

with the time of the readings.

Calculation of B.O.D. Values

Calculation of the B.O.D. values was made by use of the formulas used by Dixon (8) and Caldwell and Langelier (5). (Appendix E).

The B.O.D. value was found by multiplying the flask constants (Appendix F) by the corrected deflection readings of the open manometer tube.

Data and Results

The data obtained from the manometric B.O.D. tests of the industrial waste are presented in tabular form in Tables 5 through 16, and graphically in Figures 2 through 13. Two runs of the B.O.D. test were made for each of the variables listed in the object of this thesis and the B.O.D. results reported as 1, 2, 3, 4 and 5 day values. All the B.O.D. tests were run at a temperature of 25° C., and the B.O.D. values reported as ppm. oxygen. The standard dilution 5-day 25° C. B.O.D. values obtained from another investigator are reported with the corresponding manometric test.

V. DISCUSSION OF RESULTS

General

Throughout the investigation, only two test runs were made for each variable; therefore, the results cannot be considered conclusive but are only indicative of a trend.

The industrial waste effluent came from a plant utilizing the batch process of manufacture. While the plant operation was of such a size that the waste flow was constant and continuous, the constituents of the waste were assumed to be variable.

The waste samples were "grab" samples taken immediately before the specific B.O.D. tests were begun and successive test runs were made approximately 7 days apart.

The bottle B.O.D. values furnished by another investigator are presented in the Discussion of Results under each section.

Hydrogen Ion Concentration

The data from Test 1 is contained in Table 5 and is shown graphically in Figure 2. At an initial pH of 4.5, the waste exerted B.O.D.'s from zero to 3.5 ppm. At a different dilution in the same test run, the B.O.D.'s ranged from 4.2 to 12.7 ppm but exhibited a lag phase from 58 to 70 hours.

In Test 2 (data given in Table 6 and on Figure 3), the initial pH was 4.1 and the B.O.D.'s exerted ranged from 7.5 to 15 ppm after lag periods ranging from 80 to 90 hours; however, when the pH was raised to 7.1 the samples gave B.O.D. values from 17.9 to 33.0 ppm after lag periods of only 20 hours.

In Test 1 when the pH of the waste was 4.1, the B.O.D. values were low and were exerted only after long lag periods. In Test 2 when the pH of the waste was raised from 4.1 to 7.1, the B.O.D. values were increased and obtained after much shorter lag periods.

The only bottle B.O.D. reported was 42 ppm on a sample with a pH of 7.1.

Type of Seed

The results for Test 3 are presented in Table 7 and Figure 5. When river water, garden soil, fresh sewage, and settled sewage were used as seeds, the waste gave respective B.O.D. values of 107.8 ppm, 92.7 ppm, 89.3 ppm, and 85.0 ppm.

The same seeds were used in Test 4 as in Test 3. The analytical data for Test 4 is listed in Table 8 and illustrated graphically in Figure 6. The waste seeded with river water gave a B.O.D. of 63.0 ppm, whereas, the two B.O.D. values obtained from the sample seeded with soil were 54.2 ppm and 51.1 ppm. Samples inoculated with fresh sewage and with settled sewage exerted B.O.D.'s of 47.5 ppm and 49.1 ppm respectively.

In Tests 3 and 4 the river water seed gave the highest B.O.D. values, and garden soil seed gave the second highest B.O.D. values. The fresh sewage seed gave a higher value than settled seed in Test 3, but settled sewage seed gave a higher value in Test 4. These results indicate that the river seed may have contained bacterial organisms more suited to decomposing the waste than the other seeds used. This may be explained by the fact that the river water used in the experiment was obtained below a waste outfall, and the micro-organisms had become adapted to the waste under study.

Bottle B.O.D. tests were run by another investigator using river water, fresh sewage, and settled sewage as seeds. The values obtained ranged from 90 to 110 ppm for Test 3, and from 45 to 56 ppm in Test 4.

Age of Seed

For Test 5, soil seed 1 day of age and river water seeds varying in age from 6 hours to 67 days were used to seed the waste for the B.O.D. test. The B.O.D. values (Table 9 and Figure 7), ranged from 27.8 to 50.7 ppm.

The average B.O.D. value of the waste, using a river water seed of 67 days age, was 50.6 ppm; with a 36 day old river water seed, the average B.O.D. value was 45.9 ppm, and for a river water seed with age of 6 hours, the average B.O.D. value was 44.5 ppm. A single B.O.D. value of 32.2 ppm was obtained with river water seed that had been aged 7 days. The average B.O.D. value using soil seed prepared 1 day in advance was 34.5 ppm.

