

Techniques of Water-Resources Investigations

Book 9

Handbooks for Water-Resources Investigations

National Field Manual  
for the Collection of  
Water-Quality Data

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Chapter A7.  
**BIOLOGICAL INDICATORS**

*Edited by*  
D.N. Myers and F.D. Wilde



U.S. DEPARTMENT OF THE INTERIOR  
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## CHAPTER A7

### Replacement Pages

### *National Field Manual for the Collection of Water-Quality Data*

This package contains:

Replacement pages for the Contents, Abstract, and Introduction to Chapter A7. Chapter A7 is contained in the binder for Chapters A6–9 of the *National Field Manual for the Collection of Water Quality Data*.

U.S. Geological Survey Techniques of Water-Resources Investigations  
Book 9, Handbooks for Water Resources  
Section A. National Field Manual for the Collection of  
Water Quality Data  
**Chapter A7 Biological Indicators**

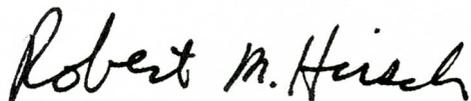
September 1999

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## FOREWORD

The mission of the Water Resources Division of the U.S. Geological Survey (USGS) is to provide the information and understanding needed for wise management of the Nation's water resources. Inherent in this mission is the responsibility to collect data that accurately describe the physical, chemical, and biological attributes of water systems. These data are used for environmental and resource assessments by the USGS, other government and scientific agencies, and the general public. Reliable and objective data are essential to the credibility and impartiality of the water-resources appraisals carried out by the USGS.

The development and use of a National Field Manual is necessary to achieve consistency in the scientific methods and procedures used, to document those methods and procedures, and to maintain technical expertise. USGS field personnel are instructed in the use of this manual to ensure that data collected are of the quality required to fulfill our mission.



Robert M. Hirsch  
Chief Hydrologist

## Techniques of Water-Resources Investigations

### Book 9

## Handbooks for Water-Resources Investigations

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# National Field Manual for the Collection of Water-Quality Data



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# Chapter A7. BIOLOGICAL INDICATORS

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## ABSTRACT

The *National Field Manual for the Collection of Water-Quality Data* (National Field Manual) provides guidelines and standard procedures for U.S. Geological Survey (USGS) personnel who collect data used to assess the quality of the Nation's surface-water and ground-water resources. This chapter of the manual presents procedures and guidelines for the collection, identification, and enumeration of fecal indicator bacteria in water and for the determination of biochemical oxygen demand using a 5-day bioassay test.

Each chapter of the National Field Manual is published separately and revised periodically. Newly published and revised chapters will be announced on the USGS Home Page on the World Wide Web under "New Publications of the U.S. Geological Survey." The URL for this page is <<http://water.usgs.gov/lookup/get?newpubs>>.

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## INTRODUCTION

As part of its mission, the U.S. Geological Survey (USGS) collects data needed to determine the quality of our Nation's water resources. A high degree of reliability and standardization of these data are paramount to fulfilling this mission. Documentation of nationally accepted methods used by USGS personnel serves to maintain consistency and technical quality in data-collection activities. *The National Field Manual for the Collection of Water-Quality Data (National Field Manual)* describes protocols (required and recommended procedures) and provides guidelines for USGS personnel who collect those data on surface-water and ground-water resources. Chapter A7 includes procedures for the collection, identification, and enumeration of fecal indicator bacteria in water and for the determination of biochemical oxygen demand using a 5-day bioassay test.

The *National Field Manual* is Section A of Book 9 of the USGS publication series "Techniques of Water-Resources Investigations" (TWRI) and consists of individually published chapters designed to be used in conjunction with each other. A list of TWRI publications is included at the end of this chapter. Chapter numbers are preceded by an "A" to indicate that the report is part of the *National Field Manual*. Other chapters of the *National Field Manual* are referred to in the text by the abbreviation "NFM" and the specific chapter number (or chapter and section number). For example, NFM 6 refers to chapter A6 on "Field Measurements" and NFM 6.4 refers to the section in Chapter A6 on field measurement of pH.

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## PURPOSE AND SCOPE

The *National Field Manual* is targeted specifically toward field personnel in order to (1) establish and communicate scientifically sound methods and procedures, (2) encourage consistency in the use of field methods for the purpose of producing nationally comparable data, (3) provide methods that minimize biasing the data and, when properly applied, result in data that are reproducible within defined limits of variability, and (4) provide citable documentation for USGS water-quality data-collection protocols.

