MANOMETRIC DETERMINATION OF THE BIOCHEMICAL OXYGEN DEMAND OF SULFITE PAPER MILL WASTES

by

Samuel E. Ketner

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

APPROVED:

Director of Graduate Studies Head of Department

Dean of Engineering In Charge of Investigation

June, 1951

Blacksburg, Virginia

TABLE OF CONTENTS

		Page
I.	INTRODUCTION	7
II.	LITERATURE REVIEW	9
	Stream Pollution	9
	History of Stream Pollution Studies	12
	Standard Biochemical Oxygen Demand Test	16
	Environmental Effects on the Standard B. O. D.	19
	Limitations of the Standard B. O. D. Test	26
	Chemical Oxygen Demand	30
	Direct Measurement of the B. O. D.	36
	Haldane Apparatus	36
	Nordell Odeometer	39
	Warburg Constant-Volume Respirometer	40
	Determination of the B. O. D. with the Warburg Respirometer	45
	Resting Cell Technic	47
	Nephelometric Analysis	48
III.	EXPERIMENTAL	49
	Purpose of Investigation	49
	Plan of Experimentation	49
	Materials	50

Page

Apparatus 53

Method of Procedure 56

Data and Results 65

IV. DISCUSSION 81

Discussion of Results 81

Limitations 96

Recommendations 97

V. CONCLUSIONS 100

VI. SUMMARY 103

VII. BIBLIOGRAPHY 106

VIII. ACKNOWLEDGEMENTS 112

IX. VITA 113

TABLES

		Page
Table I.	Chemical Oxygen Demand, Standard B. O. D., and Direct B. O. D. Values for Some Samples of Domestic Waste	33
Table II.	A Comparison of the Chemical Oxygen Demand of Some Synthetic Wastes as Obtained with Different Oxidising Agents	34
Table III.	The Standard Five-day B. O. D. and the C. O. D. Using Various Oxidizing Agents of Some Organic Compounds	35
Table IV.	Calculated Volumes of the Reaction Flasks, Graduated Sections of the Manometers, and Ungraduated Sections of the Manometers for Calculation of the Total Volume of the Respirometers ...	44
Table V.	Effect of Concentration of Blowdown Liquor on the Oxygen Uptake of 7.4 Milligrams of Lake Water Bacteria Respiring on 2.5 Milliliters of Various Dilutions of the Paper Mill Waste at 30 °C	67
Table VI.	Effect of Temperature on the Oxygen Uptake of 7.4 Milligrams of Lake Water Bacteria Respiring on 2.5 Milliliters of a 1:500 Dilution of Blowdown Liquor	69
Table VII.	Effect of Effluent Concentration on the Oxygen Uptake of 11.8 Milligrams of Lake Water Bacteria Respiring on 2.5 Milliliters of Effluent at 30 °C from a Sewage-Blowdown Liquor Digester	70

Page

Table VIII. Comparison of Manometric and Standard B. O. D. Values of Domestic Sewage and Blowdown Liquor Mixtures 72

Table IX. Effect of 0.7, 1.6, and 3.4 Milligrams of Lake Water Bacteria on the Oxygen Uptake Caused by the Presence of 2.5 Milliliters of a 1:500 Dilution of Blowdown Liquor 73

Table X. Effect of Bacterial Concentration on the Oxygen Uptake of a Suspension of 2.5 Milliliters of Lake Water Bacteria on a 1:500 Dilution of Blowdown Liquor at 30 °C 75

Table XI. Effect of Age of Culture and Time of Growth in Nutrient Broth on the Endogenous Rate and the Oxygen Uptake of Various Concentrations of Lake Water Bacteria on 2.5 Milliliters of a 1:500 Dilution of Blowdown Liquor at 30 °C 76

Table XII. Manometric Determination of the B. O. D. of Total Mill Wastes at 25 °C Using 2.5 Milliliters of Solutions for the Standard Dilution B. O. D. 77

Table XIII. Colorimeter Readings and Weights of Dry Bacterial Cells for Distilled Water Suspensions of Lake Water Bacteria 79

FIGURES

		Page
Figure 1.	Effect of Concentration of Blowdown Liquor on the Oxygen Uptake of Lake Water Bacteria	68
Figure 2.	Effect of Concentration of Effluent from a Sewage-Blowdown Liquor, Anaerobic, Digester on the Oxygen Uptake of Lake Water Bacteria	71
Figure 3.	Effect of Excessive Time on the Oxygen Uptake of Various Concentrations of Lake Water Bacteria Caused by the Presence of a 1:500 Dilution of the Blowdown Liquor	74
Figure 4.	Oxygen Uptake Caused by Various Concentrations of Total Mill Wastes in 2.5 Milliliters of Standard Dilution Water at 25 °C	78
Figure 5.	Calibration Curve for Klett-Summerson Photoelectric Colorimeter for Conversion of Colorimeter Readings to Bacterial Concentration	80

I. INTRODUCTION

In nearly all chemical industries the problem of wastes is one of vast importance, being in many cases the deciding factor in determining the economic feasibility of a given process. Not only must the waste problem be studied with the view towards the possible removal of the products, reactants, and any of the valuable constituent by-products of the main process, but also towards the final disposal of the wastes. In most cases there is no alternative, economically or physically, other than disposing of the wastes into a stream or municipal sewer.

Regardless of the means of disposal used, the concept of the B. O. D. or biochemical oxygen demand is of utmost importance. The biochemical oxygen demand of a waste refers to the amount of free oxygen utilized or demanded in the aerobic biochemical oxidation of the wastes. If in any given system the rate of biochemical oxygen utilization exceeds the rate of diffusion of free oxygen into the system, the aerobic biochemical oxidation of the wastes will change into an anaerobic process. The products of the anaerobic biochemical oxidation cause the system to become foul-smelling and poisonous to aquatic life.

The B. O. D. is usually measured by an arbitrary standard test procedure, but may be determined by manometric measurement of the free oxygen utilized. In either case the B. O. D. determination of a given waste requires at least 24 hours. Since the free oxygen involved in the B. O. D. of a waste is utilized in metabolic processes of various microorganisms present, an increase in the number of microorganisms lowers the time required for oxidation of the wastes.

The purpose of this investigation was to develop a rapid method for the determination of the B. O. D. of semichemical sulfite pulp mill wastes using the direct Warburg technic and high concentrations of microorganisms.

II. LITERATURE REVIEW

The literature was reviewed for pertinent information concerning stream pollution; the biological and chemical tests for determining the pollutional potential of wastes; and the use of the Warburg constant-volume microrespirometer. The literature was also examined to determine the results of related projects and to examine the various theories advanced.

Stream Pollution

Phelps[27] categorized the effects of stream pollution as being manifest in physical, chemical, and bacterial changes.

Physical Pollution. Physical pollution is caused by the presence of suspended solids, insoluble liquids, and coloring matter in the stream[27]. On settling, the solids often form a coating on the bottom of the stream which tends to become anaerobic. Insoluble liquids on the surface of the water prevent the diffusion of oxygen into the stream. Coloring matter, as well as all types of physical pollution, offend the aesthetic nature of people and is often prohibited on that basis.

Chemical Pollution. Chemical pollution, as defined by Phelps[27], includes the biochemical oxidation of organic matter in streams resulting in chemical changes. The chemical changes in turn cause a loss of oxygen dissolved in the water.

Ellis[13] adds that industrial and municipal wastes may be detrimental to aquatic life either indirectly by quantitative alterations in the normal characteristics of the stream such as dissolved oxygen, dissolved carbon dioxide, and hydrogen-ion concentration; or directly by specific physiological and toxic effects on the aquatic organisms themselves. In determining the effects of a given stream pollutant, which may be a simple compound or a mixture of complex organic substances whose exact composition is unknown, it is necessary to determine the modifications of the environment and the specific physiological actions attributable to each individual pollutant[17].

Ellis[13] also indicates, and it is generally accepted, that the degree of chemical pollution of most streams can be accurately determined by a measure of the molecular oxygen dissolved in the waters of the stream. If the rate of oxygen loss is greater than the rate of diffusion of the oxygen from the air into the water, the oxygen balance is affected. This change in the oxygen balance is always followed to some extent by

fluctuations in the life processes of the microorganisms, especially bacteria. A variation in the species of dominant organisms may take place. In extreme cases, chemical pollution may lead to total exhaustion of the oxygen, production of the odors of putrefaction, and the destruction of the normal flora and fauna. The capacity of the stream to cope with this type of pollution is based wholly on the free oxygen concentration; which, in turn, depends on the rate of diffusion of the oxygen into the stream. The oxygen utilized in the biochemical oxidation of the organic wastes is referred to as the biochemical oxygen demand of the wastes. When the oxygen demand of the wastes is properly embodied in a general formula involving certain stream characteristics such as volume, velocity of flow, and depth; the demand may be utilized as a measure of the influence of any given source of pollution upon the receiving stream(28). The oxygen demand of a waste is high or low, depending on the stability of the waste. The stability of a waste is a property of the organic matter present and is measured by the resistance of the organic matter to biochemical oxidation; or, inversely, by the availability of the organic matter as a bacterial food. Sewage, for instance, is considered to be highly unstable.

Paessler[26] in discussing chemical pollution states that in any given problem the volume and analysis of the waste discharged must be known; together with a knowledge of the oxygen demand of the individual waste components.

Bacterial Pollution. Phelps[27] defines bacterial pollution as the influx of pathogenic bacteria into the stream.

History of Stream Pollution Studies

During the latter half of the nineteenth century, social and economic changes brought about an increasing interest in the problem of stream pollution. Advances in the field of bacteriology by Pasteur paved the way for Dupre's[4] discovery in 1884 that the oxygen depletion in water was caused by the metabolism of microorganisms. The British Royal Commission on Sewage Disposal[31] then began a series of investigations and analytical determinations which led to the standard test for measuring the biochemical oxygen demand, or B. O. D. Prior to, and part of, the development of the B. O. D. test were the putrescibility and relative stability tests.

Putrescibility Test. Early stream pollution studies were made, Phelps[29] points out, without a basic knowledge of the concept of stability of a waste; consequently, the tests used

were related to the pollutant as a whole rather than to the organic matter which it contained. This distinction can best be demonstrated by a reference to the technic of some of the "incubation," "putrescibility," or "smell" tests, as they were variously called. In general, a tightly stoppered bottle was filled with an effluent under examination and incubated for a certain period of time, usually several days. If during that time the contents of the bottle became dark in color, developed a putrefactive odor, or gave other evidence of having developed an anaerobic condition; it was pronounced "putrescible" or "unstable." The time required for the development of the anaerobic state was noted and the effluent was then said to be "stable" for that length of time. It can be seen that this test was an indicator of the degree of pollution, in a limited sense.

Mechanism of the Putrescibility Tests. In the various putrescibility tests, the physical changes in the color and odor were manifestations of the various stages of biochemical oxidation[29]. The bottle was closed to the atmosphere and the only oxygen available, except for that chemically combined with the carbon compounds, was the molecular oxygen dissolved in the water, the nitrate and nitrite oxygen, and the sulfate oxygen. When the dissolved oxygen had been utilized, the oxygen of the nitrite,

nitrate, and sulfate compounds was used in that order. When the sulfate was reduced, hydrogen sulfide was formed which reacted with the salts that might have been present, resulting in a black or brown precipitate. The dark color could also have been caused by the organic products of the anaerobic digestion. At the oxidation potential near which hydrogen sulfide was formed, the nitrogenous and carbon compounds were decomposed yielding putrescent compounds. The end point of the test was, therefore, the level at which the sulfate decomposed and was determined by smell and appearance. Although the end point determination was improved by the addition of methylene blue, which turns colorless at the sulfate oxygen-depletion level, the putrescibility test at best merely indicated the keeping property of a pollutant in the absence of air at a certain temperature. Since this condition is seldom found in streams, the test was of little value[29].

Relative Stability Test. On the basis of the putrescibility tests, Phelps[30] developed the concept of relative stability, the forerunner of the present standard B. O. D. test. Phelps proposed a scale of "relative stability" in which a stability of 100 per cent represented a condition of equilibrium between the ultimate or long-time oxygen demand and the available oxygen. In terms of the putrescibility test, 100 per cent stability meant

that the bottle could be kept stoppered for an indefinite length of time without becoming anaerobic. On the basis of approximately 2,000 putrescivility tests, Phelps[32] postulated that the biochemical reaction was a first-order or monomolecular reaction, i. e., the rate of reaction was proportional to the concentration of the reactants present. The consequent differential relation was used to derive the following equation:

$$(1) \qquad \text{Per Cent Relative Stability} = \left[1.000 - (0.794)^{t}\right] \times 100$$

where:

t = time of incubation required for putrefaction, days

This equation indicated, and it was found to be true experimentally, that a relative stability of 50 per cent represented a supply of available oxygen one-half that required for the ultimate stability. According to the established rate of rate reaction, one-half of the ultimate stability would be obtained in a period of three days. Using equation (1), if the contents of the stoppered stability bottle turned brown or otherwise indicated that a putrefactive decomposition of the wastes present was taking place after three days, the relative stability was determined to be 50 per

cent. This figure follows since 0.794 raised to the third power is 0.50.

Present-Day Oxygen Demand Measurements. Out of the relative stability tests, the standard or dilution B. O. D. test has grown and today is used universally to determine the pollution potential of wastes. In addition, the oxygen uptake of wastes can be measured directly. Also, the chemical oxygen demand test has been developed in recent years to measure the total possible oxygen requirements of organic wastes.

Standard Biochemical Oxygen Demand Test

The standard biochemical oxygen demand test is a determination of the amount of dissolved oxygen required by a mixed population of organisms on a specific waste over a five-day period in a sample incubated at 20 °C[40]. The specifications for this test are given in the "Standard Methods of Water Analysis" published by the American Public Health Association[1]. The purpose of this arbitrary test, according to Borden[2], is to give an accurate measure of the amount of oxygen necessary to stabilize the organic content of an effluent. New developments in, and modifications of, the test are plentiful in the literature[2,4,23,40].

Paessler and Hedgepeth[26] consider the B. O. D. test to be a laboratory measurement of the chemical oxygen demand and of the oxygen taken from the stream biota in breaking down the organic matter contained in wastes discharged into it. The definition by Paessler and Hedgepeth takes in a wider field in that the oxygen demand by the chemically unstable organic compounds is also considered.

In reference to the standard B. O. D. test, Phelps[31] states, "This test introduced the element of time, but in only one arbitrary value. It actually determined the amount of oxygen that would be abstracted from the stream during the ensuing five days."

Procedure for the Standard B. O. D. Test. The B. O. D. test[26] is described briefly as follows: two identical bottles are each filled with a mixture containing known percentages of the waste sample and oxygenated water. The bottles are then inoculated with organisms similar to those found in the stream. The dissolved oxygen present in one bottle is determined immediately; the other is incubated at 20 °C for five days and the dissolved oxygen is determined. The difference in the dissolved oxygen values is found and divided by the decimal fraction of the waste in the dilution bottle. The quotient thus obtained is the B. O. D.

