FIVE-DAY BIOCHEMICAL OXYGEN DEMAND 7.0

by G.C. Delzer and S.W. McKenzie

Page

7.0 Five-Day Biochemical Oxygen Demand BOD-3

7.0.1 Equipment and supplies BOD-6

7.0.2 Sample collection and storage BOD-9

7.0.3 Five-day test for biochemical oxygen demand . BOD-11

7.0.3.A Sample preparation BOD-11

7.0.3.B Interferences BOD-13

7.0.3.C BOD_5 test procedure BOD-14

7.0.4 Calculations BOD-19

7.0.5 Troubleshooting BOD-20

7.0.6 Reporting BOD-21

Illustrations

7.0-1. Biochemical oxygen demand curves: (A) typical carbonaceous-demand curve showing the oxidation of organic matter, and (B) typical carbonaceous- plus nitrogeneous-demand curve showing the oxidation of ammonia and nitrite BOD-4

7.0-2. Example of a 5-day biochemical oxygen demand worksheet BOD-18

Tables[1]

7.0-1. Equipment, supplies, chemical reagents, and preparation of dilution water and chemical solutions used in the procedure for determination of 5-day biochemical oxygen demand..... BOD-7

7.0-2. Recommended sample volumes for the 5-day biochemical oxygen demand test..... BOD-13

7.0-3. Troubleshooting guide for the 5-day biochemical oxygen demand test..... BOD-20

[1]See Appendix A7-A, Table 1, for information on parameter codes used in the National Water Information System (NWIS) of the U.S. Geological Survey for biochemical oxygen demand.

7.0 FIVE-DAY BIOCHEMICAL OXYGEN DEMAND

The presence of a sufficient concentration of dissolved oxygen is critical to maintaining the aquatic life and aesthetic quality of streams and lakes. Determining how organic matter affects the concentration of dissolved oxygen (DO) in a stream or lake is integral to water-quality management. The decay of organic matter in water is measured as biochemical or chemical oxygen demand. Oxygen demand is a measure of the amount of oxidizable substances in a water sample that can lower DO concentrations (Nemerow, 1974; Tchobanoglous and Schroeder, 1985).

Biochemical oxygen demand represents the amount of oxygen consumed by bacteria and other microorganisms while they decompose organic matter under aerobic conditions at a specified temperature.

The test for biochemical oxygen demand (BOD) is a bioassay procedure that measures the oxygen consumed by bacteria from the decomposition of organic matter (Sawyer and McCarty, 1978). The change in DO concentration is measured over a given period of time in water samples at a specified temperature. Procedures used to determine DO concentration are described in NFM 6.2. It is important to be familiar with the correct procedures for determining DO concentrations before making BOD measurements. BOD is measured in a laboratory environment, generally at a local or USGS laboratory.

Accurate measurement of BOD requires an accurate determination of DO.

There are two stages of decomposition in the BOD test: a carbonaceous stage and a nitrogenous stage (fig. 7.0-1).

- The carbonaceous stage, or first stage, represents that portion of oxygen demand involved in the conversion of organic carbon to carbon dioxide.
- The nitrogenous stage, or second stage, represents a combined carbonaceous plus nitrogeneous demand, when organic nitrogen, ammonia, and nitrite are converted to nitrate. Nitrogenous oxygen demand generally begins after about 6 days. For some sewage, especially discharge from wastewater treatment plants utilizing biological treatment processes, nitrification can occur in less than 5 days if ammonia, nitrite, and nitrifying bacteria are present. In this case, a chemical compound that prevents nitrification should be added to the sample if the intent is to measure only the carbonaceous demand. The results are reported as carbonaceous BOD (CBOD), or as $CBOD_5$ when a nitrification inhibitor is used.

