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| **TITLE:** | **Biochemical Oxygen Demand (BOD), 5-day** |
| **ANALYTE:** | **Biochemical Oxygen Demand** |
| **FACILITY:** | **Acme WWTP** |
| **REFERENCE METHOD:** | **Standard Methods 5210 B – 2001** |
| **DATE OF ISSUE:** | **9/7/21** |

1. Applicable Matrices
   1. This method is applicable to domestic and industrial waste matrices.
2. Summary of the Test Method
   1. The BOD test indicates the organic strength of wastewater. This data is used for submittal on the electronic Discharge Monitoring Report (eDMR) and is used for compliance of discharge limits.
   2. The BOD test is a bioassay procedure that empirically measures the dissolved oxygen consumed by a microbial population during the oxidation and incorporation of organic matter. Standard conditions for the test include incubation at 20°C ± 1°C in darkness for 5 days (± 6 hours). Interpretation of the results obtained must take into account the laboratory’s ability to reproduce actual environmental conditions such as temperature, microbial population, sunlight, and water movement. The value of BOD results for raw and final domestic wastewater varies significantly between wastewater facilities depending on the industrial loading. Use past history to determine the appropriate volumes to use when preparing a sample dilution series.
3. Safety
   1. Proper personal protection equipment, such as a lab coat, safety glasses, and nitrile gloves should be worn when performing any laboratory test.
   2. When using acid solutions, wear appropriate protective clothing: rubber apron, protective sleeves, gloves, and safety goggles or glasses.
   3. Always have adequate ventilation when working with acids, bases, and solvents to minimize exposure to vapors.
   4. Refer to the Safety Data Sheet for information on a specific chemical.
4. Equipment and Supplies
   1. Optical DO Meter, ABC Company Model 123
      1. The internal barometer on the DO meter must be verified no less than once per year. To verify this (avoid if there is a storm approaching), check the sea level barometric pressure of the nearest airport or on a weather website. Correct this value by the local elevation. Record this verification (this can be done on the “Pressure Verification” tab on the spreadsheet used for the BOD bench sheet).

Document the original barometric pressure, the sea level pressure, and corrected barometric pressure. If the DO meter barometer if off more than 5 mm of Hg, adjust according to manufacturer’s instructions and note this on the barometric pressure verification log sheet. Include the date and initials.

Elevation correction = 760 mm Hg – (facility elevation in ft X 0.0254)

760 mm Hg

The elevation of Acme WWTP is 1642 ft, so the elevation correction factor is 0.946.

* 1. BOD Probe, ABC Company Model 456
  2. Incubator – Capable of maintaining a constant 20°C ± 1°C temperature range and excluding light (to eliminate O2 production from photosynthesis). Temperatures are to be recorded on days of use. If not within acceptable range, the thermostat is to be adjusted and noted on the incubator log or BOD bench sheet.
  3. 300-mL BOD bottles with glass stoppers
  4. Plastic BOD bottle over-caps
  5. Class A graduated cylinders, various sizes
  6. Wide-tip volumetric or serological pipettes, various volumes
  7. Glass bottle for preparing dilution water
  8. Glass carboys or aspirator bottles of appropriate sizes for storage of aerated DI water covered with clean coffeemaker filters. If applicable, aquarium pumps with C-Flex™ tubing attached to a glass pipette may be used for aeration. Note: attach one end of the C-Flex™ tubing to the air pump and the other to the glass serological pipette. Place the serological pipette into the DI water for aeration. Do not immerse the tubing in the DI water used for preparing BOD dilution water.
  9. Beakers
  10. Wash bottle filled with distilled water
  11. Magnetic stirrer
  12. Magnetic stir bars
  13. pH paper, narrow range (such as 5.5 – 8.0)