**Data collectors must have formal training and field apprenticeship in order to correctly implement the procedures described in this chapter.** The *National Field Manual* is meant to complement such training. The information provided in Section 7.1 (Fecal Indicator Bacteria) is to be used in conjunction with *Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples* edited by L.J. Britton and P.E. Greeson (TWRI, Book 5, Chapter A4, 1989). A description of the determination for ultimate carbonaceous biochemical oxygen demand is beyond the scope of Section 7.2 (Five-Day Biochemical Oxygen Demand), but is provided in Stamer and others (1979, 1983).

It is impractical to provide guidance that would encompass the entire spectrum of data-collection objectives, site characteristics, environmental conditions, and technological advances related to water-quality studies. The fundamental responsibility of data collectors is to select methods that are compatible with the scientific objective for the field work and to use procedures that are consistent with USGS standard procedures to the extent possible. Under some circumstances, data collectors may have to modify standard procedures. Whenever a standard procedure is modi-

fied or an alternative procedure is used, a description of the procedure used and supporting quality-assurance information are to be reported with the data.

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## REQUIREMENTS AND RECOMMENDATIONS

As used in the *National Field Manual*, the terms **required** and **recommended** have the following USGS-specific meanings.

**Required** (require, required, or requirements) pertains to USGS protocols and indicates that USGS Office of Water Quality policy has been established on the basis of research and (or) consensus of the technical staff and has been reviewed by water-quality specialists and selected District<sup>1</sup> or other professional personnel, as appropriate. Technical memorandums or other internal documents that define the policy pertinent to such requirements are referenced in this chapter. Personnel are instructed to use required equipment or procedures defined herein. Departure from or modifications to the stipulated requirements that might be necessary to accomplishing specific data-quality requirements or study objectives must be based on referenced research and good field judgment, and be quality assured and documented.

**Recommended** (recommend, recommended, recommendation) pertains to USGS protocols and indicates that USGS Office of Water Quality policy recognizes that one or several alternatives to a given equipment selection or procedure are acceptable on the basis of research and (or) consensus. References to technical memorandums and selected publications pertinent to such recommendations are cited in this chapter to the extent that such documents are available. Specific data-quality requirements, study objectives, or other constraints may affect the choice of recommended equipment or procedures. Selection from among the recommended alternatives should be based on referenced research and good field judgment, and reasons for the selection must be documented. Departure from or modifications to recommended procedures must be quality assured and documented.

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<sup>1</sup>**Distict** refers to an office of the USGS, Water Resources Division, located in any of the States or territories of the United States.

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## FIELD MANUAL REVIEW AND REVISION

Chapters of the *National Field Manual* will be reviewed, revised, and reissued periodically to correct any errors, incorporate technical advances, and address additional topics. Comments or corrections can be sent to: NFM-QW, USGS, 412 National Center, Reston, VA 20192 (or send electronic mail to: [nfm-owq@usgs.gov](mailto:nfm-owq@usgs.gov)). Information regarding the status and any errata of this or other chapters can be found at the beginning of the electronic version of each chapter, located in the Publications section of the following website: <http://water.usgs.gov/lookup/get?owq>.

Newly published and revised chapters will be announced on the USGS Home Page on the World Wide Web under "New Publications of the U.S. Geological Survey." The URL for this page is <http://water.usgs.gov/lookup/get?newpubs>.

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## ACKNOWLEDGMENTS

The information included in the *National Field Manual* is based on existing manuals, a variety of reference documents, and a broad spectrum of colleague expertise. In addition to the references provided, important source materials included unpublished USGS training and field manuals and technical memorandums. The editors and authors wish to acknowledge the following individuals in the USGS who developed the field and training manuals that provided the foundation for this *National Field Manual*: M.E. Dorsey, T.K. Edwards, W.B. Garrett, W.J. Gibbons, L.R. Kister, J.R. Knapton, C.E. Lamb, R.F. Middleburg, J. Rawson, L.R. Shelton, M.A. Sylvester, and F.C. Wells.

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# FIVE-DAY 7.2 BIOCHEMICAL OXYGEN DEMAND

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

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## CHAPTER A7

### Section 7.2

# of the U.S. Geological Survey *National Field Manual for the Collection of Water-Quality Data*

This package contains:

**Section 7.2, Five-Day Biochemical Oxygen Demand**, which is to be added to Chapter A7 contained in the binder for Chapters A6-9 of the *National Field Manual for the Collection of Water-Quality Data*. Also included in this package are replacement pages for the Contents, Abstract, and Introduction of Chapter A7.

U.S. Geological Survey Techniques of Water-Resources Investigations

Book 9, Handbooks for Water-Resources

Section A. *National Field Manual for the Collection of  
Water-Quality Data*

**Chapter A7 Biological Indicators**

**Section 7.2 Five-Day Biochemical Oxygen Demand**

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# FIVE-DAY BIOCHEMICAL OXYGEN DEMAND 7.2

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).

