Introduction to
BIOCHEMICAL OXYGEN DEMAND

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I. INTRODUCTION TO BOD

WHAT IS BOD?

Biochemical Oxygen Demand (BOD) is the amount of oxygen, expressed in mg/L or parts per million (ppm), that bacteria take from water when they oxidize organic matter. The carbohydrates (cellulose, starch, sugars), proteins, petroleum hydrocarbons and other materials that comprise organic matter get into water from natural sources and from pollution. They may be dissolved, like sugar, or suspended as particulate matter, like solids in sewage.

Organic matter can be oxidized (combined with oxygen) by burning, by being digested in the bodies of animals and human beings, or by biochemical action of bacteria. Because organic matter always contains carbon and hydrogen, oxidation produces carbon dioxide (the oxygen combining with the carbon) and water (the oxygen combining with the hydrogen).

Bacteria in water live and multiply when organic matter is available for food and oxygen is available for oxidation. About one-third of the food bacteria consumed becomes the solid organic cell material of the organisms. The other two-thirds is oxidized to carbon dioxide and water by the biochemical action of the bacteria on the oxygen dissolved in the water. To determine BOD, the amount of oxygen the bacteria use is calculated by comparing the amount left at the end of five days with the amount known to be present at the beginning. At room temperature, the amount of oxygen dissolved in water is 8 mg/L. At freezing, it increases to 14.6 mg/L; it also increases at high barometric pressures (low altitudes). At the boiling point, the solubility of oxygen is zero.

During the five-day period of a BOD test, the bacteria oxidize mainly the soluble organic matter present in the water. Very little oxidation of the solid (insoluble) matter occurs in that short time.

WHAT IS THE SIGNIFICANCE OF BOD?

Measurement of BOD has long been the basic means for determining the degree of water pollution. It is the most important measurement made in the operation of a sewage treatment plant. By comparing the BOD of incoming sewage and the BOD of the effluent water leaving the plant, the efficiency and effectiveness of sewage treatment can be judged. For example, in a typical residential city raw sewage has a BOD value of around 300 mg/L. If the effluent from the sewage treatment plant has a BOD of about 30 mg/L, the plant has removed 90 percent of the BOD.

If water of a high BOD value flows into a river, the bacteria in the river will oxidize the organic matter, consuming oxygen from the river faster than it dissolves back in from the air. If this happens, fish will die from lack of oxygen, a consequence known as a fish kill.

Thus, sewage treatment plants must remove as much BOD as possible from the sewage water. To check sewage treatment effectiveness and to study and control organic-matter pollution, millions of BOD tests are performed.

HOW IS BOD MEASURED?

Two methods are widely used for BOD measurement. One method, the dilution method, is a standard method of the American Public Health Association (APHA) and is approved by the U.S. Environmental Protection Agency (USEPA). The other method, the manometric method, has been used for over 75 years in many sewage plants and other installations throughout the world. The USEPA denied approval of this method when it selected methods for wastewater analysis, although in certain cases the USEPA has approved the manometric method.

The dilution method is conducted by placing various incremental portions of the sample into bottles and filling the bottles with dilution water. The dilution water contains a known amount of dissolved oxygen. The dilution water contains a portion of inorganic nutrients and a pH buffer. The bottles are completely filled, freed of air bubbles, sealed and allowed to stand for five days at a controlled temperature of 20 °C (68 °F) in the dark. During this period, bacteria oxidize the organic matter using the dissolved oxygen present in the water. At the end of the five-day period, the remaining dissolved oxygen is measured. The relationship of oxygen that was consumed during the five days and the volume of the sample increment is then used to calculate the BOD.

Measurement of BOD by the manometric method is easier because the oxygen consumed is measured directly rather than with chemical analysis. Because the sample is usually tested in its original state (not diluted), its behavior more closely parallels that of the waste in an actual sewage treatment plant. As the oxygen in the sample is used up, more will dissolve into the water from the air space over it. The manometer measures the drop in air pressure in the bottle. This continuous indication of the amount of oxygen uptake by the sample is an important feature of the manometric method. By graphing the results, you can find the rate of oxygen uptake at any time and thereby gain considerable insight into the nature of the sample.

HOW RELIABLE IS THE BOD TEST?

The BOD test measures only the oxygen taken up by wastewater during the biological oxidation of organic matter present. Therefore, a BOD test is a highly unreliable means of determining the amount of organic matter present in water. The test measures only the approximate amount of oxygen that will be required (absorbed or consumed) by a polluted water when it is exposed to air or oxygen for an extended period of time.

Bacteria in the water oxidize organic matter. The growth of bacteria is highly temperamental and erratic and is affected by numerous factors, many of which are unknown or difficult to control. Also, the bacteria grow somewhat slowly, so that in a five-day period, at 20 °C,

the biological oxidation is never complete. It is approximately 80 percent complete in five days but is not 100 percent complete even after 20 days.

Many other factors also affect the tests. Toxic substances in the sample inhibit or even prevent bacterial growth and, therefore, oxidation of the organic matter. When this happens, the test result is lower than the actual amount of organic matter present would suggest.

II. DILUTION METHOD OF MEASUREMENT

INTRODUCTION

The format of the APHA BOD test (dilution method) as it is ordinarily arranged is complex and carrying out the tests requires a great deal of time. The usual approach also has several inherent weaknesses, which cause numerous operating troubles.

To simplify the procedure and save time without changing the standard test, it has been possible to rearrange the format of the system and to package reagents in sterile "pillows." Stable PAO (phenylarsine oxide) solution or stabilized thiosulfate also can be substituted for unstable thiosulfate used in the dissolved oxygen (DO) determination. These changes produce a straightforward system that requires less time and is more reliable. The Hach system compensates for the oxygen demand of seed (if used) and the erratic oxygen demand of the dilution water. This system eliminates the need for running an incubated five-day dilution water blank. It also allows the use of dilution water having an oxygen demand > 0.2 mg/L without losing accuracy of the BOD measurement of the unknown samples. Such a blank would cause a direct error in the traditional system. This has been a serious problem for many years.

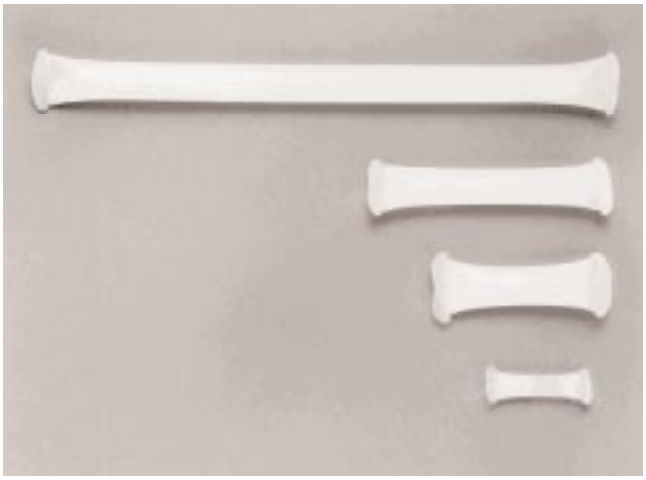
The Hach system uses an improved method of preparing the dilution water. The nutrient buffer is formulated into a slurry, packaged in "unit-dose" pillows and sterilized. The stability of the slurry in these pillows solves the problem of preserving the APHA stock phosphate buffer solution, which often develops biological growth when exposed to air.

In summary, the Hach system for the APHA BOD test uses the same reagents, sample volumes, test conditions, and principles of operation as the traditional approach. The format has merely been streamlined and made more reliable to improve accuracy and save time.

PROCEDURE FOR THE DILUTION METHOD BOD TEST

1. Select the required BOD Nutrient Buffer Pillow, shake, cut open, and add the contents to a jug containing the required amount of distilled water at 20 °C.

2. Cap the jug and shake vigorously for 1 minute to dissolve the slurry and to saturate the water with oxygen.



Slurry pillows for preparing BOD dilution water.

3. If the minimum sample volume is 3 mL or more, determine the dissolved oxygen in the undiluted sample. This step can be omitted when analyzing sewage and settled effluents known to have a dissolved oxygen content near zero.

4. With a serological pipet, measure a graduated series of at least four, but preferably five or six, portions of well-

mixed sample and transfer to BOD bottles. Stir the sample with the pipet before pipetting each sample.

5. Add two measures of Nitrification Inhibitor 0.61 liters (0.16 g) from the dispenser bottle to each BOD bottle.

6. Fill each BOD bottle to just below the lip with seeded or unseeded dilution solution. In order to prevent formation of bubbles, allow the water to flow slowly down the sides of the bottle.

7. Stopper the bottle, being careful not to trap any air bubbles. Press on the stopper of the bottle with a finger and then invert several times to mix.

8. Add enough dilution water to cover the lip of the BOD bottle to make a water seal.

9. Place a plastic cap over the lip of each BOD bottle and place in a dark incubator for five days at 20 °C ± 1 °C.

10. After the five-day incubation period is complete, determine the dissolved oxygen of each of the samples as described in the Dissolved Oxygen (DO) procedure in *Appendix II*.

11. Improved method for calculating BOD: Plot the mg/L DO remaining in each diluted sample versus the mL sample; then draw the best straight line through the plotted points. An erroneous point is visually evident at this time and can be disregarded. At least three points should lie on or very close to the line. The line should cross the mg/L oxygen scale near or below the oxygen saturation value for the altitude.*

*At sea level with a normal barometric pressure, water will dissolve 9.2 mg/L oxygen at 20 °C. The following table gives the solubility at various altitudes at 20 °C.

Table 1. How altitude influences oxygen saturation.

Altitude		Oxygen Saturation
(meters)	*(feet)*	
Sea Level	Sea Level	9.2 mg/L
305	1000	8.9
610	2000	8.6
914	3000	8.2
1219	4000	7.9
1524	5000	7.6
1829	6000	7.4
2134	7000	7.1
2438	8000	6.8
2743	9000	6.5
3048	10,000	6.3

Table 1 shows that at higher elevations less oxygen will dissolve in water because of the lower barometric pressure. This limits the amount of available oxygen at higher elevations and thus limits the BOD range of a particular sample increment taken for analysis.