1. Reagents and Standards
   1. Distilled water, purchased locally
   2. BOD nutrient buffer. Liquid nutrients are purchased (FeCl3-0.25 g/L; CaCl2-27.5 g/L; MgSO4-22.5 g/L; KH2PO4 – PO4 phosphate buffer pH 7.2). These are added to the distilled water at a rate of 1 mL per L to make the dilution water. Alternatively, pre-made nutrient buffer pillows from ABC Chemicals may be used and prepared for 300 mL, 3 L, or 4 L of BOD dilution water. Dilution water preparation is in the procedure section, “Reagents and Solution Preparation.”
   3. Glucose - Glutamic Acid solution (GGA), 198 ppm. Purchase prepared solution from ABC Chemicals. Keep refrigerated at ≤6°C. It expires according to manufacturer’s expiration date or sooner if low recoveries are observed. GGA preparation is in the procedure section, “Reagents and Solutions Preparation.”
   4. BOD seed. ABC Chemicals synthetic seed (not Polyseed Nx). It expires according to manufacturer’s expiration date. Seed is prepared on days of use. Alternatively, mixed liquor may be used. Seed preparation is in the procedure section, “Reagents and Solutions Preparation.”
   5. 1.5 N Sulfuric Acid, ABC Chemicals. Used for adjustment of samples with pH over 8.5. This is rare – use only if needed.
   6. 1.5 N Sodium Hydroxide, ABC Chemicals. Used for adjustment of samples below 6.0 pH. This is rare – use only if needed.
   7. Proficiency Testing samples from an approved provider.
2. Interferences
   1. Any constituent that is toxic to the population of microorganisms in the water sample may be considered an interference. Samples containing caustic alkalinity, acidity, or residual chlorine must be pretreated before proceeding with the BOD analysis. If this is suspected, sliding BODs may be observed. Chlorinated samples at this facility are not applicable; either chlorination is not done or the samples are collected prior to chlorination. If samples are chlorinated, see the procedure section, “Sample Pretreatment.”
   2. A positive bias may be introduced due to poor quality dilution water, which may contain material contributing to the dissolved oxygen (DO) depletion. A negative bias may be introduced due to poor quality distilled water containing inhibitory agents. Un-chlorinated or only partially dechlorinated samples, certain industrial wastes, and soap residues may also cause a negative bias in the results.
   3. Differences in the atmospheric pressure and temperature on the days of the initial and final DO readings may cause DO probe calibration error due to the differences in the partial pressure of oxygen at saturation. This problem is generally minimized if the DO meter’s on-board barometer is in good working order and a weather front is not rapidly moving through the area at the time the instrument is calibrated.
   4. Water has a finite ability to contain dissolved oxygen (DO). The amount of DO is dependent on temperature and barometric pressure and is considered the oxygen saturation level. Anything beyond this amount is considered super-saturation. Refer to the oxygen saturation chart at the end of the SOP (and in the spreadsheet used for the BOD bench sheet) to find the saturation level based on temperature and pressure. When BOD sample dilutions with initial DO readings which are super-saturated are incubated at 20°C, the DO will physically diffuse out of solution and will appear as BOD. Samples must be warmed to 20°C ± 3°C and rigorously shaken or stirred (and allowed to vent) to drive off excess DO prior to preparing samples for BOD analysis.
3. Sample Collection, Preservation, and Storage
   1. Container cleaning process: sample carboys, BOD calibration bottles, and the dilution water bottle are washed daily with hot tap water and non-phosphate detergent. The containers are rinsed weekly with 10% household bleach (not bleach that has additives). Follow the cleaning by hot tap water, rinse until all suds disappear, then rinse well with DI water. *For the containers that are also used to collect phosphorus samples, a 1% solution of laboratory grade HCl is used just prior to the rinse with DI water.*
   2. Plastic bottles and carboys may be used for sample collection. Sample material should not adhere to the container walls.
   3. Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD results. Analysis should be done within 2 hours from collection, or the samples must be placed in the refrigerator at ≤6°C. Warm chilled samples to 20°C ± 3°C before analysis (and prior to dilution).
   4. Hold times may not exceed 48 hours from the time of collection.
4. Quality Control
   1. Initial Quality Control
      1. Method Detection Limit (MDL): The Laboratory is not required to complete a MDL study for BOD. The reporting limit used, as specified by the method, is 2 mg/L if 300 mL of sample volume is used.
      2. All analysts shall perform an IDC once for each lab technician. An IDC is required of all new lab technicians including technicians performing only part of the testing (for example, technicians who only perform the read out).
   2. On-going Quality Control
      1. Analyze a dilution water blank sample with each batch of 20 samples.
      2. At least two seed control samples must be set up with each batch that have samples seeded or QC seeded. Seed strength should generally be between 0.6 - 1.0 mg/L for the seed added to each BOD bottle. However, this is not a requirement. The critical requirement is that regardless of its strength, a sufficient quantity of seed is added so that the GGA meets acceptance criteria.
      3. All pH adjusted samples, disinfected (UV or chlorinated/de-chlorinated) samples, industrial samples, and GGA must be seeded. Proficiency testing standards, samples which contain nitrification inhibitors, and samples collected post-disinfection also must be seeded.
      4. A Glucose-Glutamic Acid (GGA) sample must be analyzed every week (if less than 20 samples are analyzed in a week).
      5. Annually a Proficiency Testing (PT) sample must be obtained and analyzed from one of the Wisconsin DNR approved providers.
5. Calibration and Standardization
   1. The ABC Company Model 123 meter and probe is used. Refer to the User Manual for additional information.
   2. Calibration of the DO meter (Water-Saturated-Air method) must be performed each day BODs are set up or read out.
   3. Room temperature must be 20°C ± 3°C. The temperature from the meter is preferred instead of a separate thermometer.
   4. Shake the probe gently to remove water droplets from the tip, or using a lint-free tissue, gently dab the probe to remove water. Return the probe to a BOD bottle containing 1 inch of water, turn on the meter, and allow the probe to stabilize (this may take up to 30 min); be consistent every day. The air in the BOD bottle must be saturated with water for the calibration to be done properly.
   5. The Model 123 meter instructions for a water-saturated-air calibration are the following:

Press **Calibration**, highlight “DO %,” and press **enter** to confirm. The instrument will use the value from the internal barometer during calibration and will display this value in brackets at the top of the display. Record the pressure from the meter on the bench sheet. Highlight the barometer value and press **enter** to adjust it if needed. If the barometer reading is incorrect, it is recommended that you calibrate your barometer. Note: the barometer should be reading “true” barometric pressure (see Barometer section in “Equipment and Supplies” for more information on “true” barometric pressure). If the value is acceptable, there is no need to change it or perform a barometer calibration. Wait for the temperature and DO% values under “Actual Readings” to stabilize, then highlight “Accept Calibration” and press **enter** to calibrate.

* 1. Document the calibration result. Using the recorded pressure and recorded room temperature from the DO meter, determine the theoretical DO value. Compare the calibration result to the theoretical DO; the two values should be within about 0.2 mg/L of each other. If not, check for reasons why (such as proper saturation or membrane replacement) or recalibrate.

1. Procedure
   1. Reagents and Solutions Preparation
      1. Dilution water preparation: To the large dedicated carboy, fill to about 3.5 L, cover with a coffee filter, and secure with a rubber band. If using the aquarium pump system to aerate the water, insert the serological pipet into the water and aerate for at least one day. Alternatively, the water must be shaken extensively multiple times to ensure it is saturated; make sure there is headspace in the container, mix vigorously, and periodically vent.

On the day of use, fill to the 4 L mark (make sure it is still saturated) and add 4 mL each of the four liquid nutrients (FeCl3, CaCl2, MgSO4, KH2PO4 – PO4); refer to the section, “Reagents and Standards.” Swirl to mix the nutrients. If preparing a different amount of dilution water, adjust the reagents so that the ratios are the same. If using nutrient buffer pillows, add a nutrient pillow designed to fortify the volume you are preparing (**4 L**). **Note any preparation changes from this procedure in the reagent log book.**

* + 1. Seed preparation (on days required): In a 1000 mL beaker, add 500 mL of the prepared dilution water (with nutrients added). Add one synthetic seed capsule. Aerate by mixing the seed rigorously for 1 hour using a stir bar and magnetic stir plate (see manufacturer’s instructions for details). Carefully remove stir bar, and allow the particles to settle for at least 15 minutes. After 15 minutes, slowly decant off the top of the seed solution and pour into a clean beaker. Make sure not to include any of the settle solids (bran) into the beaker. Place the beaker on a stir plate, add a stir bar, and gently stir the decanted seed solution. Insert a wide-bore pipet to just below the surface, taking care not to disturb the particles on top, and take a portion of the seed from between the vortex and the container wall.

If the GGA does not fall in the control range, increase/decrease the amount of seed used. It is necessary to have an adequate population of microorganisms capable of oxidizing the organic matter present in a sample. Alternatively, use settle mixed liquor or raw wastewater from a domestic wastewater treatment as a seed source.

* + 1. GGA standard preparation: Prepare this standard once per week unless there are failures, then prepare it the next run as corrective action. Remove from refrigerator, pour off enough for a single day use into a clean beaker, and cover. Allow to warm to room temperature before use. Return stock GGA bottle to refrigerator immediately after pouring off amount required for the day.
  1. Sample Pretreatment
     1. Shake the sample thoroughly to disperse solids, and bring the temperature to 17°C - 23°C. For samples that tend to have (or have been measured to have) DO levels above saturation (see saturation chart\*), warm to 21°C - 22°C and strip excess DO by shaking sample vigorously in a bottle filled about ¾ full, remove the lid, and let vent 2-3 seconds; repeat as needed. DO NOT warm samples beyond 23°C. Record the sample temperature on the bench sheet.