In Test 6 (data found in Tables 10-a, 10-b, and Figure8), the aged river water seed (6 to 73 days), the fresh river seed, and the aged soil seed (1 day), gave B.O.D. values for the waste from 71.0 to 79.6 ppm. The river water seed of 73 days age gave the highest B.O.D. value, while the B.O.D. values obtained from the seeds of different ages (1/4 to 42 days) were approximately 10% less. At a different dilution of the waste sample, river water seed 6 days old gave a B.O.D. of 86.6 ppm, whereas the river water seed aged 6 hours gave a value of 96.2 ppm. The soil seed of 1 day age caused the waste to exert a B.O.D. of 96.2 ppm.

The B.O.D. values from Tests 5 and 6 do not show a definite trend toward any age of seed with respect to effect of seed age over the time range investigated.

The bottle test (6 hour and 67 day seeds) B.O.D. values given were higher than the corresponding manometric B.O.D. values for Test 5. In Test 6, the manometric B.O.D. values were higher than the bottle B.O.D. value for waste and a 6 hour seed.

Adapted Seed

The data for Test 7 is listed in Table 11 and shown in graphical form in Figure 8. The waste gave B.O.D. values from 43.8 to 210.6 ppm when seeded with river water, garden soil, adapted garden soil (Appendix D), and activated sludge. The average B.O.D. value of the waste when seeded with river water was 138.7 ppm while the waste seeded with activated sludge was 89.3 ppm; garden soil seeded waste exerted a B.O.D. of 62.8 ppm and the waste with an adapted soil seed gave a B.O.D. of 53.6 ppm.

In Test 8 (results in Table 12 and on Figure 9), the same seeds were used as in Test 7 and the B.O.D. values of the waste ranged from 93.6 to 272.0 ppm. The average B.O.D. value of the waste with adapted soil seed was 197.4 ppm, and with river water seed was 191.5 ppm. Using an activated sludge seed, the B.O.D. value was 149.0 ppm, and with garden soil seed the B.O.D. value was 98.0 ppm.

Tests 7 and 8 show that seeds of river water, adapted garden soil, and activated sludge give higher B.O.D. values than the B.O.D. results obtained when garden soil was used as a seed.

It is believed that the bacterial organisms in the river water below the waste outfall had become adapted to the waste material and, therefore, this river water when used as seed caused the waste to exert higher B.O.D. than the waste when seeded with non-adapted seed.

The bottle B.O.D. values reported in each test for river water and activated sludge were from one-fourth to one-half the manometric B.O.D. values.

Dilution Water

For Test 9 (results in Table 13 and on Figure 10), river water, demineralized tap water, and standard dilution water were used to dilute the waste samples for the B.O.D. test. The diluted waste gave values from 41.9 to 110.3 ppm. The average B.O.D. value for the waste using river water as the dilutant was 76.1 ppm and the single value obtained when demineralized tap water was used as dilution water was 54.4 ppm. The waste did not give a B.O.D. value when standard methods dilution water was used. This zero reading was probably the result of faulty technique.

The values of Test 10 (data reported in Table 14 and on Figure 11), ranged from 42.8 to 227.0 ppm and were obtained by using the same dilution waters as were used in Test 9. The average B.O.D. value obtained with standard dilution water was 145.5 ppm; the average B.O.D. value obtained with river water as the dilutant was 119.5 ppm, and the average B.O.D.

value obtained with demineralized tap water was 46.1 ppm.

These tests indicate that river water and standard dilution water gave higher B.O.D. values when used to dilute the waste than a demineralized dilution water. It is well established that certain mineral salts must be present to enable the reacting sample to exert a normal B.O.D. In this case the mineral nutrients may be present in river water due to minerals dissolved from the soil or from artificial pollution, and also some portion of the minerals needed may occur in the waste itself. The standard dilution water contained the minerals recommended by Standard Methods for the Examination of Water and Sewage (1), plus the possible minerals found in the waste. However, the demineralized dilution water had only the mineral nutrients present in the particular waste sample which was analyzed at a 10% waste dilution.

For both Tests 9 and 10, the curves show that when demineralized tap water was used the maximum B.O.D. was exerted in about 2 days; this indicates that something was limiting the B.O.D. reaction after 2 days and this may have been the lack of mineral nutrients.

The bottle B.O.D. reported was 50 ppm when standard dilution water was used with sample from Test 9.