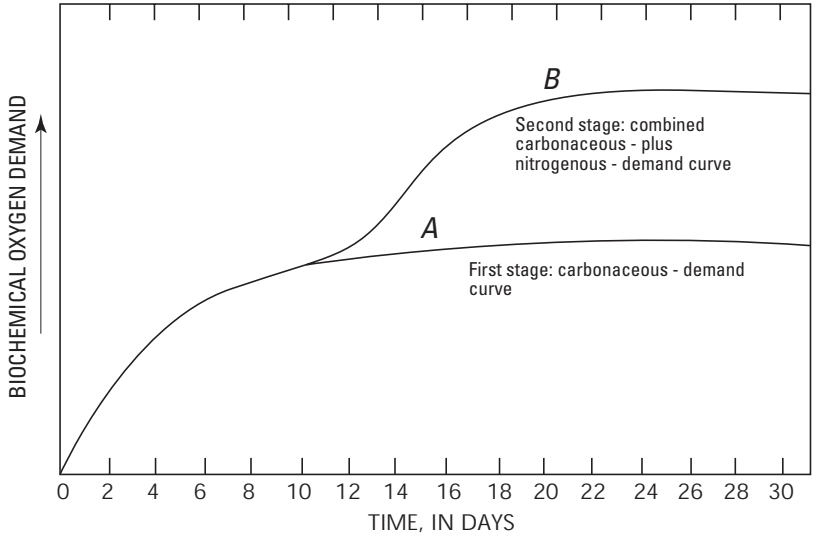

Figure 7.0-1. Biochemical oxygen demand curves: (*A*) typical carbonaceous-demand curve showing the oxidation of organic matter, and (*B*) typical carbonaceous- plus nitrogeneous-demand curve showing the oxidation of ammonia and nitrite. (Modified from Sawyer and McCarty, 1978.)

The standard oxidation (or incubation) test period for BOD is 5 days at 20 degrees Celsius (°C)(BOD_5). The BOD_5 value has been used and reported for many applications, most commonly to indicate the effects of sewage and other organic wastes on dissolved oxygen in surface waters (see TECHNICAL NOTE). The 5-day value, however, represents only a portion of the total biochemical oxygen demand. Twenty days is considered, by convention, adequate time for a complete biochemical oxidation of organic matter in a water sample, but a 20-day test often is impractical when data are needed to address an immediate concern.

- The BOD_5 and $CBOD_5$ tests have limited value by themselves in the assessment of stream pollution and do not provide all of the relevant information to satisfy every study objective (Nemerow, 1974; Stamer and others, 1983; Veltz, 1984). Additional analyses of water samples for chemical oxygen demand, fecal bacteria, and nutrients can aid in the interpretation of BOD_5.

- An ultimate carbonaceous BOD ($CBOD_u$) test is needed to obtain additional BOD information, and can be used for modeling DO regimes in rivers and estuaries (Hines and others, 1978; Stamer and others, 1983). Guidelines for the $CBOD_u$ determination are described in Stamer and others (1979, 1983).

- Note that BOD results represent approximate stream oxygen demands because the laboratory environment does not reproduce ambient stream conditions such as temperature, sunlight, biological populations, and water movement.

TECHNICAL NOTE: A 5-day duration for BOD determination has no theoretical grounding but is based on historical convention. Tchobanoglous and Schroeder (1985) provide the following background: "In a report prepared by the Royal Commission on Sewage Disposal in the United Kingdom at the beginning of the century, it was recommended that a 5-day, 18.3°C, BOD value be used as a reference in Great Britain. These values were selected because British rivers do not have a flow time to the open sea greater than 5 days and average long-term summer temperatures do not exceed 18.3°C. The temperature has been rounded upward to 20°C, but the 5-day time period has become the universal scientific and legal reference."

7.0.1 EQUIPMENT AND SUPPLIES

Table 7.0-1 lists equipment and supplies commonly used in the BOD_5 test using amperometric determination of DO. For more detailed guidance on equipment, supplies, maintenance, and calibration of the DO instrument, refer to NFM 6.2. If the iodometric (Winkler) method of DO determination is to be used, refer to table 6.2-3 in NFM 6.2 for a list of equipment and supplies. Equipment used for BOD sampling must be thoroughly cleaned with nonphosphate detergent and rinsed with tap water and deionized water, as described in NFM 3.

CAUTION: Before handling chemical reagents, refer to Material Safety Data Sheets. Wear safety glasses, gloves, and protective clothing.