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 District laboratory.

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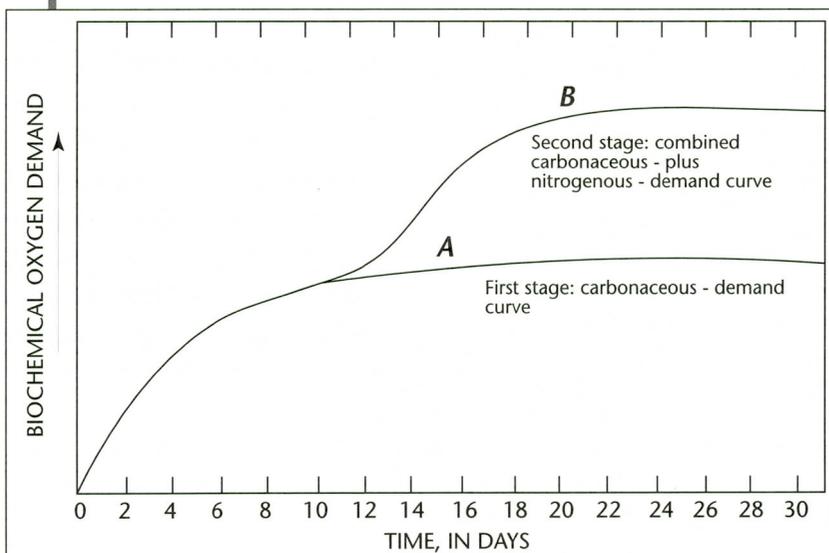
**Biochemical oxygen demand represents the amount of oxygen consumed by bacteria and other microorganisms while they decompose organic matter under aerobic conditions at a specified temperature.**

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**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. 7.2-1).

- ▶ 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 nitrogenous 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 7.2-1.** Biochemical oxygen demand curves: (A) typical carbonaceous-demand curve showing the oxidation of organic matter, and (B) typical carbonaceous- plus nitrogenous-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 ( $BOD_5$ ). 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."

## 7.2.1 EQUIPMENT AND SUPPLIES

Table 7.2-1 lists equipment and supplies commonly used in the BOD<sub>5</sub> 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 7.2-1.** Equipment, supplies, chemical reagents, and preparation of dilution water and chemical solutions used in the procedure for determination of five-day biochemical oxygen demand

[±, plus or minus; °C, degrees Celsius; BOD, biochemical oxygen demand; mL, milliliter; mm, millimeter; NFM, *National Field Manual for the Collection of Water-Quality Data*; L, liter; g, gram;  $\text{KH}_2\text{PO}_4$ , potassium dihydrogen phosphate;  $\text{KHPO}_4$ , potassium monohydrogen phosphate;  $\text{Na}_2\text{HPO}_4$ , sodium monohydrogen phosphate;  $\text{NH}_4\text{Cl}$ , ammonium chloride; *N*, normality; KCl, potassium chloride; DO, dissolved oxygen;  $\text{CoCl}_3$ , cobalt chloride]

Item	Description
<b>Equipment and supplies</b>	
Constant temperature chamber or water bath	Thermostatically controlled to maintain $20 \pm 1^\circ\text{C}$ . 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 $5\text{--}40^\circ\text{C}$ with $0.5^\circ\text{C}$ 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.
<b>Chemical reagents<sup>1</sup> and preparation of dilution water</b>	
Calcium chloride ( $\text{CaCl}_2$ ) solution <sup>2</sup>	Dissolve 27.5 g of $\text{CaCl}_2$ 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 ( $\text{FeCl}_3$ ) solution <sup>2</sup>	Dissolve 0.25 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in deionized water and dilute to 1 L.
Magnesium sulfate ( $\text{MgSO}_4$ ) solution <sup>2</sup>	Dissolve 22.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in deionized water and dilute to 1 L.
Phosphate buffer solution <sup>2</sup>	Dissolve 8.5 g of $\text{KH}_2\text{PO}_4$ , 21.8 g of $\text{KHPO}_4$ , 33.4 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , and 1.7 g of $\text{NH}_4\text{Cl}$ in about 500 mL of deionized water. Dilute to 1 L.

