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SM5210B – Biochemical Oxygen Demand in Water

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1 Scope and Application

1.1 Biochemical oxygen demand (BOD)

1.1.1 Biochemical oxygen demand (BOD) testing is used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters; its widest application is in measuring waste loadings to treatment plants and in evaluating the plants' BOD removal efficiency. BOD testing measures the molecular oxygen used during the specified incubation period to biochemically degrade organic material (carbonaceous demand), oxidize inorganic material (e.g., sulfides and ferrous iron), and/or measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless an inhibitor is added to prevent such reduction. The seeding and dilution procedures provide an estimate of the BOD at pH 6.0 to 8.0. The standard test conditions included dark incubation at 20 degrees C for 5 days. A glucose-glutamic acid standard is used to check dilution water quality, seed effectiveness, and analytical technique for this measurement system.

1.2 Carbonaceous Biochemical oxygen demand (CBOD)

1.2.1 Microorganisms can oxidize reduced forms of nitrogen, such as ammonia and organic nitrogen, thus exerting nitrogenous demand. Nitrogenous demand historically has been considered an interference in BOD testing; adding ammonia to dilution water contributes an external source of nitrogenous demand. The interference from nitrogenous demand can now be prevented by an inhibitory chemical, but if it isn't used, the measured oxygen demand is the sum of carbonaceous and nitrogenous demands. Most biological treatment plant effluents contain enough nitrifying organisms to cause nitrification in BOD tests. Because nitrogenous compounds can oxidize in such samples, nitrification inhibition is recommended for secondary-effluent samples, samples seeded with secondary effluent, and polluted water samples.

1.3 Restricted Procedure

This procedure is restricted to use by an analyst experienced in the operation of a dissolved oxygen electrode/BOD probe and meter, and a pH meter.

Additionally, the analyst must complete the requirements of the GaEPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GaEPD Chemical Hygiene Plan for additional information regarding chemicals required by this method.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Plan (see SOP reference 13.5) for Quality Control Definitions.

3 Interferences

3.1 Sample containing caustic alkalinity or acidity.
3.2 Samples containing residual chlorine compounds.
3.3 Samples containing other toxic substances often found in industrial and plating wastes such as toxic metals.
3.4 Samples supersaturated with dissolved oxygen.
3.5 Samples outside temperature range of 20 ± 3°C.
3.6 Samples containing nitrification inhibitor.
3.7 Dirty glassware.

4 Safety

4.1 Refer to the EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision. (See Section 13.7)

5 Apparatus and Equipment

5.1 Sample Container: half-gallon plastic jug.
5.2 Incubator: must be capable of maintaining an air temperature of 20°C ± 1°C. Exclude all light to prevent formation of oxygen by algae.
5.3 Bench top carbon canister for water filtration
5.3.1 10” Carbon Water filter – Evoqua Catalog No. W3T184798 or equivalent
5.4 Glassware – Class “A” volumetric flasks, graduated cylinders and pipettes
5.5 YSI 5000 Dissolved oxygen meter or equivalent.
5.5.1 YSI 5010 BOD Probe
5.6 Thermo Scientific™ Orion™ 3-Star Benchtop pH Meter with pH probe - Thermo Scientific™ Orion™ Triode™ 3-in-1 pH/ATC Probe Thermo Scientific™ 9157BNMD Catalog No.13-642-252 or equivalent.
5.7 300 mL glass or disposable BOD bottles
5.7.1 Glass stoppers
5.7.2 BOD bottle caps
5.8 Air displacement pipettes of various volumes, auto-pipettor, pipette tips in various sizes. Air displacement pipettes and auto-pipettors may also be described as mechanical pipettes.
5.8.1 Each day of use, the volume dispensed by each mechanical pipette must be verified for the specific volume for which the pipette is being used.
5.8.2 Mechanical pipette volumes are verified by measuring the weight of a volume of water dispensed by the unit. At room temperature, 1 ml of water

is equal to 1g. Mechanical pipettes must be verified to be within ± 2.5 percent of the nominal volume.

5.8.3 Mechanical pipettes must be professionally calibrated every 6 months.

5.8.4 Auto-pipettors may be verified by measuring the volume dispensed with a Class "A" graduated cylinder. The volume dispensed must be within ± 2.5 percent of the nominal volume.