Table 7.0-1. Equipment, supplies, chemical reagents, and preparation of dilution water and chemical solutions used in the procedure for determination of 5-day biochemical oxygen demand

[NH_4Cl, ammonium chloride; BOD, biochemical oxygen demand; $CoCl_3$, cobalt chloride; DO, dissolved oxygen; NFM, *National Field Manual for the Collection of Water-Quality Data*; *N*, normality; KCl, potassium chloride; KH_2PO_4, potassium dihydrogen phosphate; $KHPO_4$, potassium monohydrogen phosphate; Na_2HPO_4, sodium monohydrogen phosphate; g, gram; L, liter; mL, milliliter; mm, millimeter; ±, plus or minus; °C, degrees Celsius]

Item	Description
Equipment and supplies	
Constant-temperature chamber or water bath	Thermostatically controlled to maintain 20 ± 1°C. During incubation, exclude all light to prevent the possibility of photosynthetic production of oxygen.
Aquarium pump, plastic air tubing, and air diffusion stones	Wash tubing and air diffusion stone thoroughly with a 0.2-percent nonphosphate detergent solution and rinse thoroughly 3 to 5 times with deionized or distilled water before use.
BOD bottles	300 mL, ground glass stoppered. Wash bottles thoroughly with a 0.2-percent nonphosphate detergent solution and rinse with deionized or distilled water before each test. Label bottles appropriately for sample identification.
Glass beads	Borosilicate, solid spherical; 5-mm diameter. Wash thoroughly with a 0.2-percent nonphosphate detergent solution and rinse with deionized or distilled water before use.
Graduated cylinder	Borosilicate, 50- to 250-mL capacity, depending on the volume of sample to be tested.
Overcap	Paper or plastic cup, or aluminum foil, to be placed over BOD stoppers to prevent evaporation of the water seal.
Pipet	Bacteriological, large bore, borosilicate, volume ranging from 1 to 50 mL, depending on the volume of sample to be tested.
Thermometer	Calibrated within temperature range of approximately 5-40°C with 0.5°C graduations (NFM 6.1).
Sample container(s)	Wide mouth, screwtop lid, polyethylene, polypropylene, or borosilicate glass. Containers of 1-L capacity are sufficient for most samples.
Waste disposal container(s)	Capped, and of appropriate material to contain specified sample and chemical wastes.
Chemical reagents[1] and preparation of dilution water	
Calcium chloride ($CaCl_2$) solution[2]	Dissolve 27.5 g of $CaCl_2$ in deionized water and dilute to 1 L.
Dilution water	Deionized water of high quality; must be free from toxic substances such as chlorine or toxic metals.
Ferric chloride ($FeCl_3$) solution[2]	Dissolve 0.25 g of $FeCl_3 \cdot 6H_2O$ in deionized water and dilute to 1 L.
Magnesium sulfate ($MgSO_4$) solution[2]	Dissolve 22.5 g of $MgSO_4 \cdot 7H_2O$ in deionized water and dilute to 1 L.
Phosphate buffer solution[2]	Dissolve 8.5 g of KH_2PO_4, 21.8 g of $KHPO_4$, 33.4 g of $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g of NH_4Cl in about 500 mL of deionized water. Dilute to 1 L.

Table 7.0-1. Equipment, supplies, chemical reagents, and preparation of dilution water and chemical solutions used in the procedure for determination of 5-day biochemical oxygen demand—*Continued*

Item	Description
Chemical reagents for sample pretreatment and preparation of chemical solutions	
Sodium hydroxide (NaOH) for caustic acidity pretreatment	Add 40 g of NaOH to about 900 mL of deionized water. Mix and dilute to 1 L (1 *N* NaOH). Store in a plastic container.
Sodium sulfite (Na_2SO_3) or sodium thiosulfate ($Na_2S_2O_3$) for residual chlorine pretreatment solution	Dissolve 1.575 g of Na_2SO_3 or NaS_2O_3 in 1 L of deionized water. This solution is not stable and should be prepared daily to weekly, as needed. Store refrigerated in a dark bottle.
Sulfuric acid (H_2SO_4) for caustic alkalinity pretreatment	Slowly and while stirring add 28 mL of concentrated H_2SO_4 to about 900 mL of deionized water. Mix and dilute acid solution to 1 L (1 *N* H_2SO_4).
DO equipment and supplies (refer to NFM 6.2)	
Calibration chamber	Follow manufacturer's recommendations.
DO instrument system	Temperature and pressure compensated.
Stirrer attachment for DO sensor	Must fit in 300-mL BOD bottle.
Pocket altimeter-barometer	Calibrated, Thommen™ model 2000 or equivalent.
DO sensor membrane replacement kit	Membranes, O-rings, KCl filling solution.
Oxygen solubility table	Refer to table 6.2-6 in NFM 6.2.
Zero DO calibration solution	Dissolve 1 g Na_2SO_3 and a few crystals of $CoCl_3$ in 1 L water. Prepare fresh zero DO solution before each use.