Dilution

Table 14 and Figure 12 show the data obtained from Test 11. The 50% and 10% dilutions of the waste samples exerted a B.O.D. ranging from 16.4 to 56.7 ppm. The B.O.D. values of all the 10% dilutions were greater than the 50% dilution values, the average being 53.5 ppm for the 10% dilutions and 23.4 ppm for the 50% dilutions.

Test 12 (data in Table 15 and on Figure 13) shows B.O.D. values for the 50% and 10% dilutions from 63.2 to 266.1 ppm.

The average B.O.D. value for the 10% dilutions of waste was 191.7 ppm as compared to the average B.O.D. value for the 50% dilutions of 70.9 ppm.

The fact that the B.O.D. values increased as the waste was made more dilute indicated the presence of a substance toxic to the B.O.D. reaction. The dilution at which the B.O.D. values were not repressed by this toxicity was not determined.

No bottle B.O.D. values were obtained to accompany Test 11 and 12.

VI. CONCLUSIONS

The following trends were observed in the manometric study of the B.O.D. of a neutralized acid industrial waste under the influence of the variables pH, type of seed, age of seed, adapted seed, dilution water, and dilution:

(1) The waste exerted a normal B.O.D. when the pH was adjusted to 7.1.

(2) The waste gave the highest B.O.D. values when river water was used as a seed. The waste gave comparable B.O.D. values when seeded with garden soil, fresh sewage, and settled sewage.

(3) The age of the seed made little difference in the B.O.D. exerted for the seeds studied.

(4) The waste gave higher B.O.D. values with adapted seeds than with unadapted seeds.

(5) The presence of mineral nutrients were necessary for the waste to exert a normal B.O.D.

(6) The presence of a substance in the waste which was toxic to the B.O.D. reaction was indicated.

The bottle B.O.D. values were in the same general range as the corresponding manometric B.O.D. values.

The Warburg apparatus was well adapted for use in studying the effect of variables on the B.O.D. reaction of a neutralized acid industrial waste.

VII. SUMMARY

The purpose of this investigation was to use the manometric method to determine the effect of the following variables on the B.O.D. values of a neutralized acid industrial waste: pH, type of seed, age of seed, adapted seed, dilution water, and dilution. In order to determine the effect of each variable on the B.O.D. of the waste, the temperature and agitation of the Warburg apparatus were kept at fixed values. While the optimum condition for one variable was being determined, the remaining factors were kept constant. As the optimum condition of each variable was found, it was used as a reaction constant, and this process was continued until the list of variable factors was completed.

Since only a limited number of tests were run for each variable, no definite conclusions can be drawn. However, the following trends were indicated from the data obtained: A pH of 4.1 depressed the B.O.D. values and they were exerted only after long lag periods; the waste exerted a higher B.O.D. with much shorter lag periods when the pH was raised to 7.1. River water seed caused the waste to exert higher B.O.D. values than did garden soil seed, fresh sewage seed, and settled sewage seed. This indicated that the river water contained a microbiological population adapted to the utilization of the waste under study. The age of seed made little difference in the B.O.D. values exerted for the seeds studied. The waste gave higher B.O.D. values when adapted seeds of river water, adapted soil seed, and activated sludge were used than when regular garden soil seed was used. It is believed that the bacterial organisms in the river water below the waste outfall had become adapted to the waste material. The presence of mineral nutrients as found in river water or the addition of mineral nutrients as recommended by Standard Methods for the Examination of Water and Sewage (1) are necessary for the waste to exert a normal B.O.D. The presence of a substance toxic to the B.O.D. test is indicated because the B.O.D. values increased with increasing dilution.

The bottle B.O.D. values, obtained from another investigator, were not given for all of the tests; therefore, no conclusions were drawn except that the bottle B.O.D. values were in the same general range as the corresponding manometric B.O.D. values.

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X. VITA

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Jasper R. Sutherland

XI. APPENDICES

Table 11.--Commodity class furniture shipped from Virginia to other states by Class I Railroads, 1961

Destination[1] (state)	Volume of freight	Short-line distance[2]
	Tons	Miles
Arizona	24	2313
California	128	2781
Colorado	28	1704
Florida	40	765
Illinois	87	724
Iowa	27	1130
Massachusetts	73	695
Minnesota	41	1144
Missouri	72	892
Nebraska	30	1157
New Jersey	64	478
New York	73	562
Ohio	33	536
Texas	141	1327
Virginia	50	14
Washington	22	2859

[1] Only states with shipments of 20 tons or more included.