To calculate the BOD, use the following equation, which is mathematically derived (as shown in *Appendix I*) from the BOD equation in *Standard Methods for the Examination of Water and Wastewater (Standard Methods)*.

mg/L BOD = (slope x 300) – Y intercept = undiluted sample DO

mg/L BOD = (a x b) – c = d

a. The slope of the line is equal to the mg/L DO consumed per mL of sample taken. At any point on the line, subtract the mg/L DO remaining there from the mg/L DO where the line crosses the DO scale. Divide the difference by the mL of sample at the point chosen. This is the slope.

b. The value 300 is the volume of the BOD bottle.

c. The "Y intercept" is the DO value where the line crosses the DO scale.

d. The sample DO is the DO of the undiluted sample.

Preparing Dilution Water

Organic Free Water

APHA *Standard Methods* stresses the necessity of using high quality water in preparing BOD dilution water. The quality of dilution water is checked and a dilution water blank is included in the method. The 17th edition of *Standard Methods* does not specify distilled water. However, the DO uptake in five days at 20 °C should not exceed 0.2 mg/L. Water not meeting this criteria should not be used.

Experience has shown that demineralized water, particularly from a new demineralizer with new resin, often contains a substantial amount of organic matter that is released intermittently and is undetectable with a conductivity water-purity gauge. Also, the large surface-to-volume ratio that exists in the columns because of the resin beads encourages bacterial growth in the column.

Years of experience have proven that much of the trouble with BOD testing is caused by excessive organic matter in the water used to prepare the BOD dilution water. It has even happened that the prepared BOD dilution water itself has had an oxygen demand in excess of the dissolved oxygen present in it, resulting in total depletion in all incubated samples. It is desirable to have a near-zero oxygen demand in the dilution water.

The most practical way to consistently produce water of low organic content is by distillation with alkaline permanganate. Commercial stills can be set up to produce high quality distilled water automatically. When a still is fed with chlorinated water, some chlorine may distill over with the water. If this occurs, the chlorine must be destroyed by following the thiosulfate procedure given in the section on "Pretreating the Sample" (page 7).

Distilled Water Storage

Distilled water coming from the still is usually warm and not saturated with oxygen. The temperature of the BOD dilution water must be 20 °C at the time of use and at or near saturation with oxygen. It is recommended that the distilled water be stored in the BOD incubator until it reaches 20 °C, and the dilution water be prepared immediately before use. The distilled water can be placed in 3.8-liters (1-gallon) jugs filled to the 3.0-liter (0.8 gallons) mark or in 7.6-liters (2-gallon) jugs filled to the 6.0-liter (1.6 gallon) mark. The jugs are then capped and placed in the incubator for storage. After a day or more, the temperature will be 20 °C and the water will be at or near saturation with oxygen from the air above the water in the jugs. To saturate the water in 19-liters (5-gallon) containers, a source of filtered compressed air or an aquarium pump is required.

Preparing Dilution Water with APHA Solutions

In the APHA procedure for the preparation of BOD dilution water, 1 mL each of four different solutions is added to 1 liter (0.3 gallons) of the organic-free, oxygen-saturated water. One of the solutions is a phosphate buffer of pH 7.2. Experience at Hach indicates that if the buffer is sealed in a liter or 500-mL stock bottle and steam sterilized, it will keep. After the bottle is opened, a mold will invariably develop in the solution. This growth has been found to produce oxygen demand, which is undesirable. Mold growth is difficult to prevent, although refrigerating the phosphate buffer stock solution helps. Mold contamination has been a constant source of trouble to analysts.

Preparing Dilution Water with Concentrated Slurry Pillows

Hach has combined the APHA-specified buffer and nutrients into a slurry and packaged it in sterile pillows of 0.5, 3, 6 and 19 mL each. One mL of this slurry is added to each liter of distilled water to produce APHA BOD dilution water. A 0.5-mL pillow will prepare 300 mL (0.08 gallons) of dilution water when added to 300 mL (0.08 gallons) of distilled water in a BOD bottle. A 3-mL pillow will prepare 3 liters (0.8 gallons) of dilution water when it is added to 3 liters (0.8 gallons) of distilled water. The 6-mL pillow will prepare 6 liters (1.6 gallons) of dilution water when it is added to 6 liters (1.6 gallons) of distilled water. And the 19-mL pillow will prepare 19 liters (5 gallons) of dilution water when it is added to 19 liters (5 gallons) of distilled water..

To prepare the dilution water, first remove the jug of distilled water from the BOD incubator when it has reached 20 °C. Select the required pillow, shake, cut open, and add the contents to the jug. Then cap the jug and shake it vigorously for 1 minute to dissolve the slurry and to saturate the water with oxygen. This BOD dilution water is ready for immediate use in analyzing raw sewage, unchlorinated sewage plant effluent, and river and surface waters, all of which contain a high population of bacteria capable of oxidizing organic matter. The dilution water should not be prepared more than eight hours before use, because biological growth will develop.

Because the pillows of slurry are sterilized at the time of manufacture, they have an unlimited shelf life without need of refrigeration or fear of contamination. They are reliable, economical and convenient, and the resulting dilution water meets APHA specifications.

Seeding Dilution Water

Using seeded dilution water is not necessary when measuring the BOD of sewage, sewage plant effluent (unless it has been chlorinated) or river water. However, some samples do not contain sufficient bacteria to oxidize any organic matter that may be present. Many industrial or trade wastes are of this type. Also, many sewage treatment plants chlorinate their final effluent so that it is essentially sterile and thus impossible to test directly for BOD.

In order to test such samples, it is necessary to seed the prepared BOD dilution water. This is done by adding a small measured volume of water known to contain a good bacterial population to the dilution water that is used to prepare the samples. Polyseed® eliminates the problem of finding seed that contains sufficient bacterial populations for oxidizing biodegradable organic matter. Polyseed provides a consistent BOD seed source free of nitrifying bacteria and can be used with either the dilution method or the BODTrak method (see page 13). For more information, request literature number **1412.**

Perhaps the most common source of seed is simply raw sewage. Raw sewage used for seeding is stored at 20 °C for 24 to 36 hours before use. When using domestic sewage as the seed, it is recommended that it be allowed to stand undisturbed until most solids settle. Pipet from the upper portion of the bottle of seed material. The addition of 3.0 mL of raw domestic sewage seed to each liter of dilution water is ample. Do not add more than this. Seed that has a BOD of 200 ppm (a typical range for domestic sewage), when added at the rate of 3 mL per liter of dilution water, will deplete 0.6 ppm DO.

Seed Acclimatization

If the waste sample to be tested contains materials not readily biodegradable, or if it contains toxic materials such as phenol, formaldehyde or similar microbic inhibitory agents, an acclimated seed must be used. The acclimatization process can usually be carried out quite easily in any nonmetal 3.8-liter (1-gallon) container fitted with an aeration system. Domestic sewage is first aerated for approximately 24 hours after which the heavier material is allowed to settle. After one hour, two-thirds of the volume is siphoned from the surface and discarded. The container is then refilled to the original

level with domestic sewage containing 10 percent of the waste material in question. The sludge-activating unit is again aerated for 24 hours and the procedure is repeated, but the amount of waste material is increased by an additional 10 percent each time until the fill process is 100 percent waste material.

Often, a particular seed can be acclimated to a waste material more easily than the preceding instructions indicate, especially if the original seed is taken from a stream where the waste is present. In this case the procedure can be modified and the acclimatization time reduced. If normal acclimating processes prove ineffective, a specific waste culture should be included in the conditioning process.

The graphical method Hach has developed for BOD tests is suitable when seeded dilution water is used. The procedure automatically compensates for the oxygen demand of the seed. This method will be described later in this booklet.

Nitrification

During incubation of the samples, particularly during the later stages, oxygen can be consumed by the bacterial oxidation of nitrogen compounds to nitrite and nitrate. It is a matter of opinion whether this is desirable or undesirable. Many authorities wish to restrict BOD to carbonaceous oxygen demand only and not include nitrogen oxygen demand.

Nitrification Inhibitor does not affect the biological oxidation of carbonaceous matter. This compound is available in a ready-to-use bottle with a dispenser cap. Two measures of Nitrification Inhibitor should be added to each BOD bottle before filling it with dilution water. This material is now widely used, but the use of nitrification suppression must be clearly stated when reporting results.

Selecting the Proper Sample Volumes

Use a series of five or six dilutions prepared from graduated volumes of sample, at least three of which deplete 20 to 90 percent of the initial dissolved oxygen in the prepared dilutions. The volumes of the sample increments to be taken depend upon the BOD value of the sample. Sample sizes should be chosen so that at least 2 mg/L dissolved oxygen is consumed and 1 mg/L dissolved oxygen remains at the end of the five-day incubation period.

Table 2 is helpful in selecting the appropriate sample volume to meet these criteria.

The minimum BOD determinable is based upon a 2.0 mg/L dissolved oxygen depletion and is the same at all altitudes. The maximum BOD determinable varies with altitude (since the amount of oxygen in water varies with altitude) and is based upon depletion of all except a residual 1.0 mg/L dissolved oxygen. To use the sample size selection table, first select the probable BOD from the Sample Type and Estimated BOD columns. Then determine the minimum and maximum sample volumes for that BOD. For example, raw domestic sewage commonly runs about 300 mg/L BOD. The minimum sample volume corresponding to a BOD of 300 mg/L is 2.0 mL and the maximum sample volume for that concentration analyzed at sea level is 8 mL. This maximum sample volume will permit a maximum of 304 mg/L BOD (the value in the table closest to 300) to be analyzed. Now choose three additional sample volumes between the minimum and maximum sizes. Thus a series of five samples could be set up with increments of 2, 3.5, 5, 6.5 and 8 mL.