[1] Properly discard chemical reagents if there is any sign of biological growth or if past the expiration date.
[2] Can be purchased from the HACH™ Instrument Company in the form of nutrient buffer pillows ready for immediate use.

SAMPLE COLLECTION AND STORAGE 7.0.2

Samples can degrade significantly during extended storage. To minimize sample degradation, and thus avoid negative bias in the measurement of BOD_5, analyze samples promptly or store chilled without freezing (maintain a temperature from 1 to 4°C). Chilling the sample is not necessary if the analysis begins within 2 hours of collection (American Public Health Association and others, 1995).

- If a sample is refrigerated prior to analysis, allow the sample to warm to 20°C before starting the test. A sample may be removed from an ice chest or refrigerator during transit to allow it to warm to 20°C before analysis begins.
- It is optimum to start the BOD_5 analysis immediately after sample collection to minimize changes in bacterial concentration.
- **The maximum holding time of a sample to be analyzed for BOD is 24 hours.**

Do not freeze samples.

Bacteria are commonly associated with suspended sediment, which can vary spatially and temporally along a stream cross section (Britton and Greeson, 1989). Like suspended sediment, the oxygen-demanding compounds may not be equally distributed along a cross section. Where possible, use the equal-width-increment or equal-discharge-increment procedures described in NFM 4 to collect a BOD sample representative of the stream cross section.

When using cross-sectional, depth-integrating, or discharge-weighted methods:

1. Use a DH-81 or D-77 sampler in most situations (NFM 2). If stream depths exceed 5m (meters) (16.4 feet), use the bag version of the D-77 sampler.
2. Clean all equipment thoroughly and rinse with sample water before use (NFM 3).
3. Collect samples using appropriate procedures and pour sample water into a compositing device (NFM 4; Edwards and Glysson, 1998).
4. Withdraw a composite sample from the sample-compositing device into a clean container of sufficient capacity to perform the desired BOD tests. The volume of sample depends on the number of BOD tests to be completed and any prior knowledge of BOD for the water of interest. Generally, a 1-liter (L) sample is sufficient.
5. Cap the container securely and protect the sample from light during transport to the laboratory for analysis.
6. Store the sample on ice if not processed and analyzed within 2 hours of collection.

If depth-width-integrated or discharge-weighted methods cannot be used, collect a grab sample by the hand-dip method. A grab sample can be collected directly from the stream using a clean container of sufficient capacity (American Public Health Association and others, 1995).

When collecting a hand-dipped sample:

1. Grasp the sample container near the base on the downstream side of the bottle.
2. Plunge the bottle opening downward below the water surface. **Avoid contact with the streambed during this process.**
3. Allow the sample container to fill with the opening pointed slightly upward into the current.
4. Cap the container securely and protect the sample from light during transport to the laboratory for analysis.

FIVE-DAY TEST FOR BIOCHEMICAL OXYGEN DEMAND 7.0.3

The BOD_5 test procedure is based on DO concentration and requires an accurate DO determination. Follow procedures described in NFM 6.2 to determine DO concentration. Iodometric titration or amperometric (DO meter) methods used to measure DO are used for the BOD_5 test procedure (American Public Health Association and others, 1995). The procedures presented below incorporate the amperometric method for determining DO concentration. Refer to section 6.2.1.B in NFM 6.2 if the iodometric method will be used to determine DO.

> TECHNICAL NOTE: If using the iodometric titration method to measure DO concentration, double the sample volume, number of dilutions, and number of bottles to account for determining an initial DO and a final DO.

SAMPLE PREPARATION 7.0.3.A

Most relatively unpolluted streams have a BOD_5 that ranges from 1 to 8 mg/L (milligrams per liter) (Nemerow, 1974). If the BOD_5 value of a sample is less than 7 mg/L, sample dilution is not needed. A BOD_5 value greater than 7 mg/L requires sample dilution. Dilution is necessary when the amount of DO consumed by microorganisms is greater than the amount of DO available in the air-saturated BOD_5 sample (American Public Health Association and others, 1995). The BOD_5 analyst is responsible for determining the dilution(s) that will be needed. Table 7.0-2 provides general dilutions based on anticipated ranges of BOD_5 (Sawyer and McCarty, 1978).