[2] Short-line distance is the shortest rail route over which carload traffic can be moved without transfer of lading.

Source: Adapted from, Interstate Commerce Commission, 1963:253-254.

Appendix B

Determination of the Density of Brodie's Solution

Brodie's Solution was prepared according to directions (see Appendix A) and the density of the solution determined as follows: A small tared specific gravity bottle was filled with distilled water at 25° C. and weighed. The bottle was then filled with Brodie's Solution at 25° C. and weighed again.

The data and calculations are shown below:

Weight of bottle plus water at 25° C.	-- 43.2345 g
Weight of bottle	-- 18.3250 g
Weight of water at 25° C.	24.9095 g

Density of water at 25° C. 0.99707

Volume of bottle -- $24.9095 \div 0.99707 = 24.9827$ ml.

Weight of Brodie's Solution plus bottle	-- 44.1048 g
Weight of bottle	-- 18.3250 g
Weight of Brodie's Solution	25.7798 g

Density of Brodie's Solution -- $\frac{25.7798}{24.9827} = 1.032$

Appendix C

Preparation of Soil Seed

Approximately one pound of fresh garden soil was rolled into a ball and allowed to dry at room temperature. The dry soil was powdered and mixed thoroughly. The soil was then placed in a ground glass container, wrapped in aluminum foil to protect it from sunlight, and stored in a desiccator at room temperature.

Twenty-four hours before the soil seed was to be used, 100 milligrams of the soil were placed in 100 ml. of dilution water prepared according to the procedure outlined in Standard Methods for the Examination of Water and Sewage (1), and thoroughly shaken. This mixture was kept at 25° C. for 24 hours and the clear liquid from the upper portion of the container was used as seed.

Appendix D

Preparation of Adapted Soil Seed

One gram of prepared garden soil was added to one liter of dilution water prepared as per Standard Methods for the Examination of Water and Sewage (1). After shaking, portions of the liquid were withdrawn from this mixture and equal portions of the industrial waste were added, in the amounts and after the time intervals shown below. This mixture of soil, dilution water, and waste was kept at a constant temperature of 25° C.

Amount of waste added in ml	Time interval in days
2	0
5	3
5	1
5	1
5	1
5	1
5	1
5	1
5	1
5	3
5	2
5	1

Appendix E

Derivation of Flask Constant Formula

Dixon (8) uses the formula $X = hK$ for determining the oxygen uptake where X is the milliliters of oxygen uptake, h is the change in the open manometer leg in centimeters, and K is the reaction flask constant. The value of K is expressed as:

$$\frac{\frac{273\ Vg}{T} + Vf\alpha}{Po}$$ in which

Vg = total volume of gas in flask-manometer system to index point in ml.

T = absolute temperature.

Vf = total volume of fluid in flask in ml.

α = solubility of oxygen under test conditions (in ml./ml. at 25° C.)

Po = normal pressure in centimeters of manometer fluid.

Caldwell and Langelier (5) convert the Dixon equation from milliliters of oxygen consumed to parts per million by weight (w) of oxygen used to get:

$$w = \left(\frac{273\ Vg}{T\ Po} + \frac{Vf\alpha}{Po}\right) \times \frac{1430}{Vs} \times h .$$

Using a temperature of 25° C. and 1.032 for the density of the manometer fluid (Brodie's solution) the equation reduces to: $w = h\ 1.31\ Vg/Vs + .04V_fh/Vs$.

Since the second term of the equation is small it can be dropped and the equation used as : $w = h\ (1.31Vg/Vs)$ where $(1.31\ Vg/Vs)$ is K.

Appendix F

Calculation of Flask Constant

A flask constant (K) was calculated from the following data as shown below:

V = 17.68 ml - volume of flask-manometer system to index point.

Vf = 4.0 ml - volume of diluted waste added to reaction flask.

Va = 0.5 ml - volume of alkali added to alkali well.

Vs = 2.0 ml - volume of undiluted sample added to reaction flask.

Vg = - total volume of gas in flask-manometer system to index point.

$$
\begin{aligned}
Vg &= V - Vf - Va \\
&= 17.68 - 4.0 - 0.5 \\
&= 13.18 \text{ ml}
\end{aligned}
$$

Then:

$$
\begin{aligned}
K &= 1.31 \frac{Vg}{Vs} \\
&= 1.31 \times \frac{13.18}{2} \\
&= 8.63
\end{aligned}
$$

"K" values were calculated for other flasks and other waste dilutions in a similar manner.