Table 2. Determining Sample Sizes

A. Minimum Sample Size			B. Maximum Sample Size			
			Estimated BOD (mg/L) at:			Sample Size(mL)*
Sample Type	Est. BOD (mg/L)	Sample Size (mL)*	Sea Level	305 m (1000')	1524 m (5000')	
Strong Trade Waste	600	**1**	2460	2380	2032	**1**
	300	**2**	1230	1189	1016	**2**
	200	**3**	820	793	677	**3**
Raw and	150	**4**	615	595	508	**4**
Settled	120	**5**	492	476	406	**5**
Sewage	100	**6**	410	397	339	**6**
	75	**8**	304	294	251	**8**
	60	**10**	246	238	203	**10**
	50	**12**	205	198	169	**12**
	40	**15**	164	158	135	**15**
Oxidized	30	**20**	123	119	101	**20**
Effluents	20	**30**	82	79	68	**30**
	10	**60**	41	40	34	**60**
Polluted	6	**100**	25	24	21	**100**
River	4	**200**	12	12	10	**200**
Waters	2	**300**	8	8	7	**300**

*mL of sample taken and diluted to 300 mL in standard BOD bottle.

To further illustrate the principles for selecting the appropriate sample volumes, take the case where sewage plant effluent is to be analyzed. The general BOD range of effluent is from 10 to 50 mg/L. Assume the laboratory is at an elevation of 1524 m (5000 ft.). From the table, the smallest sample volume permitted for 50 mg/L BOD is 12 mL. The largest volume that can be accommodated at an altitude of 1524 m (5000 ft) would be 30 mL, which will permit a maximum of 68 mg/L BOD to be analyzed. A series of 12, 16, 20, 25 and 30 mL sample volumes can be taken.

Pretreating the Sample

1. Add 10 mL of 0.020N Sulfuric Acid Standard Solution and 10 mL of 100 g/L Potassium Iodide Solution to a 100-mL portion of the sample in a 250-mL Erlenmeyer flask.

2. Add 3 droppers of Starch Indicator Solution and swirl to mix.

3. Titrate from dark blue to colorless with 0.025N Sodium Thiosulfate Standard Solution. (Do not use stabilized Sodium Thiosulfate solutions for dechlorination. To do so could inhibit biological reactions.)

4. Calculate the amount of Sodium Thiosulfate Standard Solution necessary to dechlorinate the remaining sample.

$$\text{mL 0.025N sodium thiosulfate} = \frac{\text{mL used x mL to dechlorinate}}{100}$$

5. Add the required amount of 0.025N Sodium Thiosulfate Standard Solution to the sample and mix thoroughly. Allow the sample to stand for 10 to 20 minutes before running the BOD test.

Chlorine in low concentration may be dissipated by allowing the sample to stand for one to two hours at room temperature. If the chlorine concentration is high, it must be determined and an appropriate quantity of sodium thiosulfate added to destroy the chlorine. Follow Steps 4 and 5 (above), using Sodium Thiosulfate Standard Solution.

Some samples require special consideration and handling. The effluents of many industries and chlorinated sewage effluent are among the samples that must use special analytical techniques. Experimentation with the specific sample usually indicates when you must modify the routine BOD test procedure to establish reliable results.

Poisons or Toxic Materials, Including Chlorine

The presence of toxic materials in the sample will result in a diminished BOD value. Consequently, they must either be removed or their effects eliminated by diluting the sample.

The presence of other toxic materials, such as phenols, heavy metals and cyanide, must be considered. The actual concentration of these materials may be determined by methods described in Hach's *Water Analysis Handbook* (request literature number **8353**). The effect of these materials may be eliminated by diluting sample with distilled water.

Effect of pH

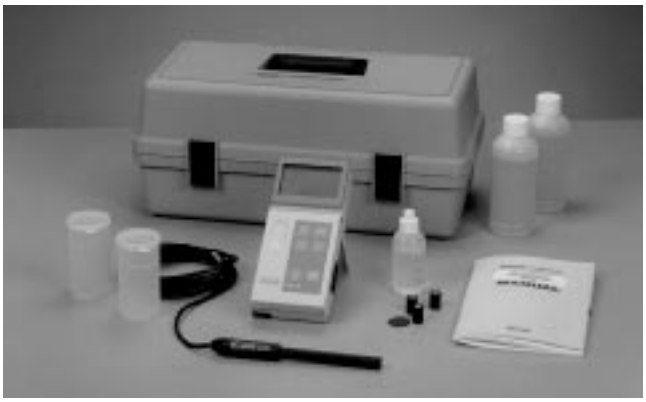
Whenever the pH of a waste material to be tested is exceedingly high or low, it should be adjusted to near neutrality. Optimum pH for biochemical oxidation is 6.5 to 8.0. Any appreciable deviation from this range tends to yield a low five-day BOD. Samples containing caustic alkalinity or acidity can be adjusted to neutrality by using 1N (or weaker) sulfuric acid or sodium hydroxide, respectively. In some industrial wastes (if an acidic or basic oxidation product is expected or extremely high dilutions are performed), dilutions should be made using a pH 7.2 phosphate-type buffer solution.

Supersaturation

Cold samples containing more than 9 mg/L dissolved oxygen (supersaturated) must be reduced to saturation by bringing the sample temperature to about 20 °C. Partly fill a bottle with sample and shake vigorously for 2 minutes or aerate with filtered compressed air for 2 hours. The solubility of oxygen in pure water at various temperatures and pressures is given in *Appendix III*.

Determining Dissolved Oxygen

The two main methods used to determine the residual DO in a series of BOD dilutions are instrumental and iodometric. The membrane electrode method using a DO175 Dissolved Oxygen Meter is the simplest. A DO Probe and BOD Accessory Kit, specially designed for use with BOD bottles, are available. No reagents are needed and most interferences experienced with other methods have little effect on the instrumental method. The azide modification of the Winkler Method is the most commonly used iodometric method. DO present in the samples oxidizes divalent manganese under alkaline conditions. With acidification, the manganese will, in turn, oxidize iodide to iodine in an amount equivalent to the original oxygen content of the sample. The iodine is titrated with thiosulfate or phenylarsine oxide. The end point is detected visually by using starch.



For fast and efficient dissolved oxygen measurements, choose the DO175 Meter with DO probe and BOD Accessory Kit.

Calculating BOD

It can be shown *(Appendix I)* that the APHA formula for calculating BOD can be rewritten as follows:

$$\text{mg/L BOD} = \left(\frac{\text{mg/L DO with smaller sample volume} - (\text{mg/L DO with larger sample volume} \times 300)}{\text{mL of larger sample volume} - \text{mL of smaller sample volume}} \right) - DO_D = S$$

DO_D and S are the Dissolved Oxygen Values of the dilution water and sample, respectively, and the value 300 represents the volume of the standard BOD bottle. If a set of BOD dilutions is run correctly with a homogeneous sample, and a graph of the mg/L DO remaining vs. the mL sample volume is plotted, the

points will fall exactly on a straight line. Thus the term in parentheses in the equation would equal the slope (i.e., the change in dissolved oxygen with respect to the change in sample volume, expressed as a positive number) and the dissolved oxygen of the dilution water would be the Y intercept. Or, in terms of the graph,

mg/L BOD = (slope x 300) - Y intercept = sample DO

For example, on a series of four dilutions of domestic sewage, the dissolved oxygen in each BOD bottle was determined after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn, see Figure 1. The point where the line intersects the Y axis indicates the DO content of the dilution water after incubation, although this was not actually measured. In this case, it was equal to 9.0 mg/L. The DO of the sample, domestic sewage, was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or potentiometrically.

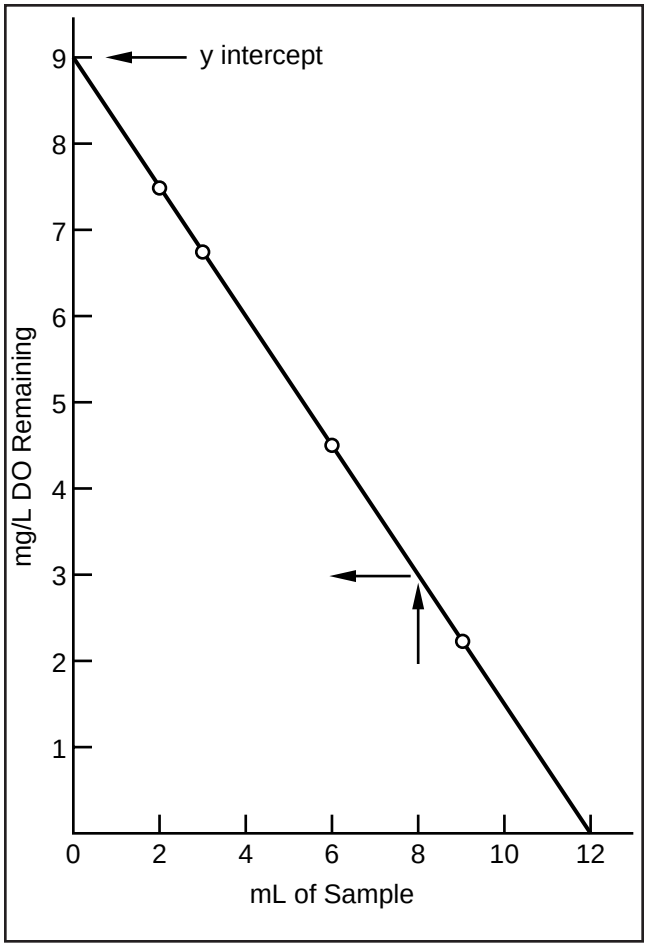


Figure 1

In our example, at 0 mL of sample the DO remaining equals 9.0 mg/L and at 8 mL of sample the DO remaining equals 3.0 mg/L. So, 8 mL of sample (8 - 0 = 8) would have consumed 6.0 mg/L DO (9 - 3 = 6) and so the slope would equal 6/8 or 0.75 and:

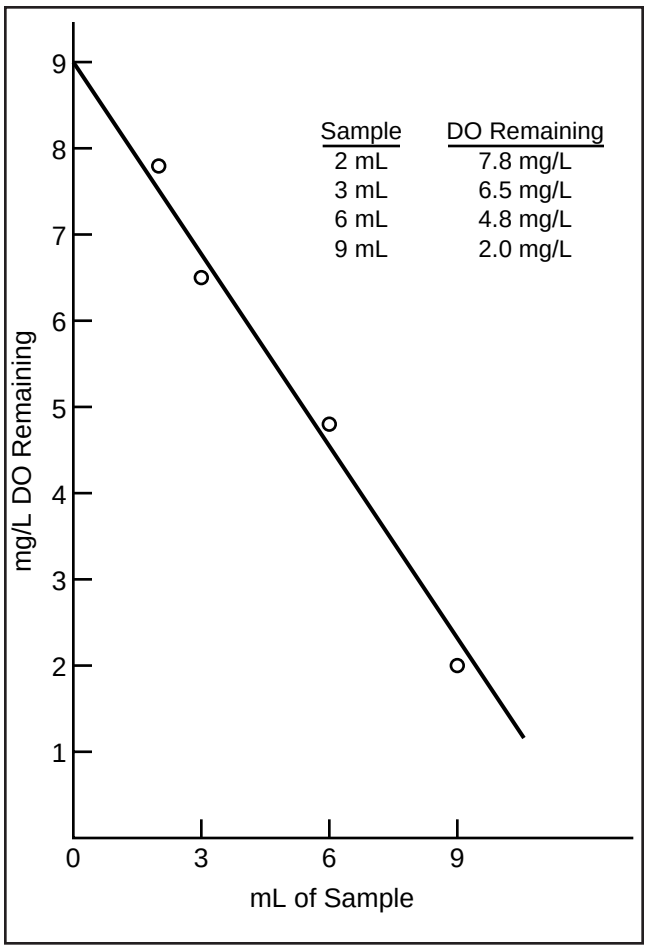


Figure 2

mg/L BOD = (slope x 300) - Y intercept + sample DO
BOD = (0.75 x 300) - 9 + 0
= 225 - 9
= 216 mg/L

However, the results found in "the real world" are not so perfect. They may not all fall on a straight line, and the BOD found by using the usual APHA equation will vary depending on which dilutions are used. Furthermore, some oxygen demand may exist in the dilution water and will not show up until the rest of the results are also complete. In other cases, some seed material might need to be added, which would increase demand and with the ordinary calculation method, would necessitate an extra series of dilutions on the seed. Instances like these demonstrate the real value of graphing the results.

For example, the sewage tested in Figure 1 might have given the results shown in Figure 2. Since there are no blanks in this case, drawing the best straight line through the points yields one BOD value, which is close to the average of the answers that would have resulted from

use of an equation. Using the results in Figure 2 and an APHA equation, the BODs found for the 3, 6 and 9-mL dilutions were 241, 201 and 224 mg/L respectively, with an average of 222.* But by using the graphical method a BOD of 216 mg/L is found—one value from one calculation. This value inspires greater confidence because it uses all the dilutions in a manner similar to that of standard additions, the best way to prove accuracy.

If there are three points on a straight line, use them instead of drawing the best straight line. If one dilution gives a result that is quite different from the general line of the others, it should be discarded. If three or more results fall on a straight line, they all can be used, even if one does not deplete more than 2 mg/L oxygen or leave more than 1 mg/L (see Figure 1).

Other information about the sample will become apparent when the results from five or six dilutions are graphed. For example, a graph in which the values of remaining DO go down and then flatten out or curve back up might indicate the presence of a toxic substance.

Compensating for Oxygen Demand from Dilution Water and/or Seed

Let us suppose, however, that a dilution water blank of 0.5 mg/L is found along with the results shown in Figure 3. The APHA equation provides no way to correct for this blank. If results cannot be thrown out, the blank has to be included. The dilutions then give BODs of 300, 255 and 250 mg/L for the 3, 6 and 9-mL sample sizes, with an average of 262 mg/L.

With the graphical method, the blank is automatically corrected and the analyst will again get the true value of 216 mg/L. Unless the graphical method is used, in fact, the BOD value will always be high when a dilution water blank is present. Because the correction for the blank is automatic, it is not necessary to determine the APHA "seed correction." The oxygen demand from the seed should be perceived as a deliberately introduced blank, which eliminates the need for a series of dilutions on the seed material. Compare Figures 2 and 3 to see the effect of a 0.5 mg/L blank on the Y intercept. With this method, there is no need to question the results when the dilution water shows a blank > 0.2 mg/L.

The graphical method is of value in other ways, too. The Y intercept gives an exact indication of the dilution water blank, including the seed correction, since the difference between the Y intercept and the theoretical oxygen saturation value (or the dilution water DO, if different) is this blank. When drawing the best straight line one also knows that this Y intercept cannot be higher than the saturation value. And, if the seed correction is known, the Y intercept cannot be above the saturation value minus the seed correction.

*Since the inter-laboratory precision found by the APHA was 17%, this 10% variation is not reasonable.

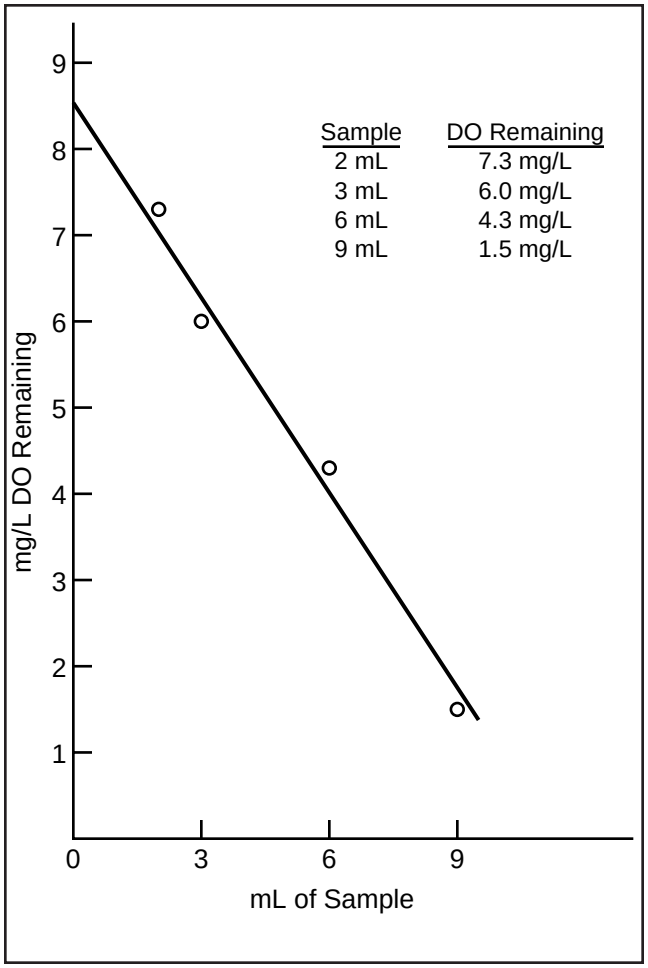


Figure 3

Summary

Numerous advantages are gained by graphing the amount of DO remaining versus the mL of sample. Bad data points are evident and can be judged as outliers and dropped. In contrast to the APHA method where only one point is used and its validity is not known, the graphical method determines BOD of the sample from all valid points. In addition, because calculating or measuring the initial DO for each dilution is no longer necessary, extra work and possible errors are eliminated. If the dilution water used a demand (from a blank or seeding), it is incorporated into the graph automatically and its effect on the results is negated.

PROVING THE ACCURACY OF THE DILUTION METHOD BOD TEST

Many factors can affect the performance of the BOD test. To determine the reliability of routine test results, BODs are run on a pure organic standard having a known or determinable BOD. The widely accepted BOD standard is a mixture of glucose and glutamic acid. Increasing increments (1, 2, 3 and 4 mL) of a BOD standard (Voluette® Ampule Standard for BOD) are added to the BOD bottles, which are then filled with seeded dilution water and incubated at 20 °C for five days. The amount

of dissolved oxygen remaining after five days is plotted against the volume of standard used and the best straight line is drawn through the accepted points. The slope of the line is determined, and this value, multiplied by the volume of the BOD bottle, gives the BOD of the standard. The sample DO and the Y intercept will be nearly equal (unless there is a substantial blank) and will cancel out in this case. Using a mixed primary standard containing 150 mg/L each of glucose and glutamic acid, the five-day BOD varies in magnitude according to the type of seed, and in precision according to the quality of seed as shown in the table below.

Type of Seed	Mean five-day BOD mg/L	Standard Deviation mg/L
Settled fresh sewage	218	±11
Settled stale sewage	207	±8
River water (four sources)	224-242	±7-13
Activated sludge effluent	221	±13
Trickling filter effluent	225	±8

Note: Data taken from *Standard Methods*, 14th ed., p. 548, 1975.

Because the BOD standard prepared by Hach contains 300 mg/L each of glucose and glutamic acid, the BOD value determined from the graph must be divided by two to be compared with values in the table.

Example of a Calculation

A series of four BOD bottles was set up with 1, 2, 3 and 4 mL samples of BOD standard; all were diluted to 300 mL with seeded dilution water (3 mL settled sewage/L). After five days, the dissolved oxygen remaining in each was determined.

BOD Standard	DO Remaining After 5 Days
1 mL	5.61 mg/L
2 mL	4.10 mg/L
3 mL	2.75 mg/L
4 mL	1.35 mg/L

The BOD of the standard is calculated from the slope multiplied by 300 minus the Y intercept and plus the sample DO. In this case the sample DO can be assumed to be saturated and nearly the same as the Y intercept, and so only the slope is needed.

