Final Report

RESPIROMETER ASSEMBLY FOR BOD MEASUREMENT

of River Waters and Biologically Treated Effluents

by

John J. Gannon
Associate Professor of Public Health Engineering

with

Jackson R. Pelton
Assistant in Research

and

James Westfield
Graduate Student

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SECTION I

SUMMARY AND CONCLUSIONS

Dissolved oxygen is one of the important measures of natural water quality where organic pollution is involved. The presence of adequate amounts of dissolved oxygen in natural waters is important from the standpoint of the protection of fish life and, also, from the standpoint of avoidance of gross nuisance.

A respirometer assembly has been developed as a laboratory device for the better measurement of the biochemical oxygen demand (BOD), including both rate and level, of river waters and biologically treated effluents. The focus of this report, therefore, is this respirometer assembly.

Section II of the report presents a simplified discussion of the nature of river oxygen balance to serve as a frame of reference for subsequent consideration of the respirometer assembly. Specifically, the river dissolved oxygen sag curve is discussed showing the interrelationship of the deoxygenation and reaeration components of the sag curve. Also considered is the classical monomolecular first stage BOD progression, together with the influence of the deoxygenation constant k_1 on the BOD progression. Following this is a description of the conventional laboratory dilution and laboratory reaeration procedures for determination of BOD

level and deoxygenation rate. In addition, the influence of nitrification in producing a second stage demand is considered including the potential influence on the first stage deoxygenation constant k_1 and, finally, a discussion is presented of the possible factors accounting for the difference between the river BOD removal constant k_r and the laboratory bottle deoxygenation constant k_1.

Section III of this report focuses specifically on the proposed respirometer assembly for BOD measurement of river waters and biologically treated effluents. The conventional dilution method and the reaeration procedure for the laboratory determination of both BOD level and deoxygenation rate, as described in the section II of this report, require considerable expenditure of effort, especially in the early stages of the rate when as many points as possible are desirable. Usually this means a laboratory attendant on duty both day and night during the first two days of a rate study, and, as a result, the availability of manpower becomes a limiting consideration as to the number of rates which can be run at a given time. Based on the experience of using these procedures, it became apparent that some type of continuous automatic, or at least semiautomatic, BOD instrumentation could be used to advantage, both in terms of more frequent data during the early time periods, and, also, in terms of reduced manpower requirements. With this stimulus and these

objectives, a respirometer assembly for BOD measurement of river waters and biologically treated effluents was developed. A discussion of some recent efforts of other investigators at improved BOD instrumentation is presented, followed by lengthy consideration of the proposed respirometer assembly, including a description of the apparatus, the general operating procedure, the precision of the oxygen electrode system, mixing considerations in the reaction flask, the BOD of any enclosed assembly components, and finally stability of electrode response during incubation.

Section IV of the report demonstrates the application of the respirometer assembly to the measurement of BOD of treated effluents and river waters. The results of two selected runs are presented, the first, consisting of an effluent sample from the Ann Arbor waste water treatment plant and, the second, consisting of a mixture of Huron River water and Ann Arbor waste water treatment plant effluent. In both cases, several controls were incorporated in the experiments allowing a comparison of the respirometer assembly results with the more conventional methods of determining BOD.

It has been demonstrated that the respirometer assembly can be effectively used for obtaining a continuous measure of BOD with time, involving considerably less effort than the more conventional dilution or reaeration produces. However, at this stage of development of the apparatus, it is desirable

to run selected parallel controls consisting of the more conventional methods so that the relationships between the old and new methods can be established.

For the reader who may wish more specific direction in the use of the assembly, a suggested operating procedure is presented in Appendix A.

SECTION II

THE NATURE OF RIVER OXYGEN BALANCE

Dissolved oxygen is one of the important measures of water quality where organic pollution is involved. The presence of adequate amounts of dissolved oxygen is important from the standpoint of the protection of fish life and, also, from the standpoint of avoidance of gross nuisance.

It is the intent of this section of the report to present a simplified discussion of the nature of river oxygen balance to serve as a frame of reference for later consideration of a respirometer assembly for measurement of river water deoxygenation rates. Specifically, the river dissolved oxygen sag curve will be discussed showing the interrelationship of the deoxygenation and reaeration components of the sag curve. Also to be considered is the classical monomolecular first stage BOD progression, together with a consideration of the influence of the deoxygenation constant k_1 on the BOD progression. Following this will be a description of the laboratory dilution and laboratory reaeration procedures for determination of BOD level and deoxygenation rate. In addition, the influence of nitrification in producing a second stage demand will be considered including the potential influence on the first stage deoxygenation constant k_1. Finally, a discussion will be presented of the possible factors accounting for the difference between the river BOD

removal constant k_r, and the laboratory bottle deoxygenation constant k_1.

River Oxygen Sag Relationships

In a river below a source of pollution dissolved oxygen concentration follows what is frequently called the "sag curve", i.e., a fairly rapid drop to a low point with a gradual recovery to a concentration equal to or greater than the original concentration, assuming no new waste sources are added. A typical sag curve is illustrated as Curve A in Figure 1. Many years ago Streeter and Phelps[1] formulated this relationship mathematically by the differential equation:

$$\frac{dD}{dt} = K_1L - K_2D$$

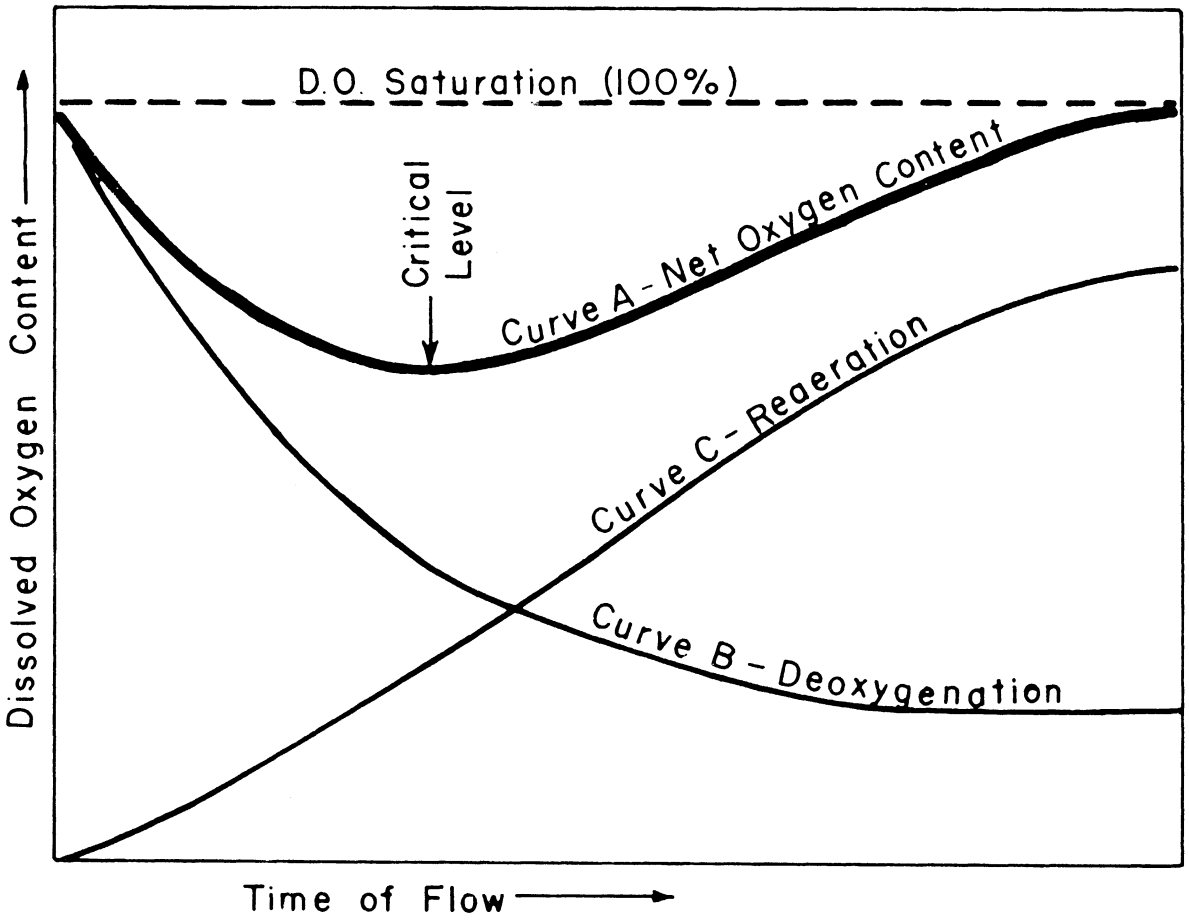
where D is the dissolved oxygen deficit below saturation, K_1L represents the deoxygenation part of the relationship, while K_2D represents the reaeration. Thus, the rate of change of the oxygen deficit is dependent on the influence of both deoxygenation and reaeration. Integration of this differential equation and expression of the constants in terms of common logarithms results in the working equation:

$$D_t = \frac{k_1L_a}{k_2 - k_1}\left(10^{-k_1t} - 10^{-k_2t}\right) + D_a \times 10^{-k_2t}$$

where D is the saturation deficit at time t in mg/l, D_a is the initial oxygen saturation deficit in mg/l, L_a is the initial BOD in mg/l, and where k_1 is the deoxygenation

Figure 1

RIVER OXYGEN SAG RELATIONSHIPS



(after Streeter and Phelps)

constant, while k_2 is the reaeration constant. The small k refers to a common log base while the capital K refers to the natural log base.

The basic elements of the oxygen sag relationships are expressed in graphical form in Figure 1 where it is seen that Curve A represents the net oxygen content, while Curve B represents the deoxygenation component and Curve C the reaeration component. Close inspection of Figure 1 will show that for a particular time of flow below a source of pollution, the net oxygen content or Curve A at that point, is the sum of the deoxygenation and reaeration Curves B and C respectively.

Inasmuch as the deoxygenation component of the oxygen balance is the subject of most attention in this study, it will be developed in more detail in subsequent sections of this report. At this point, however, consideration will be given to the sources of oxygen to meet the demands of the oxidizing organic material.

Dissolved oxygen is or becomes available in a river to meet the needs of decomposing organic material from two main sources, (1) the oxygen dissolved in the river water above the waste source and in the waste source itself, together with new tributary dilution water, and (2) oxygen which dissolved in the water as a result of reaeration from the atmosphere. Quantitative evaluation of the oxygen available from the first source, i.e., upstream river water, the waste source, and tributary water, involves knowledge of the runoff in each

case, together with the oxygen concentration. The maximum or saturation capacity of dissolved oxygen in fresh water exposed to normal atmosphere is inversely proportional to temperature—the higher the temperature the lower the saturation capacity, the lower the temperature the higher the saturation capacity. Table 1 is an abridged form of more complete tables which are available showing the influence of temperature on dissolved oxygen saturation in fresh water.

TABLE 1

SATURATION CAPACITY OF DISSOLVED OXYGEN IN FRESH WATER EXPOSED TO AIR

Temperature in °C	D.O. Concentration in Milligrams/liter
0	14.6
5	12.8
10	11.3
15	10.2
20	9.2
25	8.4
30	7.6
35	7.1
40	6.6

An important factor to be appreciated here is the significance of river runoff or flow in the oxygen balance of that

river. Generally speaking the more water, the more oxygen available for waste assimilation purposes, the less water, the less oxygen available for this purpose. Also, as the temperature goes up there is less oxygen available in the water together with an increase in the deoxygenation rate to be considered later, and as the temperature goes down there is more oxygen available together with a decrease in the deoxygenation rate. In the Great Lakes Region of the United States, therefore, the most unfavorable conditions in relation to oxygen balance usually occur in the late summer when the river flows are low and the temperatures are high.