**Table 7.2-1.** Equipment, supplies, chemical reagents, and preparation of dilution water and chemical solutions used in the procedure for determination of five-day biochemical oxygen demand—*Continued*

Item	Description
<b>Chemical reagents for sample pretreatment and preparation of chemical solutions</b>	
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 <sub>2</sub> SO <sub>3</sub> ) or sodium thiosulfate (Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ) for residual chlorine pretreatment solution	Dissolve 1.575 g of Na <sub>2</sub> SO <sub>3</sub> or Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> 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 <sub>2</sub> SO <sub>4</sub> ) for caustic alkalinity pretreatment	Slowly and while stirring add 28 mL of concentrated H <sub>2</sub> SO <sub>4</sub> to about 900 mL of deionized water. Mix and dilute acid solution to 1 L (1 N H <sub>2</sub> SO <sub>4</sub> ).
<b>DO equipment and supplies (refer to NFM 6.2)</b>	
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 <sub>2</sub> SO <sub>3</sub> and a few crystals of CoCl <sub>2</sub> in 1 L water. Prepare fresh zero DO solution before each use.

<sup>1</sup>Properly discard chemical reagents if there is any sign of biological growth or if past the expiration date.

<sup>2</sup>Can be purchased from the HACH™ Instrument Company in the form of nutrient buffer pills ready for immediate use.

## SAMPLE COLLECTION 7.2.2 AND STORAGE

Samples can degrade significantly during extended storage. To minimize sample degradation, and thus avoid negative bias in the measurement of BOD<sub>5</sub>, 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<sub>5</sub> 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.**

**Never 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 5 meters, 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-L sample is sufficient.
5. Cap container securely and protect the sample from light during transport to the laboratory for analysis.
6. Store 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 a 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 7.2.3 BIOCHEMICAL OXYGEN DEMAND

The BOD<sub>5</sub> 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<sub>5</sub> 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 BOD<sub>5</sub> that ranges from 1 to 8 mg/L (milligrams per liter) (Nemerow, 1974). If the BOD<sub>5</sub> value of a sample is less than 7 mg/L, sample dilution is not needed. A BOD<sub>5</sub> 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<sub>5</sub> sample (American Public Health Association and others, 1995). The BOD<sub>5</sub> analyst is responsible for determining the dilution(s) that will be needed. Table 7.2-2 provides general dilutions based on anticipated ranges of BOD<sub>5</sub> (Sawyer and McCarty, 1978).

***BOD<sub>5</sub> 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 7.2-2.** Recommended sample volumes for the five-day biochemical oxygen demand test[Adapted from Sawyer and McCarty, 1978. BOD<sub>5</sub>, 5-day biochemical oxygen demand]

Anticipated range of BOD <sub>5</sub> (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 BOD<sub>5</sub> test procedure:*

1. Prepare dilution water 3 to 5 days before initiating BOD<sub>5</sub> 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 (H<sub>2</sub>SO<sub>4</sub>) 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<sub>2</sub>SO<sub>3</sub>) 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 BOD<sub>5</sub> 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 BOD<sub>5</sub> 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  $\pm$  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°  $\pm$  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  $\pm$  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<sub>5</sub> 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 7.2-2.
  - 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.

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  $\text{BOD}_5$  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<sub>5</sub>) 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

$D_1$  = 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 7.2-2.** Example of a five-day biochemical oxygen demand worksheet.

## CALCULATIONS 7.2.4

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

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

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).

## 7.2.5 TROUBLESHOOTING

The troubleshooting suggestions in table 7.2-3 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 7.2-3.** Troubleshooting guide for the five-day biochemical oxygen demand test

[DO, dissolved oxygen; BOD<sub>5</sub>, 5-day biochemical oxygen demand; mg/L, milligram per liter; HCl, hydrochloric acid]

Symptom	Possible cause and corrective action
DO readings drift downward	<ul style="list-style-type: none"> <li>Weak batteries for stirring unit result in inadequate flow across membrane—replace batteries.</li> </ul>
BOD <sub>5</sub> demand in dilution water is greater than the acceptable 0.2 mg/L	<ul style="list-style-type: none"> <li>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.</li> <li>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.</li> <li>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.</li> </ul>
Sample BOD values are unusually small in the diluted sample (BOD <sub>5</sub> dilution water is within the acceptable range)	<ul style="list-style-type: none"> <li>Dilution water contains interferences inhibiting the biochemical oxidation process—increase purity of dilution water or obtain from another source.</li> <li>Use deionized water that has been passed through mixed-bed resin columns. <b>Never use copper-lined stills.</b> Distilled water may be contaminated by using copper-lined stills or copper fittings—obtain from another source.</li> </ul>

## REPORTING 7.2.6

When reporting results of a BOD<sub>5</sub> test, be sure to use the correct parameter code.

- ▶ Report BOD<sub>5</sub> values less than 2 mg/L as <2 mg/L rather than as 2.0 mg/L.
- ▶ Report BOD<sub>5</sub> values less than 10 mg/L to the nearest 0.1 mg/L.
- ▶ Report BOD<sub>5</sub> 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<sub>5</sub> results.

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