From the graph (Figure 4)

Y intercept = 7.05 mg/L DO

Chosen point = 3.5 mg/L DO at 2.5 mL sample

$$\text{Slope} = \frac{7.05 - 3.5}{2.5 - 0} = \frac{3.55}{2.5} = 1.42$$

BOD = 1.42 x 300 = 426 mg/L

Dividing by 2 (because twice the concentration is used as is called for in *Standard Methods*) gives 213 mg/L BOD, as compared to 218 ± 11 mg/L BOD in the table above. Since these two values are comparable, there can be confidence in the technique.

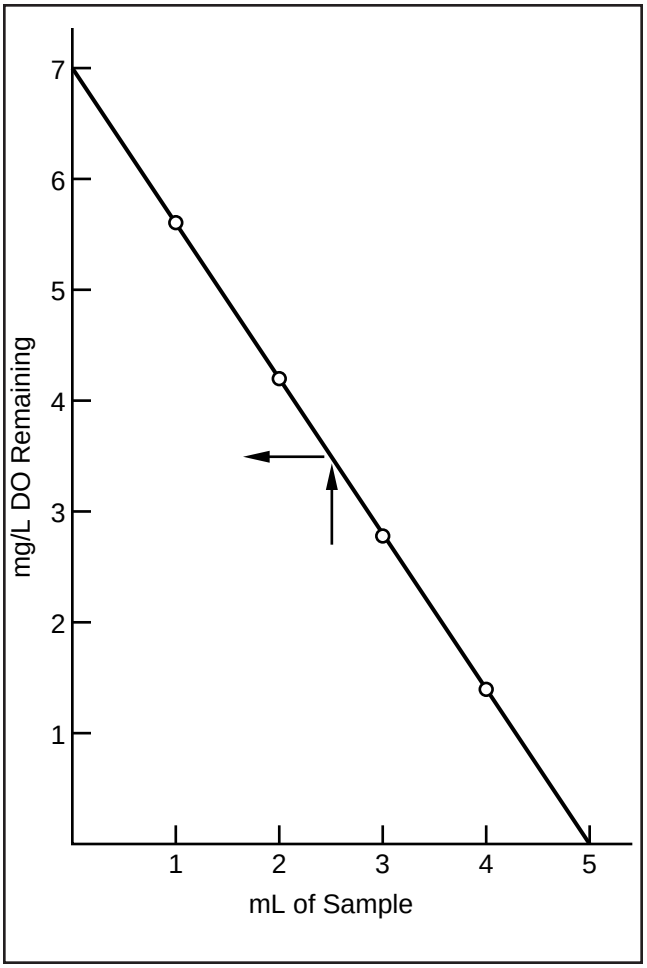


Figure 4

The BOD Incubator Model 205 holds up to 62 standard BOD bottles or one BODTrak Apparatus (see page 13).

REAGENTS AND APPARATUS FOR DILUTION METHOD

Cat. No.	Description	Unit
14160-66	BOD Nutrient Buffer Pillow makes 300 ml of dilution water	50
14861-98	BOD Nutrient Buffer Pillow makes 3 L of dilution water	25
14862-98	BOD Nutrient Buffer Pillow makes 6 L of dilution water	25
14862-98	BOD Nutrient Buffer Pillow makes 19 L of dilution water	25
50182-00	BOD Accessory Kit (Use with DO175 Meter, DO Probe and Stirrer)	each
50175-00	DO175 Dissolved Oxygen Meter	each
50180-00	DO Probe, Model 50180	each
26162-00	Incubator, BOD, Model 205, 120 Vac, 50/60 Hz	each
26162-02	Incubator, BOD, Model 205, 240 Vac, 50/60 Hz	each
26198-00	Incubator, BOD/Refrigerator, Model 207, 120 Vac, 50/60 Hz	each
26198-02	Incubator, BOD/Refrigerator, Model 207, 240 Vac, 50/60 Hz	each
2597-00	IncuTrol/2 Temperature Regulator, 115 Vac, 60 Hz	each
2597-02	IncuTrol/2 Temperature Regulator, 220 Vac, 50 Hz	each
26318-00	Still, water, 2-3 L/hr, 110 Vac	each
14867-00	Still, water, 3 L/hr, 208/240 Vac	each
45300-01	Stirrer Stand, electromagnetic, 115 Vac	each
45300-01	Stirrer Stand, electromagnetic, 230 Vac	each
Optional Reagents and Apparatus		
14865-10	BOD Standard, 10-mL Voluette Ampules	16
621-00	Bottle, glass-stoppered, BOD, 300 mL	6
620-11	Bottle, poly, wash, 500 mL	each
14868-17	Bottle, aspirator, poly, 4 L, with spigot	each
14868-58	Bottle, aspirator, poly, 10 L, with spigot	each
431-53	Buffer Solution, phosphate, pH 7.2, APHA	1 L
681-40	Buret, Teflon stopcock, 25 mL	each
428-53	Calcium Chloride Solution, APHA	1 L
459-01	Cap, dispenser, for Nitrification Inhibitor	each
2419-06	Cap, for BOD bottle	6
968-00	Clippers, for opening pillows	each
508-42	Cylinder, graduated, 100 mL	each
429-53	Ferric Chloride Solution	1 L
505-46	Flask, Erlenmeyer, 250 mL	each
328-00	Holder, double buret	each
430-53	Magnesium Sulfate Solution	1 L
2533-35	Nitrification Inhibitor	35 g
532-35	Pipet, serological 1 mL	each
532-37	Pipet, serological, 5 mL	each
532-38	Pipet, serological 10 mL	each
2066-40	Pipet, serological, 25 mL	each
12189-00	Pipet filler	each
24712-00	Polyseed® Inoculum, capsules	50
12289-49	Potassium Iodide Solution, 10%	500 mL (pt)
168-01	Potassium Permanganate	454 g (lb)
427-00	Sampler, sewage	each
352-53	Sodium Thiosulfate Standard Solution, 0.025 N	1 L
349-32	Starch Indicator Solution, MDB	100 mL
203-53	Sulfuric Acid Standard Solution, 0.020 N	1 L
1270-53	Sulfuric Acid Standard Solution, 1.000 N	1 L
329-00	Support, buret	each
1877-01	Thermometer, -20 to 105 °C (-4 to 221 °F)	each

III. USING THE BODTRAK METHOD TO MONITOR BOD

INTRODUCTION

The easiest and most direct way to measure BOD is by the BODTrak™ method. Because it takes a direct physical measurement of the oxygen consumed by a sample of the waste, chemical analysis is not necessary. In addition, the apparatus continuously indicates the amount of oxygen taken up by the sample; by graphing the results one can know the rate of oxygen uptake at any time. From this, one can gain a great deal of added insight into the nature of the sample.

The Hach BODTrak Apparatus is based on the manometric principle of operation. This apparatus has been compared with the standard dilution method under controlled test conditions in the laboratory and in routine analysis. Results are equivalent in terms of both accuracy and precision.

The advantages of the Hach BODTrak Apparatus over the dilution method are:

1. Minimal sample preparation time is required.

2. Titrations are unnecessary, no dilutions are required, and total testing time is reduced.

3. Since no dilutions are required, and the sample is stirred continuously, the sample is maintained under natural conditions.

4. The BODTrak Apparatus stores data over a selectable 5-, 7-, or 10-day time period and then shuts off each channel automatically after the selected test period.

5. Oxygen depletion is greatly reduced because the dissolved oxygen is continuously replenished. Biochemical oxidation in the BODTrak Apparatus mimics natural conditions better than in the dilution method. Also, when using the dilution method, samples may show depletion of the dissolved oxygen by as much as 89 percent.

6. At any time during the test period, the analyst can check the BOD rate for each bottle.

7. RS232 interface port enables the analyst to download data to a computer. This data is enhanced with the available HachLink™ Software.

PRINCIPLES OF OPERATION

A measured sample of sewage or wastewater is placed in one of the amber bottles on the apparatus and the bottle is connected the instrument. Above the sewage or water sample is a quantity of air, which contains 21 percent oxygen. Over a period of time, bacteria in the sewage consume dissolved oxygen to oxidize organic matter present in the sample. The air in the closed sample bottle replenishes the used oxygen, resulting in a drop in air pressure in the sample bottle. The BODTrak Apparatus measures the drop in pressure and displays results directly as mg/L BOD. During the test period (usually five days) the sample is continually agitated by a magnetic stirring bar. Carbon dioxide is produced by the oxidation of organic matter and must be removed from the system so that the pressure difference observed is proportional only to the amount of oxygen used. This is accomplished by the addition of a few crystals of lithium hydroxide in the seal cup of each sample bottle.

The electromagnetic stirring mechanism provides adequate agitation to effectively maintain rapid transfer of oxygen from the liquid sample to the air above. The BODTrak Apparatus is free of leaks and has an effective carbon dioxide absorption system. The instrument also has accurate pressure sensors for reading pressure changes. The BODTrak Apparatus is a practical, convenient and economical answer to BOD testing.

The BODTrak Apparatus operates unattended and provides analysts with a convenient method for tracking the 5-, 7-, or 10-day BOD test.

PROCEDURE FOR THE BODTRAK METHOD

1. Carefully follow the complete directions for initial setup that came with your instrument.

2. Heat or cool a sample volume (e.g., 420 mL for 0 to 35mg/L range) to within 2 °C of its incubation temperature, typically 20 °C (68 °F).

3. Using a clean graduated cylinder, pour the required sample volume into a clean sample bottle.

4. Place a 3.8-cm (1-1/2-inch) magnetic stirring bar in each sample bottle (included with the apparatus).

5. Add the contents of one BOD Nutrient Buffer Pillow to each bottle for optimum bacterial growth.

6. Apply stopcock grease to the seal lip of each bottle and seal cups.

7. Using the funnel, add the contents of one Lithium Hydroxide Powder Pillow to each seal cup. Place a seal cup in the neck of each sample bottle. Do not allow lithium hydroxide particles to fall into the sample. If this occurs, discard the sample and prepare a fresh one.