Quantitative evaluation of the oxygen obtained from the second source, i.e., reaeration from the atmosphere, is a much more complicated task but can be accomplished with reasonable accuracy. The rate of reaeration is directly proportional to the dissolved oxygen deficit, but also is dependent on water temperature, depth, velocity and the physical characteristics of the stream. In the oxygen sag equation previously presented the factors of temperature, depth, velocity, and the physical characteristics of the stream are incorporated in the reaeration coefficient k_2. Recognizing that it was difficult to include all of the factors influencing reaeration into a single constant, Velz[2] proposed a more refined approach, while in recent years O'Connor and Dobbins[3] and Churchill[4] have developed relationships for estimating k_2 based on temperature and channel and flow characteristics. Each of these

methods are, however, refinements of the basic oxygen sag relationship.

The Biochemical Oxygen Demand (BOD) Concept

When organic matter in the form of waste water is discharged into a river it oxidizes biochemically using oxygen present in the river water thereby depleting the oxygen level of this water. Counterbalancing this deoxygenation, of course, as previously discussed, is the process of natural reaeration, with the actual amount of dissolved oxygen present at a given point in the river being the net result of the interaction of these two processes. The capacity of an organic waste water to utilize oxygen biochemically, in contrast to straight chemical oxidation, is referred to as its biochemical oxygen demand (BOD).

Streeter and Phelps[1] in 1925 described this type of reaction as follows: "The rate of biochemical oxidation of organic matter is proportional to the remaining concentration of unoxidized substance, measured in terms of oxidizability."

Expressed in differential form it would be as follows:

$$-\frac{dL}{dt} = KL$$

integrating to

$$\log_e \frac{L_t}{L} = -Kt$$

or

$$\log_{10} \frac{L_t}{L} = -kt$$

or

$$\frac{L_t}{L} = 10^{-kt}$$

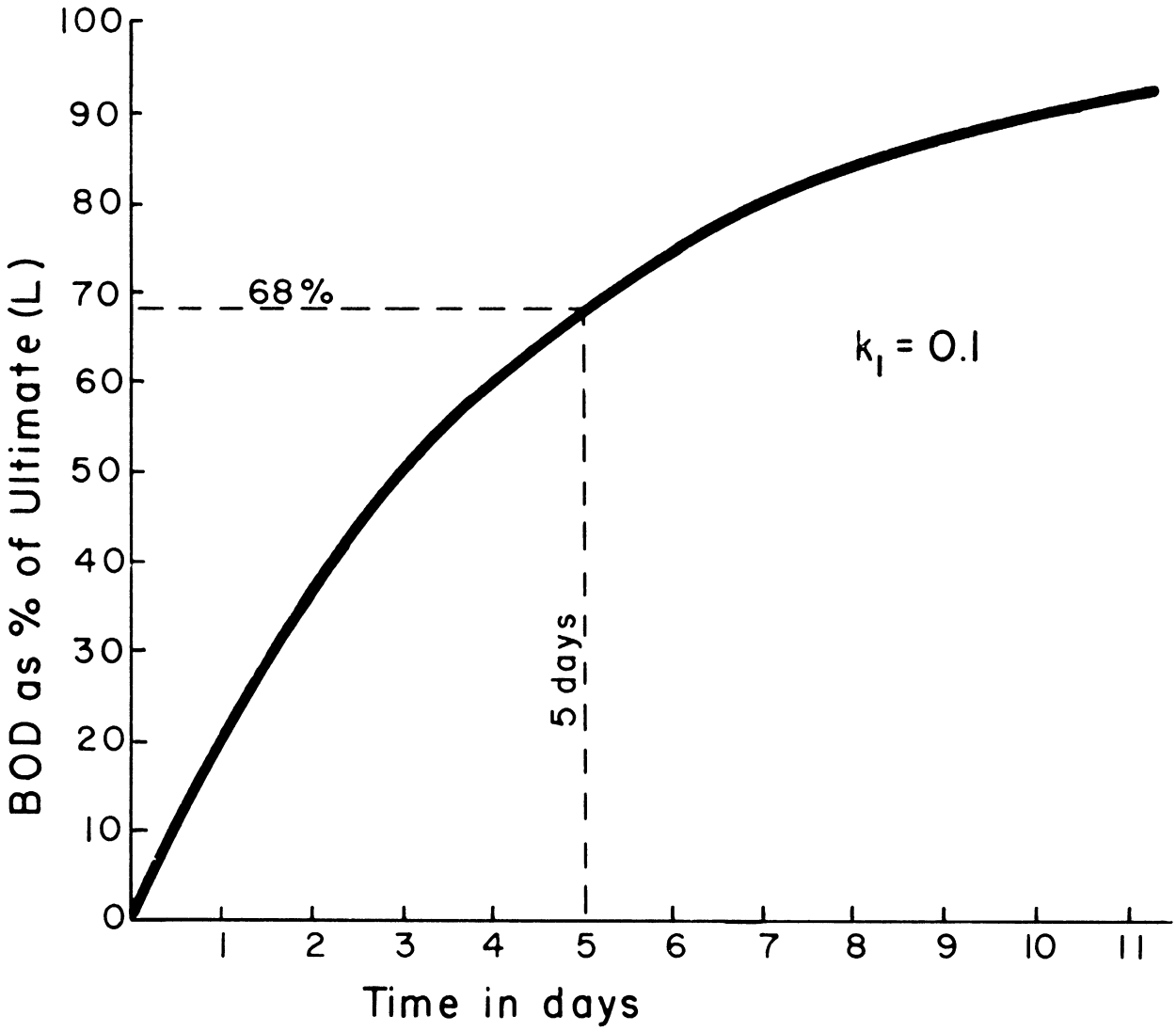
In this expression L is the total carbonaceous oxidizable material present at the beginning of the reaction, or, looking at it in another way, this is the ultimate or total first stage demand. Furthermore, the expression L_t represents the amount left at time t, while k is a measure of the speed of the reaction and is sometimes called the velocity constant, generally expressed with the subscript k_1 to distinguish it from the reaeration coefficient k_2. With a mixed sewage and river water, the value of k_1 is normally assumed to be 0.1 at 20°C. This was developed mostly from laboratory bottle experiments performed many years ago by Phelps, and seems to hold for the larger rivers such as the Ohio and the Willamette. Where a temperature correction is needed generally the expression: $k_T = k_{20^o}\ 1.047^{(T-20)}$ is used with T representing the adjusted temperature in °C.

The expression $\frac{L_t}{L}$ gives the fraction left after time t while $1 - \frac{L_t}{L}$ gives the fraction oxidized.

Figure 2 is a graphical expression of the per cent BOD oxidized versus time following the classical monomolecular expression of Streeter and Phelps, where it is seen that for a $k_1 = 0.1$ in 5 days, 68 per cent of the total first stage demand is exercised. Five days is an important time period because Standard Methods[5] suggests that routine BOD samples should be incubated for 5 days. Thus, under the classical conditions, 68

Figure 2

CLASSICAL MONOMOLECULAR 1st STAGE BOD PROGRESSION



per cent of the total demand should be exercised in 5 days and an estimate of the ultimate first stage demand L could be made by dividing the 5-day BOD by 0.68.

Figure 3 is a graphical expression of the influence of the deoxygenation constant k_1 on the first stage BOD progression. Of particular interest is the amount of BOD utilized in 5 days. It is seen, for example, that for a $k_1 = 0.01$ only 11 per cent of the ultimate would be utilized in 5 days, for a $k_1 = 0.05$ approximately 44 per cent would be utilized in 5 days, while for a $k_1 = 0.15$ about 82 per cent would be utilized in 5 days. Thus, it is necessary to know both the k_1 and the 5-day BOD figure in order to estimate the ultimate BOD L which is used in oxygen balance calculations, and an assumption that the k_1 had the expected value of 0.1 when in fact it had a value of 0.01 could cause a serious underestimation of the ultimate first stage BOD.

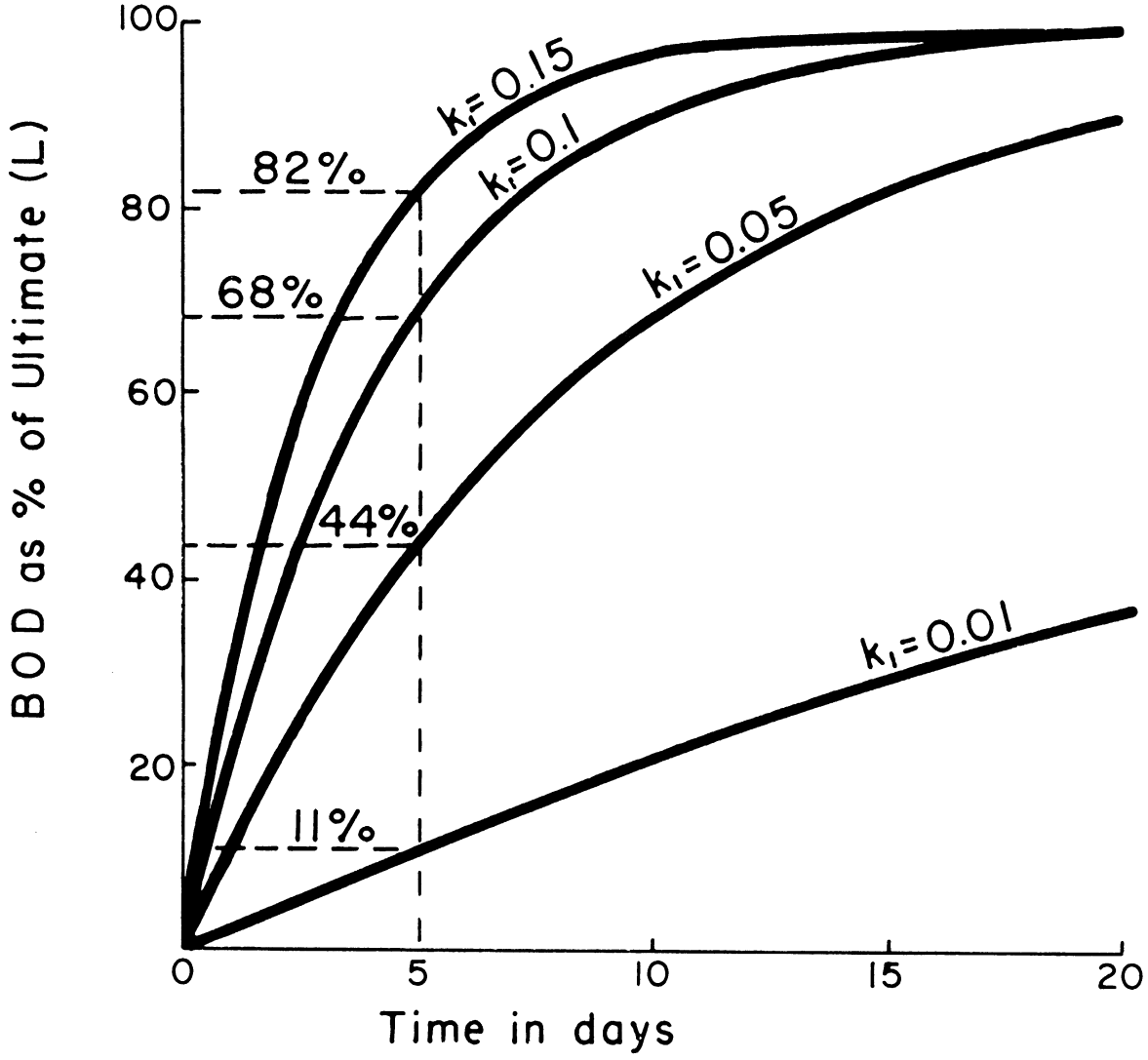
Another method of expressing the BOD reaction is in terms of the amount or the fraction left $\left(\frac{L_t}{L}\right)$ versus time rather than the amount oxidized, and this will plot as a straight line on semi-logarithmic grid, with BOD left on the log scale and time on the linear scale. Such a plot will be illustrated later in this report as Figure 5.

Laboratory Determination of BOD Level and Deoxygenation Rate

Up to the present time, the two most commonly used methods for the laboratory determination of BOD level and deoxygenation

Figure 3

INFLUENCE OF DEOXYGENATION CONSTANT k_1 ON 1st STAGE BOD PROGRESSION



rate of river waters or biologically treated effluents, were the dilution procedure recommended by Standard Methods,[5] or the reaeration procedure proposed by Elmore[6] of the T.V.A. staff. Both procedures have been used extensively by staff members of this laboratory.