In many cases the above procedure is modified[40]. Instead of the organisms being added to the bottles separately, the

dilution water is seeded with organisms. The dilution water is incubated also for five days and then the dissolved oxygen is determined.

Rate of Reaction in the Standard B. O. D. Test. As has been stated previously concerning the relative stability test, it has been postulated[32] that the rate of oxidation of the organic wastes by the microorganisms is proportional to the concentration of wastes present at any time under consideration. Differentiating the consequent first-order equation, the following relation was obtained:

$$(2) \qquad L_{t/L} = 10^{-kt}$$

where:

L = the concentration of waste at beginning of test

L_t = the concentration of waste at any time, t.

t = the time of incubation at 20 °C in days.

The relationship for relative stability was derived from this equation. Equation (2) is used today to predict, from a known three-day B. O. D., for instance, the five-day B. O. D. The ultimate B. O. D. is also predicted from equation (2). The

ultimate B. O. D. is the oxygen demanded in the complete biochemical oxidation of the waste in question and corresponds to the 100 per cent stability concept.

Environmental Effects on the Standard B. O. D.

The speed and extent of the biological action of microorganisms on wastes in the standard B. O. D. test[26] depends on the environmental conditions provided for the microorganisms. The major environmental conditions which have been found to affect the B. O. D. values are the type of organic matter in the waste, the concentration of waste in the dilution bottle, the type of organisms in the inoculum or dilution water, the temperature of incubation, and the type of dilution water.

Effect of Food Concentration on the Standard B. O. D.

Phelps[37] states, "There are no obvious reasons why a biochemical reaction of this sort should proceed in accordance with the monomolecular formula." This apparent dilemma is explained by the "implication that the metabolic activities of the bacteria proportion themselves exactly to the concentration of the available organic matter remaining at any time[37]. This explanation applies only after the bacteria have passed the

logarithmic growth phase, since on experiment it was found that the rate of growth of the bacteria determined the rate of reaction, rather than the mere number of bacteria present during the logarithmic phase. However, since the rate of growth of bacteria is considered to be a first-order reaction, the explanation followed. The exact cause for the equilibrium between the bacteria and the food source has not been determined. While the number of bacteria reaches a ceiling value for a corresponding concentration of food, this ceiling value is increased with an increase in the concentration of the food. The increase in the ceiling value causes a consequent decrease in the food concentration. The experiment of adding more bacteria has not been tried, but it is known that bacteria tend to die slowly after reaching saturation values[37].

Although there seems to be no really substantial justification for holding that the rate of decrease of B. O. D. is exactly monomolecular, in practical applications it appears[36] that the observed rates justify the use of equation (2). On the other hand, there are some facts that suggest that the monomolecular law does not exactly express the situation. Butterfield's[6,30] data indicate ceiling values that are proportional to about the eight-tenths power of the concentration. This relationship indicates

a corresponding decrease of about the same order in the rate of multiplication. This means that at different dilutions of the wastes, there would be a difference in the ceiling values of the number of bacteria present. Therefore, for varying dilutions of the same waste, the standard five-day B. O. D. would vary.

Phelps[38] indicates that it is a matter of common experience among sewage works chemists that high dilutions do give higher B. O. D. values. While it is known that the effect of increasing concentration on the B. O. D. values is to depress the B. O. D. throughout the ranges studied, the depression is not nearly as high as the eight-tenths power of the concentration. The degree of depression varies with the environmental conditions and the waste under consideration and no generalization can be made[38,52].

To summarize briefly, the rate of oxidation of organic matter is to a large extent determined by the existing bacteriological population and the rate of metabolic activity. If these factors are increased, the rate of oxidation is increased likewise. The basic fact, as Phelps[39] indicates, which underlies the observed consistent rate of biochemical oxidation of organic matter is that the oxidizing activity of the bacteria is proportional to the food concentration in a direct relation[53]. This relation holds only

within the range of experimental technic and the conditions naturally existing in streams.

Effect of Temperature on the Standard B. O. D. Just as any chemical or biochemical reaction rate is a function of the temperature, so is the rate of the B. O. D. reaction. The value for the rate constant, k, as given in equation (2), therefore, varies with temperature and is defined by the following equation which has a theoretical derivation[34], but has been modified empirically to fit experimental data.

$$k_1/k_2 = \phi^{(T_1-T_2)} \tag{3}$$

where:

k = the rate constant as defined in equation (2)

T = the temperature, °C

ϕ = the temperature coefficient

Subscripts 1 and 2 indicate states 1 and 2.

The temperature coefficient, ϕ, as given by Streeter and Phelps[34], is 1.047. However, the temperature coefficient itself varies with the temperature, but not over 10 per cent of the ranges used.

The temperature may also affect the physiological characteristics of the microorganisms so that the end product of the metabolic processes may be altered or the type of organic waste preferably digested may change. In addition, the ultimate oxidizibility increases with temperature[35] in the region of 20 °C at approximately two per cent per degree. However, the increases are not constant for different types of waste.

Theriault[36] has presented an equation to determine the concentration of waste in the B. O. D. test at any temperature, T, as related to the concentration of waste at the standard temperature of 20 °C. This equation corrects for the two per cent increase in the ultimate oxidizibility of the waste per degree Centigrade temperature change.

$$L_t = L_{20} \cdot \left[1 + 0.02(T - 20) \right] \qquad (4)$$

where:

L = the concentration of the waste at any time, t

T = temperature of test, °C

Subscripts t and 20 °C indicate the temperatures of the tests.

By use of equations 1, 2, 3, and 4, the B. O. D. as determined at 20 °C over any time interval greater than one day can be converted to the value at any other temperature and time of incubation.

Effect of Inorganic Ions in Dilution Water. The water used to dilute the wastes in the standard B. O. D. test is of vital importance[33]. The B. O. D. test is an attempt to determine the degree to which bacteria will utilize an organic compound or compounds as food by measuring the oxygen requirements of the bacteria. However, not only must the organic substance be a bacterial food, but it must be a complete food or combined with other essential food requirements. In the B. O. D. determination on many industrial wastes, small quantities of the ingredients essential to bacterial metabolism such as nitrogen, potassium, sodium, calcium, magnesium, and phosphorus, have to be added to the dilution water[33].

Effect of Toxic Wastes on the Standard B. O. D. Bacterial metabolism[33] may be inhibited by substances that are toxic. A bactericidal or bacteriostatic influence may be exerted at certain concentrations and may not at other concentrations. For some wastes, an adverse influence may be exerted at any concentration used in the dilution test. In most cases of toxicity, the

B. O. D. increases with the dilution. This variation of the B. O. D. with dilution is referred to as the "sliding scale" effect[16,18].

Significance of the B. O. D. of Toxic Wastes. Heukelekian[17] points out that, although the deoxygenation of sewage is independent of concentration or dilution, in the case of certain industrial wastes the deoxygenation values vary with dilution. Further Heukelekian states[17], "The use of mineralized dilution water fortified with nitrogen and phosphorus, the correction of the pH value, and the proper seeding do not always give consistent values with such wastes. The B. O. D. sometimes varies with dilution... and therefore has little meaning unless the dilution used is specified. ...The dilution method cannot be used to determine the inhibiting effects (of toxic wastes) over a wide range of dilutions. Furthermore, the B. O. D. exerted in a treatment plant or stream may be entirely different unless the dilution corresponds to the dilution used in the B. O. D. determinations."

Elimination of Toxic Effects on B. O. D. Toxicity can be overcome sometimes by seeding the dilution water with organisms which have been acclimatized to the toxic element. Borden[2] found that the toxicity of paper mill waste, for instance, caused a distorted B. O. D. value, and that this distortion could be

eliminated by growing the seeding organisms in the presence of the paper mill waste.

Interferences in the Standard B. O. D. Determination. Borden[2] indicates that; while such substances as compounds of mercury, phenols, and some dyes affect variations in the B. O. D. by toxicity, other substances affect B. O. D. values that cannot be considered toxic. Rodgers[40] points out that certain salts, acids, and bases affect the oxygen solubility, and that at certain pH values the life of the organisms may be affected. The effect of such chemical additives are referred to as interferences[16].

Limitations of the Standard B. O. D. Test

For determinations of the B. O. D. by the standard dilution method, the following factors are involved[7,17]:

1) Varying dilutions of waste involving the use of several dilution bottles.

2) Especially prepared, aerated dilution water, the composition of which is dependent upon the waste under consideration.

3) An incubation period of five days.

The main defects of the test are two[17]: the different dilutions oft times do not agree and the amount of oxygen available is limited by the solubility of oxygen in the water limiting the

concentration of waste studied. In addition, the bacteria in the dilution water may not represent the type of bacteria in the stream.

Accuracy of the Standard B. O. D. Burtle[4] states that the standard B. O. D. values seldom confirm each other more closely than five per cent and often miss as far as 15 to 20 per cent. Burtle adds, "The dilution B. O. D. is distinctly an empirical determination. It measures only the oxygen taken up from a dilution water of a certain type in a certain definite length of time under a particular set of conditions of incubation. The fixed values obtained have meaning only as long as the conditions remain fixed."

Paessler[26] points out, "This test (B. O. D. by dilution), while indicative of the relative intensity of the pollution is not a high precision method..."

Use of the Monomolecular Equation. While the monomolecular equation is considered by some (Phelps[31]) to have theoretical significance, it has been stated by Ruchoft[41] that, "...There is nothing fundamental about the monomolecular equation in relation to the B. O. D. satisfaction. The application of the equation to the B. O. D. data is empirical in nature. Its success in this

fitting is essentially due to the tremendous flexibility of this exponential equation."

Moore, et al[23], point out that the purpose of any B. O. D. test is to determine the oxygen strain on the stream, and further: "However useful the usual 5-day B. O. D. may be as a practical means of controlling plant operation, it is general conceded that it does not give an answer to the problem of subsequent oxygen depletion in the stream. What is needed to be known is both the total ultimate oxygen-consuming power of the waste and the rate." Moore[23] regards the monomolecular equation as a good first approximation to be developed empirically for each waste. From the developed empirical equation, which follows the monomolecular form, the rate (k) and ultimate oxygen demand values are determined.

Ruchoft[41] points out that when the equation applies, it has the following advantages:

1) The equation is dimensional.

2) The value of the reaction rate, k, indicates the rate for comparison purposes.

3) The equation limits the ultimate demand, which is entirely theoretical, but is useful for comparison.

Toxic and High B. O. D. Wastes. Heukelekian[16] notes that the concentration employed in the B. O. D. determinations are limited by the B. O. D. of the waste. If the waste contains large quantities of oxidizible material in addition to some specific toxic ingredient, higher dilutions than usual will have to be employed, which may or may not overcome the inhibition, depending on the concentration and potency of the toxic material. However, at extremely high dilutions the B. O. D. values are usually very erratic and the dilution in the river may not be as high as in the dilution bottle.

In determining the B. O. D. of various activated sludges, Hurwitz et al[19], and Dawson and Jenkins[12] report that the determination of the B. O. D. by the dilution method gives false values because of the active nitrification and point out that it is impossible to use the dilution method with activated sludges.

Standard B. O. D. of Industrial Wastes. Lyon[22], in studying the effect of synthetic organic chemical wastes on the Kanawha River in West Virginia, stated, "Results ... certainly indicate that the standard B. O. D. test is not applicable to the evaluation of many industrial organic wastes. Six out of the 17 organic compounds tested gave results which were acceptable. No attempt was made to determine the k (the reaction

rate) values as it was evident that they would be of little value." An example of Lyon's results are given in Table III.

Chemical Oxygen Demand

A determination of the stoichiometric amount of oxygen required in the complete oxidation of a waste is referred to as the chemical oxygen demand[26]. While there are several methods for determining the chemical oxygen demand, or C. O. D.; most determinations involve the use of potassium permanganate or potassium dichromate with varying amounts of 95 per cent concentrated H_2SO_4 and 85 per cent H_3PO_4[4,20]. The organic wastes of known quantities are added to the oxidizing solution and refluxed for about one hour. After refluxing, usually in the presence of a catalyst, the solution is cooled and an excess of potassium iodide is added. The solution is back-titrated with sodium thiosulfate[20] to determine the unreacted potassium iodide. From this data the amount of carbon oxidized can be calculated.

Comparison of B. O. D. and C. O. D. Values. A comparison of C. O. D., dilution B. O. D., and direct B. O. D. values is given in Table I. The effect of various oxidizing agents on the C. O. D. values of wastes, together with the corresponding dilution B. O. D.

values, are given in Table II and in Table III. Ingols[20] points out that the values for the organic carbon present, obtained by titration, represents the ultimate B. O. D. and, in order to approximate the five-day dilution B. O. D., the C. O. D. should be multiplied by 0.68 to give an approximation of the five-day B. O. D. The 0.68 is the fraction of organic matter oxidized in the five-day B. O. D. as indicated by the monomolecular equation.

Uses of the C. O. D. Besides being recommended as a control means in waste treatment plants, the C. O. D. can be used as a check on the dilution B. O. D. values[26]. Weston[49] indicates that a determination of the C. O. D. is valuable to use with the dilution B. O. D. in forming an idea of the characteristics of the waste being treated.

Criticism of the C. O. D. Only in a few wastes do the C. O. D. values coincide with the B. O. D. determinations. Gehm[15], in discussing the problems faced in interpreting the C. O. D. values, points out that, "No chemical oxidation test will measure the oxygen utilized by microorganisms under natural conditions."

Phelps[33] states, "It (the biochemical oxygen demand) is not at all related to the complete oxygen requirements in

chemical combustion but is determined wholly by the availability of the material as a bacterial food and by the amount of oxygen utilized by the bacteria during its oxidation."

An excellent example of the discrepancy in the dilution B. O. D. and the C. O. D. values is given in the case of formaldehyde and oxalic acid. The B. O. D. of formaldehyde is usually twice the stoichiometric value while the B. O. D. of oxalic acid is zero. The C. O. D. values, for the cases cited, correspond closely to the stoichiometric values[26,49].

The C. O. D. values, in many cases, however, do not agree with the stoichiometric values and, in general, vary with the oxidizing agent used. Lyon[22] studied the C. O. D. values obtained from synthetic organic wastes, and states, "Results were obtained from a series of tests to compare the acid permanganate and the acid dichromate oxidation tests with the standard five-day B. O. D. test in an effort to obtain some yardstick to evaluate the organic wastes. Here again 17 different organics were tested, but no trend acceptable for all organic compounds was indicated. The results which would be obtained from the oxidation of a combination of the organics tested, no doubt, would be different but cannot be anticipated." Lyon's results for a few of the typical organics are given in Table III.