8. Place the bottles on the chassis base. Connect the appropriate tube to the sample bottle and firmly tighten the cap. Start the instrument (connect the electrical plug and turn on the power switch on the side panel).

9. Make sure all stirring bars are rotating.

To select a test duration, simultaneously press and hold the **<** (left) and the **>** (right) arrow keys until the time menu appears. Press the **CHANNEL 6** key to activate the test length parameter. Use the arrow keys to choose a 5-, 7-, or 10-day test (test length is shown on the last line of the screen). Press **OFF** to save selections and exit the menu.

10. To start a test, press the channel number of the bottle, then press the ON key. A menu for selecting the BOD range is displayed. For 0 to 35 mg/L range, press the **<** key. For 0 to 350 mg/L, press the **>** key. To cancel a test, press OFF. (Each channel needs to be started individually.)

11. Place the BODTrak Apparatus in an incubator.

12. The BODTrak Apparatus will automatically stop each channel after the selected time period.

13. Read the BOD results directly from the BODTrak Apparatus display by pressing the number of each sample.

For more information about BODTrak request literature number **4555.**

INTERPRETATION OF RESULTS

If carried out as described, a five-day, 20 °C BOD test should show the following trends:

1. The BOD reading should be increasing on each succeeding day of the test.

2. The rate of increase of readings on successive days should be decreasing, at least for the first five days.

BOD Curves

Figure 7 shows some examples of BOD curves that might be found.

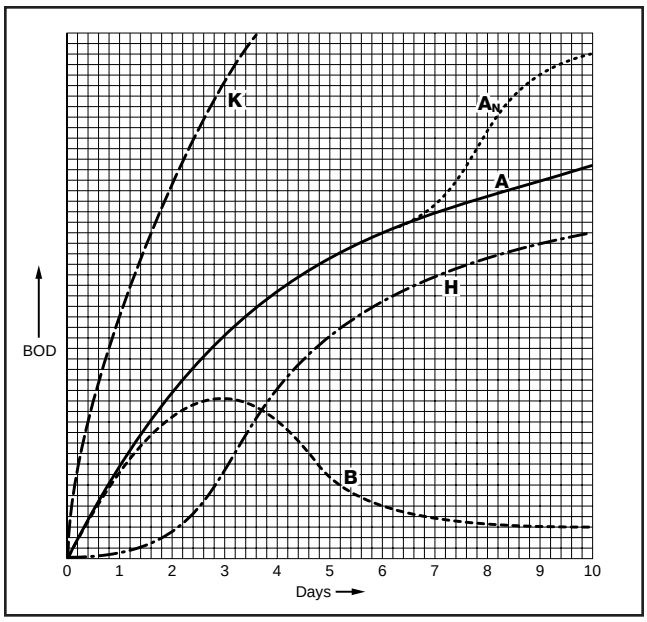


Figure 7. Example of BOD curves

Curve A shows a typical plot of a BOD test run at 20 °C. The BOD value increased each succeeding day but at a decreasing rate. The anomaly shown by curve AN is an example of nitrification. Biological oxidation of organic nitrogen usually occurs after five days with normal domestic waste, because it takes that long for the carbon oxidation to near completion and the nitrogen bacteria seed to develop.

Curve B indicates that a leak developed in the system. A poor seal at the bottle or caps would be the most likely cause.

Curve H shows a delayed start in the oxidation process. This could be the result of insufficient numbers of bacteria at the start of the test period, or it could be an adjustment period during which the bacteria are being acclimated to the sample.

Curve K indicates the sample has a BOD value too high to measure in the undiluted state. The sample should be diluted with distilled or demineralized water. The scale readings should then be multiplied by the dilution factor. Whenever the BOD range of a sample is unknown, it is recommended that the COD test be performed and the results used to establish the range. (Write for information on Hach's simplified COD Method, literature number **3901**.)

Nitrification

Nitrifying bacteria usually are not considered a problem in manometric BOD determinations. Because nitrification usually occurs between the fifth and seventh days of incubation, it causes only a minor error in the normal five-day BOD. However, an abnormally high

uptake of oxygen (especially when testing final effluent with the manometric apparatus) is evidence that nitrifying bacteria are adding appreciably to the oxygen demand. This problem can be alleviated by chemically inhibiting nitrification with Nitrification Inhibitor. The inhibitor should be added directly to the wastewater sample by dispensing the powder into the empty bottle before the sample is poured in. If a small Hach dispenser cap is used, two measures 0.6 liters (0.16 g) of Nitrification Inhibitor are adequate.

Dilutions

When high oxygen demands are encountered, the sample must be diluted. This is done with distilled water that is of very high quality and free of all toxic substances (such as small amounts of chlorine, copper, mercury, etc.) and all organic matter. Demineralizers can erratically release undetected organic matter, which will create an objectionable oxygen demand. The most practical way to produce water of low organic content on a consistent basis is by distillation from alkaline permanganate. Commercial stills which can produce high-quality distilled water automatically are available. Enough distilled water should be made to fill a 3.8-liters (1-gallon) jug to the 3.0-liters (0.8-gallon) mark, and the water temperature should be brought to 20 °C. The jug should then be capped and shaken vigorously for two minutes to saturate the dilution water with oxygen from the air in the jug. Store at 20 °C.

When several identical samples are desired, a single dilution should be performed and the samples dispensed from this dilution. After dilution, the scale reading must be multiplied by the dilution factor. For example, if 1 volume of original sample is diluted to 2 volumes, the scale reading is multiplied by 2, or if 1 part of the original sample is diluted to 10, the scale reading is multiplied by 10. In some samples, especially those with a high or low pH or where insufficient nutrients are suspected, "dilution water" should be considered. *See the sections on the dilution method for a more complete discussion of dilution water.*

SEEDING SAMPLES

Certain types of BOD samples, including many industrial discharges, do not contain sufficient bacteria to oxidize the organic matter present in the sample. Some sewage treatment plant effluents are chlorinated to the extent that they are essentially sterile, making it impossible to perform a direct BOD test. In order to test such samples, it is necessary to seed each bottle. This is done by adding a small, accurately measured volume of water known to contain a good bacterial population. Polyseed provides a reliable source of seed. The BOD of the seed must be known in order to calculate the BOD of the sample. The following relationship exists:

$$\text{BOD} = \frac{(\text{BOD observed}) - (\text{Fraction seed} \times \text{BOD of seed})}{\text{Fraction Sample}}$$

For example, if a 10 percent seed (by volume) is added to a sample and the BOD observed is determined to be 285 mg/L, and the BOD of the pure seed is found to be 150 mg/L:

$$\frac{285\ \text{mg/L} - (0.10 \times 150\ \text{mg/L})}{0.90} = 300\ \text{mg/L BOD}$$

A concentration of seed that is too low is more critical than one that is too high. Low seed concentrations will cause an excessive lag in the start of oxidation and, thus, a low five-day BOD. The trial and error method is the most reliable technique for determining the optimum concentration of seed for a specific waste material. However, it has been found that the use of Polyseed or addition of 1 mL settled domestic sewage seed to each BOD sample is usually sufficient. Various concentrations of seed should be tried and the respective BODs determined on the waste sample as well as on the seed itself. The seed concentration yielding the highest corrected waste sample BOD should be chosen. This seed percentage can range between 2 and 30 percent depending on the waste material being tested.

TEMPERATURE CONSIDERATIONS

The APHA recommends a temperature of 20 °C ± 1 °C (68 °F) for the BOD test. A refrigerator can be converted easily and inexpensively into an incubator by connecting a Hach IncuTrol/2® Temperature Regulator and adjusting the temperature for 20 °C. Request literature number **1402** for more information. Hach also offers a BOD Incubator and BOD Incubator/Refrigerator. This incubator holds up to 92 BOD bottles. Request literature number **4555** for more information.

The BOD test can be run at temperatures other than 20 °C. Results at 35 °C, shown by Tool[1], indicate the five-day, 20 °C value can be reached in two and a half days. Middlebrooks presents nomographs for converting BOD tests at other temperatures to 20 °C.

When the temperature of the wastewater at the time of collection is above 49 °C (120 °F), or if it is known that the wastewater temperature exceeds the value, the sample should be cooled to incubation temperature and the seed procedure followed before doing the actual BOD test. This will prevent time lag in the test.

1.H. R. Tool. (1976). *Water and Sewage Works*, 114, 211.
2. E. J. Middlebrooks. (1965). *Water and Sewage Works*, 112, R230.

INDUSTRIAL WASTES

Industrial samples and chlorinated samples often require special consideration and handling. Experimentation with the specific sample usually indicates modification of the routine BOD test procedure and is necessary to establish reliable BOD test results.

Poisons or Toxic Materials, Including Chlorine

The presence of toxic substances in the sample will result in a diminished BOD value. Consequently, either

they must be removed or their effects eliminated by dilution of the sample.

Low concentrations of chlorine may be dissipated by allowing the sample to stand for one to two hours at room temperature. Where high concentrations are present, the concentration of chlorine must be determined and an appropriate quantity of sodium thiosulfate added to destroy the chlorine as follows:

1. Add 10 mL of Sulfuric Acid Standard Solution (0.020 N) and 10 mL of Potassium Iodide Solution (100 g/L) to a 100-ml portion of the sample in a 250-mL Erlenmeyer flask.

2. Add three droppers of Starch Indicator Solution and swirl to mix.

3. Titrate from dark blue to colorless with 0.025N Sodium Thiosulfate Standard Solution.

4. Using the following relationship, calculate the amount of Sodium Thiosulfate Standard Solution necessary to dechlorinate the remaining sample.

$$\text{mL 0.025N sodium thiosulfate} = \frac{\text{mL used x mL to dechlorinate}}{100}$$

5. Add the required amount of 0.025N Sodium Thiosulfate Standard Solution to the sample and mix thoroughly. Allow the sample to stand for 10 to 20 minutes before running the BOD test.