5.9 Titration Burette

5.9.1 Burette clamp

5.9.2 Stopcock

5.10 Lab supplied air for aerating sample

5.11 Magnetic stir plate and stir bars

5.12 10 L Nalgene Carboy

5.13 NIST Certified Barometer

5.14 Chlorine Test Strips – Hach AquaCheck Catalog No. 27450-50 or equivalent

5.15 YSI 5906 Membrane Kit – 1 ml Teflon – Catalog No. 059880

5.15.1 Contains: Oxygen Probe Electrolyte Solution and 6 membranes

5.16 Glass Erlenmeyer flasks and beakers

6 Reagents

6.1 Reagent Water:

6.1.1 Purified water which does not contain any measurable quantities of target analytes or interfering compounds for each compound of interest (Deionized, HPLC, Milli-Q water, or equivalent. Milli-Q water has a resistivity of 18.2[MΩ.cm] @ 25oC and a TOC of 50 ug/L or less).

6.1.2 Tap water is run through a bench top carbon canister to remove organics for the BOD water (see 5.3 above).

6.2 Glucose-Glutamic Acid (GGA) Solutions(300 mg/L Glucose/ Glutamic Acid):

6.2.1 Purchased from HACH.

6.2.2 HACH BOD Standard Solution, Catalog No. 1486510 or equivalent

6.3 Nutrient Buffer Water:

6.3.1 Purchased from Environmental Express.

6.3.2 BOD SimpleWater™ Nutrient Buffer Dilution Vials Catalog No. D4003L or equivalent.

6.3.3 Use the contents of 1vial per 3 liters of filtered tap water.

6.3.3.1 Prepare 9L of buffer water most days, so add 3 vials for 9L of dilution water (filtered tap water).

6.3.4 Aerate for one hour.

6.3.5 Prepare fresh daily.

6.4 Seeding Solution:

6.4.1 InterLab P110 Polyseed purchased from HACH, Catalog No. 2918700 or equivalent.

6.4.2 To set up seed, pour 450 ml of prepared buffer water into an Erlenmeyer flask.

6.4.3 Aerate and stir (at lowest speed) the Polyseed solution for 1 hour, then let the solution settle for 15 minutes. Finally decant the supernatant carefully so as not to allow any bran in the solution.

6.4.4 Pour the decanted polyseed dilution water into a clean 500 ml beaker with sterile stir bar, place on magnetic stirrer and gently stir for the remainder of the test.

6.4.5 For best results, the Polyseed solution should be used within 6 hours of rehydration of the capsule. Always follow the manufacturer's recommendations.

6.4.5.1 The DO uptake attributable to added seed generally should be between 0.6 and 1.0 mg/L.

6.5 Sodium Sulfite Solution:

6.5.1 Dissolve 1.575 g of Sodium Sulfite Anhydrous(Na_2SO_3) in reagent water and dilute to one liter in Class A volumetric flask.

6.5.2 Prepare fresh daily.

6.5.3 VWR Catalog No. 0628-500G or equivalent

6.6 Manganous sulfate solution(364 g/L):

6.6.1 Purchased from Ricca.

6.6.2 Ricca Catalog No. 4620-32 or equivalent.

6.6.3 This purchased chemical is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store at room temperature.

6.7 Alkali-Iodide – Azide Reagent:

6.7.1 Purchased from Ricca.

6.7.2 Ricca Catalog No. 541-16

6.7.3 This purchased chemical is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store at room temperature.

6.8 Concentrated Sulfuric Acid:

6.8.1 Purchased from Fisher, Catalog No. A300C-212 or equivalent

6.8.2 This purchased chemical is stable until expiration date on bottle or within 2 year of opening date, whichever is sooner. Store at room temperature.