BOD_5 values are acceptable only if the following criteria are met:

- The DO concentration after 5 days must be at least 1 mg/L and at least 2 mg/L lower in concentration than the initial DO (American Public Health Association and others, 1995).

- At least three different dilutions are set per sample to cover the anticipated range of BOD. The three sample volumes used are selected to provide an overlapping range in expected BOD concentrations. For example, if the BOD_5 is known to range from 3 to 28 mg/L for a particular stream, then the sample volumes used for the test would be 50 mL, 100 mL, and 300 mL (no dilution). If there is no prior knowledge of the BOD_5 of the stream water, use a minimum of four volumes to accommodate a range of BOD_5 from 0 to 210 mg/L.

When less than a 300-mL sample is to be analyzed, sample volumes are added to a standard solution of dilution water to bring the total sample volume to 300 mL. Because bacteria need nutrients and micronutrients to survive, these compounds are added to the dilution water. Similarly, the pH of the dilution water needs to be maintained in a range suitable for bacterial growth (6.5 to 7.5). Consequently, sulfuric acid or sodium hydroxide may need to be added to the dilution water to lower or raise the pH, respectively.

Some types of sewage, such as untreated industrial wastes, disinfected wastes, and wastes that have been heated to a high temperature contain too few bacteria to perform the test. Thus, the samples must be seeded with a population of microorganisms to produce an oxygen demand. Discussion of the seeding procedure is beyond the scope of this chapter. Most natural waters contain an adequate amount of microorganisms. For guidance on seeding procedures, including the BOD_5 equation when dilution water is seeded, refer to American Public Health Association and others (1995).

Table 7.0-2. Recommended sample volumes for the 5-day biochemical oxygen demand test

[Adapted from Sawyer and McCarty, 1978. BOD_5, 5-day biochemical oxygen demand]

Anticipated range of BOD_5 (in milligrams per liter)	Milliliters of sample	Milliliters of dilution water
0–7	300	0
6–21	100	200
12–42	50	250
30–105	20	280
60–210	10	290
120–420	5	295
300–1,050	2	298
600–2,100	1	299

INTERFERENCES 7.0.3.B

Certain constituents present in a water sample can inhibit biochemical oxidation and interfere with the BOD analysis. Interferences in the BOD analysis include caustic alkalinity or acidity; the presence of residual chlorine; or the presence of toxic elements, including trace elements such as copper, lead, chromium, mercury, and arsenic, or compounds such as cyanide. Procedures for pretreating samples for some common interferences are described in this chapter. Refer to American Public Health Association and others (1995) for further guidance on sample seeding and pretreatment.

The following preparations are needed before implementing the BOD_5 test procedure:

1. Prepare dilution water 3 to 5 days before initiating BOD_5 tests to ensure that the BOD of the dilution water is less than 0.2 mg/L. **Discard dilution water if there is any sign of biological growth.**
2. Determine sample pH. Adjust sample to a pH between 6.5 and 7.5, if necessary, using sulfuric acid (H_2SO_4) for samples with pH greater than 7.5 or sodium hydroxide (NaOH) for samples with pH less than 6.5 (American Public Health Association and others, 1995).
3. Add sodium sulfite (Na_2SO_3) to remove residual chlorine, if necessary. Samples containing toxic metals, arsenic, or cyanide often require special study and pretreatment (American Public Health Association and others, 1995). Samples must be seeded after pretreatment.

7.0.3.C BOD_5 TEST PROCEDURE

Troubleshooting suggestions are provided in section 7.0.5 (table 7.0-3).

1. Determine the amount of sample to be analyzed; if available, use the historical results of a previous test of BOD_5 for a particular sampling site, and refer to table 7.0-2.
2. Place a clean, calibrated thermometer into the constant temperature chamber. (See NFM 6.1 for thermometer care and calibration.)
3. Turn on the constant temperature chamber to allow the controlled temperature to stabilize at 20°C ±1°C.
4. Turn on the DO instrument, but not the stirring attachment. Some DO instruments need to be turned on 30 to 60 minutes before calibration—check the manufacturer's instruction manual.
5. Aerate dilution water before adding nutrient solutions.