TABLE I[a]

Chemical Oxygen Demand, Standard B. O. D., and Direct B. O. D. Values for Some Samples of Domestic Sewage

Oxygen Consumed, (C.O.D. from $KMnO_4$) ppm	Standard B. O. D. ppm	Direct B. O. D. (Nordell Odeeometer) ppm
114	121	123
26	60	65
63	125	113
71	153	141
84	180	137

(a) Burtle, J. and A. M. Boswell: Oxygen Demand Studies, Sewage Works Journal, 9, Table VI, page 237, (1937).

TABLE II[(a)]

A Comparison of the Chemical Oxygen Demand of Some Synthetic Wastes as Obtained with Different Oxidizing Agents

Synthetic Waste	Per Cent of Theoretical Oxygen Necessary for Complete Oxidation			
	Oxidizing Agent CrO_3, H_2SO_4, H_3PO_4	Oxidizing Agent $KMnO_4, H_2SO_4$	Oxidizing Agent $K_2S_2O_8$	Oxidizing Agent $KMnO_4$ NaOH
Acetic Acid	99.0	98.6	98.3	13.8
Benzene	32.3	12.2	25.1	1.6
Glycerol	97.0	94.7	96.2	92.0
Sucrose	98.2	96.8	98.3	97.4

(a) Burtle, J. and A. M. Buswell: "Oxygen Demand Studies," Sewage Works Journal, 9, Table III, page 232 (1937).

TABLE III[a]

The Standard Five-Day B. O. D. and the C. O. D., Using Various Oxidizing Agents, of Some Organic Compounds

Compound	Per Cent of Theoretical Oxygen Demand Satisfied		
	Acid Permanganate	Acid Dichromate	Standard 5-Day B. O. D.
2-Butanol	2.9	23.9	0.0
n-Butyraldehyde	5.5	11.5	44.3
Isopropyl Ether	0.0	0.6	0.3
Ethylene Glycol	8.8	80.8	1.8
Acetone	2.3	5.5	55.4
Ethanolamine (Mono)	5.4	44.9	0.0
Methanol	4.8	90.7	53.4

(a) Lyon, R. D.: Disposal of Synthetic Organic Wastes, Ch. E. Progress, 46, 392-3 (1950).

Direct Measurement of the B. O. D.

In an effort to overcome the difficulties of the standard dilution B. O. D. test, several means have been devised for the direct measurement of the gaseous oxygen taken up in the biochemical oxidation of the bacterial food. Four types[8] of apparatus, the Fenn, Barcroft, Haldane, and Warburg, are in common use. These various methods are discussed in the literature by Burtle et al[5,8]. Since the Fenn, Barcroft, and Warburg equipment are all microrespirometers, only one, the Warburg microrespirometer, will be presented. The Nordell Odeometer is unimportant, relatively speaking, but is considered since the investigators utilizing this equipment have presented data comparing the three major methods of B. O. D. determination.

Haldane Apparatus

In 1928, Sierp[24] used a modified Haldane apparatus for the measurement of molecular oxygen taken up by undiluted domestic sewage. In Sierp's experiments, sewage was maintained in contact with pure oxygen and the rate of absorption was measured in a calibrated eudiometer tube. The carbon dioxide liberated was absorbed from the oxygen by a concentrated sodium

hydroxide solution which was used to read the volume changes in the eudiometer. The apparatus is similar to the Orsat equipment for gas analysis. To keep the sewage saturated with oxygen, it became necessary[14,24] to shake the apparatus at ten-minute intervals. Sierp obtained approximately ten per cent higher results with his apparatus than by the dilution method.

Neomeyanov[24] utilized the Haldane apparatus to determine the B. O. D. of sewage and anaerobic river-bottom mud, and found the results to be accurate within five per cent.

Industrial Waste Studies. Falk[14] determined the direct B. O. D. with almost the same type of equipment as that used by Sierp. Falk studied the direct B. O. D. of sewage, slaughterhouse wastes, and sulfite liquors. The dilution and direct B. O. D. values tallied within plus or minus ten per cent for the slaughterhouse wastes and the sewage. The direct values were repeated with an accuracy of five per cent.

However, in determining the direct B. O. D. for sulfite liquors, the results were found to be lower than the dilution values and to vary widely. Since in the Sierp method the waste is not diluted much, if at all, the discrepancy in the values for the sulfite liquors is explained by the presence of a higher concentration of solids[14]. In addition, it was thought that the

higher concentration of waste would increase the inhibitory effects of any toxic elements which might be present.

The direct B. O. D. also varied with the type of seeding used. For sulfite liquors which was unseeded or seeded with sewage, the direct B. O. D. was found to be 1,000 parts per million. The dilute B. O. D. was found to be 17,000 parts per million. When the sulfite liquor was seeded with a soil inoculum, the direct B. O. D. was found to be only 730 parts per million. A seed was prepared also by growth in the presence of the sulfite liquor, and this adapted or acclimatized seed gave a direct B. O. D. of 6,200.

Criticism of the Haldane Method. Falk[14] points out that the Haldane determinations are affected by the toxicity of the industrial wastes, and that incomplete and slow absorption of the carbon dioxide by the sodium hydroxide necessitates correction factors which are not known to be reliable when high concentrations of carbon dioxide are evolved.

Nordell Odeometer

The Nordell Odeometer is presented by Burtle and Buswell[3,4] as a direct means of determining the B. O. D. of sewage and industrial wastes. A comparison of the C. O. D., standard dilution B. O. D., and the Nordell B. O. D. values of some wastes are presented in Table I. As it can be seen from Table I, the Nordell values are in the same range as the standard B. O. D. values, but that the degree of agreement is erratic.

The method for the determination of the Nordell B. O. D. within ten hours applies only to certain types of wastes, notably sewage, which give a constant oxygen uptake after the first ten hours or whatever time interval is used. The B. O. D. is obtained by assuming that the rate of oxygen uptake remains constant after the first ten hours, and multiplying the constant rate by the time of the five-day period remaining. The apparatus is calibrated so that the oxygen uptake can be determined from a direct reading of a gage. Two criteria are fundamental for the determination of the B. O. D. by this method in a time period less than five days:

1) The digestion of the waste studied must stabilize itself after a preliminary logarithmic phase to give a constant oxygen uptake.

2) The digestion must not enter a nitrification stage, a definite possibility where the rate of reaction is higher than that in the dilution determination[3,4].

Warburg Constant-Volume Respirometer

The Warburg constant-volume respirometer has found wide use in metabolic studies of microorganisms by determining small amounts of gaseous absorption or evolution. The pressure change because of variations in the amount of gas present at constant volume and temperature can be used to calculate the quantity of gas exchanged. Langelier and Caldwell[9] first made use of the Warburg apparatus to determine the B. O. D. of sewage by direct means.

Description of Warburg Apparatus. The Warburg respirometer consists of a constant temperature bath to which a shaking apparatus is attached. Reaction flasks are connected to manometers which are in turn attached to a frame on the shaking mechanism. Each reaction flask is attached to a U-type capillary manometer with one end open to the atmosphere and the other to the closed reaction flask. The flask is thus closed to the atmosphere and any changes in the pressure in the flask will affect the height of

the manometer fluid. The height of the manometer fluid is controlled by a screw clamp on a rubber or plastic tube which acts as a fluid reservoir.

Calculation of the Gaseous Exchange. The essential principle of the measurement of the gaseous exchange in the reaction flasks is that, if the volume and temperature of a gas are held constant, the changes in the amount of gas can be measured by the changes in the pressure[43]. Since pressure and quantity of gas present are the only variables, the ideal gas law will indicate:

(5) $$x \propto h \quad \text{or} \quad x = kh$$

where:

x = the microliters of gas at standard conditions

h = the millimeters change in height of manometer fluid

k = the proportionality constant or flask constant.

The flask constant, k, is determined at calibration so that the gaseous exchange is expressed at standard conditions.

Determination of Flask Constant. The flask constant can be determined from the following equation which is derived in the literature[43]:

$$k = \frac{V_g \frac{273}{T} - V_f \alpha}{P_o} \quad (6)$$

where:

V_f = volume of gas phase in flask including connecting manometer tubes down to the fluid, microliters

T = temperature of flask, °K

P_o = standard pressure of one atmosphere expressed in terms of the manometer fluid, millimeters of manometer fluid

α = solubility of oxygen at temperature, T, expressed as milliliters of oxygen, at one atmosphere and 0 °C, per milliliter of water.

The volume of the flask and of the capillary in the manometer tubing may be determined by weighing the amount of mercury

required to fill them (see Table IV). The technics and various methods of calibration are described by Burris and Umbreit[45].

Determination of the Oxygen Uptake. The oxygen uptake of microorganisms in the reaction flask can be determined merely by providing a 20 per cent by weight solution of potassium hydroxide in a center well of the reaction flask to absorb all of the carbon dioxide evolved. Burris[43] indicates that the assumption that the only gaseous product of anaerobic metabolism is carbon dioxide has been verified thoroughly. Thus, the only gaseous exchange which takes place is the absorption of oxygen. The constant shaking of the reaction flasks results in the bacterial suspension remaining saturated with molecular oxygen.

Correction for Temperature and Pressure Changes. If changes in the atmospheric temperature or pressure occur during the experimental work, corrections must be made in the manometer readings. The correction factor is obtained from the thermobarometer, a respirometer with water in the reaction flask instead of a suspension of microorganisms. Since in the thermobarometer manometer, no gas is evolved or absorbed, the change in the manometer reading must be caused by a change in the temperature or pressure of the atmosphere. The pressure change in the thermobarometer manometer is added to or subtracted from the

TABLE IV

Calculated Volumes of the Reaction Flasks, Graduated Sections of the Manometers, and Ungraduated Sections of the Manometers for Calculation of the Total Volume of the Respirometers

Companion Manometer No.	Flask No.	Average Calculated Volume		
		Flask	Manometer	
		ml	Ungraduated Section ml	1 cm of Graduated Section ml
1	XI	16.66082	0.51696	0.01787
2	II	13.38797	0.57596	0.02024
3	III	13.69120	0.60622	0.02255
4	4a	17.45409	0.55515	0.02158
5	VII	13.93247	0.55356	0.02090
6a	VI	13.39413	0.64845	0.01987
8	XVII	17.44369	0.37440	0.01483
9	XIX	18.82468	0.36105	0.01354
10	XX	17.17540	0.40184	0.01486

Watkins, P. H.: Personal Communication, July 1, 1950.

observed pressure change of the other manometers, depending on the change in the atmospheric conditions.

Determination of the B. O. D. with the Warburg Respirometer

The Warburg apparatus has been used to measure the biochemical oxygen demand of wastes directly[7]. Values for industrial wastes and sewage were obtained which, in general, were higher than the standard dilution B. O. D. values. In addition, the Warburg apparatus has been used by Dawson and Jenkins[12] to determine the B. O. D. of activated sludges and the effects of temperature, pH, and various concentrations of synthetic wastes on the B. O. D. of activated sludges.

Reaction Rate of the Direct B. O. D. Langelier and Caldwell[9] found that the manometric method gave both higher reaction rate and ultimate demand values of the B. O. D. It was found that the one-day direct B. O. D. was approximately 75 per cent of the five-day value. At 25 °C, the temperature which Langelier and Caldwell used, the standard dilution one-day B. O. D. is only 33 per cent of the five-day value.

Langelier and Caldwell concluded that the reaction rate of the direct method was more than double that of the standard rate.

Effect of Concentration on the Direct B. O. D. When, in the Warburg method, concentration is made the only variable, it is found that the dilution lowers the calculated demand and that the effect of dilution is most noticeable at the lower concentrations[9]. The proportionately higher rate of oxygen demand in the Warburg method of undiluted sewage compared to various degrees of diluted sewage is verified by the accepted theories of reaction velocity[10] which indicate that the reaction rate is dependent upon the concentration of the reactants.

Standardization of Manometric Method. Langelier and Caldwell[11] suggest the use of a 24-hour incubation period and a 25 °C incubation temperature. The 24-hour or one-day B. O. D. was recommended since in that time 75 per cent of the five-day value had been reached. The 25 °C incubation temperature was used because around 30 °C nitrification is believed to affect the B. O. D., and at lower temperatures the reaction rates are less.

In addition, it was suggested[11] that the following method be used to eliminate the seeding problem in the determination of the direct B. O. D. of industrial wastes: the B. O. D. of a

mixture of sewage and the industrial waste is determined, together with the B. O. D. of the sewage alone. The B. O. D. of the industrial waste is then the B. O. D. of the sewage and industrial waste mixture minus the B. O. D. of the sewage alone.

Criticism of the Warburg Method. Langelier and Caldwell[9] indicate that the Warburg apparatus is well adapted for the study of reaction velocity because all of the variables are subject to ready control, and readings at various time intervals can be made without disturbing the reaction. However, it is pointed out that the equipment is fragile and expensive, requiring an experienced analyst as operator.

Resting Cell Technic

In bacteriological uses of the Warburg apparatus, high concentrations of nonproliferating or resting cells are often used in determining the effect of environmental factors on cell respiration. Resting cells without any carbon food source are said to be in an endogenous state. Endogenous or starving cells still utilize oxygen, but at a lower rate than the resting cells. The oxygen uptake of the endogenous cells is subtracted usually from the oxygen uptake of the suspension of resting cells to

give the oxygen uptake resulting from the bacterial digestion of the carbon source. However, in some investigations the endogenous uptake may not be subtracted, but merely be reported along with the other uptake values. The resting cell technic, used in conjunction with the Warburg respirometer, is described in the literature by Wilson(50).

Nephelometric Analysis

Since, in the resting cell method for the study of bacterial metabolism, the number of organisms present in the tests must be held constant or be known, the washed suspension of resting cells must be analyzed to determine the concentration. The nephelometric methods of quantitative analysis have been found to be advantageous in many cases because of speed, simplicity, precision, and accuracy over a wide range(44). The results of the nephelometric analyses have been found to correspond with colony counts(52). The use of the photoelectric colorimeter as a nephelometer is advocated by Umbreit(44).

III. EXPERIMENTAL

The experimental part of this investigation is described by an indication of the purpose of the investigation, an outline of the plan followed in the experimentation, a list of materials and apparatus used, a description of the methods of procedure, and a presentation of the data and results obtained.

Purpose of the Investigation

The purpose of this investigation was to develop a rapid method for the determination of the B. O. D. of semichemical sulfite pulp mill wastes using the direct Warburg technic and high concentrations of microorganisms.

Plan of Experimentation

The preliminary experimentation consisted of a review of the literature, calibration of the photoelectric colorimeter, and the procurement and growth of the organisms. After the optimum temperature and concentration of wastes were determined, the manometric B. O. D. values were found for four wastes. The wastes studied were blowdown liquor and total mill wastes from a

semichemical pulp mill, raw sewage from a sewage treatment plant, and effluent from a sewage-blowdown liquor anaerobic digester. To determine the significance of the manometric B. O. D. values obtained, the effects of waste and bacterial concentration were studied.

Materials

The materials used in the experimental part of this investigation to determine the manometric B. O. D. of various wastes utilizing high concentrations of lake water bacteria are listed in the following section.