Briefly, the dilution method involves preparing suitable dilutions of the sample to be tested with specially fortified dilution water so that some dissolved oxygen will be left in the mixture at the end of the selected incubation period. This presumes, therefore, some approximate prior knowledge of the BOD level, or if this is not available, more dilutions must be used. Duplicate sample bottles must be filled from the dilution vessel, followed by an immediate dissolved oxygen determination on one of these sample bottles, with the other sealed bottle incubated for the desired time, usually in the dark at 20°C. At the end of the incubation period, the second sample is removed from the incubator and the remaining dissolved oxygen in the sample is measured, with the BOD consisting then of the loss in dissolved oxygen multiplied by the appropriate adjustments for dilution. The dilution water recommended by Standard Methods[5] is a specially fortified water, but is a drastic change from the natural environment of the river and no doubt has an influence on the BOD results.

Enough samples must be initially prepared and incubated for variable time periods, so that curves similar to those of Figure 2 and Figure 3 may be developed from the experimental results.

This imposes, therefore, considerable burden initially on the laboratory staff. Also, the river water sample as collected is diluted and thereby changed, and is, in addition, broken down and incubated in a whole series of small sealed BOD bottles. The assumption is made that the same rate of reaction is occurring in each of these small bottles which may be unrealistic.

To improve on the dilution method, especially for river waters and treated effluents with low BOD values, Elmore[6] proposed a special reaeration procedure. Briefly, the technique consists of collecting a large volume of sample generally in the 3 to 5-gallon range, bringing it into the laboratory and aerating it with compressed air to bring the oxygen concentration close to saturation, filling a series of sealed BOD bottles and then incubating these small bottles together with the larger remaining part of the sample. The dissolved oxygen in the sealed bottles is measured initially and at various selected time intervals. When the dissolved oxygen level in the sealed bottle approaches 2 mg/l, the large unsealed bottle is aerated and another set of sealed bottles is prepared as initially, with the process then repeated through any desired time interval. The successive oxygen depletions can be summed, resulting in the availability of a BOD vs. time relationship, with each point obtained from a dissolved oxygen determination on a single sealed bottle.

The main advantage, of course, of this procedure over the dilution approach is that the river water sample is used as it is collected without alteration by dilution, buffering, etc. Also, there is a saving in initial setup time, together with a saving in time resulting from the fact that no seed, blanks, or dilution water is needed.

Some of the disadvantages include the fact that the procedure is not recommended for samples having a BOD_5 greater than 25 mg/l because of the frequent need for reaeration. Also, there is a need for a larger sample volume together with the need for more incubator capacity. In addition, there is a need for more attention after the initial setup than would be needed in the case of the dilution procedure.

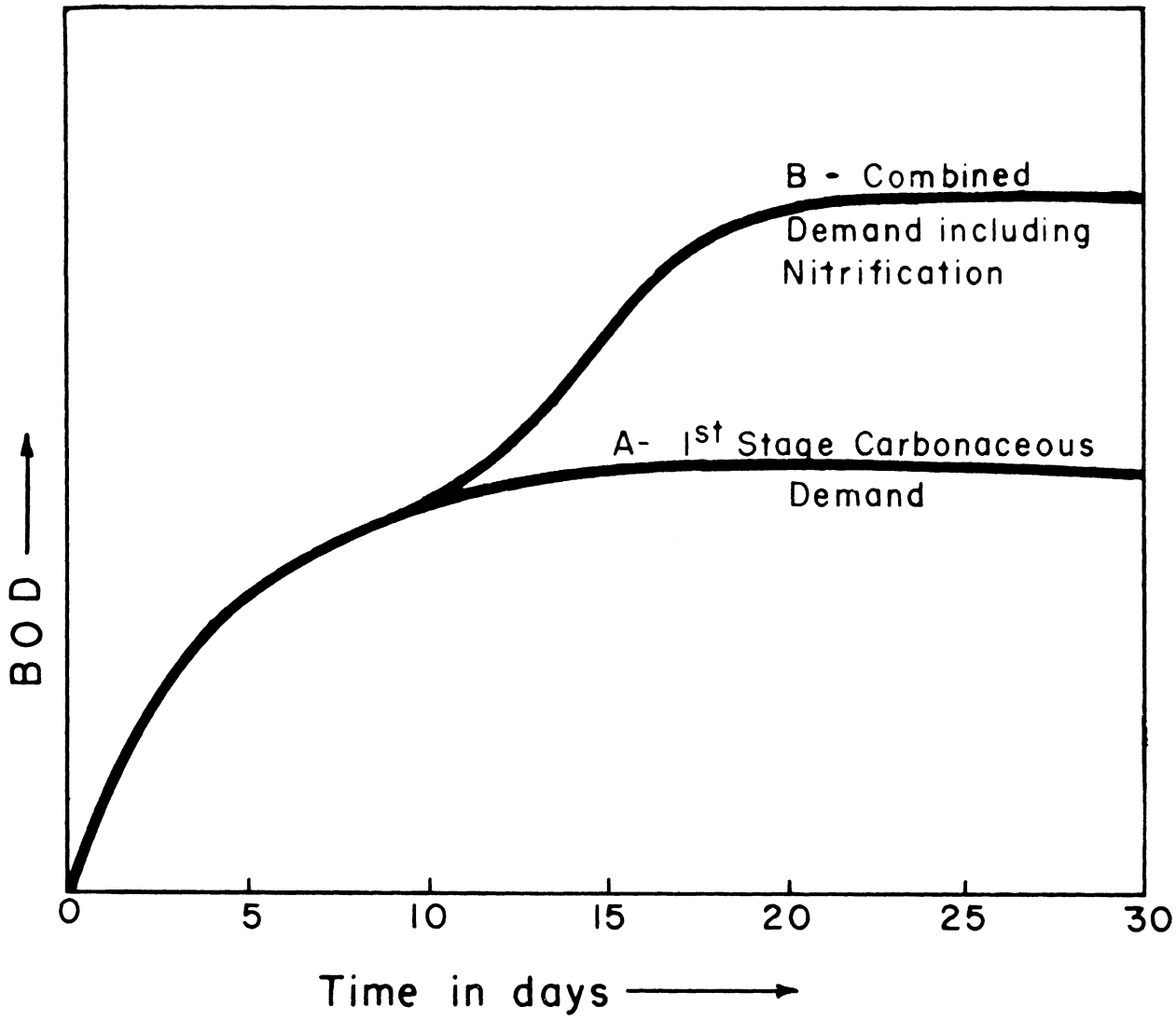
Of the two methods presented here, it appears that the reaeration method of Elmore is to be recommended over the dilution method where the sample consists of river water or biologically treated effluents.

Nitrification Influence on BOD Results

For a normal mixed domestic waste it has been found that at the end of eight to ten days a second stage demand develops usually attributable to nitrification. Nitrogen sources present in the waste utilize oxygen in going from NH_3 to NO_2 to NO_3, the latter being a more stable form from an oxygen consuming standpoint. Figure 4 illustrates the classical first and second stage BOD progression where it is seen that Curve A

Figure 4

CLASSICAL 1st AND 2nd STAGE BOD PROGRESSION INCLUDING NITRIFICATION



represents the first or carbonaceous demand while Curve B represents the combined demand including nitrification. Thus, under normal conditions as a result of nitrification, it is rarely possible to measure in the laboratory the first stage ultimate carbonaceous demand L, and generally it is a calculated value from the earlier parts of the BOD curve before nitrification. Several methods are available for calculating both the k_1 and the ultimate first stage BOD value L, and it has been brought out by the writer[7] elsewhere that the method of computation used can have an influence on the resulting magnitudes of both k_1 and L.

Under most instances of a normal mixed waste, nitrification generally starts after the first five days of the laboratory incubation period and, therefore, exerts no significant influence on the standard 5-day BOD. However, there are exceptions, particularly if the waste is a biologically treated effluent of the activated sludge type of treatment. It has been demonstrated by the writer[7] that in the case of the Pontiac, Michigan activated sludge waste treatment plant effluent, and, also, the Clinton River receiving this effluent, that active nitrification frequently started within the normal 5-day laboratory incubation period. Thus, under these circumstances the standard 5-day BOD result must be interpreted with care.

In the case of the Grand River below the Lansing, Michigan activated sludge plant, Courchaine[8] has reported on the

presence of active nitrification progressing in the river at the same time as the carbonaceous first stage demand, resulting in a significant influence on the oxygen balance in the river immediately below the treatment plant, including the low or critical point of the oxygen sag curve. For the Clinton River below the Pontiac, Michigan treatment plant, the writer [7] has shown that nitrification had an influence on the standard 5-day laboratory BOD test but was not an influence in the river at least as far downstream as the low point in the oxygen sag curve.

Several investigators have reported on efforts to inhibit nitrification including the work of Sawyer and Bradney[9] using pasteurization and chlorination, and the work of Hurwitz et al.[10] describing the use of acidification. There is a need for additional work in the area of nitrification, and studies are currently underway in the Department of Environmental Health to explore further the basic mechanism involved, together with a more critical evaluation of nitrification inhibition techniques.

River BOD Reduction

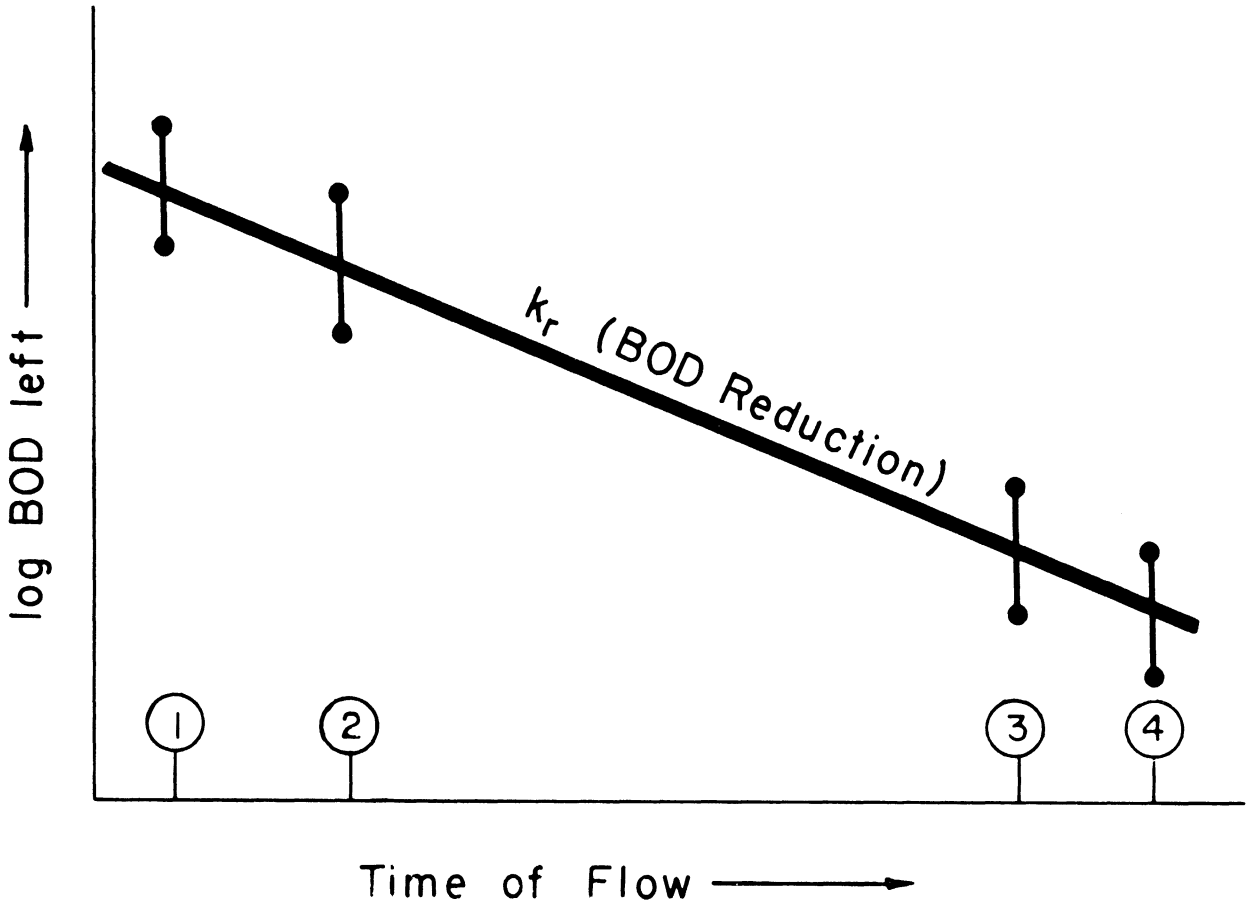
One of the important reasons for studying deoxygenation rates in the laboratory is to use this information in predicting the deoxygenation rates in the river for purposes of calculating oxygen balance. Much effort has been and is still being expended in developing reliable laboratory procedures for predicting river deoxygenation rates.

