DWR–NPDES–SOP–G–06-BOD/cBOD 5210 B-01012024
Biochemical Oxygen Demand (BOD), SM 5210 B-2016, 5-Day BOD Test

DISCLAIMER: This document is guidance only and does not create legal rights or obligations. Agency decisions in any particular case will be made applying applicable laws and regulations to the specific facts.

1) **EFFECTIVE DATE: 01/01/2024**

2) **SIGNATURES:**

April Grippo (May 30, 2024 16:25 CDT)

April Grippo, DWR Interim Director

Brianne Begley, Environmental Consultant
Drafter / Preparer

Stephanie Durman, Deputy General Counsel
Reviewer

Initial Demonstration of Capability (DOC)

- 1020 B. 1 – As a minimum, include a reagent blank and at least 4 LFBs (laboratory fortified blanks)
- 5020 B.3. – Each analyst in the laboratory should conduct an initial demonstration of capability at least once before analyzing any sample to demonstrate proficiency in performing the method and obtaining acceptable results for each analyte. As a minimum, include a reagent blank and at least four laboratory fortified blanks (LFB) at a concentration between one and ten times the MDL and the midpoint of the calibration curve (or other level specified in the method)
    - Ensure that precision and accuracy (percent recovery) calculated for LFBs are within the acceptance criteria listed in the method of choice or generated by the laboratory (if there are no established mandatory criteria)
- **Summary: Each operator running this test needs to analyze 4 samples of glucose-glutamic acid (GGA) at a concentration of 198 ± 30.5 mg/L**
    - **Keep a folder for each analyst**
    - **Maintain documentation (signed form) that each analyst has read and understands all appropriate SOPs and Methods**
    - **A backup analyst should complete a DOC once a year**

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves
- Hach's Method: 7.1.1. – Add approximately 1 inch (2.54 cm) of reagent water to a clean BOD bottle and stopper
- 7.1.2. – Shake vigorously for ~10 seconds
- 7.1.3. – Allow for the BOD bottle and its contents to equilibrate to room temperature. Room Temperature should be approximately 20 $\pm$ 3°C
- 7.1.4. – The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes
- **Summary: Calibrate DO probe daily (day of) in accordance with manufacturer's instructions**

Method Blank

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure
- 5020 B.5. – As a minimum, include one reagent blank (method blank) with each sample set (batch) or on a 5% basis, whichever is more frequent. Analyze a blank after the initial CCV standard and before analyzing samples
- 5210 B.6.c. – With each batch of dilution water, incubate two or more bottles of dilution water containing nutrient, mineral, and buffer solutions but no seed or nitrification inhibitor. Dilution water checks must be analyzed with each batch of samples
    - Determine initial and final DO for each bottle and average results
    - The average DO uptake in 5 days must not be greater than 0.20 mg/L and preferably less than 0.10 mg/L, before making seed corrections
- **Summary: Analyze two dilution water blanks *daily* (day of), preferably one at beginning and one at the end of the day**
    - **The target average value is less than 0.20 mg/L (preferably less than 0.10 mg/L)**

Laboratory Fortified Blank (LFB)

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added
    - Sample batch = 5% basis = 1 every 20 samples
- 5020 B.6. – Evaluate the LFB for percent recovery of the added analytes by comparing results to method-specified limits, control charts, or other approved criteria (See Control Charts below)
    - As a minimum, include one LFB with each sample set (batch) or on a 5% basis, whichever is more frequent
- 5210 B.6.b. – Add sufficient amounts of standard glucose and glutamic acid solutions (5210B.3*h*) to give 3.0 mg glucose/L and 3.0 mg glutamic acid/L in each of three test bottles (20 mL GGA solution/L seeded dilution water, or 6.0 mL/300-mL bottle). Commercial solutions may contain other GGA concentrations; adjust doses accordingly
    - Add nitrification inhibitor if seed is obtained from a source that is nitrifying, and also to all CBOD GGA checks
    - The resulting average BOD/CBOD for the three bottles, after correcting for dilution and seeding, must fall into the control-limit range established in 5210B.8*a*
- **Summary: analyze three GGA samples with a resulting average of 198 ± 30.5 mg/L**
    - **Run on a 5% basis (see batch size for more information)**
    - **If permit requires CBOD, add nitrification inhibitor (NI) to all GGA bottles**

- **Plot percent recoveries on a control chart**

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 5020 B.8. – As a minimum, include one duplicate sample with each sample set (batch) or on a 5% basis, whichever is more frequent, and process it independently through the entire sample preparation and analysis
- **Summary: analyze two samples for BOD or CBOD**
  - **On duplicate days, choose one dilution concentration to run in duplicate and set up an extra sample bottle**
  - **The target value is to get close to the first value and have a small RPD**
- **Report the average of the results that meet method criteria**
- **A precision control chart is required for duplicates (see control chart section below for more information)**
- **Influent and effluent are two different samples, so a duplicate will need to be run on each**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Continuing Calibration Verification (CCV)