6.9 Starch Solution:

6.9.1 Purchased from Ricca.

6.9.2 VWR Catalog No. BDH5102-500 ml or equivalent

6.9.3 This purchased chemical is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store at room temperature.

6.10 Potassium Iodide:

6.10.1 VWR Catalog No. BDH9264-1250 or equivalent.

6.10.2 Manufactured by MP Biomedicals, Inc. Catalog No.152562

6.10.3 This purchased chemical is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store at room temperature.

6.11 Sodium Thiosulfate solution 0.0375N:

6.11.1 Purchased from Ricca, Catalog No. 7925-32 or equivalent

6.11.2 This purchased chemical is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store at 0 - 6°C (not frozen).

6.12 1:100 H_2SO_4 for adjusting pH:

6.12.1 In a 100 ml volumetric flask containing approximately 50 ml of reagent water, carefully pipette 1 ml of concentrated sulfuric acid, swirl carefully to mix and bring to final volume with reagent water.
6.12.2 This reagent is stable for one year. Store at room temperature.
6.13 1:100 50% NaOH for adjusting pH:
6.13.1 In a 100 ml volumetric flask containing approximately 50 ml of reagent water, pipette 1 ml of 50% NaOH solution and bring to volume with reagent water.
6.13.2 This solution is stable for one year. Store at room temperature.
6.14 Nitrification Inhibitor:
6.14.1 Purchased from Hach
6.14.2 HACH Catalog No. 253335 or equivalent.

7 Sample Collection

7.1 Samples are collected in a plastic half-gallon container.
7.2 No chemical preservative is required.
7.3 Samples should be cooled on ice as soon as possible and stored at 0 - 6°C (not frozen) until analysis.
7.4 Holding time is 48 hours.

8 Calibration

8.1 Calibration
The BOD probe is calibrated using a Winkler titration.
8.2 Calibration Curve
Not applicable.
8.3 Calibration Verification
8.3.1 A Glucose/Glutamic Acid standard is analyzed with every batch of samples.
8.3.1.1 When using HACH Glucose/Glutamic Acid Solution for the LCS/LCSD, the true value of the LCS's should be 3.96 mg/L for BOD. The acceptance criteria is 85 -115% recovery.
8.3.1.2 When using HACH Glucose/Glutamic Acid Solution for the LCS/LCSD, the true value of the LCS's should be 3.28 mg/L for CBOD. The acceptance criteria is 81 -118% recovery.
8.3.1.3 If the Glucose/Glutamic acid (GGA) does not meet the acceptance criteria, all samples in the affected batch have to be flagged with a "J."

9 Quality Control

9.1 Refer to Table 14.1 for Reporting Limits (RLs), Table 14.2 for Quality Control Acceptance Criteria, and Table 14.3 for Quality Control Procedures associated with this method and Standard Operating Procedures for Control Charts and Control Limits.
9.2 Refer to reference 13.3 for training and certification procedures.
9.3 Refer to reference 13.4 for control charting procedures.
9.4 All BOD acceptance criteria are static (i.e. not based on control charts).

9.5 Quality control limits are static, but control charts are created and reviewed twice per year for trend monitoring.

9.6 BOD analyses are exempt from the requirement to perform MDL studies.

9.7 LCS's are to be analyzed at a frequency rate of one LCS/LCSD per batch of 20 samples. GGA standards are used for the LCS/LCSD.

9.8 Sample Duplicates must be analyzed at a frequency of 5% of all samples.

9.9 Dilution water result of < 0.20 mg/L is required

9.9.1 If the prepared nutrient buffer dilution water blank D.O. drops more than 0.2 mg/L, the data should be flagged and commented using the following comment. BOD-SM – SM5210B -<B> -Dilution water blank exceeds method required limit of 0.20 mg/L (Corrective Action Number).

9.10 Minimum Reporting Limit of 2 mg/L is independent of any dilutions made to sample.

9.11 If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/l and DO depletion of at least 2 mg/l and there is no evidence of toxicity at the higher sample concentration or the existence of an obvious anomaly, average the results in the acceptable range.

9.12 If all the dilutions have an final dissolved oxygen reading below 1.0 mg/L, use the dilution with the smallest sample volume(highest dilution) as the final result and report with a "J" qualifier and the comment, " Result estimated. Sample depleted to less than 1.0 mg/L for final DO measurement. Result is an estimate and may be higher than the result reported.

10 Procedures

10.1 Remove sample bottles, standards, and reagents from cold storage and allow them to equilibrate to room temperature prior to sample preparation and/or analysis.

10.2 Procedure for setting up BOD water:

10.2.1 Fill carboy to the top with filtered tap water that is run through a bench top carbon canister. This will be approximately 12L. Place the filled carboy in the BOD incubator the night before setting up or reading BOD samples. This allows the water to equilibrate to the correct temperature.