Field procedures are available, of course, for evaluating river BOD removal rates from standard 5-day BOD analyses run on samples collected at frequently located downstream sampling stations, together with a knowledge of the river time of passage between these stations. Figure 5 is a typical chart showing the log of the river BOD remaining versus time of passage, which, in accordance with the BOD monomolecular reaction previously presented, results in a straight line on semi-log paper. The slope of this line is the river BOD removal constant k_r, to be distinguished from the laboratory deoxygenation constant k_1. To define this river k_r it is highly desirable to conduct an around-the-clock survey over a period of 48 to 72 hours, under representative steady state conditions of low flow and waste discharge involving at least 12 to 15 BOD samples at each station. This is to be contrasted with the more usual undesirable practice of collecting just one or two BOD samples at each sampling station.

The classic work of Streeter and Phelps[1] on the Ohio River published in 1925 and more recently summarized by Phelps[11] showed that during the summer period of 1914 the Ohio River below Cincinnati had an average river k_r value of 0.12 which corresponded very closely with the laboratory deoxygenation constant k_1 of 0.1 derived from bottle experiments. This agreement appears to be particularly true for the larger rivers, where there is relatively less contact with the river bottom and sides, and where there is no gross dispersed

Figure 5

EVALUATION OF RIVER BOD REDUCTION



type of growth. A second illustration of agreement is the case of the Willamette River[12] in Oregon where the river BOD removal rate k_r of 0.125 was determined from field data which corresponded to the normal laboratory $k_1 = 0.1$ at 20°C with appropriate temperature adjustment.

Unfortunately not all rivers exhibit this type of agreement, and there are several river conditions which cause k_r to be much higher than k_1 including such things as: sludge deposition and accumulation, immediate demand, biological extraction and accumulation, and increased river oxidation rates particularly in small shallow streams.

It is beyond the scope of this presentation to consider all of these possible influences in detail although certain of them can be predicted in a more quantitative way than others. Generally, immediate demand can be evaluated from a study of the waste characteristics, while Velz[13] has presented a procedure for calculating the influence of sludge deposition and accumulation. Velz and Gannon[14] have discussed more recently the problem of biological extraction and accumulation, but must rely on observed river BOD data for evaluation of the river k_r; while the writer[7] has illustrated the situation of an increased river BOD oxidation rate for the Tittabawassee River in Michigan, but again relying on observed river BOD data for evaluation of k_r. Thus, it is apparent that a need exists to develop more appropriate laboratory procedures for quantitative prediction of the river k_r for the several river

situations involving biological extraction and accumulation and, also, for increased river oxidation situations such as the Tittabawassee River.

The reader should appreciate, however, the need for knowledge of both the laboratory k_1 and the river k_r —the laboratory k_1 so that the standard 5-day BOD can be converted to an ultimate BOD (L), since oxygen balances are calculated in terms of ultimate BOD, and the river k_r so that appropriate river BOD removal rates can be used in the oxygen balance calculations.

If the river k_r is different from the laboratory k_1, then the oxygen sag equation as presented by Streeter and Phelps[1] must be modified and appropriate substitution of k_r must be made for k_1 as presented by Eckenfelder and O'Connor.[15]

Summary

This section of the report has presented a simplified discussion of the nature of river oxygen balance to serve as a frame of reference for later consideration of a respirometer assembly for measurement of river water deoxygenation rates. The river oxygen sag curve has been discussed showing the interrelationship of the deoxygenation and reaeration components of the curve. In addition, the BOD concept has been presented including the mathematical formulation of Streeter and Phelps, and also showing the relative influence of the magnitude of the deoxygenation constant k_1. Following this is a description of the laboratory dilution and laboratory reaeration procedures for determination of the BOD level and deoxygenation rate. In addition, a consideration of nitrification influence on BOD results has been presented and, finally, a discussion has been given of the situation where river BOD removal rates k_r are higher than the laboratory deoxygenation rates k_1, due possibly to such things as sludge deposition and accumulation, immediate demand, biological extraction and accumulation, and increased river oxidation rates particularly in small streams.

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SECTION III

RESPIROMETER ASSEMBLY FOR BOD MEASUREMENT

The dilution method and the reaeration procedure for the laboratory determination of both BOD level and deoxygenation rate as described in the previous section of this report require considerable expenditure of effort, especially in the early stages of the rate when as many points as possible are desirable. Usually this means a laboratory attendant on duty both day and night during the first two days of a rate study, and, as a result, the availability of manpower becomes a limiting consideration as to the number of rates which can be run at a given time. Based on the experience of using these procedures, it became apparent that some type of continuous automatic, or at least semi-automatic, BOD instrumentation could be used to advantage, both in terms of more frequent data during the early time periods, and, also, in terms of reduced manpower requirements. With this stimulus and these objectives, a respirometer assembly for BOD measurement of river waters and treated waste treatment plant effluents was developed.

This section of the report will start with a discussion of some recent efforts of other investigators at improved BOD instrumentation, and then will consider at length the proposed respirometer assembly, including a description of the

apparatus, the general operating procedure, the precision of the oxygen electrode system, mixing considerations in the reaction flask, the BOD of any enclosed assembly components, and finally stability of electrode response during incubation.

Some Recent Efforts at Improved BOD Instrumentation

In recent years several investigators have published reports of efforts toward improved BOD instrumentation, or on instruments that might be adapted for such use. One inventive approach was reported by J.W. Clark [1][2][3] of New Mexico State University using a sealed digestor assembly, involving a procedure for producing oxygen by electrolysis and measuring the amount of this oxygen added to the assembly to replace oxygen utilized in the substrate. Thus, the amount of oxygen added is totaled with time, and, in effect, becomes the BOD. The applications reported had BOD_5 in the range of 1000 to 5000 mg/l, although the author reported that modifications were underway to run tests within the range of 0 to 1000 mg/l. Also, certain components of the assembly were not readily available through normal laboratory equipment supply channels involving, therefore, customized construction of each assembly requiring skilled instrument personnel.

A second proposed system was reported by Eye[4] et al. of the University of Cincinnati involving a sealed Erlenmeyer flask containing a platinum electrode system for oxygen

measurements. All of the illustrations reported by Eye involved BOD_5 levels of 7.0 mg/l or less, which means that the oxygen initially present in the sample was adequate to meet the BOD_5 demand. The Eye concept of including an oxygen electrode in a sealed reaction flask was appealing, but there needed to be some method of reaerating the sample if the oxygen level fell below 2 mg/l.

Following Eye's work, Mancy, Westgarth and Okun[5] described a respirometer cell assembly used for determining oxygen uptake rates of activated sludge, involving a galvanic oxygen analyzer as a sensing probe, continuously immersed in a reaction vessel. Provision was made for aerating the liquid content and for continuously recording the amount of dissolved oxygen present with time. Because of the high oxygen demand of a normal activated sludge mixture, oxygen present in the mixture as a result of aeration at the beginning of a run is quickly utilized, and complete depletion is a matter of minutes rather than hours or days. The apparatus to be described by the writer in this report is similar to that reported by Mancy, Westgarth, and Okun[5] with a few modifications, and with the application made to low BOD waters such as biologically treated effluents and river waters, rather than to an activated sludge mixture.

While not developed for or directly related to BOD determination, an interesting reaction vessel called a photosynthetic gas exchanger using mass cultures of algae for O_2

production was described by Hannan and Patouillet.[6][7] The impetus for the study was the consideration of the algal system as a means of oxygen generation in submarines. Basically the unit consists of a glass reaction cylinder with six high intensity lamps placed vertically through the cylinder in cooling jackets, with the CO_2-air mixture forced through the algal suspension in the vessel through the base of the unit. The gas was allowed to pass freely through the unit and then through holes in the top of the culture vessel and was collected periodically at one of these holes and analyzed for oxygen and carbon dioxide. Thus, the vessel was used for oxygen production rather than oxygen utilization, but had features which might be incorporated in a BOD measuring apparatus, especially if interest centered in evaluating the influence of algae on BOD.

Another type of reaction vessel or culture chamber was described recently by Sanders[8] who developed it to study the relationship between the oxygen utilization of heterotrophic slime organisms and the wetted perimeter. His chamber was designed as a continuous culture system rather than a batch type system; the batch system is, of course, the type characteristically used in the standard BOD determination. Thus, the apparatus cannot be used for the usual BOD determination as it is generally performed. A slime mass was developed in the reaction vessel and an aerated medium was pumped through the chamber at a constant rate of 5 ml/minute, with continuous

recordings made of the dissolved oxygen concentrations in the influent and effluent media streams, using a galvanic cell oxygen analyzer. With this assembly, the dissolved oxygen drop or oxygen utilized could be related to the slime buildup, which was measured on a removable slide.

Respirometer Assembly

Assembly Description

The respirometer assembly consists of a standard 2-liter sealed reaction flask with a specially constructed galvanic cell oxygen electrode as proposed by Mancy, Westgarth, and Okun[5] tapered to fit through one of the standard port openings. Ports are also available to permit aeration of the contents of the flask when the D.O. level drops to 2 mg/l and for temperature observation. A typical assembly is shown in Figure 6a where the oxygen electrode is connected to a standard strip chart recorder to permit continuous recording of residual oxygen concentration with time. Also, it is seen that a magnetic stirrer is used to keep the contents of the flask under continuous mixing to provide adequate velocity for electrode response, and that an asbestos pad is used to control the transfer of heat from the magnetic stirrer to the reaction flask. Not all the heat transfer is eliminated by this procedure, but it is possible to compensate for this heat by appropriate adjustment of a standard BOD incubator so as to maintain the temperature of the sample at $20^{o} \pm 1.0^{o}C$.

The galvanic cell oxygen analyzer as proposed by Mancy, Westgarth, and Okun[5] and by Mancy and Westgarth[9] was constructed by the Gelman Instrument Company of Ann Arbor in accordance with the general specifications provided by Mancy and Westgarth[10] with some modification. This oxygen analyzer consists of a silver-lead galvanic couple separated from the test sample by a polyethylene membrane with a 1 M KOH solution between the couple and the membrane. The current generated is directly proportional to the oxygen content in the sample, assuming no interfering substances, without the need of an external applied voltage. This analyzer is temperature dependent, but in BOD studies where the temperature is held constant at 20°C this is not a problem.

One modification of the analyzer consisted in the substitution of one per cent carboxymethylcellulose (Dow Chemical Co.) in place of the lens paper as a carrier for the 1 M potassium hydroxide to increase the sensitivity of the electrode. Also, the membrane material used consisted of a cross-linked heat-shrinkable type L polyethylene film of 0.5 mil thickness, manufactured by the Cryovac Company, Cambridge 40, Massachusetts as suggested by Mancy.[11] After repeated use of the reaction flask, it was found the cover did not seat as well as it did when new, suggesting the need for a gasket to prevent leakage. Several different materials were investigated and finally gaskets were made of silicone rubber material (S-2000) manufactured by Dow Chemical Company of Midland, Michigan which has been reported to have no BOD of its own.