Bacteria. Lake water, obtained from Lake Solitude on the campus of the Virginia Polytechnic Institute, Blacksburg, Va. Used in all determinations of the B. O. D.

Benzene. Crude, obtained from the stockroom of the Chemical Engineering Department, Virginia Polytechnic Institute, Blacksburg, Va. Used to dissolve grease on the flask joints.

Blowdown Liquor. Semichemical, sulfite, 145,000 parts per million total solids, obtained from Mead Corporation, Lynchburg, Va., through the courtesy of the National Council for Stream Improvement project on semichemical pulp mill waste treatment

of the Virginia Polytechnic Institute, August, 1950. Used in determining the effects of waste and bacterial concentrations.

Detergent. "Tide," manufactured by the Procter and Gamble Co., Inc., Baltimore, Md. Distributed by the Great Atlantic and Pacific Tea Co., Blacksburg, Va. Used as a cleaning agent for the Warburg flasks.

Effluent. From an anaerobic, sewage-blowdown liquor, digester, obtained from the National Council for Stream Improvement project on semichemical pulp mill waste treatment of the Virginia Polytechnic Institute, August, 1950. Used in determining effects of waste and bacterial concentration on effluent.

Lanolin. Anhydrous, U. S. P. grade, lot No 499143, manufactured by the Fisher Scientific Co., Pittsburgh, Pa. Used in preparation of glass joint lubricant.

Nutrient Broth. Difco dehydrated, control No 387625, formula of "Standard Methods of Water Analysis" of the American Public Health Association. Manufactured by the Difco Laboratories, Inc., Detroit, Mich. Used as a broth medium for growth and storage of the lake water bacteria.

Potassium Hydroxide. Reagent grade, lot No 481635, catalogue No P-250, manufactured by the Eimer and Amend Co., New York, N. Y. Used to absorb carbon dioxide evolved in the reaction flasks.

Potassium Phosphate, Monobasic. KH_2PO_4, analytical grade, lot No 1.548, manufactured by the J. T. Baker Chemical Co., Phillipsburg, N. J. Used in the preparation of the buffer solution.

Raw Sewage. Obtained from the Sewage Disposal Plant of the Virginia Polytechnic Institue, August, 1950. Used in determining manometric B. O. D. values of raw sewage.

Sodium Hydroxide. Reagent grade, code No 2325, lot No E2241, manufactured by the General Chemical Co., New York, N. Y. Used in preparation of the buffer solution.

Total Mill Wastes. Semichemical, sulfite, 2,000 parts per million total solids. Obtained from Mead Corporation Lynchburg, Va., through the courtesy of the National Council for Stream Improvement project on the semichemical pulp mill waste treatment of the Virginia Polytechnic Institute, November, 1950. Used in determining dilution B. O. D. of total mill wastes by manometric means.

Vaseline. White petroleum jelly, manufactured by the Chesebrough Manufacturing Co., New York, N. Y. Used to prepare glass joint lubricant for reaction flasks.

Apparatus

The apparatus used in the experimental part of this investigation to determine the manometric B. O. D. of various wastes utilizing high concentrations of lake water bacteria are listed in the following section.

Autoclave. Serial No 94863, manufactured by the American Sterilizer Co., Erie, Pa. Used for sterilization of materials, equipment, and solutions.

Balance. Analytical, 0 to 100 grams, chain-o-matic, manufactured by the Seederer-Kohlbusch Co., Inc., Jersey City, N. J. Used for all analytical determinations.

Balance. Triple-beam, 0 to 610 grams, patent No 1732612, manufactured by the Ohaus Co., Newark, N. J. Used for weighing nutrient broth.

Blender. Waring, 115 v, 3 amp, 25 to 60 cy, ac or dc, catalogue No 700, manufactured by the Waring Products Corp., New York, N. Y. Used for mixing bacteria to remove clumps.

Centrifuge. Chemical, maximum speed 3,000 rpm, 110 v, ac or dc, No X1249, manufactured by the International Equipment Co., Boston, Mass. Used for harvesting organisms.

Colorimeter. Klett-Summerson photoelectric, No 7093, 115 v, ac, manufactured by the Klett Manufacturing Co., New York, N. Y. Used for quantitative analysis of the bacterial suspensions.

Glassware. The glassware used was standard equipment. Obtained from the stockroom of the Chemical Engineering Department of the Virginia Polytechnic Institute.

Incubator. Model 70, 400 w, 115 v, manufactured by the Electric Hotpack Co., Inc., Fox Chase, Pa. Used for incubation of the organisms.

Oven. Drying, 40 to 95 °C, 110 v, 660 w, manufactured by the Will Corporation, Rochester, N. Y. Used for drying glassware and in the analysis of the dry bacterial weight of bacterial suspensions.

Refrigerator. Household "Frigidaire," 7.7 cu ft, Pc No 5858351, 10-2-48-295M(33), model ML-93, manufactured by the Frigidaire Division of General Motors, Dayton, Ohio. Used for storage of nutrient broth and stock cultures.

Stirrer. Varispeed, 110 v, 60 cy, manufactured by the Precision Scientific Co., Chicago, Ill. Used for aerating and mixing the bacterial suspensions.

Timer. "Precision Time-it," 0 to 9999.9 seconds, 0.1 second increments, 115 v, 60 cy, 5 w, manufactured by the Precision Scientific Co., Chicago, Ill. Used to determine the time of the tests.

Warburg Flasks. Catalogue No BW-125, manufactured by the E. Machlett and Son, New York, N. Y., and catalogue No 5-202, manufactured by the American Instrument Co., Silver Spring, Md. Used as reaction vessels in the Warburg respirometer.

Warburg Respirometer. "Precision," catalogue No 66706, serial No C-6, 115 v, 13 amp, 1,500 w, single phase, 60 cy, 10 units, including stand for supporting the manometers, manufactured by the Precision Scientific Co., Chicago, Ill. Used to determine the oxygen uptake of the bacterial suspensions.

Warburg Manometers. Catalogue No CBW-155, manufactured by the E. Machlett and Son, New York, N. Y., and catalogue No 5-200, manufactured by the American Instrument Co., Silver Spring, Md. Used to determine the pressure changes in the Warburg flasks.

Method of Procedure

The method of procedure consists of the procedure for sterilization of materials and equipment, growth and harvesting of the organisms, nephelometric analysis, preparation of the flasks, determination of the waste concentration and manometric B. O. D., and cleaning the reaction flasks.

Sterilization. Sterilization of the media and equipment was accomplished by autoclaving at 15 pounds per square inch, gage, steam pressure at a temperature of 250 to 260 °F for 15 minutes.

Growth of Organisms. The lake water bacteria, obtained from Lake Solitude on the campus of the Virginia Polytechnic Institute, were grown on nutrient broth containing 3.2 grams of anhydrous Difco nutrient broth and 400 milliliters of distilled water. The organisms were stocked in tubes containing five milliliters of nutrient broth at 5 °C. In preparing organisms for studies with blowdown liquor, two milliliters of the blowdown liquor were added to the one-milliliter bacterial inoculum. The three-milliliter inoculum was added to 400 milliliters of nutrient broth. The organisms were grown for 24 hours at 33 °C.

Harvesting of Organisms. The organisms were harvested by centrifugation in 50-milliliter centrifuge tubes in an International Chemical centrifuge. The speed of rotation of the centrifuge was 2,000 revolutions per minute and the time of rotation was approximately 30 minutes. After centrifuging, the supernatant liquid containing the remainder of the nutrient broth and waste inoculum was discarded and the settled organisms were resuspended in 200 milliliters of a 0.05 M phosphate buffer solution with a pH of 6.8. The buffer was prepared according to data given in the literature(21).

The organisms were resuspended to dilute further the remaining nutrient broth present with the organisms. The resuspended bacteria were agitated with a laboratory stirrer for 20 minutes to insure aeration so that the organisms might digest as much of the remaining nutrient broth as possible in an aerobic condition. This suspension was then recentrifuged to remove the cells from the solution containing the diluted nutrient broth which had not been digested. The supernatant liquid was again discarded and the organisms were resuspended in the phosphate buffer solution. The solution was then stirred while being made up to the desired concentration of organisms per milliliter of suspension. The bacterial suspension was then

placed in the Waring blender for a minute to remove any clumps of bacteria which may have formed.

Nephelometric Analysis. The desired concentration of bacteria per milliliter was obtained by use of the Klett-Summerson photoelectric colorimeter and a previously determined calibration curve relating the bacterial concentration and the colorimeter reading.

Calibration of the Colorimeter. For calibration of the Klett-Summerson photoelectric colorimeter, the centrifuged organisms were resuspended in distilled water instead of the phosphate buffer. After two washings in distilled water, as highly concentrated a bacterial suspension as possible was made up and analyzed with the colorimeter, and the reading recorded. Then volumetrically prepared dilutions of the bacterial suspension were analyzed with the colorimeter and the data recorded, until no variation in the colorimeter reading with further dilution was obtained.

To determine the weight of dry bacterial cells per milliliter of the original highly concentrated bacterial suspension, five-milliliter aliquot portions were dried at 85 °C for 24 hours. The dried samples were weighed and

placed in a desiccator over calcium chloride. The samples were weighed every 24 hours until the weight remained constant. The weights of dry bacterial cells per milliliter corresponding to the colorimeter readings for the various dilutions were then calculated. The calibration curve was plotted from the data relating dry bacterial weight per milliliter and the colorimeter reading (see Figure 4).

Determination of the Bacterial Concentration. The bacterial suspension was diluted to the proper concentration by means of the colorimeter and the calibration curve relating colorimeter reading and the dry bacterial weight per milliliter. The desired volume of dilution buffer solution to be added to the bacterial suspension was estimated by the following equation[46]:

$$\frac{\text{Concentration A}}{\text{Concentration B}} = \frac{\text{Volume B}}{\text{Volume A}}$$

where:

A = the actual bacterial suspension

B = the desired bacterial suspension.

Determination of the Waste Concentration. The determination of the concentration of waste to be used in each test was important since the experimental work was primarily a measurement of the oxygen uptake of a high concentration of lake water bacteria on dilute solutions of a given waste. The waste concentration to be used was controlled by two factors: the total oxygen uptake and the rate of the reaction. A low concentration of the waste was desirable so that less time, or a high reaction rate, would be involved in the test. Yet, the concentration of the waste, for best accuracy, had to be high enough to cause an optimum uptake of from 100 to 200 microliters per hour, according to Umbreit[44]. The proper waste concentration was determined by experimentation.

Determination of the Manometric B. O. D. Values. The manometric B. O. D., in milligrams of oxygen per liter of waste or parts of oxygen used per million parts of waste, was calculated from the oxygen uptake values in microliters. The oxygen uptake values increase slightly after an initial rapid uptake of oxygen. Therefore, the manometric B. O. D. was calculated from an arbitrarily taken value which varied less than two per cent over a ten-minute increment of time.

Preparation of the Warburg Respirometers. In preparing the Warburg respirometers for the experimental tests[44], the initial

step was the lubrication of the glass joints. To prevent creeping by the alkali, the top part of the center wells of each of the clean, dry Warburg reaction flasks was greased. A piece of cotton saturated with grease was used for this operation. Then, the attachment joint of the manometer and the side arm plug were lubricated. The grease used in these operations was a mixture of anhydrous lanolin and vaseline in a 1:1 ratio by weight.

The potassium hydroxide, 0.2 milliliter at a 20 per cent by weight concentration, was added to the center well. Two milliliters of a suspension of lake water bacteria, prepared at the desired concentration, were added to the body of the reaction flask. One-half milliliter of the waste, or food source, was pipetted into the side arm. A square piece of filter paper, one inch on the side, was folded to form a rectangle 1/4 by 1/4 inch. This folded paper was inserted into the center well. After the plug was inserted into the side arm, the flasks were attached to the manometers and placed on the Warburg apparatus.

To bring the temperature of the flasks to that of the bath and to insure saturated oxygen conditions in the bacterial suspension, the flasks were shaken for 15 minutes in the constant temperature bath. The oscillating apparatus was stopped. By means of the screw clamp, the manometer fluid was adjusted to

25 centimeters on the flask side of the manometer with the stopcock open. The stopcock was closed and readings were taken of the level of the manometer fluid in the open end. The respirometers were removed from the constant temperature bath. The contents of the side arm were dumped into the main body of the reaction flask, and any remaining liquid in the side arm was removed by backwashing. In this operation the open end of the manometer was closed with the forefinger to prevent movement of the manometer fluid. After the respirometers were returned to the constant temperature bath, shaking was initiated and differential pressure readings were taken at the time interval desired.

Determination of the Oxygen Uptake. The pressure changes in the reaction flasks as indicated by the height of the manometer fluid were read every 15 minutes for the first hour. The time interval for the readings after the first hour depended upon the rate of oxygen uptake. In cases where the effect of time on the oxygen uptake was not being studied, the readings could be taken at hourly intervals.

The pressure change caused by variations in the atmospheric temperature and pressure were corrected for by use of the thermobarometer. The pressure change in the thermobarometer manometer was added or subtracted, depending on the variations in the atmosphere, to the pressure change of the other manometers.

The oxygen uptake of the control containing distilled water instead of the food source, or waste, was subtracted from the total oxygen uptake of the microorganisms digesting the waste. The difference in the two oxygen uptake values was taken to represent the oxygen involved in the biochemical oxidation of the waste or the B. O. D. The oxygen uptake of the control was referred to as the endogenous uptake, since no food source was present.

Cleaning the Reaction Flasks. Umbreit[44] presents several methods for cleaning the reaction flasks. In this investigation the flasks were removed from the manometers, and the side arm plugs were removed from the flasks. The paper in the center well and the fluid contents of the flask were discarded. Benzene-saturated cotton was used to dissolve as much of the grease as possible from the reaction flasks. To remove the alkali, the flasks were flushed with water from the Virginia Polytechnic Institute mains, and placed in a beaker of boiling water and "Tide" detergent for 30 minutes. The flasks were removed from the boiling water, rinsed thoroughly with water from the mains, and finally with distilled water before being placed in the drying oven.

Nomenclature. In this investigation the following nomenclature was used to distinguish between the various biochemical tests for B. O. D. Standard dilution B. O. D. refers to the standard test for all sanitary and industrial wastes[1]. Manometric dilution B. O. D. refers to the direct measurement of the oxygen uptake by manometric means of the standard dilution test samples as outlined by Langelier and Caldwell[7]. Manometric B. O. D. refers to the direct measurement used in this investigation of the oxygen uptake of high concentrations of microorganisms because of the presence of the sanitary or industrial waste. All B. O. D. values are expressed in milligrams of oxygen per liter of waste[1], or parts per million. The microliters of oxygen are expressed at 0 °C and one atmosphere.

Data and Results

The data and results determined in this investigation are presented in the following sections.

Effect of Temperature on the Manometric B. O. D. The results of tests made to determine the optimum temperature for the lake water bacteria are presented in Table VI.