6. After aeration,

   a. Add to dilution water

      - 1 mL each of the potassium phosphate, magnesium sulfate, calcium chloride, and ferric chloride solutions per 1 L of dilution water, or
      - Hach Company nutrient buffer pillows to a selected volume of dilution water per the manufacturer's recommendation.

   b. Shake the container of dilution water for about 1 minute to dissolve the slurry and to saturate the water with oxygen.

   c. Place the dilution water in the constant temperature chamber to maintain a temperature of 20°C until sample dilutions and analyses begin.

   d. The initial and final (after 5 days ± 4 hours) DO tests of the dilution water is determined and recorded simultaneously with each batch of environmental samples.

7. Check the temperature of the air incubator or water bath using a laboratory thermometer to ensure that the temperature has been maintained at 20° ± 1°C. A minimum/maximum recording thermometer can be used to audit the temperature during times when checks cannot be made.

8. Place the sample container in the constant-temperature chamber or water bath to begin warming the sample to 20°C ± 1°C. While the sample is warming, insert the air diffusion stone into the container and aerate the sample for about 15 minutes. After removing the air diffusion stone, allow several minutes for excess air bubbles to dissipate. The initial DO of the BOD sample needs to be at or slightly below saturation.

9. **Prepare dilutions as required—**Measure the appropriate amounts of sample necessary for the analysis. BOD_5 dilutions should result in a DO residual of at least 1 mg/L and a DO depletion of at least 2 mg/L after a 5-day incubation to produce the most reliable results. Prepare the dilutions to obtain a DO uptake in this range using the dilution water prepared earlier.

   a. For each subsample, mix thoroughly by inverting 20 times.

      - Use a large-bore pipet for sample volumes less than 50 mL. Withdraw a subsample that is representative of all the particle sizes present.

- Use a graduated cylinder for sample volumes greater than or equal to 50 mL.

b. Dilute two additional samples to bracket the appropriate dilution by a factor of two to three. Prepare at least three samples diluted according to volumes specified in table 7.0-2.

c. Pour the sample from the pipet or graduated cylinder into a clean BOD bottle.

- Agitate the dilution water and fill the remaining portion of the BOD bottle with dilution water.
- Prepare three samples containing only dilution water. These samples serve as blanks for quality control. If two of the three samples meet the blank-water criterion, accept the data.

10. Calibrate the DO instrument in accordance with the procedures outlined in NFM 6.2.

11. After bringing the samples to saturation and preparing the dilutions (steps 8 and 9 above), measure the initial DO concentration (D_1) of each sample and each dilution blank.

    a. Carefully insert the self-stirring sensor into the BOD bottle, avoiding air entrapment.

    b. Turn on the stirrer and allow 1 to 2 minutes for the DO and temperature readings to stabilize.

12. Record the bottle number, date, time, and D_1 on a form similar to that shown in figure 7.0-2.

13. Turn off the stirrer and remove the sensor from the BOD bottle. Rinse the sensor and stirrer with deionized water from a wash bottle. Discard rinse water into a waste container.

14. Add glass beads to the BOD bottle, if necessary, to displace the sample up to the neck of the bottle so that inserting a glass stopper will displace all air, leaving no bubbles.

15. Carefully cap the BOD bottle with the ground-glass stopper. Tip the bottle to one side and check for an air bubble.

    - If an air bubble is present, add glass beads to the bottle until the bubble is removed. Cap the bottle and check again for an air bubble. Repeat if necessary.
    - If no bubble is present in the sample, create a water seal by adding distilled or deionized water to the top of the BOD bottle around the glass stopper. Then place the overcap over the stopper on the BOD bottle to minimize evaporation from the water seal.

16. Place the sealed BOD sample in the air incubator or water bath and incubate the sample at 20°C ± 1°C for 5 days.

17. At the end of 5 days ± 4 hours, remove the BOD bottles from the incubator, remove the overcap, pour off the water seal, remove the ground-glass stopper, and measure the final DO concentration (D_2).

