



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L306

Biochemical Oxygen Demand (BOD₅)

Standard Operating Procedure

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1. Purpose

This SOP describes the determination of five-day biochemical oxygen demand (BOD₅) in water and wastewater samples using the manometric method (WTW OxiTop system). BOD₅ is a measure of the dissolved oxygen consumed by microorganisms during the aerobic decomposition of organic matter over a five-day incubation period at 20 °C and is the standard regulatory indicator of organic pollution load in water (APHA et al., 2012; U.S. Environmental Protection Agency, 1978).

2. Scope

- **Applies to:** Surface water, wastewater, and effluent samples analyzed for BOD₅ using the WTW OxiTop manometric method in the EA Program laboratory
- **Does not apply to:** Carbonaceous BOD (CBOD) with nitrification inhibitor; BOD by Winkler titration or dissolved oxygen probe methods; chemical oxygen demand (COD)
- **Location:** Biogeochemistry Laboratory, Pomona College

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Approve SOP; review QC data; maintain equipment
Trained Personnel	Prepare reagents, run analyses, record results
New Students	Complete training before independent use

4. Definitions

- **Biochemical oxygen demand (BOD):** The mass of dissolved oxygen consumed per unit volume of sample by microbial oxidation of organic matter under aerobic conditions at 20 °C; expressed in mg/L (APHA et al., 2012).
- **BOD₅:** BOD measured over a 5-day incubation period at 20 °C; the standard regulatory measure of organic pollution load in surface water and effluent (APHA et al., 2012; U.S. Environmental Protection Agency, 1978).
- **Carbonaceous BOD (CBOD):** BOD attributable to carbon-containing organic compounds; measured in the presence of a nitrification inhibitor (allylthiourea) to prevent oxygen consumption by nitrifying bacteria from contributing to the result.

- **Dilution water:** Aerated, buffered, nutrient-supplemented deionized water used to dilute concentrated samples to bring the expected BOD₅ into the measurable range of the OxiTop system (APHA et al., 2012).
- **Glucose-glutamic acid (GGA) check standard:** A standard seed-check solution prepared at 150 mg/L glucose + 150 mg/L glutamic acid; expected BOD₅ of 198 ±30.5 mg/L; used to verify seed quality and method performance (APHA et al., 2012, Method 5210 B).
- **Manometric method (OxiTop):** A closed-system BOD measurement technique in which O₂ consumption is tracked by measuring the resulting decrease in headspace pressure; CO₂ produced during microbial respiration is absorbed by NaOH pellets, ensuring that pressure changes reflect only oxygen consumption (WTW (Xylem Analytics), 2019).
- **Nitrification:** Microbial oxidation of ammonia to nitrate by nitrifying bacteria; consumes dissolved oxygen and can inflate BOD values if not inhibited in carbonaceous BOD analyses.
- **Seeding:** Addition of a microbial inoculum (e.g., diluted wastewater or settled sewage) to dilution water to ensure adequate microbial activity when samples lack native microorganisms (APHA et al., 2012).

5. Safety and Hazards

Hazards

- **Chemical:** Nutrient buffer solutions contain phosphate and ammonium salts; NaOH pellets (CO₂ absorbent) are corrosive. Handle with gloves and avoid skin or eye contact.
- **Biological:** Wastewater samples may contain pathogens. Treat all samples as potentially infectious.
- **Physical:** Glass BOD bottles are fragile. Handle with care to avoid breakage.

5.1. Required PPE

- ☐ Safety glasses or goggles
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

In case of chemical spill or exposure, refer to [SOP-L004 Chemical Spill Response](#) or call Campus Safety. For NaOH contact with skin or eyes, flush immediately with copious water for at least 15 minutes and seek medical attention.