Effect of Endogenous Uptake on the Manometric B. O. D. The varying endogenous uptake values for 24-hour and 48-hour cultures at several concentrations are presented in Table XI.

Effect of Dilution of Blowdown Liquor on Manometric B. O. D. The oxygen uptake of lake water bacteria on various dilutions of blowdown liquor is tabulated in Table V. The oxygen uptake of the bacteria caused by the presence of the waste is plotted versus time in Figure 1.

Effect of Dilution of Effluent on the Manometric B. O. D. The oxygen uptake of lake water bacteria on various dilutions of effluent from a sewage-blowdown liquor, anaerobic, digester is tabulated in Table VII. The oxygen uptake of the bacteria caused by the presence of the waste is plotted versus the time of contact in Figure 2.

Comparison of Manometric and Standard B. O. D. The manometric and standard B. O. D. values of blowdown liquor, domestic sewage,

and mixtures of these two wastes are tabulated in Table VIII for comparison.

Effect of Time on the Manometric B. O. D. The oxygen uptake of various concentrations of lake water bacteria on a 1:500 dilution of blowdown liquor as measured at various time intervals is tabulated in Table IX, and the data plotted in Figure 3.

Effect of Bacterial Concentration on the Manometric B. O. D. The manometric B. O. D. of a 1:500 dilution of blowdown liquor as determined with varying concentrations of lake water bacteria is presented in Table X.

Effect of the Age of Culture on the Manometric B. O. D. The effect of the age of the lake water bacterial culture on the manometric B. O. D. is shown in Table XI.

Standard Manometric B. O. D. The standard manometric B. O. D. obtained with standard dilution water at various time intervals up to five days is presented for total mill wastes in Table XII, and the B. O. D. is plotted versus time in Figure 4.

Calibration of Colorimeter. The data for the calibration of the Klett-Summerson photoelectric colorimeter showing the colorimeter readings of the bacterial suspensions and the corresponding weight of dry bacterial cells are tabulated in Table XIII. The calibration curve is presented in Figure 5.

TABLE V

Effect of Concentration of Blowdown Liquor on the Oxygen Uptake of 7.4 Milligrams of Lake Water Bacteria Respiring on 2.5 Milliliters of Various Dilutions of the Paper Mill Waste at 30 °C

Elapsed Time minutes	Oxygen Uptake at 1:1000 Dilution microliters	Oxygen Uptake at 1:500 Dilution microliters	Oxygen Uptake at 1:250 Dilution microliters	Oxygen Uptake at 1:125 Dilution microliters
5	7.26	7.06	7.26	6.89
10	12.46	18.28	18.72	22.91
15	13.45	25.36	33.44	38.93
20	16.04	29.38	46.91	55.62
25	14.96	29.86	58.33	67.31
30	15.45	31.80	64.64	86.46
35	16.47	31.74	65.93	102.45
40	16.97	33.19	68.88	122.27
45	15.86	32.56	68.03	129.91
50	17.41	33.02	68.66	134.83
55	15.85	32.94	69.51	137.22
60	17.36	34.45	71.23	140.35

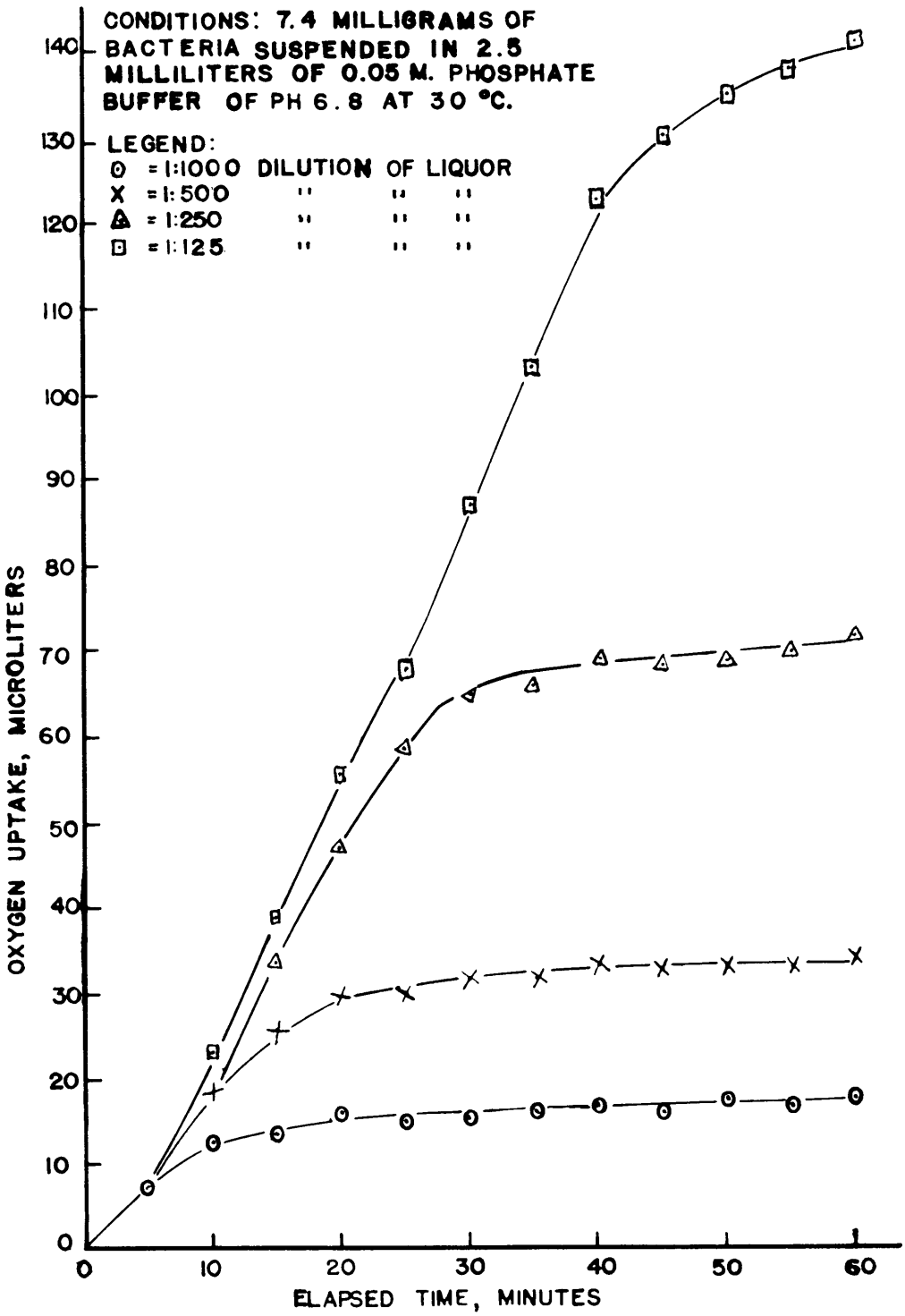

FIGURE 1. EFFECT OF CONCENTRATION OF BLOWDOWN LIQUOR ON THE OXYGEN UPTAKE OF LAKE WATER BACTERIA.

TABLE VI

Effect of Temperature on the Oxygen Uptake of 7.4 Milligrams of Lake Water Bacteria Respiring on 2.5 Milliliters of a 1:500 Dilution of Blowdown Liquor

Elapsed Time minutes	Oxygen Uptake at 30 °C microliters	Oxygen Uptake at 33 °C microliters	Oxygen Uptake at 36 °C microliters
20	29.07	36.38	28.50
40	32.25	37.60	33.90
60	33.50	39.34	35.02
80	34.24	39.11	38.74

TABLE VII

Effect of Effluent Concentration on the Oxygen Uptake of 11.8 Milligrams of Lake Water Bacteria Respiring on 2.5 Milliliters of Effluent at 30 °C from a Sewage-Paper Mill Waste Digester

Elapsed Time minutes	Oxygen Uptake, 1:125 Dilution microliters	Oxygen Uptake, 1:50 Dilution microliters
10	2.19	11.50
20	21.32	42.26
30	22.44	50.75
40	23.56	56.25
50	23.62	57.79
60	23.68	59.55
70	24.70	60.02
80	25.90	60.46
90	24.90	62.47
100	24.94	62.47
110	26.05	62.10
120	25.04	61.69
130	25.09	61.97

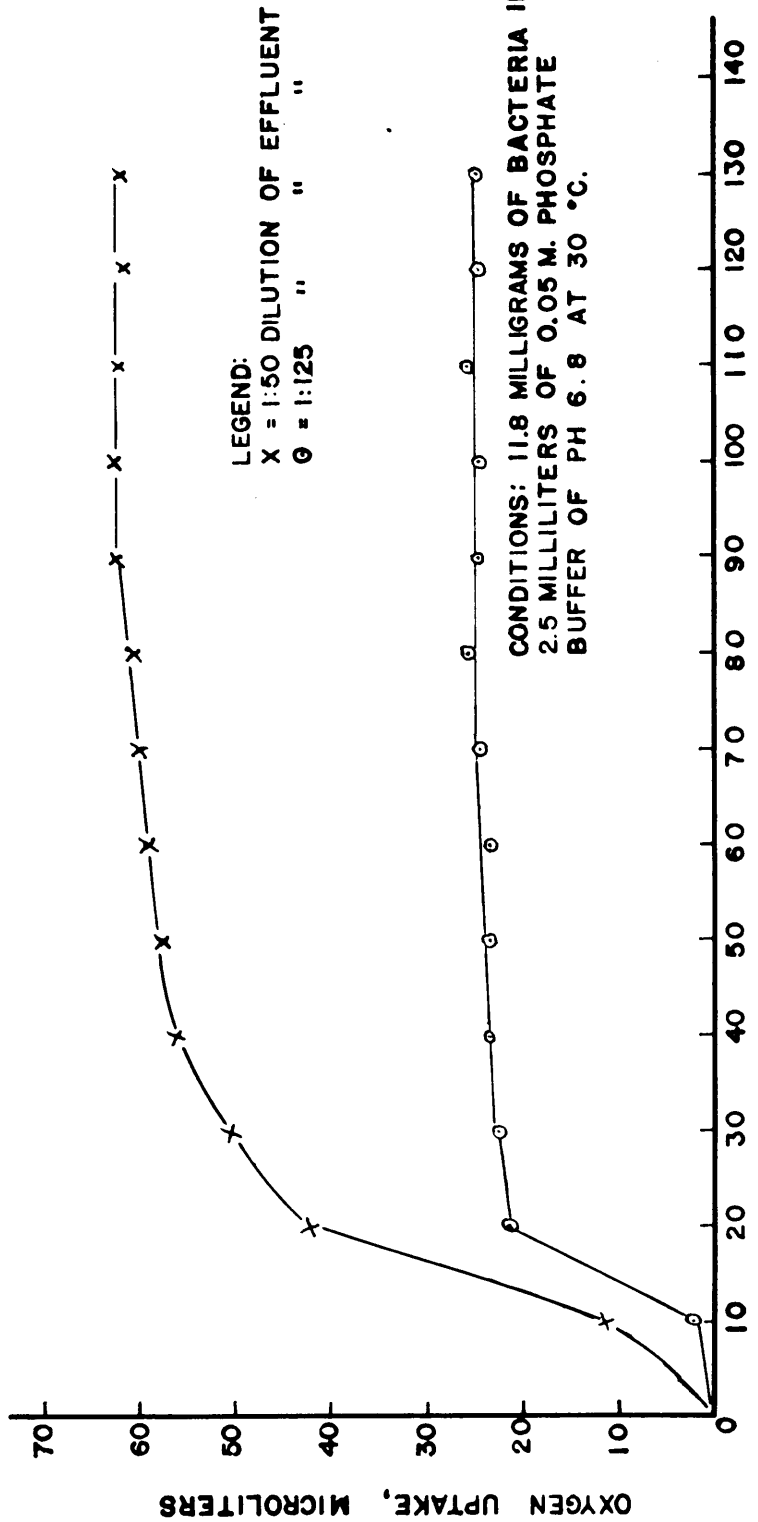

FIGURE 2. EFFECT OF CONCENTRATION OF EFFLUENT FROM A SEWAGE-BLOWDOWN LIQUOR, ANAEROBIC DIGESTER ON THE OXYGEN UPTAKE OF LAKE WATER BACTERIA.

TABLE VIII

Comparison of Manometric[a] and Standard[b] B. O. D. Values of Domestic Sewage and Blowdown Liquor Mixtures

Composition of Waste,		Manometric B. O. D.	Standard B. O. D.[b]
Domestic Sewage %	Blowdown Liquor %	ppm	ppm
100	0	413	495
95	5	703	3,170
90	10	1,306	4,240
75	25	3,325	8,840
65	35	4,420	19,900
50	50	9,900	31,200
35	65	11,900	31,600
25	75	9,710	46,500
0	100	13,760	37,800

(a) Manometric B. O. D. determined using 10.2 milligrams of lake water bacteria suspended in 2.5 milliliters of a 0.05 M. phosphate buffer of pH 6.8 at 30 °C.

(b) Personal Communication: Robert Opferkuch, National Council for Stream Improvement project on semi-chemical pulp mill waste treatment at the Virginia Polytechnic Institute, Blacksburg, Virginia. August, 1950.