The presence of other toxic materials, such as phenols, heavy metals, and cyanide, must also be considered. The actual concentration of these materials may be determined by methods described in Hach's *Water Analysis Handbook* (request literature number **8353**). The effect of these materials may be eliminated by dilution of the sample with distilled water. The correct BOD is obtained when two successive dilutions result in the same BOD value.

SEED ACCLIMATIZATION

The importance of selecting the proper seed for a particular waste is emphasized in *Standard Methods*, 18th edition. Polyseed provides a reliable source of seed for most samples. A suitable seed may also be obtained from domestic sewage. However, if the waste sample to be tested contains materials not readily biodegradable, or if it contains toxic materials such as phenol, formaldehyde or similar microbic inhibitory agents, an acclimated seed must be used.

The acclimatization process usually can be carried out quite easily in any nonmetal or stainless steel gallon container fitted with an aeration system. Polyseed eliminates the problem of finding seed that contains sufficient bacterial populations for oxidizing biodegradable organic matter. Polyseed provides a consistent BOD seed source—free of nitrifying bacteria. Use Polyseed with either the dilution method or the BODTrak method. For more information, request literature number **1412.**

Domestic sewage is first aerated for approximately 24 hours after which the heavier material is allowed to settle. After one hour of settling time, two-thirds of the volume is siphoned from the surface and discarded. The container is then refilled to the original level with domestic sewage containing 10 percent of the waste material in question. The sludge-activating unit is again aerated for 24 hours and the procedure is repeated, but the amount of waste material is increased by an additional 10 percent each time until the fill process is 100 percent waste material.

Often, a particular seed can be acclimatized to a waste material more easily than the above procedure indicates, especially if the original seed is taken from a stream where the waste is present. In this case the procedure can be modified and the acclimatization time reduced. If normal acclimatization processes prove ineffective, a specific waste culture should be included in the conditioning process.

PROVING THE ACCURACY OF THE BODTRAK METHOD BOD TEST

Occasional analysis of a standard BOD sample will assure the operator that the BODTrak Apparatus is functioning properly and the procedure is correct. Running a standard sample can be the simplest way to prove the existence of one or more of the mechanical, physical, chemical or biological effects described previously. The BOD standard that has been widely accepted is a mixture of 150 mg/L each of glucose and glutamic acid. A prepared standard solution is available as the Voluette Ampule Standard, which when diluted 1:20 gives the required concentration. A procedure for analyzing standard BOD samples follows.

Analyzing Standard BOD Samples

1. Prepare approximately 3 liters (0.8 gallons) of oxygen-saturated water by shaking distilled water in a partially filled container for one minute. Add the contents of one BOD Nutrient Buffer Pillow and invert several times to mix.

2. Snap the neck off a Voluette Ampule Standard for Manometric BOD, and pipet 7 mL of standard into a sample bottle.

3. Add 133 mL of the nutrient buffer solution and 14 mL seed. This will give 10 percent by volume of the seed in solution. Mix well. Refer to the section on Seeding Samples, page 16.

4. Follow the general procedure for the BOD test 0 to 350 mg/L BOD range.

5. Perform a BOD test (full strength) on the seed at the same time as the sample.

6. Correct the BOD result for the seed effect as described previously. The corrected BOD of the standard solution should be 220 ± 22 mg/L.

REAGENTS AND APPARATUS FOR THE MANOMETRIC METHOD

Cat. No.	Description	Unit
14160-66	BOD Nutrient Buffer Pillow makes 300 mL of dilution water	50
14861-98	BOD Nutrient Buffer Pillow makes 3 L of dilution water	25
14862-98	BOD Nutrient Buffer Pillow makes 6 L of dilution water	25
14862-98	BOD Nutrient Buffer Pillow makes 19 L of dilution water	25
26197-00	BODTrak Apparatus, 115 Vac, 50/60 Hz	each
26197-02	BODTrak Apparatus, 230 Vac, 50/60 Hz	each
26162-00	Incubator, BOD, Model 205, 120 Vac, 50/60 Hz	each
26162-02	Incubator, BOD, Model 205, 240 Vac, 50/60 Hz	each
26198-00	Incubator, BOD/Refrigerator, Model 207, 120 Vac, 50/60 Hz	each
26198-02	Incubator, BOD/Refrigerator, Model 207, 240 Vac, 50/60 Hz	each
2597-00	IncuTrol/2 Temperature Regulator, 115 Vac, 60 Hz	each
2597-02	IncuTrol/2 Temperature Regulator, 220 Vac, 50 Hz	each
14163-69	Lithium Hydroxide Pillows	100
Optional Reagents and Apparatus		
14866-10	BOD Standard, 10-mL Voluette Ampules	16
14868-17	Bottle, aspirator, poly, 4 L, with spigot	each
459-01	Cap, dispenser, for Nitrification Inhibitor	each
508-40	Cylinder, graduated, 25 mL	each
508-46	Cylinder, graduated, 250 mL	each
508-49	Cylinder, graduated, 500 mL	each
2533-35	Nitrification Inhibitor	35 g
20934-38	Pipet, Mohr, 10.00 mL	each
24712-00	Polyseed® Inoculum, capsules	50
427-00	Sampler, sewage	each
26318-00	Still, water, 2-3 L/hr, 110 Vac	each
14867-00	Still, water, 2-3 L/hr, 208/240 Vac	each
1877-01	Thermometer, -20 to 105 °C (-4 to 221 °F)	each

APPENDIX I

DERIVATION OF EQUATION TO CALCULATE BOD

From *Standard Methods*:

$$BOD = \frac{D_c - D_f}{P}$$

Where:

D_f = Final DO of incubated dilution
D_c = $D_o p + SP$ = calculated initial DO of dilution
S = DO of undiluted sample
D_o = DO of dilution water

$$P = \frac{V}{300} = \text{fraction of sample used}$$

$$p = \frac{300 - V}{300} = \text{fraction of dilution water used}$$

So, substituting for D_c

$$BOD = \frac{D_o p + SP - D_f}{P}$$

Substituting for p and P for dilution 1

$$BOD_1 = \frac{D_o\left(\frac{300 - V_1}{300}\right) + \frac{SV_1}{300} - D_{f1}}{\frac{V_1}{300}}$$

Multiply through by $V_1/300$:

$$BOD_1\left(\frac{V_1}{300}\right) = \frac{D_o 300 - V_1 D_o + SV_1 - D_{f1}300}{300}$$

Multiply through by 300:

$$BOD_1 V_1 = D_o 300 - V_1 D_o + SV_1 - D_{f1}300$$

Subtracting the equation for dilution 1 from dilution 2 ($V_2 > V_1$)

$$BOD_2 V_2 - BOD_1 V_1 = [D_o 300 - V_2 D_o + D_{f2}300] - [D_o 300 - V_1 D_o + SV_1 - D_{f1}300]$$

or

$$BOD_2 V_2 - BOD_1 V_1 = -V_2 D_o + V_1 D_o + SV_2 - SV_1 - D_{f2}300 + D_{f1}300$$

but $BOD_2 = BOD_1 = BOD$ since they are from the same sample:
so, with collection of terms,

$$BOD(V_2 - V_1) = D_o(V_1 - V_2) + S(V_2 - V_1) + 300(D_{f1} - D_{f2}).$$

Since $DO(V_1 - V_2) = -D_o(V_2 - V_1)$,

$$BOD(V_2 - V_1) = 300(D_{f1} - D_{f2}) - (D_o - S)(V_2 - V_1)$$

and dividing through by $(V_2 - V_1)$:

$$BOD = 300\left(\frac{D_{f1} - D_{f2}}{V_2 - V_1}\right) - D_o + S$$

When the remaining DOs of a series of dilutions are graphed versus the mL of sample the Y intercept = D_o – (Demand of Dilution Water). This demand is due to the dilution water blank and/or the seed added to the dilution water. Therefore, for either the seeded or unseeded case:

$$BOD = 300\left(\frac{D_{f1} - D_{f2}}{V_2 - V_1}\right) - Y\text{ int} + S$$

if we define $\frac{D_{f1} - D_{f2}}{V_2 - V_1}$ as the slope

then

BOD = 300 (slope) – Y int + S

APPENDIX II

DISSOLVED OXYGEN METHOD

Azide Modification of Winkler Method
***APHA Standard Methods*, 16th edition, 418 (1985)**

Introduction

The dissolved oxygen test is one of the most important analyses in determining the quality of natural waters. The effect of oxidation wastes on streams, the suitability of water for fish and other organisms, and the progress of self-purification can all be measured or estimated from the dissolved oxygen content. In aerobic sewage treatment units, the minimum objectionable odor potential, maximum treatment efficiency, and stabilization of wastewater depend on maintaining adequate dissolved oxygen. Frequent dissolved oxygen measurement is essential for adequate process control.

The azide modification of the Winkler Method is the standard test for dissolved oxygen. Test reagents have been formulated into dry, premeasured powder pillow form for increased stability and convenience. The standard APHA reagents in solution form also are available as described in Note C of the dissolved oxygen procedure.

An Iodate-Iodide Standard Solution, 10 mg/L as dissolved oxygen, is available from Hach for checking the strength of the titrating solutions.

Procedure

1. Drop one glass bead into the BOD bottle.

2. Add the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Reagent Powder Pillow. Carefully insert the stopper so that no air is trapped in the bottle. Pour any excess water off the bottle rim and invert several times to mix. A flocculent precipitate will form which will be brownish orange if dissolved oxygen is present or white if oxygen is absent.

3. Allow the sample to stand until the floc has settled and leaves the top half of the solution clear. Again invert the bottle several times to mix and let stand until the upper half of the solution is clear. *See Note A*.

4. Remove the stopper and add the contents of one Sulfamic Acid Powder Pillow. Replace the stopper, being careful not to trap any air bubbles in the bottle, and invert several times to mix. The floc will dissolve and leave a yellow color if dissolved oxygen is present.

5. Measure 200 mL of the prepared solution by filling a clean 250-mL graduated cylinder to the 200-ml mark. Pour the solution into a clean 250-mL Erlenmeyer flask.

