

5210 B. 5-Day BOD Test

1. General Discussion

The BOD test is an indirect measurement of organic matter; it measures the change in DO concentration caused by microorganisms as they degrade organic matter in a sample held in a stoppered bottle incubated for 5 d in the dark at 20°C. Analysts measure DO before and after incubation, and compute BOD using the difference between DO measurements. Because initial DO is determined shortly after dilution, all oxygen uptake occurring after this measurement is included in the BOD measurement.

For sampling and storage procedures, see 5210B.4a.

2. Apparatus

a. Incubation bottles: Use 60-mL, glass bottles or larger (300-mL bottles with a flared mouth and ground-glass stopper are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use. Alternatively, use disposable plastic BOD bottles that are capable of meeting all method quality-control (QC) checks.

b. Air incubator or water bath, thermostatically controlled at 20 ±1°C. Exclude all light to prevent the possibility of photosynthetic production of DO.

c. Oxygen-sensitive membrane electrode, polarographic or galvanic, or *oxygen-sensitive optical probe* with appropriate meter.

3. Reagents

Discard reagents if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable, and different stock concentrations may be used if doses are adjusted proportionally. Use reagent grade or better for all chemicals and use distilled or equivalent reagent-grade water (see Section 1060) to make all solutions.

a. Phosphate buffer solution: Dissolve 8.5 g monopotassium phosphate (KH_2PO_4), 21.75 g dipotassium phosphate (K_2HPO_4), 33.4 g disodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$), and 1.7 g ammonium chloride (NH_4Cl) in about 500 mL reagent-grade water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH_2PO_4 and 1.7 g NH_4Cl in about 700 mL reagent-grade water. Adjust pH to 7.2 with 30% sodium hydroxide (NaOH) and dilute to 1 L.

b. Magnesium sulfate (MgSO_4) solution: Dissolve 22.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in reagent-grade water and dilute to 1 L.

c. Calcium chloride (CaCl_2) solution: Dissolve 27.5 g CaCl_2 in reagent-grade water and dilute to 1 L.

d. Ferric chloride (FeCl_3) solution: Dissolve 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in reagent-grade water and dilute to 1 L.

e. Acid and alkali solutions, 1N, to neutralize caustic or acidic waste samples.

1) *Acid*—Slowly and while stirring, add 28 mL conc sulfuric acid (H_2SO_4) to reagent-grade water. Dilute to 1 L.

2) *Alkali*—Dissolve 40 g NaOH in distilled water. Dilute to 1 L.

f. Sodium sulfite (Na_2SO_3) solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL reagent-grade water. This solution is unstable; prepare daily.

g. Nitrofurantoin inhibitor:

1) 2-chloro-6-(trichloromethyl) pyridine (TCMP)—Use pure TCMP or commercial preparations.*

2) Allylthiourea (ATU) solution—Dissolve 2.0 g allylthiourea ($\text{C}_3\text{H}_7\text{N}_2\text{S}$) in about 500 mL reagent-grade water and dilute to 1 L. Store at 4°C. The solution is stable for 2 weeks when stored at 6°C without freezing.

h. Glucose-glutamic acid (GGA) solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to reagent-grade water and dilute to 1 L. Prepare fresh immediately before use unless solution is maintained in a sterile container. Store all GGA mixtures at 6°C without freezing unless manufacturer recommendations state otherwise. Commercial preparations may be used but concentrations may vary. Discard solutions if evi-

dence of contamination is indicated (e.g., growth occurs in the stock bottle or GGA test results are consistently low).

i. Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL reagent-grade water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

j. Source water for preparing BOD dilution water: Use demineralized, distilled, or equivalent reagent-grade water, tap, or natural water to make sample dilutions (see 5210B.4c).

4. Preparatory Procedures

a. Sampling and storage: Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values.

1) *Grab samples*—If analysis begins within 2 h of collection, cold storage is unnecessary. Otherwise, keep sample at 6°C between collection and analysis. Ideally, begin analysis within 6 h of sample collection; if impossible due to distance between sampling site and laboratory, then begin analysis within 24 h of collection. The recommended holding time is 24 h; however, the U.S. Environmental Protection Agency (EPA) allows for a 48-h holding time.

2) *Composite samples*—Limit compositing period to 24 h, and keep samples at 6°C during process. Store for the same time and temperature as grab samples, although in this case, holding time begins when the compositing period ends.

b. Sample preparation and pretreatment:

1) *All samples*—Check pH; if it is not between 6.0 and 8.0, adjust sample temperature to 20 ±3°C, then adjust pH to between 6.5 and 7.5 using an H_2SO_4 or NaOH solution strong enough not

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