TABLE IX

Effect of 0.7, 1.6, and 3.4 Milligrams of Lake Water Bacteria on the Oxygen Uptake Caused by the Presence of 2.5 Milliliters of a 1:500 Dilution of Blowdown Liquor Over a 37-Hour Period at 30 °C

Elapsed Time	Oxygen Uptake of 0.7 Mgm of Bacteria	Oxygen Uptake of 1.6 Mgm of Bacteria	Oxygen Uptake of 3.4 Mgm of Bacteria
hours	microliters	microliters	microliters
1.0	4.20	32.10	42.89
2.0	11.50	43.91	51.00
3.0	20.33	50.73	55.41
4.0	27.04	57.04	61.50
5.0	30.16	54.14	57.59
6.0	34.31	57.79	60.31
7.0	36.39	60.94	62.78
8.0	37.43	61.46	63.00
9.3	39.53	63.55	61.54
11.3	38.56	65.28	65.04
13.1	43.75	67.88	68.76
14.2	42.74	64.70	67.11
17.9	43.81	69.42	73.31
21.9	51.11	71.74	74.78
25.5	58.44	70.88	72.75
29.4	64.78	68.15	64.13
37.4	36.16	46.10	49.95

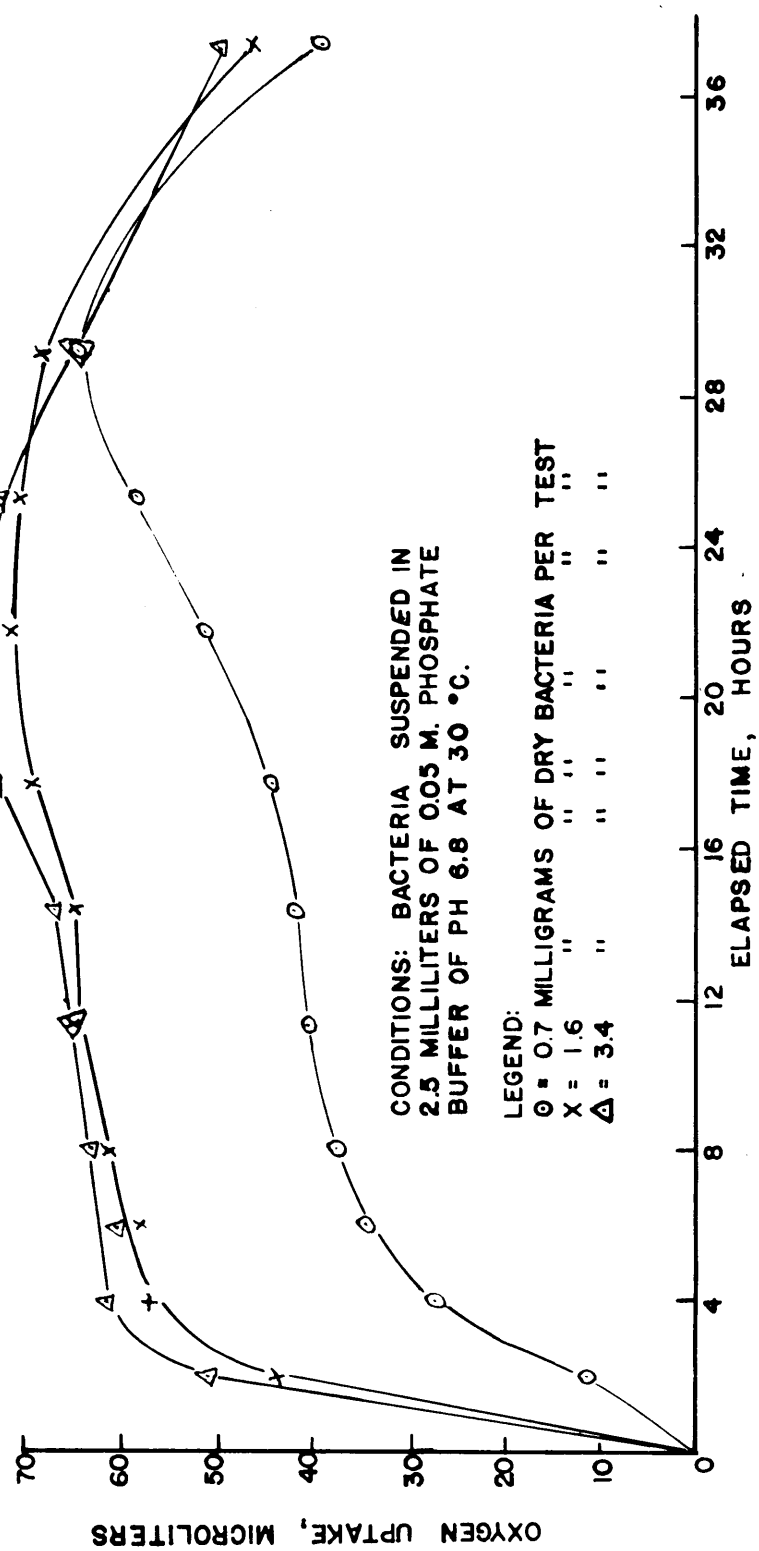

FIGURE 3. EFFECT OF EXCESSIVE TIME ON THE OXYGEN UPTAKE OF VARIOUS CONCENTRATIONS OF LAKE WATER BACTERIA CAUSED BY THE PRESENCE OF A 1:500 DILUTION OF BLOWDOWN LIQUOR.

TABLE X

Effect of Bacterial Concentration on the Oxygen Uptake of a Suspension of 2.5 Milliliters of Lake Water Bacteria Respiring on a 1:500 Dilution of Blowdown Liquor at 30 °C

Test No.	Total Bacterial Weight mg	Oxygen Uptake[1] microliters
16	3.4	41.05
20	3.4	36.03
17	5.4	40.31
18	5.4	37.98
16	8.8	39.22
18	8.8	38.80
19	13.6	40.32
20	13.6	39.99
19	17.0	42.80
20	17.0	38.32

(1) The oxygen uptake for each run was determined by averaging two values. The oxygen uptake was taken arbitrarily as the value which varied less than two per cent for a 10-minute increment of time.

TABLE XI

Effect of Age of Culture and Time of Growth in Nutrient Broth on the Endogenous Rate and the Oxygen Uptake of Various Concentrations of Lake Water Bacteria on 2.5 Milliliters of a 1:500 Dilution of Blowdown Liquor at 30 °C

	Test Number							
	20	20	20	21	21	21	22	22
Total Bacterial Wgt., Milligrams	3.4	13.6	17.0	3.4	13.6	17.0	3.4	12.0
Age of Culture, Hours	24	24	24	48	48	48	24	24
Time Transferred, Months	3	3	3	3	3	3	2/30	2/30
[a]Total Oxygen Uptake, Microliters	77.6	138.8	148.3	55.7	89.9	104.1	--	--
[a]Endogenous Uptake, Microliters	41.6	98.8	110.0	14.0	52.6	65.0	--	--
[b]Oxygen Uptake (BOD), Microliters	36.0	40.0	38.3	41.7	37.3	39.1	39.0	42.6

[a] Total oxygen uptake and endogenous oxygen uptake values were obtained over a two-hour period.

[b] The oxygen uptake caused by the presence of the waste was obtained by subtracting the endogenous from the total oxygen uptake.

TABLE XII

Manometric Determination of the B. O. D. of Total Mill Wastes at 25 °C Using 2.5 Milliliters of Solutions for the Standard Dilution B. O. D.(a)

Elapsed Time	Oxygen Uptake of 1:10 Dilution	Oxygen Uptake of 1:10 Dilution	Oxygen Uptake of 1:20 Dilution	Oxygen Uptake of 1:20 Dilution
hours	microliters	ppm	microliters	ppm
4	14.55	83	3.12	36
8	32.00	183	12.48	143
10.5	34.91	199	24.96	286
20	50.91	291	31.20	357
24	59.64	341	36.40	405
28	64.01	368	38.48	440
32	71.28	417	39.52	452
36	69.83	398	44.72	511
44	78.56	449	48.88	560
48	84.38	482	47.84	548
58	85.83	490	50.96	584
68	91.64	524	55.12	630
76	98.92	564	54.08	620
84	98.92	564	55.12	630
93	98.92	564	55.12	630
100	104.74	598	55.12	630
130	104.74	598	55.12	630

(a) The solutions were made up just as if the standard B. O. D. were being determined except that the oxygen uptake was measured directly by manometric methods.

(b) The standard five-day B. O. D. determined according to specifications found in the literature(1) was found to be 360 ppm at 25 °C(25).

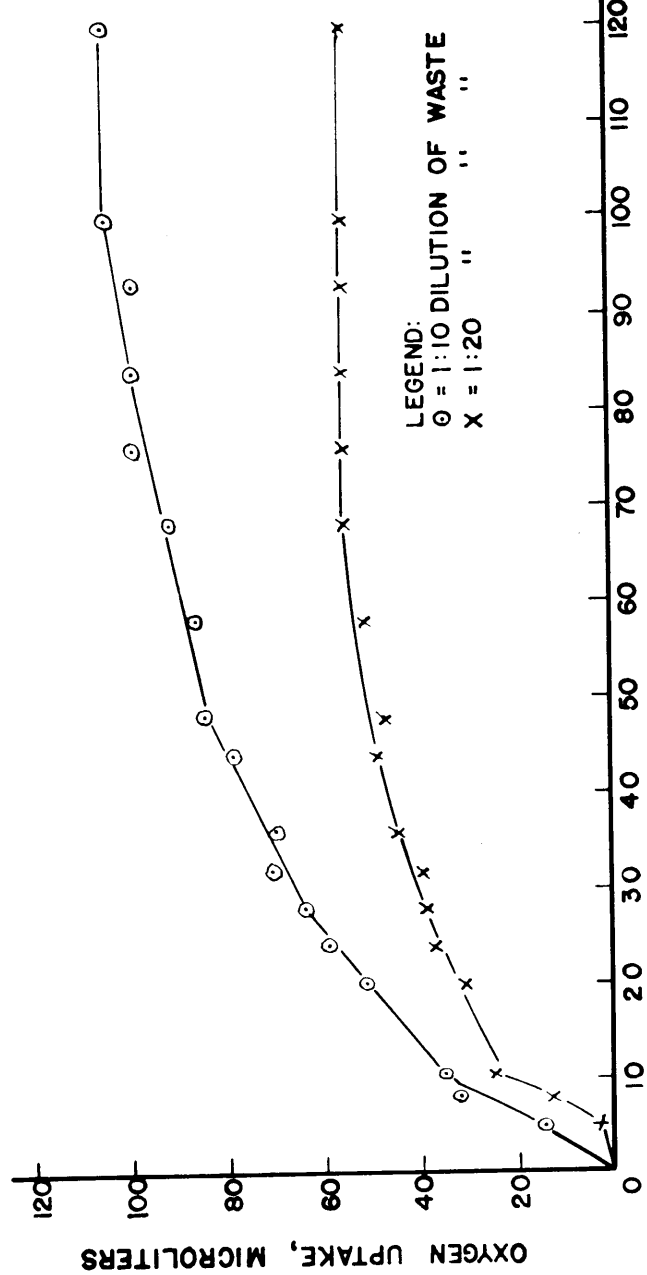

FIGURE 4. OXYGEN UPTAKE CAUSED BY VARIOUS CONCENTRATIONS OF TOTAL MILL WASTES IN 2.5 MILLILITERS OF STANDARD DILUTION WATER AT 25 °C.

TABLE XIII

Colorimeter Readings and Weights of Dry Bacterial Cells for Distilled Water Suspensions of Lake Water Bacteria

Colorimeter Readings	Dry Bacterial Weight mg/ml
730.0	9.36
450.0	4.68
330.0	3.12
268.0	2.34
222.0	1.87
190.0	1.56
143.0	1.17
121.0	1.04
96.5	0.72
82.0	0.60
60.0	0.45

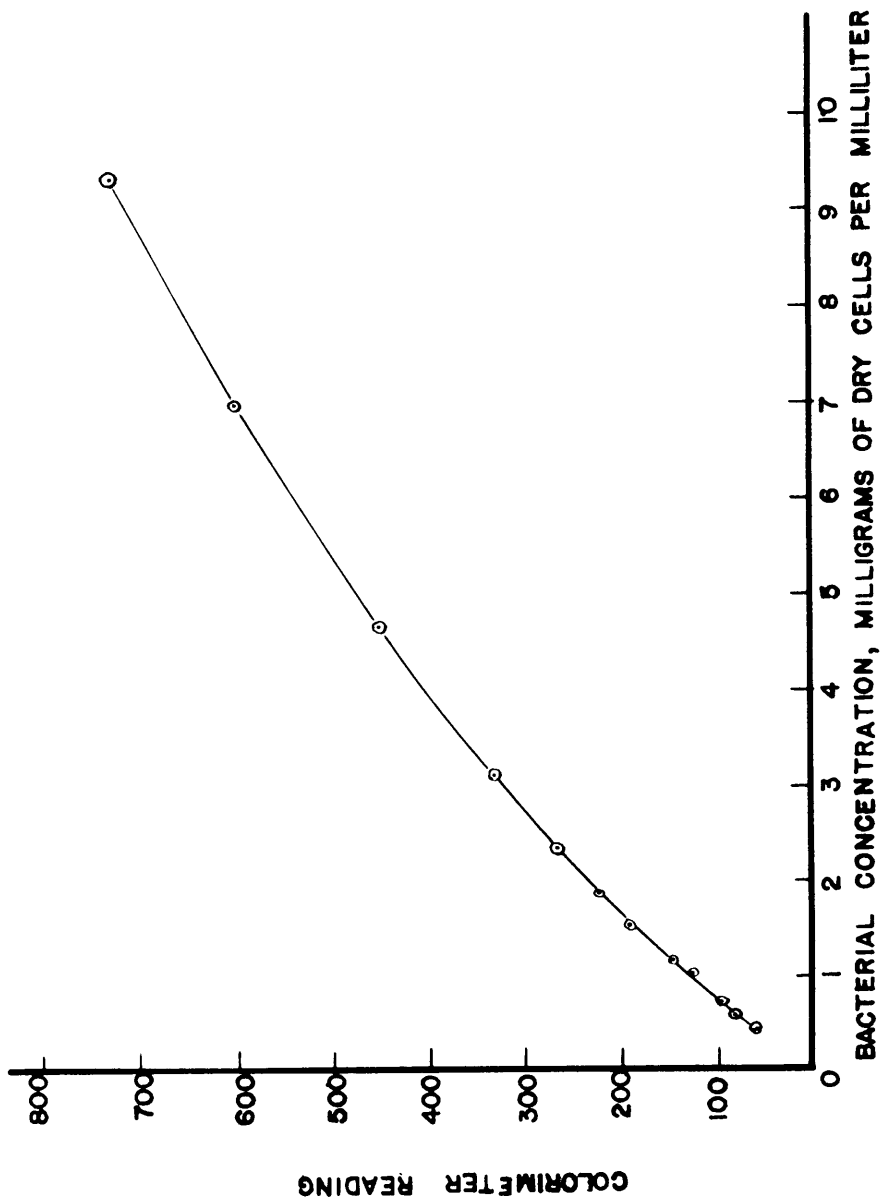

FIGURE 5. CALIBRATION CURVE FOR KLETT-SUMMERSON PHOTOELECTRIC COLORIMETER FOR CONVERSION OF COLORIMETER READINGS TO BACTERIAL CONCENTRATION FOR LAKE WATER BACTERIA.

IV. DISCUSSION

The purpose of this investigation was to develop a rapid method for the determination of the B. O. D. of semichemical sulfite pulp mill wastes using the direct warburg technic and high concentrations of microorganisms. In the following sections a discussion of the results and the limitations of this investigation, together with recommendations for future studies, are presented.

Discussion of Results

The following topics are discussed as results of this investigation: the effects of temperature, waste and bacterial concentrations, age of culture, endogenous uptake, and time on the manometric B. O. D.; the effect of the manometric method on the standard B. O. D. values; the reproducibility of results and factors affecting accuracy; and the use of manometric methods in industrial waste studies.

Effect of Temperature on the Manometric Rate of Oxygen Uptake. Tests were made to determine the optimum temperature for the lake water bacteria respiring on 2.5 milliliters of a 1:500 dilution of blowdown liquor at temperatures of 30,

33, and 36 °C. After a one-hour interval, the oxygen uptake was 33.5 microliters at 30 °C, 39.3 microliters at 33 °C, and 35.0 microliters at 36 °C.