6. Equipment and Materials

6.1. Equipment

Item	Notes
WTW OxiTop manometers (3 per run)	One blank + two samples minimum; check battery (WTW (Xylem Analytics), 2019)
BOD ₅ incubation bottles (nominal 500 mL)	Brown glass with neck adapter
Rubber quivers (CO ₂ absorber holders)	One per bottle
Incubator at 20.0 ±1.0 °C	Verify temperature daily
Magnetic stirrer platform (in incubator)	Low-speed continuous stirring
Magnetic stir bars	One per bottle
500 mL graduated cylinder	
Micropipette, 100–1000 µL	With disposable tips
pH meter	Calibrated per SOP-L300
Air pump with diffuser stone	For aerating dilution water

6.2. Consumables

Item	Storage	Notes
NaOH pellets	Desiccator	CO ₂ absorbent; 2 pellets per bottle
Phosphate buffer stock	Refrigerator	See §6
MgSO ₄ , CaCl ₂ , FeCl ₃ solutions	Shelf	See §6
Seed inoculum (if needed)	Fresh	Settled domestic wastewater or commercial seed

7. Reagents and Dilution Water

7.1. Nutrient Stock Solutions

Prepare the following four stock solutions; add 1 mL of each per liter of dilution water (APHA et al., 2012):

1. **Phosphate buffer:** Dissolve 8.5 g KH₂PO₄, 21.75 g K₂HPO₄, 33.4 g Na₂HPO₄ · 7H₂O, and 1.7 g NH₄Cl in ~500 mL deionized water and dilute to 1 L (pH 7.2).
2. **Magnesium sulfate:** Dissolve 22.5 g MgSO₄ · 7H₂O in deionized water; dilute to 1 L.
3. **Calcium chloride:** Dissolve 27.5 g CaCl₂ in deionized water; dilute to 1 L.
4. **Ferric chloride:** Dissolve 0.25 g FeCl₃ · 6H₂O in deionized water; dilute to 1 L.

7.2. Dilution Water Preparation

1. Fill a clean 1 L vessel with deionized water.
2. Add 1 mL of each stock solution.
3. Aerate with an air pump for at least 1 hour to bring DO near saturation.
4. Verify pH is 6.8–7.2; adjust with dilute HCl or NaOH if necessary.
5. Allow to equilibrate to 20 °C before use.

7.3. Measuring Range and Sample Volume Selection

Table 1: Volume selection for WTW OxiTop BOD₅ analysis based on expected concentration (WTW (Xylem Analytics), 2019).

Expected BOD ₅ (mg/L)	Total volume (mL)	Sample (mL)	DI water (mL)
0–40	432	216	216
0–80	365	182.5	182.5
0–200	250	125	125
0–400	164	82	82
0–800	97	48.5	48.5
0–2000	43.5	21.75	21.75

For most EA Program surface water samples, the 0–200 mg/L range is a reasonable starting point. If the expected concentration is unknown, run samples at two dilutions.

8. Procedure

8.1. Preparation (Day 0)

1. Calibrate the pH meter per [SOP-L300](#).
2. Prepare dilution water as described above; make enough for all blanks and samples.
3. Allow refrigerated samples to equilibrate to 20 °C. Add seed inoculum to both sample and blank bottles if seeding is required.
4. Verify that all OxiTop manometers are functional (battery check, reset previous readings) (WTW (Xylem Analytics), 2019).

8.2. Bottle Setup

1. Place a clean magnetic stir bar in each BOD₅ bottle.
2. Measure the appropriate sample volume into each sample bottle (see Table 1).
3. Add the corresponding volume of dilution water.
4. For the blank bottle, add dilution water only (same total volume as sample bottles).
5. Place 2 NaOH pellets in each rubber quiver. Insert the quiver into the bottle neck above the liquid surface.
6. Screw the OxiTop manometer head onto each bottle; ensure a gas-tight seal.

Labeling

Label each bottle (Blank, Sample A-1, Sample A-2, etc.) on both the bottle and the manometer before starting incubation. Record bottle assignments in the lab notebook.

8.3. Incubation

1. Place all bottles on the magnetic stirrer platform inside the incubator at 20.0 ± 1.0 °C.
2. Activate the OxiTop manometers to begin recording pressure.
3. Incubate for five days (120 ± 2 hours) in the dark; keep the incubator door closed except for daily temperature checks (APHA et al., 2012).
4. Verify incubator temperature at least once daily; record in the instrument logbook.