All of the equipment illustrated in Figure 6a is readily available through ordinary laboratory supply channels with the exception of the galvanic cell oxygen probe as used in this assembly and the silicone rubber gasket. This particular probe was constructed at a cost of approximately $55.00. Thus, the assembly, exclusive of the recorder, normally should cost somewhere in the range of $125-150 putting this unit easily within the price range of most research organizations. It should be reported that since the development of this assembly, the Precision Scientific Company has put on the market a galvanic cell oxygen analyzer. However, it does not appear that the commercial probe can be readily adapted to the assembly described in this report because of size.

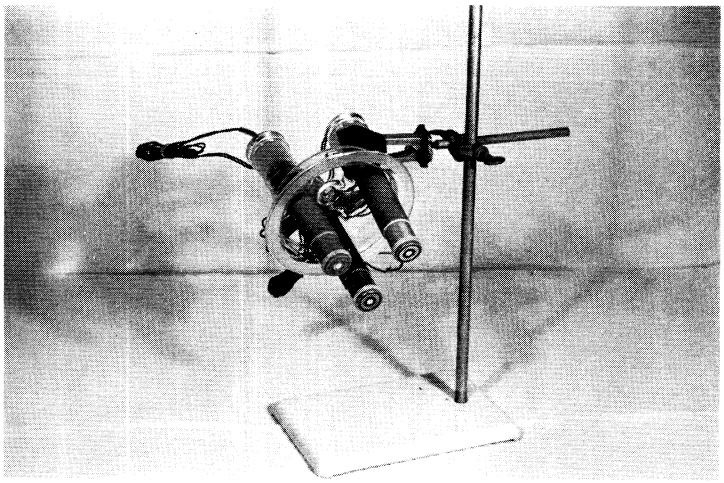
Temperature control is accomplished in a standard BOD incubator with the recorder unit generally placed outside the incubator. Normally, it is possible to fit as many as three respirometer assemblies in one standard incubator and maintain adequate temperature control.

General Operating Procedure

Operation of the assembly for BOD measurement is a modification of the standard reaeration technique previously described, i.e., the sample is aerated and placed in the sealed respirometer cell with the galvanic cell oxygen electrode. The whole assembly is then placed in a standard BOD incubator on a magnetic stirrer to provide the necessary velocity for

GALVANIC CELL OXYGEN ELECTRODE RESPIROMETER ASSEMBLY

a. Normal Assembly including Recorder



b. Special Three Electrode Respirometer Cover

electrode response, with the oxygen electrode connected to a continuous recorder. When the oxygen concentration in the flask drops to 2 mg/l, the flask is aerated by means of compressed air and the whole procedure is then repeated as long as it is desired to keep the rate running. The BOD then as with the standard reaeration technique, is the sum of the oxygen depletion with time. Obviously this procedure has the same advantages as the reaeration technique in that no dilution is involved but, also, has the added advantage of keeping the original sample intact, rather than splitting it up into several sealed BOD bottles. A detailed suggested operating procedure is presented in Appendix A of this report for the reader who may wish more specific directions.

Adequate temperature control is important both in terms of influence on electrode response and, also, in terms of influence on the BOD reaction itself. It has been the experience of this laboratory that a temperature range of about $\pm 1^{o}C$ can be maintained in the sample in a standard BOD incubator, and it has not been felt necessary to go to a water bath type of temperature control.

Another possible operating problem that can develop is leakage around the cover of the reaction flask, particularly if the unit has been used for some time. This problem can be eliminated by the use of the silicone rubber gasket previously described.

At the beginning of a run it has been observed in several instances that a 2-4 hour stabilization period is necessary

before the strip chart record of oxygen concentration assumes a regular pattern. In some cases the chart may show a temporary rise, while in other cases it shows a short drop within this stabilization period, and it appears desirable not to include this data in the BOD results. This early erratic pattern has developed even in the case where the electrode has been stored in the substrate to be used for a period of approximately 24 hours before the beginning of the run.

Precision of Oxygen Electrode System

During certain of the preliminary runs where two assemblies were operated in parallel on equal parts of the same original sample, some difference in BOD was noted in the results of the parallel runs. To test the precision of the electrode system itself, a special respirometer cover was constructed to accommodate three similar galvanic cell oxygen electrodes as illustrated in Figure 6b with each electrode connected to a separate recorder-amplifier as illustrated in the normal assembly of Figure 6a. Thus, the three electrodes were independently sensing and recording the dissolved oxygen concentration in the same substrate, providing a measure of the precision of the oxygen electrode system and recorder.

An experimental run was made using a standard soluble substrate consisting of a glucose-glutamic acid 1:1 mixture, together with a sewage seed consisting of one per cent Ann Arbor waste water treatment plant influent, settled for 24 hours and filtered through Whatman No. 2 filter paper as

suggested by Busch.[12] Results for selected time values in the BOD progression for the three electrodes are presented in Table 2, together with the average of the three observations presented in the last column. Variation among the electrode results is well within the variation reported by Elmore[13] and, also, by the writer[14] for replication of the jug reaeration procedure.

Figure 7 is a plot of the BOD progression with time, with the BOD shown as the average of the three electrode responses rather than as three separate lines, because of the graphical difficulty of portraying three lines that are so close to one another. This curve is characteristic of BOD progression of a soluble substrate as reported by Busch[12] involving a two stage reaction, with the first part a period of rapid oxygen uptake corresponding to the conversion of the material into cell substance, and the second part a period of utilization of stored decomposition products and cell substance at a slower oxygen utilization rate. Thus, it is seen in Figure 7 that a plateau of oxygen utilization occurs in approximately 20 hours, with this substrate and method of seed handling.

The focus in this experiment has been on the precision of the oxygen sensing and recording equipment, rather than the accuracy of the procedure itself. Results of other experiments will be presented later in this report showing comparisons between the respirometer assembly method and the jug reaeration method.

TABLE 2

PRECISION OF OXYGEN ELECTRODE SYSTEM

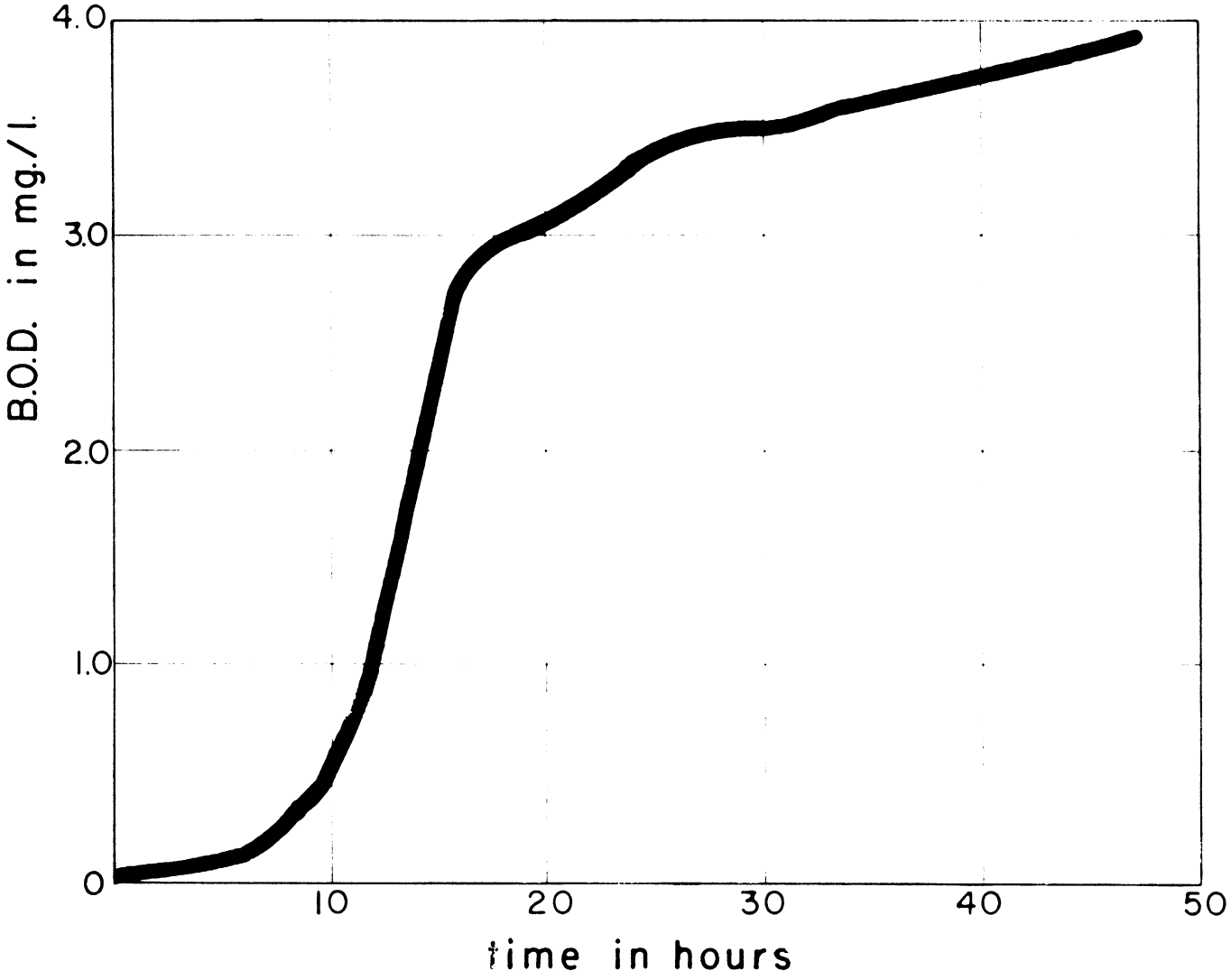
BOD PROGRESSION

GLUCOSE - GLUTAMIC ACID MIXTURE

Time Hours	Electrode Number 1 mg/l	Electrode Number 2 mg/l	Electrode Number 3 mg/l	Average of 3 Electrodes mg/l
0	0	0	0	0
6	0.1	0.1	0.1	0.1
7	0.2	0.1	0.2	0.2
8	0.3	0.2	0.2	0.2
9	0.4	0.3	0.4	0.4
10	0.6	0.5	0.5	0.5
11	0.8	0.8	0.8	0.8
12	1.1	1.1	1.0	1.1
13	1.5	1.5	1.4	1.5
14	1.9	1.9	1.8	1.9
15	2.4	2.4	2.4	2.4
16	2.7	2.8	2.8	2.8
17	2.9	2.9	2.9	2.9
18	3.0	3.0	2.9	3.0
19	3.0	3.0	3.0	3.0
20	3.1	3.0	3.0	3.0
22	3.2	3.2	3.2	3.2
25	3.4	3.4	3.4	3.4
30	3.5	3.5	3.5	3.5
33	3.6	3.6	3.5	3.6
36	3.6	3.7	3.6	3.6
40	3.7	3.8	3.7	3.7
44	3.8	3.8	3.8	3.8
47	3.9	3.9	4.0	3.9

Figure 7

B.O.D. PROGRESSION
GLUCOSE-GLUTAMIC ACID MIXTURE
(AVERAGE OF 3 ELECTRODE RESPONSES)