*\*Samples are considered super-saturated if the DO is more than a few tenths above the theoretical saturation point. The theoretical saturation point is based on the temperature and pressure at the time of calibration.*

* + 1. Check sample pH using pH paper or a calibrated pH meter and record on the bench sheet. If the sample pH is not between 6.0 and 8.5, then bring a portion of the sample that will be used for BOD analysis to a pH of 6.5 – 7.5 prior to analysis by adding no more than 0.5% of either the acid or the hydroxide (see “Reagents and Standards” section). Record the volume of the acid/base used and the final pH on the bench sheet. If the sample is adjusted, then it must be seeded.
    2. If the sample was subjected to chlorination, check the sample for residual chlorine with chlorine test strips that can detect down to 0.1 mg/L or less. If there is chlorine, treat with sodium sulfite by following instructions in SM 5210 B (4)(b)(2). Document the residual chlorine check and sodium sulfite ID on the benchsheet.
  1. Sample Setup (Read In)
     1. Be sure the following are documented on the bench sheet: sample pH, sample temperature, room temperature, barometric pressure, calibration DO, theoretical DO, analyst initials, and date. Also record the IDs or lot numbers of all chemicals used during analysis.
     2. Rotate the BOD bottles being used so that the same bottles are not used for the same samples each time. Write the start time for the analysis on the bench sheet.
     3. Add some dilution water to each bottle (except sample dilutions using 300 mL of sample).
     4. QC Sample Setup:
        1. Seed Controls: Once per week (or if any samples or QC are seeded), prepare three dilutions using **15, 20,** and **25** mL of the synthetic seed solution (see “Seed Preparation”) to BOD bottles. The volumes of seed solution may be adjusted as needed to produce the 2.0 mg/L depletion and 1.0 mg/L residual DO at the end of the test. Only one seed control is required, but preparing multiple dilutions is typical.
        2. GGA: Once per week (if <20 samples), using a 6 mL class A volumetric pipet, add 6 mL of room temperature GGA to a BOD bottle that is mostly filled with prepared dilution water. Add **4 mL** of seed (or an appropriate amount). *Strive for a seed correction in the 0.6 - 1.0 mg/L range (the mL of seed added may be adjusted in order to obtain a passing GGA result in the 198 ± 30.5 mg/L range).*
        3. Method Blank: With every batch set up, prepare one dilution water blank by filling the BOD bottle all the way to the top with dilution water.
     5. Sample Setup:
        1. Document the sample IDs and volumes.
        2. Shake to mix (stir if using pipettes) the samples well to disperse solids. See section on “Sample Pretreatment.”
        3. Using a wide-tipped pipette for samples volumes less than 25 mL or a graduated cylinder for larger sample volumes, measure the proper amount of well-mixed sample into 300 mL BOD bottles.

Add the appropriate amount of sample to BOD bottles. Typical for this lab are: **150 & 200 mL for effluent (final) and 4 & 6 mL for influent (raw).** *These are adjusted to obtain the depletion of 2 mg/L and residual of 1 DO in mg/L. See previous bench sheets for amounts. Try to obtain at least 2 dilutions with DO uptake in this range. Note: always strive to take the maximum sample volume possible.*