- Hach Method 10360 7.2 and 9.4 – Calibration Verification for membranes and LDO probes
  - 7.2.1. – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
  - 7.2.2. – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 $\pm$ 3°C
  - 7.2.3 – With a steady gentle stream of filtered air (≈10 – 40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes
  - 7.2.4. – At the completion of aeration, let water re-equilibrate to room temperature (20 $\pm$ 3°C) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric temperature reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water
  - 7.2.5. – Transfer the aerated water to a BOD bottle until overflowing and stopper
  - 7.2.6. – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman

- 9.4.3. – Initially, and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated

- **Summary: Prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration ± 0.2 mg/L)**
  - **Theoretical dissolved oxygen can be found at USGS's website at https://water.usgs.gov/software/DOTABLES/ or by using a DO saturation Table**

Control Charts

- 1020 B.13.a. – The accuracy chart for QC samples… is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest. The accuracy chart includes upper and lower warning levels (WLs) and upper and lower control levels (CLs). Common practice is to use ±2*s* and ±3*s* limits for the WL and CL, respectively, where *s* represents standard deviation
- 1020 B.13.b. – The precision chart is also constructed from the average and standard deviation of a specified number of measurements [e.g., %RSD or relative percent difference (RPD)] for replicate or duplicate analyses of the analyte of interest… Perfect agreement between replicates or duplicates results in a difference of zero when the values are subtracted, so the baseline of the chart is zero. Therefore for precision charts, only upper WLs and upper CLs are meaningful
- **Summary: Create and maintain control charts once you have 20-30 data points**
  - **If you do not yet have 20-30 data points, follow the QC Acceptance Criteria below until control charts can be created and maintained**

Corrective Action - 1020 B.5., B.8., & B.15.

- 5210 B.7.b. – Identify results in the test reports when any of the following quality control conditions occur:
  - Dilution water blank average is greater than 0.20 mg/L (5210B.6.c.)
  - Glucose-glutamic acid check falls outside of acceptable limits (5210B.6.b.)
  - Test replicates show more than 30% difference between highest and lowest values
  - None of the seed control samples meet the above criteria (5210B.6.d.) or
  - All dilutions result in a residual DO less than 1.0 mg/L (5210B.7.a.3.)

QC Acceptance Criteria

- Blanks < 0.20 mg/L
- GGA = 198 ± 30.5 mg/L
- RPD < 20%

- Minimum of two dilutions for each sample, at least one sample must have valid data with at least 2.0 mg/L depletion and a residual of 1.0 mg/L

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
- *Influent and Effluent are 2 different samples*
    - If a permit stated that 3 analyses per week, that would be 6 samples per week, we would allow for a duplicate to be analyzed at least twice a month
        - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
    - If a permit stated 5 analyses per week, that would be 10 samples per week, we would suggest once a week
        - Pick a date and be consistent, every Monday. Mark your calendar!!
    - If sampling only once a month, need to run QC once a month

Calculations

- % Recovery for LFB =

$$\left(\frac{LFB\ concentration}{expected\ concentration}\right)\text{X100}$$

- RPD – relative percent differences for duplicates =

$$\left(\frac{|sample\ result\ -\ duplicate\ result|}{(sample\ result + duplicate\ result)/2}\right)\text{x100}$$

- Unseeded – BOD_5, mg/L =

$$\left(\frac{D_1 - D_2}{P}\right)$$

- Seeded – BOD_5, mg/L =

$$\left(\frac{(D_1 - D_2) - (B_1 - B_2)f}{P}\right)$$

- Where:
  - D_1 = Initial Dissolved Oxygen Concentration in Sample, mg/L
  - D_2 = Final Dissolved Oxygen Concentration in Sample, mg/L
  - B_1 = Initial Dissolved Oxygen Concentration in Seed Control, mg/L
  - B_2 = Final Dissolved Oxygen Concentration in Seed Control, mg/L
  - f = $\frac{\text{Seed in Sample, \%}}{\text{Seed in Seed Control, \%}}$

Revision Number	Date	Brief Summary of Change
0		Initial issuance of the Guidance
1	August 2, 2017	
2	July 5, 2018	Method editorial revision date changed from 2001 to 2011. Footnote graphic updated.
3	January 24, 2020	QC Acceptance Criteria changed to include "With the addition of NI, the GGA = 164 ± 30.7 mg/L."
4	September 29, 2021	Method editorial revision date changed from 2011 to 2016 to reflect changes to 40 CFR 136 that became effective July 19, 2021. Two blanks required, with target average value less than 0.20 mg/L and three GGA standards required with resulting average of 198 +/- 30.5 mg/L.
5	December 11, 2023	Grammatical and word choice changes, effective date