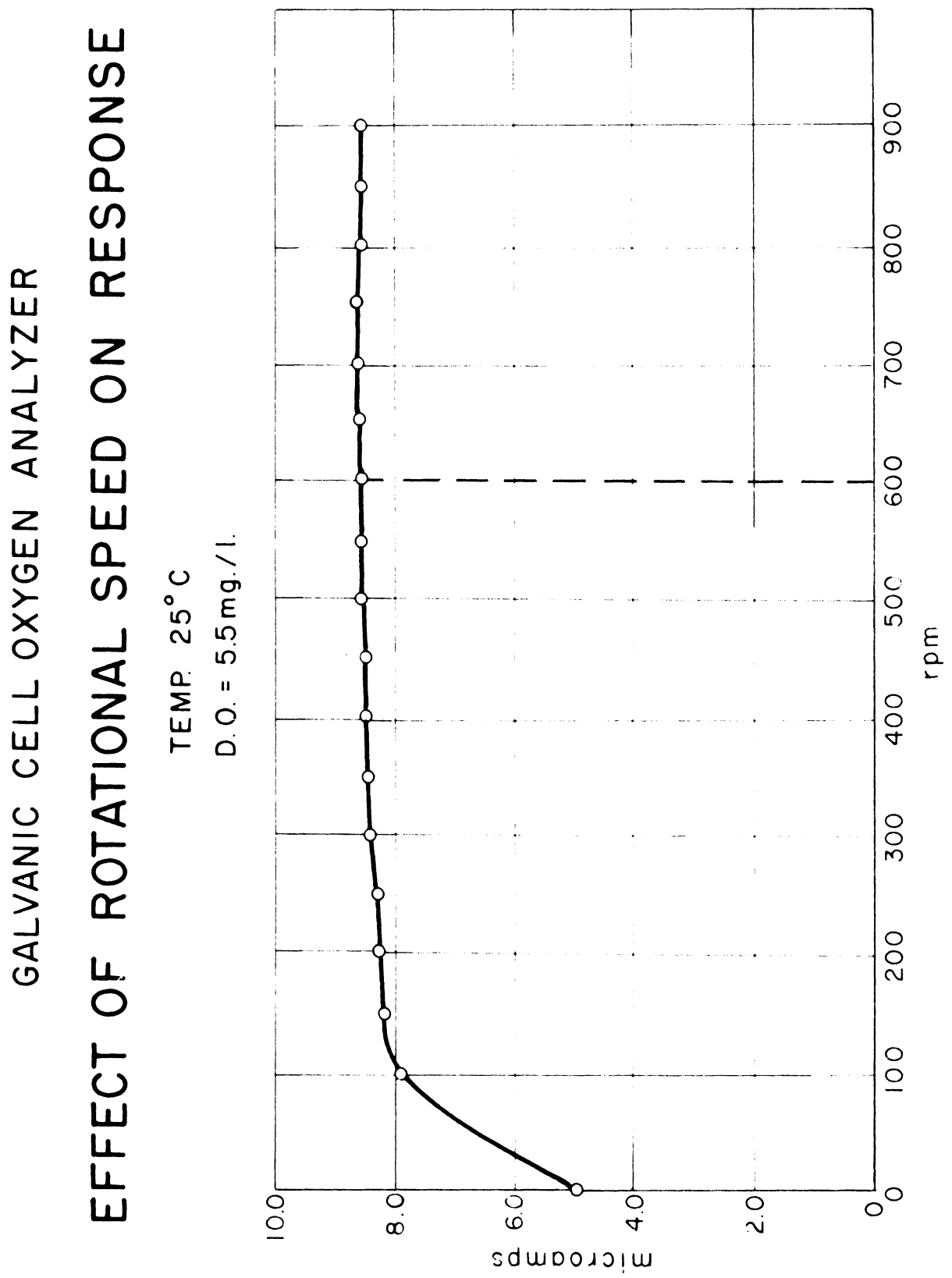


Mixing and Velocity Considerations

The galvanic cell oxygen electrode requires either movement of the electrode in the test solution, or movement of the test solution past the tip of the electrode to insure that the oxygen concentration at the membrane surface is equal to that of the bulk of the solution. This, of course, can be accomplished in several ways, but the most convenient in terms of the proposed respirometer assembly used in the laboratory is by means of a magnetic stirrer, using a teflon covered stirring bar in the enclosed test solution as illustrated in Figure 6a. An important item to know is the minimum speed of rotation necessary to give a maximum stable response corresponding to the actual amount of dissolved oxygen present. To obtain this information, an experiment was performed using a normal respirometer assembly, with electrode response measured under varying speeds of rotation for a dissolved oxygen concentration of 5.5 mg/l at 25°C. The speed of rotation was determined in revolutions per minute (rpm) using an electronic stroboscope-Strobotac type 1531-A- manufactured by the General Radio Co.

Figure 8 shows the results of this experiment with a plot of revolutions per minute (rpm) versus electrode microamp response. It is seen that a minimum speed of rotation of about 125 rpm is necessary to insure a normal stable electrode response, and that above this speed there is little change in response with an increase in speed. Also, it should be reported that for this assembly it has been difficult to maintain a speed above 600 rpm and that all experimental results presented in this report have been in the 500-600 rpm range.

Figure 8


EFFECT OF ROTATIONAL SPEED ON RESPONSE
GALVANIC CELL OXYGEN ANALYZER
TEMP. 25°C
D.O. = 5.5 mg./l.
microamps
10.0
8.0
6.0
4.0
2.0
0
0
100
200
300
400
500
600
700
800
900
rpm

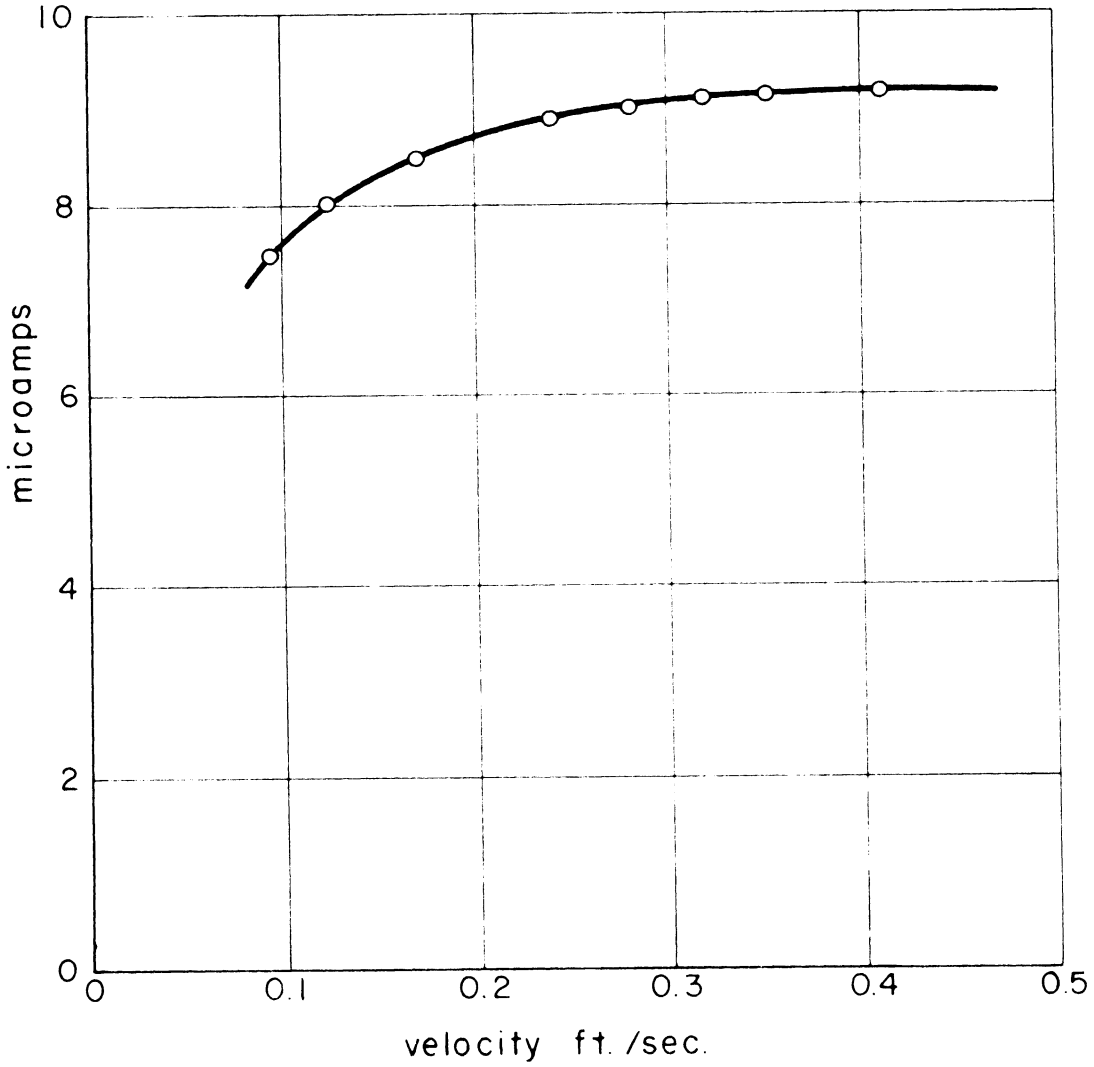
For field use of the galvanic cell oxygen analyzer (GCOA), in many cases it has been more convenient to move the electrode through the test solution such as in a river, or to pump the test solution past the electrode, rather than using a magnetic stirrer suggested for laboratory installations. Under this type of use, the question might be raised as to the minimum linear velocity necessary to insure a maximum stable response for a given dissolved oxygen concentration. To answer this question, Schwerha,[15] a graduate student working in this laboratory under the direction of the writer and using an open channel hydraulic installation available in the basement of the School of Public Health, demonstrated that a minimum linear velocity of about 0.3 ft/sec was necessary. His results are presented in Figure 9, where it is seen that a plateau is reached for velocities of about 0.3 ft/sec and above. These velocities corresponded to discharge measurements in the range of 75 gallons per minute (gpm) to about 400 gallons per minute (gpm). For purposes of taking these readings, the lower part of the galvanic cell oxygen analyzer (GCOA) was submerged directly in the water in the channel.

It is interesting that the Precision Scientific Company through the Sargent Company,[16] in promotional literature used for their commercial model of the analyzer (GCOA), suggest a minimum flow velocity of one ft/sec past the probe which is certainly on the conservative side. The minimum velocity requirement of 0.3 ft/sec is well below the suggested minimum

Figure 9

GALVANIC CELL OXYGEN ANALYZER

EFFECT OF LINEAR VELOCITY ON RESPONSE



velocities of the other oxygen electrode system currently available through commercial channels, making this an attractive feature of the GCOA analyzer.

In addition to the influence of mixing on the oxygen electrode response, it must be appreciated that mixing also can affect both the BOD level and deoxygenation rate. It has been demonstrated by the writer[14] that for both the Clinton River and Tittabawassee River (Michigan) waters, mixing definitely caused an increase in BOD level, at least in terms of the 5-day BOD. Apparently the more settleable material present, the greater the influence on the BOD, following the supposition that mixing keeps the solids in suspension, thereby allowing a more intimate contact between the solids and the test solution. The influence of mixing on increased deoxygenation of polluted waters was further confirmed by a paper presented by Lordi and Heukelekian[17] at the 16th Purdue Industrial Wastes Conference in May, 1961. Their experimental apparatus was different from the proposed respirometer assembly presented in this report, and involved an open vessel which then necessitated a calculated correction for the influence of reaeration.

Up to the present time, controlled experiments to evaluate the influence of mixing under a range of mixing rates and with different substrates using the proposed respirometer assembly, have not been conducted. However, it is anticipated such experiments will be carried on in the future which should further document the influence of mixing. As stated previously,

all respirometer runs presented in this report were made in the 500 to 600 rpm range.

BOD of Enclosed Respirometer Components

A series of experiments were conducted to check on the existence and, if any, on the magnitude of BOD contribution from the enclosed respirometer components. The test assembly was the same as illustrated in Figure 6a, except that no connection was made to the strip chart recorder. For purposes of discussion and documentation, the results of a typical run started on January 30, 1964 are presented in Table 3. Three test assemblies were used, two on a non-mixed basis, and one on a mixed basis, together with seed and BOD dilution water controls with the seed material consisting of a one per cent solution of Ann Arbor waste water treatment plant influent, which was settled and gauze filtered, and then diluted in standard BOD dilution water. No additional organic material was added to the test solution, except the one per cent sewage seed and BOD dilution water.

Inspection of the results presented in Table 3 shows the BOD_5 of the assembly components to be in the range of 0.43 to 0.58 mg/l for the non-mixed assemblies, and about 0.58 mg/l for the mixed assembly. These BOD figures, of course, represent the total BOD, with no breakdown as to the contribution of the different parts of the enclosed assembly, but because of the relatively low BOD values involved, and, also, because of the difficulty of making reliable BOD determinations at these low

levels, no further efforts were directed along these lines. Also, it is seen that the BOD differences between the mixed and non-mixed assemblies were slight. This experiment was repeated two additional times with minor variation, and in no instance did the BOD of the assembly exceed 0.65 mg/l, and in most instances was considerably less than this value.