* + - 1. If less than 3 mL of sample would be added to a 300 mL bottle, as a preliminary step, prepare a 10x dilution of the sample by pipetting 10 mL of sample into a 100 mL graduated cylinder, and fill to volume with BOD dilution water. 10 mL of this diluted sample = 1 mL of sample. Record the dilution on the bench sheet.
      2. If a dilution of more than 200 mL (201 – 300 mL) of undiluted sample is prepared, add 0.3 mL of each of the liquid nutrients (or a nutrient pillow designed to fortify 300 mL). Document the added nutrients on the bench sheet.
      3. Add seed to samples that were collected after disinfection, if pH adjusted, untreated industrial wastes, or if treated with a nitrifying inhibitor for cBOD. Use **4 mL** of seed or the same amount as used for GGA.
    1. Slowly add enough prepared dilution water half way up the frosted neck on each bottle.
    2. Measure the initial DO using the calibrated DO meter. Carefully place the probe into the BOD bottle and turn on the stirrer (uniform stirring is essential for correct DO measurement). Sample analysis is done with the bottle stirring and the mode switched to H2O. Allow the reading to stabilize (1-2 min).
    3. Record the initial DO reading for each of the BOD bottles on the BOD bench sheet. The initial DO value should be comparable to the meter calibration value (within a few tenths). If the sample is not properly saturated, follow instructions for saturating in section, “Sample Pretreatment.”
    4. The DO probe is thoroughly rinsed with DI water after each reading. Repeat this process until the initial DO measurements have been made on all of the BOD bottles.
    5. Fill each BOD bottle with additional dilution water (if necessary) to a level where insertion of the glass stopper displaces all air, leaving none trapped in the neck of the bottle.
    6. Fill the space around the bottle stopper with distilled or DI water to form a water seal. Place a plastic cap over the stopper to prevent evaporation of the water seal while the sample is being incubated. The water seal and cap are very important in preventing oxygen flow to/from the bottle during incubation.
    7. Place all samples in the BOD incubator, and incubate at 20°C ± 1°C for 5 days ± 6 hours. Document the temperature of the incubator.
  1. Samples Read Out
     1. After 5 days (± 6 hours), calibrate the meter and document calibration information (see “Calibration and Standardization”).
     2. Take samples out of the incubator and document the analyst initials, date, and time out.
     3. Read and record the final DOs, making sure to rinse the probe between the sample readings.
  2. Clean the BOD bottles by washing with hot tap water and mild non-phosphate detergent. Rinse with hot tap water until all the suds disappear then rinse with DI water.

1. Calculations
   1. ***Unseeded*** Samples

BOD (mg/L) = volume of bottle (mL) x (IDO – DO5)

Volume of sample (mL)

Where: IDO = Initial DO measurement on setup day (day 0)

DO5 = DO measurement on read out day (day 5)

* 1. ***Seeded*** Samples and GGA

BOD (mg/L) = volume of bottle (mL) x [(IDO – DO5) – seed correction]

Volume of sample (mL)

* 1. ***Seed Correction***

Seed CF (mg/L) = Seed IDO – Seed DO5

Seed correction (mg/L) = average Seed CF x volume seed used (mL)

* 1. Perform all calculations and record the calculated results on the bench sheet. The DNR supplied spreadsheet may also be used to perform calculations.
  2. Average all results that meet the depletion (2 mg/L) and residual criteria (1 mg/L).
     1. If a 300 mL dilution was analyzed and the results are less than 2 mg/L, report “<2 mg/L.” See “Method Detection Limits and Reporting.”
     2. If all dilutions fail, see the “WDNR BOD Reporting Protocols” at the end of this SOP.

1. Method Detection Limits and Reporting
   1. The method detection limit does not need to be determined for BOD. This limit is defined in SM 5210 B as 2 mg/L. The 2 mg/L reporting limit is based on a minimum oxygen consumption of 2 mg/L during the 5-day test period and a volume of 300 mL. This is also the reporting limit which is specified in the DNR guidance document, “Recommendations of the BOD LOD Technical Group 12/17/99.” The reporting limit is increased by the dilution factor; for example, if 150 mL of sample is used, then the RL is 4 mg/L. Use the dilution factor of the largest sample volume to determine the reporting level.
   2. If all dilutions fail, refer to the “WDNR BOD Reporting Protocols” at the end of this SOP.
2. QC Data Assessment, Acceptance Criteria, and Corrective Actions and Contingencies for Out-of-Control QC Measures
   1. Quality control samples summary:

|  |  |
| --- | --- |
| QC Test | Criteria |
| Method Blank | ≤ 0.24 mg/L |
| LCS (GGA) | 167.5 – 228.5 mg/L |