6. Titrate the prepared solution with Sodium Thiosulfate Standard Solution, 0.025N, to a pale yellow color.

7. Add two dropperfuls of Starch Indicator Solution and swirl to mix. A dark blue color will develop.

8. Continue the titration until the solution changes from blue to colorless.

9. The total number of mL Sodium Thiosulfate Standard Solution used is equal to the mg/L dissolved oxygen.

Notes

A. Allowing the floc to settle twice ensures reaction of the chemicals with all the dissolved oxygen present. The floc will settle very slowly in salt water; an additional five minutes is usually required before the analyst can proceed with Step 4. Results will not be affected if the floc refuses to settle.

B. A stabilized Sodium Thiosulfate Standard Solution, 0.025N, is more stable than conventional solutions of sodium thiosulfate and is not affected by bacterial action. The strength of the solution can be checked as a titrant for dissolved oxygen by using an Iodate-Iodide Standard Solution which is equivalent to 10 mg/L as dissolved oxygen. The check can be performed by adding one Sulfamic Acid Powder Pillow to 200 mL of Iodate-Iodide Standard Solution and titrating as described in Steps 6 through 9. The volume of titrant used should be 10 mL. If more than 10.5 mL is necessary to reach the end point (colorless), the titrant should be discarded.

C. Standard APHA solutions for dissolved oxygen can be used in place of the powder pillow reagents by substituting 2 mL of Manganous Sulfate solution and 2 mL of Alkaline Iodide-Azide Reagent, respectively, in Step 2, and 2 mL of Sulfuric Acid (concentrated) in Step 4. These solutions must be dispensed below the surface of the liquid.

D. An alternate method for determining oxygen content is the membrane electrode method. No reagents are needed and most interfering substances experienced with other methods have little effect on the electrode determination. The Model DO175 Portable Dissolved Oxygen Meter by Hach is the ideal instrument for measuring DO in the field.

REAGENTS AND APPARATUS FOR DISSOLVED OXYGEN METHOD

Cat. No.	Description	Unit
1072-68	Alkaline Iodide-Azide Reagent Powder Pillows	25
621-00	Bottle, glass-stoppered, BOD, 300 mL	each
968-00	Clippers, for opening pillows	each
508-46	Cylinder, graduated, 250 mL	each
505-46	Flask, Erlenmeyer, 250 mL	each
2596-00	Glass beads	100
1071-68	Manganous Sulfate Powder Pillows	25
349-32	Starch Indicator Solution, MDB*	100 mL
1073-99	Sulfamic Acid Powder Pillows	100
For titration with a Digital Titrator, include:		
16900-01	Digital Titrator	each
22675-01	Sodium Thiosulfate Standard Solution Cartridge, 0.2000 N	each
For titration with a buret, include:		
22614-40	Buret, automatic, 25 mL	each
352-53	Sodium Thiosulfate Standard Solution, 0.025 N	1 L
Optional Reagents and Apparatus		
14681-40	Buret, Class A, Teflon stopcock plug, 25 mL	each
328-00	Buret holder, double	each
326-00	Clamp holder	each
357-49	Copper Sulfate-Sulfamic Acid Inhibitor Solution	500 mL
508-38	Cylinder, graduated, 10 mL	each
508-53	Cylinder, graduated, 1000 mL	each
50175-00	DO175 Dissolved Oxygen Meter, portable, with probe and battery	each
515-36	Pipet, transfer, 2 mL (two required)	each
12189-00	Pipet filler	each
401-49	Potassium Iodate-Iodide Standard Solution, 10 mg/L as DO	500 mL
427-00	Sampler, sewage	each
24093-16	Sodium Thiosulfate Standard Solution, stabilized, 0.025 N	946 mL (qt)
329-00	Support base and rod	each
1864-41	Syphon copper tube	each
7134-00	Syphon rubber tubing	each
19400-00	TitraStir, 115 Vac	each
19400-10	TitraStir, 230 Vac	each
Optional Standard APHA Solutions		
277-49	Alkaline Iodide-Azide Reagent Solution	500 mL
275-49	Manganous Sulfate Solution	500 mL
352-53	Sodium Thiosulfate Standard Solution, 0.025 N	1 L
349-32	Starch Indicator Solution, MDB	100 mL
979-49	Sulfuric Acid, ACS	500 mL

*Marked Dropping Bottle

Safety Information

As part of good laboratory practice, please familiarize yourself with the reagents used in the preceding procedure. Read product labels and Material Safety Data Sheets (MSDS) for all chemicals before using them.

APPENDIX III

SATURATED DISSOLVED OXYGEN LEVELS

The following table lists the mg/L dissolved oxygen in water at saturation for various temperatures and atmospheric pressures. The table was formulated in a laboratory using pure water; thus the values given should be considered as only approximations when estimating the desired oxygen content of a particular body of surface water.

Temp.		Pressure in Millimeters and Inches Hg							
		775	760	750	725	700	675	650	625 mm
°F	°C	30.51	29.92	29.53	28.54	27.56	26.57	25.59	24.61 inches
32.0	0	14.9	14.6	14.4	13.9	13.5	12.9	12.5	12.0
33.8	1	14.5	14.2	14.1	13.6	13.1	12.6	12.2	11.7
35.6	2	14.1	13.9	13.7	13.2	12.9	12.3	11.8	11.4
37.4	3	13.8	13.5	13.3	12.9	12.4	12.0	11.5	11.1
39.2	4	13.4	13.2	13.0	12.5	12.1	11.7	11.2	10.8
41.0	5	13.1	12.8	12.6	12.2	11.8	11.4	10.9	10.5
42.8	6	12.7	12.5	12.3	11.9	11.5	11.1	10.7	10.3
44.6	7	12.4	12.2	12.0	11.6	11.2	10.8	10.4	10.0
46.4	8	12.1	11.9	11.7	11.3	10.9	10.5	10.1	9.8
48.2	9	11.8	11.6	11.5	11.1	10.7	10.3	9.9	9.5
50.0	10	11.6	11.3	11.2	10.8	10.4	10.1	9.7	9.3
51.8	11	11.3	11.1	10.9	10.6	10.2	9.8	9.5	9.1
53.6	12	11.1	10.8	10.7	10.3	10.0	9.6	9.2	8.9
55.4	13	10.8	10.6	10.5	10.1	9.8	9.4	9.1	8.7
57.2	14	10.6	10.4	10.2	9.9	9.5	9.2	8.9	8.5
59.0	15	10.4	10.2	10.0	9.7	9.3	9.0	8.7	8.3
60.8	16	10.1	9.9	9.8	9.5	9.1	8.8	8.5	8.1
62.6	17	9.9	9.7	9.6	9.3	9.0	8.6	8.3	8.0
64.4	18	9.7	9.5	9.4	9.1	8.8	8.4	8.1	7.8
66.2	19	9.5	9.3	9.2	8.9	8.6	8.3	8.0	7.6
68.0	20	9.3	9.2	9.1	8.7	8.4	8.1	7.8	7.5
69.8	21	9.2	9.0	8.9	8.6	8.3	8.0	7.7	7.4
71.6	22	9.0	8.8	8.7	8.4	8.1	7.8	7.5	7.2
73.4	23	8.8	8.7	8.5	8.2	8.0	7.7	7.4	7.1
75.2	24	8.7	8.5	8.4	8.1	7.8	7.5	7.2	7.0
77.0	25	8.5	8.4	8.3	8.0	7.7	7.4	7.1	6.8
78.8	26	8.4	8.2	8.1	7.8	7.6	7.3	7.0	6.7
80.6	27	8.2	8.1	8.0	7.7	7.4	7.1	6.9	6.6
82.4	28	8.1	7.9	7.8	7.6	7.3	7.0	6.7	6.5
84.2	29	7.9	7.8	7.7	7.4	7.2	6.9	6.6	6.4
86.0	30	7.8	7.7	7.6	7.3	7.0	6.8	6.5	6.2
87.8	31	7.7	7.5	7.4	7.2	6.9	6.7	6.4	6.1
89.6	32	7.6	7.4	7.3	7.0	6.8	6.6	6.3	6.0
91.4	33	7.4	7.3	7.2	6.9	6.7	6.4	6.2	5.9
93.2	34	7.3	7.2	7.1	6.8	6.6	6.3	6.1	5.8
95.0	35	7.2	7.1	7.0	6.7	6.5	6.2	6.0	5.7
96.8	36	7.1	7.0	6.9	6.6	6.4	6.1	5.9	5.6
98.6	37	7.0	6.8	6.7	6.5	6.3	6.0	5.8	5.6
100.4	38	6.9	6.7	6.6	6.4	6.2	5.9	5.7	5.5
102.2	39	6.8	6.6	6.5	6.3	6.1	5.8	5.6	5.4
104.0	40	6.7	6.5	6.4	6.2	6.0	5.7	5.5	5.3
105.8	41	6.6	6.4	6.3	6.1	5.9	5.6	5.4	5.2
107.6	42	6.5	6.3	6.2	6.0	5.8	5.6	5.3	5.1
109.4	43	6.4	6.2	6.1	5.9	5.7	5.5	5.2	5.0
111.2	44	6.3	6.1	6.0	5.8	5.6	5.4	5.2	4.9
113.0	45	6.2	6.0	5.9	5.7	5.5	5.3	5.1	4.8
114.8	46	6.1	5.9	5.9	5.6	5.4	5.2	5.0	4.8
116.6	47	6.0	5.9	5.8	5.6	5.3	5.1	4.8	4.7
118.4	48	5.9	5.8	5.7	5.5	5.3	5.0	4.8	4.6
120.2	49	5.8	5.7	5.6	5.4	5.2	5.0	4.7	4.5
122.0	50	5.7	5.6	5.5	5.3	5.1	4.9	4.7	4.4

About the Authors

The late Clifford C. Hach, founder of Hach Company, was a graduate of Iowa State University. Widely respected in the water analysis industry as an inventor, progressive innovator and research scientist, Hach held numerous patents and many of his papers appeared in technical industry publications.

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