TABLE 3

BOD OF ENCLOSED RESPIROMETER COMPONENTS
Experiment Started on January 30, 1964
Seed-1% Ann Arbor influent
Settled and gauze filtered

Sample	Initial D.O. mg/l	Final D.O. mg/l	D.O. Depletion in 5 days mg/l	BOD_5 with seed correction mg/l
Dilution Water Control				
A	9.2	9.0		
B	9.2	9.0		
Ave.	9.2	9.0	0.20	
Seed Control				
A	9.2	6.85		
B	9.2	6.90		
Ave.	9.2	6.88	2.32	
Test Assembly				
A (non-mixed)	9.2	6.30	2.90	0.58
B (non-mixed)	9.2	6.45	2.75	0.43
C (mixed)	9.0	6.10	2.90	0.58

Inasmuch as there appears to be a slight BOD contribution from the enclosed components, it is desirable for each investigator who might use this assembly to satisfy himself as to the

exact magnitude of this contribution for his assembly, by running one or two experiments to obtain data similar to that presented in Table 3.

Stability of Electrode Response During Incubation

One of the important considerations in using the galvanic cell oxygen analyzer as the sensing unit in the respirometer assembly is the stability of the electrode response during the normal incubation period. Mancy, Westgarth and Okun[5] show the long term sensitivity over an 80-day period, with good relative stability after the first 10 days. To evaluate the electrode stability in a typical respirometer assembly as illustrated in Figure 6a, an experiment was conducted for a normal 5-day incubation period with daily calibration of the electrode. This was accomplished by removing the electrode daily from the assembly and placing it in a sample of similar substrate, i.e., Huron River water which had been brought close to saturation by aeration using a laboratory compressed air source and then, of course, returning it to the assembly after taking a reading. Thus, each day one of the high points of the calibration curve was determined. The results of this experiment are presented in Table 4 as follows.

It is apparent from inspection of the data of Table 4 that a decrease in sensitivity resulted with time, i.e., there was less current output for the same D.O. level meaning that each recorder unit represented a greater amount of D.O. While there are reversals of this trend on two days, the trend itself is

quite apparent. All of the experiments using the respirometer assembly for a 5-day period showed the same decrease in sensitivity even though it was only possible to make a sensitivity check at the beginning and end of the five days.

TABLE 4

DAILY CALIBRATION OF GALVANIC CELL OXYGEN ELECTRODE IN RESPIROMETER ASSEMBLY

Experiment started May 6, 1964 using aerated Huron River water

Date	Time	D.O. in mg/l (Winkler)	Recorder Units	D.O. in mg/l per Recorder Unit
May 6	4:00 P.M.	8.3	90	.0922
May 7	4:20 P.M.	8.5	84	.1012
May 8	4:30 P.M.	8.0	83	.0964
May 9	3:00 P.M.	8.2	83	.0988
May 10	4:30 P.M.	8.3	71	.1169
May 11	4:00 P.M.	8.5	75	.1133

Under routine use, it generally is not possible to make sensitivity checks each day but certainly they should be made at least every five days. It has been the practice of this laboratory to make such a check at the beginning and end of each 5-day incubation period, and then to calculate a daily sensitivity adjustment based on a linear interpolation.

Also, where full range dissolved oxygen calibration curves were run from D.O. saturation to zero D.O., relating current output to D.O. concentration in mg/l, they were always found to be linear as suggested by Mancy, Westgarth and Okun.[5]

Summary

A respirometer assembly for BOD measurement of river waters and biologically treated effluents has been described. This section of the report started out with a consideration of some recent efforts at improved BOD instrumentation, followed by a detailed discussion of the respirometer assembly, including the assembly description, suggested general operating procedures, the precision of the oxygen electrode system, mixing and velocity considerations, the BOD of the enclosed respirometer components, and finally stability of electrode response during incubation.

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SECTION IV

APPLICATION OF RESPIROMETER ASSEMBLY FOR BOD MEASUREMENT

To demonstrate the application of the respirometer assembly to the measurement of BOD of treated effluents and river waters, several runs were made with these types of substrate. Two of the runs were selected for presentation in this section of the report, the first, consisting of an effluent sample from the Ann Arbor waste water treatment plant and, the second, consisting of a mixture of Huron River water and Ann Arbor waste water treatment plant effluent. In addition to the respirometer assembly results, data collected from several controls which were incorporated in the experiments will be presented allowing a comparison of the respirometer assembly results with the more conventional methods of determining BOD.

Effluent Sample from Ann Arbor Waste Treatment Plant

The respirometer assembly was used for BOD measurement of a grab sample of the effluent of the Ann Arbor waste water treatment plant collected on November 16, 1963. This particular plant is of the activated sludge type, and is well operated with a low effluent BOD. In addition to the part of the sample placed in the respirometer assembly, a parallel run was made on a second part using the jug reaeration procedure. Thus, it became possible to make comparisons between the results

obtained on the same test solution using different procedures. Moreover, the three-electrode respirometer cover was used as illustrated in Figure 6b connected to three separate strip chart recorders, making it possible not only to compare different methods of running BOD rates but, also, to further evaluate the precision of the oxygen electrode system with an effluent substrate.

The results of this experiment are presented in Figure 10 where it is seen that there is good agreement among the results obtained using the three different electrode systems, and also, good agreement between the results using the respirometer assembly and the jug reaeration procedure in the early time periods. As the time progressed, however, it is seen that the reaeration procedure gave results lower than the respirometer assembly possibly due to the influence of mixing. It is interesting to observe that both curves generally follow the monomolecular type of reaction characteristic of a mixed type of waste, in contrast to the S type curve illustrated in Figure 7 for a soluble substrate of glucose and glutamic acid.

One type of analysis performed on the data consisted of the use of the "moments" method of Moore, et al.[1] for the evaluation of the deoxygenation constant k_1 and, also, the ultimate BOD value L assuming, of course, that the monomolecular reaction applies. In the use of this method only single daily BOD values are needed, and where these values were not observed as such, interpolated figures were used. The results of this analysis are presented in Table 5 as follows.

Figure 10

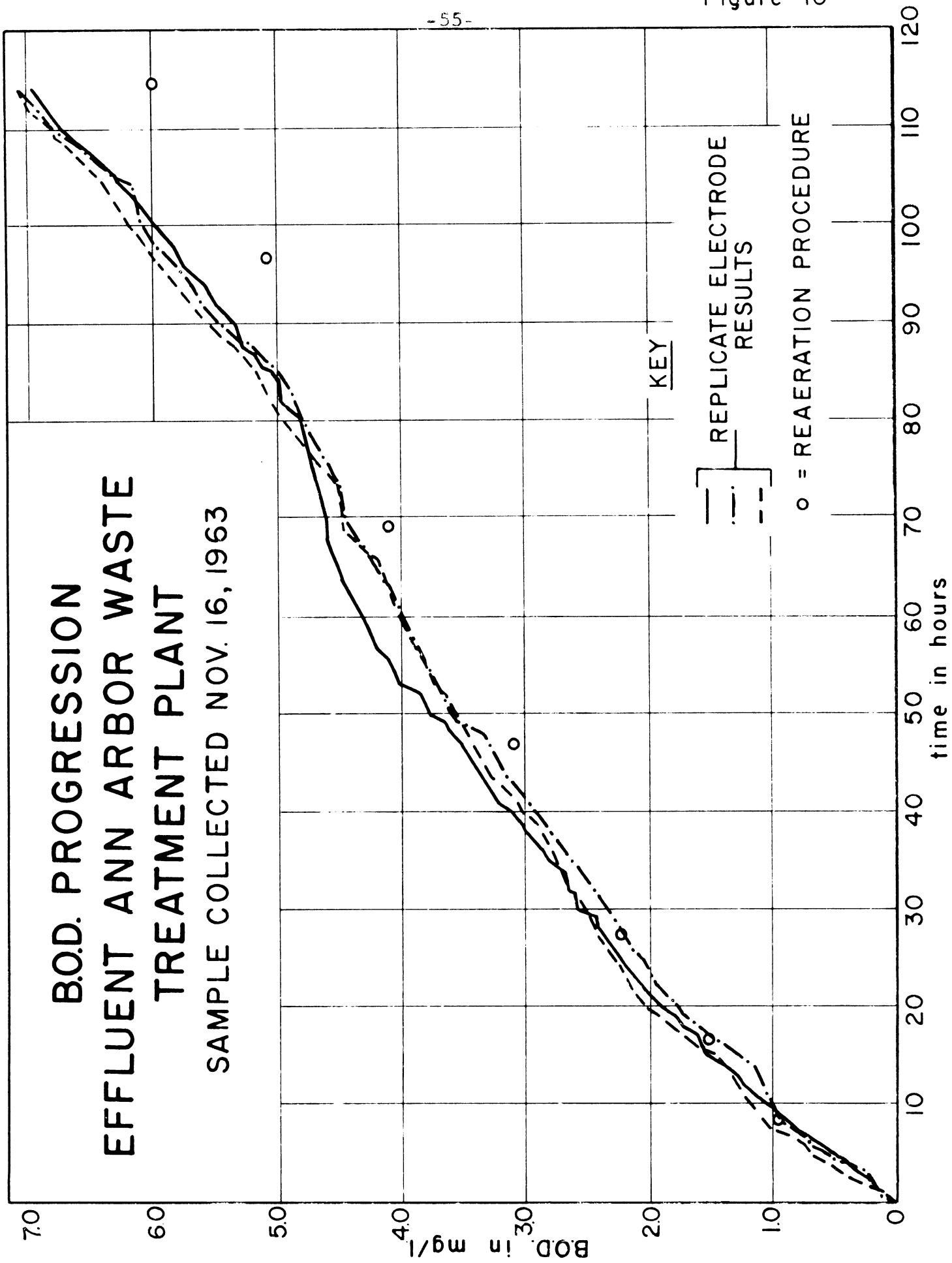

B.O.D. PROGRESSION
EFFLUENT ANN ARBOR WASTE
TREATMENT PLANT
SAMPLE COLLECTED NOV. 16, 1963
KEY
REPLICATE ELECTRODE RESULTS
o = REAERATION PROCEDURE
B.O.D. in mg/l
time in hours
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
10
20
30
40
50
60
70
80
90
100
110
120

TABLE 5

BOD PROGRESSION CONSTANTS k_1 AND L, ANN ARBOR EFFLUENT SAMPLE COLLECTED NOV. 16, 1963

Method	k_1	L in mg/l
Respirometer Assembly Average of three electrodes	.07	15.3
Jug Reaeration Method	.083	11.9

It is apparent that in both cases the calculated k_1 was less than the accepted value of $k_1 = 0.1$, but certainly much higher than the k_1 effluent values of the order of .01 to .04 generally found by the writer[2] for the Pontiac, Michigan activated sludge plant, or the Dow Chemical Company activated sludge plant at Midland, Michigan. Inasmuch as this sample represented only a grab sample on one day, it would be dangerous to assume that these k_1 values would be constant for other times.