    - The DO uptake ($DO_{0\ days}$ - $DO_{5\ days}$) in the dilution water should not be greater than 0.2 mg/L and preferably not more than 0.1 mg/L. **Exceeding the 0.2-mg/L criterion could be grounds for rejecting results of the BOD analysis of the environmental sample**.
    - Dilution water of poor quality will cause an oxygen demand and appear as sample BOD. Improve purification or get the dilution water from another source if DO uptake exceeds 0.2 mg/L (see section 7.0.5, Troubleshooting).

18. Complete the field form by recording the date, time, and D_2 for each respective sample bottle (fig. 7.0-2).

Quality control. The BOD_5 test can be quite variable. Collect sufficient field and split replicates (10 to 20 percent) to provide an estimate of method variability.

5-Day Biochemical Oxygen Demand (BOD_5) Worksheet

Site/station:_____ **Collection date and time:**_____
Project:_____ **Personnel:**_____

Dilution-water blanks

Bottle number	Initial DO reading (D_1)	Date/time of reading	Final DO reading (D_2)	Date/time of reading	BOD (D_1-D_2)	BOD average (<0.2 mg/L)

Environmental sample

Bottle number	Sample size (mL)	Initial DO reading (D_1)	Date/ time of reading	Final DO reading (D_2)	Date /time of reading	BOD $\frac{D_1 - D_2}{P}$	BOD average (mg/L)

If dilution-water demand is <0.2 milligrams per liter (mg/L), use

$$BOD_5\ (mg/L) = \frac{D_1 - D_2}{P}$$

where

D_1 = initial sample dissolved-oxygen (DO) concentration (in mg/L)
D_2 = sample DO (in mg/L) after 5 days
P = decimal volumetric fraction of sample used

Figure 7.0-2. Example of a 5-day biochemical oxygen demand worksheet.

CALCULATIONS 7.0.4

The general equation for the determination of a BOD_5 value is:

$$BOD_5\,(in\ mg/L) = \frac{D_1 - D_2}{P}$$

where D_1 = initial DO of the sample,
D_2 = final DO of the sample after 5 days, and
P = decimal volumetric fraction of sample used.

If 100 mL of sample are diluted to 300 mL, then $P = \frac{100}{300} = 0.33$. Notice that if no dilution was necessary, $P = 1.0$ and the BOD_5 is determined by D_1 - D_2.

If more than one dilution of the sample results in residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L, and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average the results that are in the acceptable range (American Public Health Association and others, 1995).

7.0.5 TROUBLESHOOTING

The troubleshooting suggestions in table 7.0-3 are not all-inclusive. Refer to the troubleshooting suggestions for DO instruments (table 6.2-4 in NFM 6.2). Remember that faulty batteries can cause erratic readings.

Table 7.0-3. Troubleshooting guide for the 5-day biochemical oxygen demand test

[DO, dissolved oxygen; BOD_5, 5-day biochemical oxygen demand; mg/L, milligrams per liter; HCl, hydrochloric acid]

Symptom	Possible cause and corrective action
DO readings drift downward	• Weak batteries for stirring unit result in inadequate flow across membrane—replace batteries.
BOD_5 demand in dilution water is greater than the acceptable 0.2 mg/L	• Deionized water contains ammonia or volatile organic compounds—increase purity of dilution water or obtain from another source. Age water for 5-10 days before use. • Deionized water contains semivolatile organic compounds leached from the resin bed—increase purity of dilution water or obtain from another source. Age water for 5-10 days before use. • Bacterial growth in reagents and poorly cleaned glassware—more vigorous cleaning of glassware, including washing followed by a 5- to 10-percent HCl rinse followed by 3-5 rinses with deionized water. Discard reagents properly.
Sample BOD values are unusually low in the diluted sample (BOD_5 dilution water is within the acceptable range)	• Dilution water contains interferences inhibiting the biochemical oxidation process—increase purity of dilution water or obtain from another source. • Use deionized water that has been passed through mixed-bed resin columns. **Never use copper-lined stills.** Distilled water may be contaminated by using copper-lined stills or copper fittings—obtain from another source.

REPORTING 7.0.6

When reporting results of a BOD_5 test, be sure to use the correct parameter code.

- Report BOD_5 values less than 2 mg/L as <2 mg/L rather than as 2.0 mg/L.
- Report BOD_5 values less than 10 mg/L to the nearest 0.1 mg/L.
- Report BOD_5 values greater than or equal to 10 mg/L to two significant figures.
- Report the results of replicate samples and dilution blanks with the BOD_5 results.