These results, shown in Table VI, page 69, indicate that the temperature affected the rate of oxygen uptake only slightly over the ranges studied. Since increased temperatures would possibly affect the endogenous controls adversely, 30 °C was taken as the optimum temperature although the rate of oxygen uptake at this temperature during the first hour interval was approximately 15 per cent less than the rate at 33 °C.

Effect of Age of Culture. After various tests were made over a period of three months, it was thought possible that the B. O. D. values obtained were characteristic of the bacterial culture which had been transferred for the previous months on nutrient broth. A new sample of bacteria was obtained from Lake Solitude on the campus of the Virginia Polytechnic Institute. These lake water bacteria were transferred every 24 hours for two days on nutrient broth. Tests were then made to determine the B. O. D. of blowdown liquor from a semichemical pulp mill utilizing the bacterial cultures which had been transferred for three months and those transferred for two days.

The oxygen uptake was determined for the two cultures because of the presence of a 1:500 dilution of the blowdown liquor in 2.5 milliliters of the bacterial concentrations of 3.4, 13.6, and 17.0 milligrams at 30 °C. The results of these tests, as tabulated in Table XI, page 83, show that the oxygen uptake values obtained because of the presence of the waste do not vary, within experimental error, from an average value of 39.3 microliters. These results indicate that the B. O. D. values obtained in this investigation are not dependent upon the possible adaptation of certain types of bacteria to the waste, or selective growth of a certain species of bacteria.

Effect of Bacterial Concentration. The data tabulated in Table X, page 75, show that the oxygen uptake by 2.5 milliliters of lake water bacterial suspensions in the presence of a 1:500 dilution of blowdown liquor does not vary over the ranges of bacterial concentration studied. The bacterial weights extended from 3.4 to 17.0 milligrams of dry bacterial cells over 2.5 milliliter sample. The data presented in Table IX, page 73, indicate that below 1.6 milligrams the rate of oxygen uptake is so low that the control endogenous uptake begins to increase rapidly before a correct value for the oxygen uptake of the waste can be obtained. The rapid increase in the oxygen uptake of the

endogenous cells began after 24 hours, causing a decrease in the manometric B. O. D. This increase in the endogenous uptake is probably caused by autolysis of the endogenous cells. The oxygen uptake caused by the presence of the waste does not vary appreciably from an average value of 39.5 microliters for bacterial weights from 1.6 to 17.0 milligrams of dry bacterial cells, as shown in Figure 3, page 74, and Table IX, page 73. Therefore, studies above 17.0 milligrams were not made.

Effect of Waste Concentration on the Manometric B. O. D. The effect of various dilutions of blowdown liquor and of the effluent studied indicate that the oxygen uptake caused by the presence of the wastes was directly proportional to the waste concentration. The manometric B. O. D., when calculated for the various dilutions of each waste, remained constant within two per cent of the average value.

After a 60-minute interval, the oxygen uptake caused by the presence of the blowdown liquor was 17.4 microliters for a dilution of 1:1000, 34.5 microliters for a dilution of 1:500, 71.2 microliters for a dilution of 1:250, and 140.5 microliters for a dilution of 1:125. All dilutions were made on a volumetric basis. These data are presented in Table V, page 67, and in Figure 1, page 68.

After a 130-minute interval, the oxygen uptake caused by the presence of the effluent from a sewage-blowdown liquor digester was 25.1 microliters for a dilution of 1:125 and 62.0 microliters for a dilution of 1:50. These data are presented in Table VII, page 70, and in Figure 2, page 71.

Effect of Endogenous Uptake on the Manometric B. O. D. The endogenous uptake for a 24-hour culture of 3.4 milligrams of lake water bacteria in a total volume of 2.5 milliliters was found to be 20.8 microliters of oxygen per hour over a two-hour period at 30 °C. The endogenous uptake of a 48-hour culture of lake water bacteria under the same conditions was found to be 7.0 microliters of oxygen per hour over a two-hour period. However, when the endogenous uptake values were subtracted from the total uptake values, the manometric B. O. D. of the paper mill wastes studied was found to be constant at 11,000 parts per million, within experimental error, when determined with these two cultures. These data are presented in Table XI, page 76.

These results indicate that the endogenous uptake of the lake water bacteria studied has no effect on the oxygen uptake caused by the presence of the waste. In addition, the data indicate the desirability of subtracting the endogenous uptake

from the total oxygen uptake, since otherwise agreement between determinations is poor and the manometric B. O. D. becomes a function of the culture age.

Effect of Time on the Manometric B. O. D. To understand the experimental limits present in the investigation, the oxygen uptake was determined over a 38-hour period. The oxygen uptake was measured for 3.4, 1.6, and 0.7 milligrams of dry bacterial cells in 2.5 milliliters of a 1:500 volumetric dilution of blow-down liquor at 30 °C.

The oxygen uptake, for 3.4 and 1.6 milligrams of lake water bacteria as shown in Figure 3, page 74, increased rapidly for four hours to an average value of 59 microliters. From 4 to 25 hours, the oxygen uptake increased slightly at about 0.6 microliter per hour to 72 microliters. After 25 hours, the oxygen uptake decreased at 1.8 microliters per hour to 48 microliters at 38 hours. After the 25-hour period, the endogenous rate increased sharply, possibly because of autolysis, and the oxygen uptake in the presence of the waste decreased rapidly. This change is indicated graphically in Figure 3, page 74.

These data indicate that the waste and bacterial concentration used in this type of determination must be such that the bacteria shall utilize the waste in less than a 24-hour

period. In this connection, the oxygen uptake of 0.7 milligram of lake water bacteria on the 1:500 dilution of blowdown liquor is to be noted.

The oxygen uptake of the 0.7 milligram of bacteria increased at 5.7 microliters of oxygen per hour to 34.5 microliters in the first six hours. From 6 to 29 hours, the oxygen uptake increased from 34.5 to 64.5 microliters at a rate of 1.3 microliters per hour. From 29 to 38 hours, the oxygen uptake decreased from 64.5 to 37.0 microliters at a rate of 3.0 microliters of oxygen per hour. This sharp decrease was probably caused by autolysis of the lake water bacteria in the control reaction flasks.

Rate of Reaction. In the manometric B. O. D. determination, as shown in Figure 1, page 68, and Figure 2, page 71, 7.4 and 11.8 milligrams of microorganisms were used per 2.5 milliliter sample and the rate of reaction is greater than in the five-day dilution B. O. D. as shown in Figure 3, page 74. The time required for an average determination for the wastes studied was one hour, with some values requiring as little as 20 minutes. These rates are presented graphically in Figure 1, page 68, and in Figure 2, page 71.

In the standard dilution B. O. D. the oxygen uptake during the logarithmic growth phase of the microorganisms is at a peak. In the manometric B. O. D. the high concentrations of cells are,

for the most part, "resting," and the metabolic activities are lowered drastically, relative to the phase of logarithmic growth[37]. The extremely high concentration of microorganisms used overcomes this tendency to decrease the reaction rate.

Another factor to be considered in the rate of reaction for the manometric B. O. D. determination is the high concentration of phosphate buffer (0.05 molar) used in the tests. In recommending that supplementary inorganic phosphate be used in the dilution water for the five-day dilution B. O. D., Langelier and Caldwell[11] point out that phosphate in enzymatic reactions acts as a catalyst, promoting rapid biochemical oxidation.

Comparison of Manometric and Standard B. O. D. Values. The manometric B. O. D. of sewage, blowdown liquor, and mixtures of the two wastes was determined. The values obtained were compared with the standard dilution B. O. D. as shown in Table VIII, page 72. The manometric B. O. D. of the sewage and blowdown liquor was 413 and 13,760 parts per million, respectively. The standard five-day B. O. D. of the sewage and the blowdown liquor was 495 parts per million and 37,800 parts per million, respectively. The manometric B. O. D. of a 1:1 mixture of the two wastes was 9,900 parts per million, while the standard

five-day B. O. D. was 31,200 parts per million. These results indicate that the sewage mixed with the blowdown liquor caused an increase in the B. O. D. resulting from the presence of the blowdown liquor.

A similar discrepancy between the standard dilution B. O. D. values and direct measurements of the oxygen uptake for semi-chemical pulp mill wastes of similar composition to the blowdown liquor is reported by Falk[14]. Falk found that the standard five-day B. O. D. of sulfite liquors was 17,000 parts per million. With the Haldane apparatus and an acclimitized seed, a direct B. O. D. of 6,200 parts per million was obtained. The B. O. D. values obtained by Falk and in this investigation are of the same order. The direct measurement of the B. O. D. results in oxygen uptake values approximate one-third of the standard dilution B. O. D. This large discrepancy obtained in the results by the two methods is probably caused by two factors[14]: the high concentration of the paper mill wastes used in the manometric method, and the extreme dilution necessary to determine the five-day dilution B. O. D. for wastes with a large B. O. D.

If toxic elements which are inhibitory to the activity of the microorganisms are present in the sulfite liquors, then the toxicity would be greater at the higher concentrations used in the manometric method.

The accuracy of the standard B. O. D. determination decreases at high dilutions[17]. In this investigation, the blowdown liquor used had 145,000 parts per million total solids present. Dilutions used in determining the standard B. O. D. ranged around 1:10,000. The dilution used for the manometric B. O. D. determinations was a maximum of 1:500.

Effect of Manometric Method on Standard B. O. D. Values. The correlation between the standard dilution B. O. D. and the manometric B. O. D. of the blowdown liquor was off by a factor of about three, as shown in Table VIII, page 72. Therefore, a determination was made of the B. O. D. using the standard dilution method as outlined in the literature[1] except that the dilution water and waste were placed in the Warburg reaction flask and the oxygen uptake measured directly following the technic of Langelier and Caldwell[7]. A suspension of bacterial cells was not added in this method. Bacterial cells were present in the dilution water, as in the standard method.

The effect of dilution of total mill wastes from a semi-chemical sulfite pulp mill and time on the standard manometric oxygen demand, as shown in Figure 4, page 78, was studied over a five-day period. The five-day B. O. D. at a dilution of 1:10 was approximately five per cent lower than at 1:20. The average

standard five-day B. O. D. of the total mill wastes was 360 parts per million, while the manometric dilution B. O. D. was 611 parts per million. From the manometric values of the oxygen uptake for the 1:10 dilution at 24 hours, the manometric B. O. D. for the total mill wastes was found to be 530 parts per million, or approximately 95 per cent of the standard five-day B. O. D. These results are similar to the findings of Langelier and Caldwell[7] and Woolridge[51]. Langelier and Caldwell obtained manometric dilution B. O. D. values at 24 hours which were approximately 75 per cent of the standard five-day values.

These manometric results indicate that the rate of oxygen uptake is not only increased by increasing the bacterial concentration, but also by a general characteristic of the Warburg apparatus. This characteristic is the maintenance of an oxygen-saturated condition in the diluted waste by the constant shaking of the reaction flasks. It is thought that with a saturated-oxygen solution the metabolism, and in the case of the manometric B. O. D. test the growth, of the organisms present would be at peak conditions.

In relation to this constant shaking of the reaction flasks, consider a single bacterium surrounded by an extremely dilute solution of some carbon compound. The carbon source in the

immediate vicinity of the cell would be in the process of being absorbed. Two hypothetical films can be assumed to be present. One film would be surrounding the cell, and could possibly be considered to be the cell wall for simplification. In this first film, the carbon source would be in the process of being absorbed and broken down by the enzymes of the bacterium. The second film would be outside the bacterium and would have a concentration gradient across it. The carbon source would then have to diffuse through the two films because of the concentration differential. Since it is known[12,51] that the bacteria will absorb the carbon source beyond its capacity to digest it, the film outside the cell wall can be assumed to be controlling as far as the reaction rate is concerned in an extremely dilute solution such as is present in the standard B. O. D. test. Therefore, an increase in the reaction rate of the B. O. D. would be expected with agitation since this stirring will decrease the outer film thickness.

Manometric Methods in Industrial Waste Studies. Other possible conclusions can be drawn from this variation in the reaction rate with agitation. These conclusions further substantiate the possible use of the manometric apparatus and high concentrations of bacteria in the study of industrial waste problems.

The results just discussed and shown in Figure 4, page 78, indicate that the reaction rate of the manometric dilution B. O. D. is much higher than that of the standard dilution B. O. D., and furthermore, this reaction rate is increased by the agitation of the solution tested. These conclusions can be extrapolated to actual stream conditions. It would follow from the above discussion that the rate of reaction in a stream increases with the degree of turbulency of the stream. However, neither the standard dilution B. O. D., where no agitation exists at all, nor the manometric dilution B. O. D., where the agitation is extreme, actually reproduce the conditions in a given stream. This failure in both cases to reproduce the actual stream conditions is a result not only of the fundamental nature of the two tests, but also because in any given stream the degree of turbulency changes, in general, as the stream approaches its mouth, and across its width.

Since the rate of reaction in one river would not be the same as that obtained in another river, a relative measure of the rates of reaction would be of value in studying the pollutional qualities of a waste. Therefore, to decrease the testing time an abnormally high concentration of bacteria could be used in a modification of the resting cell technic with the Warburg

respirometer to determine the relative rates of reaction of industrial wastes, as well as to measure the pollutional potential of the wastes as was done in this investigation.

In summation, it has been suggested that the rates of reaction in the tests for the B. O. D. are relative values since the actual conditions in the river which determine the rate of reaction are not reproduced. Although the relative rates as determined by the dilution method are probably close to the correct values within a factor of ten at the most, these dilution tests require from 24 hours to 5 days. In some instances a measure of the rate of reaction in the river is not required, but rather a measure of the pollutional potential of the waste under consideration. In such cases, as in digester control work, an immediate determination of the pollutional potential or the B. O. D. can be made by the method used in this investigation.

The following details are of importance in determining the manometric B. O. D.: the bacterial inoculum should be taken from the stream or digester to which the waste would be added, and the bacteria should be grown for a 24-hour period or longer, if possible, in the presence of the waste being studied.

In addition, it has been pointed out that a study of the relative rates of reaction are of value. Such a study can be

made in a short period of time, around one hour, by the use of the resting cell technic with concentrations of carbohydrates above the saturation point in conjunction with the Warburg respirometer. Studies following this technic, with interpretations as applied to industrial wastes, have been previously made by Watkins[48].

Reproducibility of Results. Since the B. O. D. of the wastes studied decrease on storage, the reproducibility of results can only be discussed as far as tests made on the same waste over a short period of time, preferably not greater than 24 hours, is concerned. The results of any two given tests averaged 40 microliters, and five per cent for oxygen uptake values of 75 microliters and above.

Factors Affecting Accuracy. Besides the ordinary analytical technics used in preparing the tests, the most probable source of error was in the manometer readings. At low oxygen uptake values, the manometer fluid was slow to react to small changes in the pressure. In addition, if the bacterial suspension had a high endogenous uptake compared to the oxygen uptake caused by the presence of the waste, then the endogenous uptake might easily be off by the normal five per cent experimental error. This error in the endogenous uptake could then

be as large as the oxygen uptake values caused by the presence of the waste. The error in such an instance would be as high as 100 per cent. Therefore, when a waste was first studied several preliminary tests were made to determine the optimum waste and bacterial concentration.