Mixture of Huron River Water and Ann Arbor Waste Treatment Plant Effluent

A second application of the respirometer assembly for BOD measurement was made on a sample consisting of a mixture of 50 per cent Huron River water and 50 per cent Ann Arbor waste water treatment plant effluent collected on June 3, 1964. The basic respirometer assembly as illustrated in Figure 6a was prepared in duplicate with equal parts of the original mixture, thus, allowing an opportunity to make comparisons of the

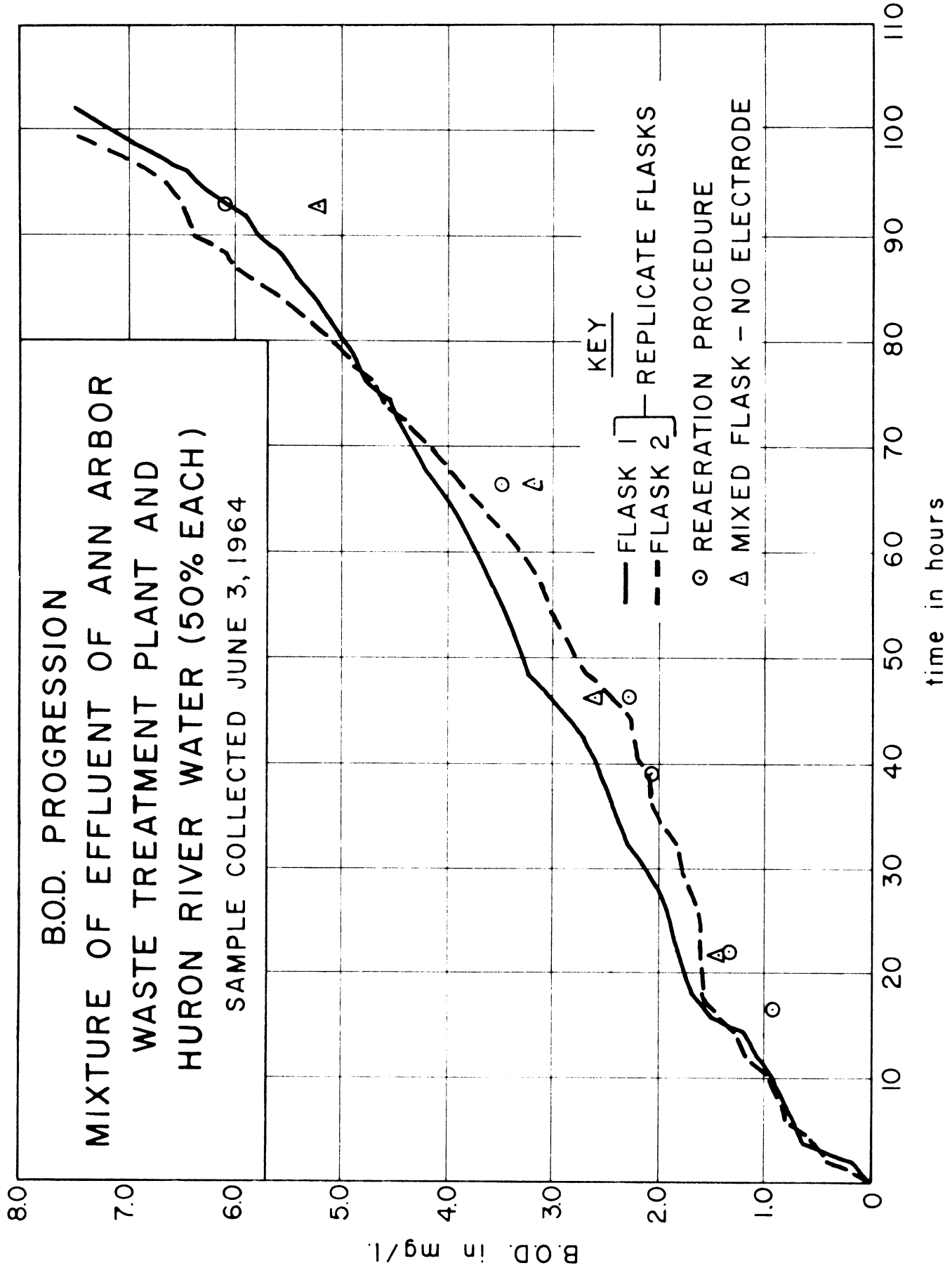
results using similar independent assemblies on the same substrate. Also, for the purposes of comparison five reaction flasks were filled with the original mixture and incubated under continuous mixing but with no electrode present. These flasks, therefore, were the same as the respirometer assembly in all respects except for the absence of a galvanic cell oxygen electrode and the associated strip chart recorder.

Each day one flask was removed and the residual dissolved oxygen present was determined by the Winkler method. In addition to these two methods of handling the sample, a parallel run was made on a third part using the non-mixed jug reaeration procedure. Thus, it became possible to make comparisons between the results on the same test solution using three different procedures, with the normal respirometer assembly run in duplicate.

The outcome of this experiment is presented in Figure 11 where it is seen there is a reasonable agreement among the results using the three methods of handling the original mixture. No data beyond the first 100 hours of incubation were used because of an accidental change in the resistance of one of the recorders, thereby influencing the comparability of the data. Also, it appears that after about 75 hours the sample is entering the nitrification stage of the BOD reaction involving a period of more rapid oxygen utilization.

It is interesting to note that good agreement is observed between the data obtained using the duplicate respirometer assemblies, suggesting that the method is reproducible. Also,

Figure 11


B.O.D. PROGRESSION
MIXTURE OF EFFLUENT OF ANN ARBOR
WASTE TREATMENT PLANT AND
HURON RIVER WATER (50% EACH)
SAMPLE COLLECTED JUNE 3, 1964
KEY
FLASK 1
FLASK 2
REPLICATE FLASKS
⊙ REAERATION PROCEDURE
Δ MIXED FLASK – NO ELECTRODE
B.O.D. in mg/l.
time in hours
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
10
20
30
40
50
60
70
80
90
100
110

it appears that for this particular sample, mixing does not seem to exert a significant influence on the results, where the non-mixed reaeration data is similar to the respirometer assembly data.

In addition to the illustrations presented in this and the preceding section of this report, several other runs have been made on effluent and river water samples. As previously mentioned, one of the distinct benefits of the respirometer assembly over the conventional bottle experiments is the ability to measure on a continuous basis the dissolved oxygen level in the assembly with time, with a minimal amount of effort by laboratory personnel. Thus, a considerable amount of additional data can be collected with the same laboratory staff. On the other hand, it must be appreciated that this is a new method of measuring BOD, and it is highly desirable at this stage of development of the procedure to run either the jug reaeration or the standard dilution method in parallel with the respirometer assembly, so that relationships between the old and new methods can be established.

Summary

This section of the report has presented two applications of the respirometer assembly for BOD measurement, the first, consisting of an effluent sample from the Ann Arbor waste water treatment plant and, the second, consisting of a mixture of Huron River water and Ann Arbor waste water treatment plant effluent. In both cases, several controls were incorporated in the experiments allowing a comparison of the respirometer assembly results with the more conventional methods of determining BOD. It has been demonstrated that the respirometer assembly can be effectively used for obtaining a continuous measure of BOD with time; however, at this stage of development of the apparatus it is desirable to run parallel controls consisting of the more conventional bottle methods so that relationships between the old and new methods can be established.

References

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2. Gannon, John J., "River BOD Abnormalities." Final Report, U.S.P.H.S., project WP 187, Office of Research Administration, The University of Michigan, 270 pp. (November, 1963).

SECTION V

ACKNOWLEDGEMENTS

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Mr. Jackson R. Pelton, an assistant in research, participated in the project on a part-time basis while supported first by U.S.P.H.S. project WP 187 - River BOD Abnormalities, and at the present time by U.S.P.H.S. project WP 91 - Biological Extraction and Accumulation in Stream Self-Purification.

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APPENDIX A

SUGGESTED PROCEDURE FOR USING RESPIROMETER ASSEMBLY

by

Jackson R. Pelton

1. Apparatus and Materials Required

   1.1 Reaction flask, complete with 4-port cover and cover clamp -- 2-liter capacity (center port = No. 32 S, outside ports = No. 24 S).

   1.2 Galvanic cell oxygen analyzer, with top of probe having a S No. 32 to fit middle port.

   1.3 Ground glass stoppers S No. 24--to fit in three ports.

   1.4 Magnetic stirrer and a teflon covered magnetic stirring bar 1½ inches in length.

   1.5 1/16 inch thick silastic gaskets (may be necessary to minimize leakage from flask).

   1.6 0.5 ml thick irradiated polyethylene membrane material (Cryovac--available from the Cryovac Division, W.R. Grace & Co., Cambridge 40, Mass.).

   1.7 Ultrasensitive millivolt recorder-amplifier having the following specifications:

   - Voltage ranges: 1 mv, 5 mv, 10 mv, 100 mv, 1 V (input resistance, 1 megohm).
   - Current ranges: 0-1 μa, 1 μa.
   - Strip chart recorder with a chart speed of 1 inch

per hour equipped with a 2-watt type AB potentiometer having a resistance of 100 ohms.

2. Reagents Required

2.1 Electrolyte solution in a methylcellulose carrier. 1 M KOH solution containing 1 per cent methylcellulose (Dow Methylcellulose, powdered, technical grade, Dow No. 28718-"Methocel"). Follow the manufacturers directions in the preparation of the methylcellulose solution.

3. Preparation

3.1 Reaction flask and cover, magnetic stirring bar, glass stoppers and electrode are thoroughly cleaned by using a brush and household cleanser. It is especially important that all lead oxide is removed from the lead anode. All components are thoroughly rinsed with tap water, followed by a rinse with distilled or deionized water.

3.2 1-2 drops of fresh electrolyte solution is placed on the GCOA sensing surface and an approximately four square inch piece of membrane material is stretched tightly over the GCOA sensing surface and secured with a teflon retaining ring. Excess membrane material is trimmed off down to the retaining ring.

3.3 The GCOA is then placed in the reaction flask which has been filled with aerated, distilled or deionized water or preferably with the substrate that is going

to be used in the test run. This flask is then placed in a 20°C incubator under continuous mixing conditions on a magnetic stirrer for a period of 24 to 48 hours in order for the electrode to stabilize.

4. Procedure

4.1 A clean reaction flask and the stabilized electrode are rinsed with the substrate to be used in the test run and the flask is then filled with the aerated substrate, making sure that all air bubbles have been displaced and that the flask cover is securely clamped onto the flask.

4.2 The flask is then placed in a BOD incubator that has had a small hole drilled through the side to allow passage of the electric cord leading to the magnetic stirrer and, also, for the leads from the electrode to the recorder. A piece of asbestos is placed on the magnetic stirrer in order to minimize heat transfer to the flask. The temperature of the incubator is dropped several degrees below 20°C so that the solution in the flask is held at 20°C.

4.3 The millivolt recorder is set to operate on the 10 μa range. The electrode leads are attached to the proper terminal on the recorder and the continuous recording is started. The recorder striking bar is adjusted with the potentiometer so as to give a reading in the air saturated substrate of between 90 to 100 per cent

of full scale deflection. Prior to starting the test, the electrode is immersed in saturated sodium sulfite solution and the recorder striking bar is adjusted to read between 0 to 5 per cent by the zeroing adjustment on the recorder. Following this step, it is very important to thoroughly rinse off all traces of the sodium sulfite solution.

4.4 A dissolved oxygen determination is run on the air saturated substrate using the Alsterberg (azide) modification of the Winkler method in order to determine the actual oxygen concentration of the substrate under test in the reaction flask. This oxygen concentration, by this reference method, represents the oxygen concentration at the start of the continuous recording test.

4.5 The speed of rotation of the magneting stirring bar is set at 600 rpm by adjusting the magnetic stirrer reostat so that the motion of the stirring bar appears to be stopped when viewed during operation of a stroboscope light, that has been set to flash at a rate of 600 flashes per minute. This rotation rate is checked at intervals during the test period and adjustments are made, if necessary.

4.6 Temperature readings are taken periodically during the test run to make certain that the substrate is maintaining a constant temperature of 20°C.

4.7 If the oxygen concentration of the substrate drops to an estimated 2 mg/l, one of the ports is opened and compressed air is bubbled through the solution through a gas diffuser, until the oxygen is brought back up to near saturation. Following this step, it is usually necessary to add some additional substrate to the reaction flask.

4.8 At the completion of the test run, the calibration of the electrode is rechecked by the Winkler method following reaeration of the substrate.

4.9 The sum of the oxygen utilized during the test run may be determined in the following manner:

Calculate a dissolved oxygen factor using the high and low dissolved oxygen points from the calibration data. The following formula may be applied:

$$f = \frac{D.O.}{U}$$

where: D.O. = the dissolved oxygen concentration by the reference method

U = range in recorder units from the zero point to the high point on the strip chart

This factor represents the dissolved concentration in mg/l for each unit on the recorder strip chart.

It should be noted that it may be necessary to make adjustments in this D.O. factor if a change in

sensitivity of the electrode occurred during the test run. This can be detected by observing for any differences in the factor between the initial and final calibrations of the electrode. A change in sensitivity may be corrected by interpolating a total change in the factor on a daily basis. The BOD then is the sum of the dissolved oxygen depletion with time.