* 1. Method blank results must be ≤0.24 mg/L. If results are greater than the limit, qualify results on the eDMR. Complete the corrective action log documentation.
  2. GGA results must be in the range of 198 ± 30.5mg/L (167.5 – 228.5 mg/L). If not within the acceptable range, qualify results on the eDMR and repeat the GGA the next time BOD analyses are performed. Complete the corrective action log documentation.
  3. Re-analyzing is not practical for BOD due to the holding time being less than the time required for the completion of the test.
  4. Qualify all BOD results with blank or GGA exceedances on the bench sheet and the eDMR.
     1. Samples that fail the Quality Control will have to be qualified back to the last date that the quality control met the above conditions. Include a lab comment on the DMR.
  5. If all dilutions fail to meet depletion and residual criteria then set up more dilutions the next time.
  6. The Proficiency Testing (PT) sample must be within the criteria of the provider. If the criteria limits are not met, the technician must immediately order another sample to be analyzed.
  7. For any of the above items or if there are any other obvious errors or deviations from the standard operating conditions, complete the Corrective Actions Log and resolve the problem. Notify the Supervisor.
     1. If results are unacceptable, take appropriate corrective action. This may include replacing the DO probe membrane, replacing the probe, acid washing all containers, checking the water source, checking expiration dates, and documenting any changes or adjustments made.
     2. Complete the Corrective Actions in the log sheets. Enter in all information as completely as possible, even if the short-term reasons for failures are not clear.
     3. Seek help from an outside source if specific QC issues cannot be resolved. These sources may be another lab, the Wisconsin Rural Water Association wastewater trainer, or the facility’s lab auditor.
     4. Extensive troubleshooting information can be found <http://dnr.wi.gov/regulations/labcert/Resources.html>

1. Pollution Prevention
   1. Consider environmental impact when purchasing materials, handling chemicals, and disposing of wastes.
   2. Prevent pollution at the source whenever possible.
2. Waste Management
   1. All laboratory waste, excess reagents, and samples must be disposed of in a manner that is consistent with applicable rules and regulations.
3. References
   1. Standard Methods for the Examination of Water and Wastewater, Method 5210 B, 2001.
   2. Recommendations of the BOD LOD Technical Group 12/17/99. <http://www.dnr.state.wi.us/org/es/science/lc/Outreach/3Guid/-LOD4BOD&TSS.pdf>
   3. Ultimate BOD Resource. <http://www.dnr.state.wi.us/org/es/science/lc/OUTREACH/BODresource/Index.html>
   4. Low GGA results: Alternative to the use of commercially prepared BOD seeds. Revision 1, February, 2014. Wisconsin Department of Natural Resources, Laboratory Certification Program.
4. Disclaimer
   1. The mentioning of company or product names does not constitute endorsement by the Wisconsin Department of Natural Resources or the authors.

**BOD Reporting Protocols when all dilutions fail method criteria** [version 9/18/19]

BOD method criteria for dilutions

* At least 2.0 mg/L DO depletion
* At least 1.0 mg/L residual DO

Case 1 – All dilutions under-deplete (less than a 2.0 mg/L DO change from initial to final DO reading)

* In this case you determine the reportable BOD result using the dilution with the largest sample volume.
* Assume a 2.0 mg/L DO change (even though it didn’t really happen – the method requires that we use the 2.0 mg/L minimum).
* Report the result as a less than ( < ).
* Qualify the result indicating that all dilutions under-depleted.
* If this happens often you need to increase your sample volume to 300 mL.

Example:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample Volume (mL) | DO Depletion (mg/L) | Which dilution to use (largest volume) | Assumed DO Depletion (mg/L) | Reported BOD result (mg/L) |
| 25 | 0.3 |  |  | 2.0 mg/L \* 300 mL/200 mL  < 3.0 mg/L |
| 50 | 0.9 |  |  |
| 100 | 1.3 |  |  |
| 150 | 1.6 |  |  |
| 200 | 1.8 | Pick this one | 2.0 |

Case 2 – All dilutions over-deplete (less than 1.0 mg/L DO residual)

* In this case you determine the reportable BOD result using the dilution with the highest residual DO.
* Report the result as a greater than ( > ).
* Qualify the result indicating that all dilutions over-depleted.
* If this happens often you need to decrease your sample volume.

Example: Assume initial DO was 8.5 mg/L for all dilutions

|  |  |  |  |
| --- | --- | --- | --- |
| Sample Volume (mL) | Final DO (mg/L) | Which dilution to use (smallest volume) | Reported BOD result (mg/L) |
| 25 | 0.9 | Pick this one | > 91 mg/L  8.5 – 0.9 mg/L =  7.6 mg/L \* 300 mL/25 mL |
| 50 | 0.7 |  |
| 100 | 0.5 |  |
| 150 | 0.4 |  |
| 200 | 0.3 |  |

In all of these cases, if you seeded your samples you will need to subtract the average seed control factor as appropriate.

On a slightly different note, when evaluating seed controls, the same method criterion that applies to sample dilutions applies to seed control dilutions. Each seed control dilution must have a minimum of 2.0 mg/L DO depletion and 1.0 mg/L residual DO. If any of the seed controls fail to meet either one of these criteria, you don’t use that seed control dilution. If all seed control dilutions fail, then use the one seed control dilution that comes closest to meeting all of the criteria.