8.4. Reading Results (Day 5)

1. Read the BOD₅ value from each OxiTop manometer; the instrument converts pressure decrease to mg/L automatically based on the selected measuring range (WTW (Xylem Analytics), 2019).
2. Record all readings in the lab notebook: setup and reading dates/ times, incubator temperature log, measuring range, sample IDs, and replicate values.
3. Remove bottles from the incubator. Discard sample water per §9. Clean bottles and stir bars thoroughly with hot water and detergent; rinse with deionized water.

9. Data Analysis and Calculations

9.1. OxiTop Direct Reading

The OxiTop system calculates BOD₅ internally from the headspace pressure decrease (WTW (Xylem Analytics), 2019). Record the displayed mg/L value directly.

9.2. Blank Correction

$$\text{BOD}_{5,\text{corrected}} = \text{BOD}_{5,\text{sample}} - \text{BOD}_{5,\text{blank}}$$

The blank must be less than 0.2 mg/L for unseeded dilution water (APHA et al., 2012). If the blank exceeds 0.2 mg/L, investigate dilution water quality and consider re-running the analysis.

9.3. Dilution Factor

For highly concentrated samples diluted beyond the standard OxiTop protocol:

$$\text{BOD}_{5,\text{final}} = \text{BOD}_{5,\text{corrected}} \times \frac{V_{\text{total}}}{V_{\text{sample}}}$$

For standard OxiTop runs, the dilution factor is already incorporated into the displayed result.

10. Quality Control

- Run at least one blank per analytical batch (APHA et al., 2012).
- Run duplicate sample bottles for each sample; RPD must be less than 20%.
- If available, include a GGA check standard at 300 mg/L combined (150 mg/L each); expected BOD₅ = 198 ±30.5 mg/L (APHA et al., 2012, Method 5210 B).
- Blank BOD₅ must be less than 0.2 mg/L (unseeded) or less than 0.6 mg/L (seeded) (APHA et al., 2012).
- Verify incubator temperature is 20.0 ±1.0 °C throughout incubation.
- Log all QC results; flag and consult the PI if criteria are not met.

11. Troubleshooting

Problem	Possible Cause	Corrective Action
No pressure change after 5 days	Dead battery; poor seal	Check battery; reseal manometer
Blank BOD ₅ too high	Contaminated dilution water	Reclean bottles; prepare fresh dilution water
Replicate RPD > 20%	Heterogeneous sample	Mix samples well; check pipette calibration
BOD ₅ exceeds range	Sample more concentrated than expected	Re-run at higher range (lower volume)
Negative BOD ₅	Leak in bottle seal; NaOH saturated	Inspect seal; use fresh NaOH pellets

12. Waste Disposal

Waste Type	Disposal Method
Analyzed sample water	Drain with copious water (if non-hazardous)
NaOH pellets (used)	Dissolve in water, neutralize, drain
Dilution water	Drain
Broken glass	Sharps container

13. References

- APHA et al. (2012)
- U.S. Environmental Protection Agency (1978)
- WTW (Xylem Analytics) (2019)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

U.S. Environmental Protection Agency. (1978). *Method 405.1: Biochemical oxygen demand (5 days, 20 °C)*. U.S. EPA, Environmental Monitoring; Support Laboratory. https://www.epa.gov/sites/default/files/2015-08/documents/method_405-1_1978.pdf

WTW (Xylem Analytics). (2019). *OxiTop control system: Operating manual*. <https://www.xylen.com/en-us/brands/wtw/>

14. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L300	pH and Conductivity (Bench)	pH meter calibration for dilution water
F209	Dissolved Oxygen Meter (Field)	Related field DO measurement
F300	Surface Water Sampling	Sample collection context

15. Training Record

Before performing BOD₅ analysis independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Review the WTW OxiTop operating manual (WTW (Xylem Analytics), 2019)
4. Observe a trained operator perform the complete procedure (setup through Day 5 reading)
5. Perform the analysis under supervision with acceptable QC results (duplicate RPD within 20%; blank within criteria)
6. Be signed off by the PI or lab manager