Limitations

The limitations present in the experimental part of this investigation were temperature, hydrogen-ion concentration, types and concentrations of wastes and microorganisms, and the modified resting cell technic.

Temperature. The effect of temperature on the manometric B. O. D. was studied over a range from 30 to 36 °C. All other tests were made at 30 °C.

Hydrogen-ion Concentration. The hydrogen-ion concentration was maintained at a pH of 6.8, which was assumed to be the average optimum pH for aerobic bacterial concentration.

Types of Wastes. The effluent from a sewage-blowdown liquor, anaerobic digester, sewage from the Virginia Polytechnic Institute sewage treatment plant, and total mill wastes and blowdown liquor from a sulfite semichemical pulp mill were the types of wastes studied in this investigation.

Types and Concentrations of Bacteria. Concentrations of microorganisms studied ranged from 0.7 to 17.0 milligrams of dry bacterial protoplasm per 2.5 milliliters of suspension in each flask. Lake water bacteria from Lake Solitude on the campus of the Virginia Polytechnic Institute were used throughout the investigation to determine both the manometric and standard B. O. D. No tests were made to determine the actual species of microorganisms present.

Resting Cell Technic. A modified resting cell technic, in that small quantities of compounds of nitrogen were present in the wastes studied, was used in conjunction with the Warburg respirometer.

Recommendations

The following recommendations are made as topics for future study:

Synthetic Industrial Wastes. The manometric B. O. D. of synthetic wastes should be determined and compared with the standard and known theoretical values of the B. O. D. as obtained by Lyon[22].

Carbon Dioxide Determination. A determination of the amount of carbon dioxide evolved by the organisms due to the presence of a waste should be made. By measuring the carbon dioxide evolved, whether or not the waste is just absorbed by the slurry of microorganisms or is actually digested could be determined. Indications of the absorption of sewage were found by Woolridge and Standfast[51] in studies of the oxygen uptake of sludges from sewage digesters. The carbon dioxide determination could easily and best be made in tests of the synthetic organis wastes, as cited above, where the values of the known theoretical demand could be used to determine the actual extent of oxidation as opposed to absorption.

Mechanism of the B. O. D. Reaction. To determine the value and significance of the manometric B. O. D. more fully, studies should be made of a synthetic waste by the manometric and standard B. O. D. tests. This synthetic waste should be composed of a carbohydrate, an amino acid, and some inorganic nitrogen source such as ammonium chloride. Standard and manometric B. O. D. tests should be made of this synthetic waste as a whole, and of the individual components. It is believed that the results of such experimentation would reveal desirable information concerning the various oxidative stages of the standard B. O. D. test, in

addition to the versatility of the manometric test used in this investigation.

Percentage Solids Versus B. O. D. Values. To determine the effect of the sewage on the B. O. D. of the blowdown liquor, mixtures of the two wastes should be tested with the composition based on per cent total solids and per cent volatile solids.

V. CONCLUSIONS

The B. O. D. of several wastes was determined by measuring the change in the oxygen uptake of a high concentration of microorganisms caused by the presence of the wastes. These determinations involved a modification of the resting cell technic used in conjunction with the Warburg respirometer. The effects of waste and microorganism concentration, and environmental conditions were studied. The lake water bacteria used were suspended in 2.5 milliliters of a 0.05 molar phosphate buffer at a pH of 6.8.

The wastes studied were raw sewage, blowdown liquor and total mill wastes from a semichemical sulfite pulp mill, and effluent from an anaerobic, sewage-blowdown liquor digester. The concentration of lake water bacteria used ranged from 3.4 to 17.0 milligrams of dry bacterial cells in a total volume of 2.5 milliliters. The incubation time for the lake water bacteria used in the tests was varied from 24 to 48 hours. The dilution B. O. D. was determined by manometric means for total mill wastes at various time intervals over a five-day period. The dilution water used followed specifications for the standard dilution B. O. D.

From this investigation, the following conclusions were drawn:

1. The manometric B. O. D. of a 1:500 dilution of blowdown liquor remained constant at 11,300 parts per million when determined utilizing bacterial concentrations from 1.4 to 6.8 milligrams of dry cells per milliliter.

2. The manometric B. O. D. remained constant at 1,785 parts per million for 1:50 and 1:125 volumetric dilutions of effluent from a sewage-blowdown liquor, anaerobic digester. The manometric B. O. D. remained constant at 10,200 parts per million for volumetric dilutions ranging from 1:125 to 1:1000 for a sample of blowdown liquor.

3. The manometric B. O. D. of a 1:500 dilution of blowdown liquor did not vary from 10,700 parts per million with the temperature over the range studied from 30 to 36 °C.

4. The manometric dilution B. O. D. of total mill wastes reached a value after 24 hours of 342 parts per million or 95 per cent of the standard five-day dilution B. O. D. The five-day manometric dilution B. O. D. was 614 parts per million, while the standard five-day B. O. D. was 360 parts per million.

5. The manometric B. O. D. of a sewage examined was 413 parts per million, while the corresponding standard five-day B. O. D. was 495 parts per million.

6. The manometric B. O. D. of blowdown liquor was found to be 13,760 parts per million, while the corresponding standard five-day B. O. D. was 37,800 parts per million.

7. The manometric B. O. D. of a 1:1 volumetric mixture of the sewage and blowdown liquor was 9,900 parts per million, while the standard five-day B. O. D. was 31,200 parts per million.

8. The average time necessary to determine the manometric B. O. D. of the wastes studied was one hour, with a minimum value for the effluent of 20 minutes.

VI. SUMMARY

In nearly all chemical industries the problem of wastes is one of importance, and in any scientific study of waste disposal, the concept of the B. O. D. of the waste is vital. The B. O. D. is usually measured by an arbitrary standard test procedure, but may be determined by manometric measurement of the free oxygen utilized. In either case, the B. O. D. determination requires at least 24 hours. Since the free oxygen involved in the B. O. D. of a waste is utilized in metabolic processes of various microorganisms present, an increase in the number of microorganisms lowers the time required for oxidation of the wastes.

In this investigation, the B. O. D. of several wastes was determined by measuring the change in the oxygen uptake of high concentrations of microorganisms because of the presence of the waste. These determinations involved a modification of the resting cell technic used in conjunction with direct Warburg technics. High concentrations of washed cells were prepared and small amounts of the waste added. A control was prepared with distilled water. The effects of waste concentration, bacterial concentration, and temperature were studied.

The wastes studied included raw sewage, blowdown liquor and total mill wastes from a semichemical pulp mill, and effluent from an anaerobic, sewage-blowdown liquor, digester. The concentrations of microorganisms used ranged from 3.4 to 17.0 milligrams of dry bacterial cells in a total volume of 2.5 milliliters of a 0.05 molar phosphate buffer at a pH of 6.8. The manometric B. O. D. was determined at 30 °C.

The manometric B. O. D. remained constant at 1,785 parts per million for 1:50 and 1:125 volumetric dilutions of the effluent. The manometric B. O. D. remained constant at 10,200 parts per million for volumetric dilutions ranging from 1:125 to 1:1000 for a sample of the blowdown liquor.

The manometric B. O. D. of sewage, blowdown liquor, and mixtures of the two wastes was determined. The values obtained were compared with the standard five-day B. O. D. The manometric B. O. D. of the sewage and the blowdown liquor was 413 parts per million and 13,760 parts per million, respectively. The standard five-day B. O. D. of the sewage and the blowdown liquor was 495 parts per million and 37,800 parts per million, respectively. The manometric B. O. D. of a 1:1 by volume mixture of the two wastes was 9,900 parts per million, while the standard five-day B. O. D. was 31,200 parts per million.

The average time necessary to determine the manometric B. O. D. of the wastes studied was one hour, with a minimum value of 20 minutes for the effluent.

The dilution B. O. D. was determined by manometric means for total mill wastes at various time intervals over a five-day period. The dilution water used followed specifications for the standard B. O. D. The manometric dilution B. O. D. of total mill wastes reached a value after 24 hours of 342 parts per million, or 95 per cent of the standard five-day B. O. D. The five-day manometric dilution B. O. D. was 614 parts per million, while the standard five-day B. O. D. was 360 parts per million. The dilution tests were made at 25 °C.

VII. BIBLIOGRAPHY

1. American Public Health Association: "Standard Methods of Water Analysis," p. 154. American Public Health Association, New York, N. Y., 1935.

2. Borden, G. C. and Irene Woodcock: Reseeding B. O. D. Bottles, TAPPI, 32, 506 (1949).

3. Burtle, J. and A. M. Boswell: A Comparison of Potassium Permanganate Oxygen Demand, B. O. D., and Direct Absorption of Oxygen, Sewage Works Journal, 7, 839 (1935).

4. _____: Oxygen Demand Studies, Sewage Works Journal, 9, 224 (1937).

5. Buswell, A. M., I. V. Meter, and J. R. Gerke: Study of the Nitrification Phase of the B. O. D. Test, Sewage and Industrial Wastes, 22, 1297 (1950).

6. Butterfield, C. T., W. C. Purdy, and R. J. Theriault: Experimental Studies of Natural Purification in Polluted Waters, Pub. Health Rep., 46, 363 (1931).

7. Caldwell, D. H. and W. F. Langelier: Manometric Measurement of the Biochemical Oxygen Demand of Sewage, Sewage Works Journal, 22, 202 (1948).

8. ibid, p. 203.

9. Caldwell, D. H. and W. F. Langelier: Manometric Measurement of the Biochemical Oxygen Demand of Sewage, Sewage Works Journal, 22, 212 (1948).

10. ibid, p. 214.

11. ibid, p. 217.

12. Dawson, P. S. S. and S. H. Jenkins: Oxygen Requirements of Activated Sludge Determined by Manometric Methods, Sewage Works Journal, 21, 643 (1949).

13. Ellis, M. M.: "Detection and Measurement of Stream Pollution," U. S. Department of Commerce, Bureau of Fisheries, Bulletin No 22, 1937.

14. Falk, L. L. and Willem Rudolphs: Factors Affecting the Direct Oxygen Utilization Method, Sewage Works Journal, 19, 1000 (1947).

15. Gehm, H. W.: Problems in the Application of the B. O. D. Test to Pulp and Paper wastes, Sewage Works Journal, 19, 865 (1947).

16. Heukelekian, H.: The Use of the Dilution Method for Determining the Effect of Industrial Wastes on Deoxygenation, Sewage Works Journal, 19, 614 (1947).

17. Heukelekian, H.: Use of the Direct Method of Oxygen Utilization in Waste Treatment Studies, Sewage Works Journal, 19, 875 (1947).

18. _____: Method for Studying the Toxicity of Industrial Wastes, Water and Sewage Works, 95, 285 (1948).

19. Hurwitz, E., G. R. Barnett, R. E. Boaudoin, and H. P. Kramer: Nitrification and B. O. D., Sewage Works Journal, 19, 995 (1947).

20. Ingols, R. S.: An Oxygen Consumed Test for Sewage, Water and Sewage Works, 95, 113 (1948).

21. Lange, N. A.: "Handbook of Chemistry," p. 1172. Handbook Publishers, Inc., Sandusky, Ohio, 1946, 6th ed.

22. Lyon, H. D.: Disposal of Synthetic Organic Wastes, Chem. Engr. Progress, 46, 388 (1950).

23. Moore, E. W., H. A. Thomas, and W. B. Snow: Simplified Method for the Analysis of B. O. D. Data, Sewage Works Journal, 22, 1343 (1950).

24. Nesmeyanov, S. A.: Direct Determination of the Oxygen Requirements of Waste Water and Precipitates, Gigiena i Sanit., 13, 1 (1948).

25. Opferkuch, Robert: Personal Communication, November, 1950, National Council for Stream Improvement Project on Semichemical Pulp Mill waste Treatment at the Virginia Polytechnic Institute, Blacksburg, Virginia.

26. Paessler, A. H. and L. L. Hedgepeth: Stream Pollution from Pulp Mills in Virginia, TAPPI, 32, 559 (1949).

27. Phelps, E. B.: "Public Health Engineering," p. 396. John Wiley and Sons, Inc., New York, N. Y., 1948, Volume I.

28. _____: "Stream Sanitation," p. 62. John Wiley and Sons, Inc., New York, N. Y., 1944.

29. ibid, p. 63.

30. ibid, p. 64.

31. ibid, p. 66.

32. ibid, p. 69.

33. ibid, p. 70.

34. ibid, p. 72.

35. ibid, p. 74.

36. ibid, p. 75.

37. ibid, p. 77.

38. ibid, p. 81.

39. ibid, p. 85.

40. Rodgers, J. F.: The Technic of the B. O. D. Determination, TAPPI, 32, 481 (1949).

41. Ruchoft, C. C.: Discussion, Sewage Works Journal, 22, 1353 (1950).

42. Sierp, F.: A New Method for Determination of the B. O. D., Ind. Eng. Chem., 20, 247 (1928).

43. Umbreit, W. W., R. H. Burrus, and J. F. Stauffer: "Manometric Technics and Related Methods for the Study of Tissue Metabolism," p. 4. Burgess Publishing Co., Minneapolis, Minnesota, 1945.

44. ibid, p. 12.

45. ibid, p. 50.

46. ibid, p. 159.

47. Watkins, Peter H.: Personal Communication, August, 1950, Virginia Polytechnic Institute, Blacksburg, Virginia.

48. _____: The Oxygen Uptake of Pseudomonas fluorescens on Glucose, Xylose, Arabinose, and Acetate Under Varying Conditions of Substrate Concentration and Environmental Temperature." Unpublished Ph. D. Thesis, Library, Virginia Polytechnic Institute, Blacksburg, Virginia, (1951).

49. Weston, R. F.: Problems in the Application of the B. O. D. Test in Paper and Pulp Wastes, Sewage Works Journal, 19, 871 (1947).

50. Wilson, J. B.: The Resting Cell Technique as a Method of Study of Bacterial Metabolism, J. Bact., 35, 601-23 (1938).

51. Woolridge, W. R. and F. B. Standfast: Use of the Barcroft Differential Manometers in Estimating Oxygen Absorption of Sewage, Biochemical J., 30, 147 (1936).

52. Zobel, C. E. and C. W. Grant: Bacterial Utilization of Low Concentrations of Organic Matter, J. Bact., 45, 555-64 (1943).

VIII. ACKNOWLEDGMENTS

The author is indebted to for instruction in the general principles of microbiology, helpful suggestions in the experimental problems encountered, and patient guidance, without which this investigation would not have been possible. Appreciation is expressed to
of the National Council for Stream Improvement project at Virginia Polytechnic Institute for making available sewage and paper mill waste samples, and by furnishing the standard dilution B. O. D. data.

The two page vita has been removed from the scanned document. Page 1 of 2

The two page vita has been removed from the scanned document. Page 2 of 2