

FIVE-DAY TEST FOR 7.2.3 BIOCHEMICAL OXYGEN DEMAND

The BOD₅ 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 BOD₅ 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 7.2.3.A

Most relatively unpolluted streams have a BOD₅ that ranges from 1 to 8 mg/L (milligrams per liter) (Nemerow, 1974). If the BOD₅ value of a sample is less than 7 mg/L, sample dilution is not needed. A BOD₅ 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 BOD₅ sample (American Public Health Association and others, 1995). The BOD₅ analyst is responsible for determining the dilution(s) that will be needed. Table 7.2-2 provides general dilutions based on anticipated ranges of BOD₅ (Sawyer and McCarty, 1978).

BOD₅ 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 BOD₅ 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 BOD₅ of the stream water, use a minimum of four volumes to accommodate a range of BOD₅ from 0 to 210 mg/L.

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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 BOD₅ equation when dilution water is seeded, refer to American Public Health Association and others (1995).

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Table 7.2-2. Recommended sample volumes for the five-day biochemical oxygen demand test

[Adapted from Sawyer and McCarty, 1978. BOD₅, 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 7.2.3.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 BOD₅ test procedure:

1. Prepare dilution water 3 to 5 days before initiating BOD₅ 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 from 6.5 to 7.5, if necessary, using sulfuric acid (H₂SO₄) 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 (Na₂SO₃) 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.

7.2.3.C BOD₅ TEST PROCEDURE

Troubleshooting suggestions are provided in section 7.2.5 (table 7.2-3).

1. Determine the amount of sample to be analyzed; if available, use the historical results of a previous test of BOD₅ for a particular sampling site, and refer to table 7.2-2.
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 its 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™ 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. BOD₅ 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.

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- 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 7.2-2. +
 - 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 criteria, 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. Insert the self-stirring sensor into the BOD bottle carefully, 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 7.2-2.
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.
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- + 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 $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 days.
17. At the end of 5 days \pm 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 ($\text{DO}_{0 \text{ days}} - \text{DO}_{5 \text{ days}}$) 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 criteria 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 7.2.5, Troubleshooting).
- + 18. Complete the field form by recording the date, time, and D_2 for each respective sample bottle (fig. 7.2-2).

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.

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5-Day Biochemical Oxygen Demand (BOD₅) 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

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

$$\text{BOD}_5 \text{ (mg/L)} = \frac{D_1 - D_2}{P}$$

where

- D₁ = initial sample dissolved-oxygen (DO) concentration (in mg/L)
- D₂ = sample DO (in mg/L) after 5 days
- P = decimal volumetric fraction of sample used

Figure 7.2-2. Example of a five-day biochemical oxygen demand worksheet.