10.2.2 Allow the water to adjust to 20 ± 3°C.

10.2.3 Fill 4 BOD bottles with water from the carboy. Add a stopper and cap to two of these bottles and place them in the incubator. These bottles will be used for your calibration and probe checks. The other two bottles will be used for the Winkler titration.

10.3 Procedure for Dissolved Oxygen by Modified Azide Winkler (to be performed twice):

10.3.1 To the two 300 ml BOD bottles containing filtered tap water, add 2 ml of manganous solution followed by 2 ml of alkali-iodide-azide solution.

10.3.2 Place a stopper on the bottle and mix well by inverting it several times.

10.3.3 When the precipitate has settled to more than half way leaving a clear supernate, add 2 ml of concentrated sulfuric acid. Restopper and mix by

inverting again.

10.3.4 Pour the entire contents of the bottle into a 500 ml Erlenmeyer flask. Titrate with 0.0375N sodium thiosulfate solution. When a pale straw color is reached, add 2 drops of starch solution, which produces a blue color, and then titrate to a clear end-point with the thiosulfate.

10.3.5 The mg/L DO can be read directly from the volume of titrant used since one milliliter of thiosulfate solution (0.0375N) is equivalent to one mg/l DO when the sample size is 300 ml. Ex: 8.0 ml of titrant is used to obtain the clear end-point so the DO of the sample is 8.0 mg/l.

10.3.6 After performing the Winkler twice, the two dissolved oxygen values should not differ by more than 0.3 mls. If the results are within 0.3 ml's then average the results. If they are not within 0.3 ml's, then a third Winkler titration needs to be analyzed.

10.4 Procedure for preparing nutrient buffer water and seed:

10.4.1 At least one hour before setting up BOD's, prepare the seed and buffer water.

10.4.2 To set up prepared buffer water, add 1 Environmental Express SimpleWater™ Nutrient Buffer Dilution Vial per 3 liters of dilution water (filtered tap water). Aerate for 1 hour. Most of the time 9L of buffer water is needed, so add 3 Environmental Express SimpleWater™ Nutrient Buffer Dilution Vials for 9L of dilution water.

10.4.3 To set up seed, pour 450 ml of prepared buffer water into an Erlenmeyer flask. Aerate and stir (at lowest speed) the Polyseed solution for 1 hour, then let the solution settle for 15 minutes. Finally decant the supernatant carefully so as not to allow any bran in the solution. Pour the decanted polyseed solution into a clean 500 ml beaker with sterile stir bar, place on magnetic stirrer and gently stir for the remainder of the test. For best results, the Polyseed solution should be used within 6 hours of rehydration of the capsule.

10.4.4 Seed all samples that have been pH adjusted.

10.4.5 Seed and dilute all WPCP, Park and Hatchery samples even if pH adjustment is not needed.

10.4.6 Seed all diluted samples.

10.5 Procedure for Calibrating pH meter:

10.5.1 Refer to SOP 3-003 – SM4500-H+B-pH for calibration instructions.

10.5.2 An IDC and or CDC in SM4500-H+B-pH is required to analyze BOD samples.

10.6 Chlorine check and pH adjustment of samples:

10.6.1 Adjust sample temperature to 20 ± 3°C by warming up ½ gallon sample container in sink filled with warm water.

10.6.2 Check all samples that are labeled as being chlorinated effluents on the chain of custody or samples suspected of being chlorinated (i.e. samples that smell of chlorine) for chlorine using Chlorine Test Strips – Hach

AquaCheck Catalog No. 27450-50 or equivalent. If chlorinated, follow SOP sect.10.9 for de-chlorinating a sample.

10.6.2.1 All State Park effluents should be checked for the presence of chlorine residual. If chlorine is present, see Section 10.9.

10.6.3 Pour samples into a 400 ml beaker (may have to use a 600 ml beaker if making dilutions).

10.6.4 Place on pH meter and begin stirring.

10.6.5 The pH must be between 6.0 and 8.0. If so, record pH and pour up sample using appropriate dilutions.

10.6.6 If the pH falls out of the above range, record the original pH, and then adjust with either 1:100 H_2SO_4 or 1:100 50% NaOH solutions to a pH range of 6.5–7.5.

10.6.7 Record pH once adjusted and pour according to dilutions. While recording pH, add seed to correct bottles.

10.6.8 Always seed samples that have had pH adjusted.

10.7 Procedure for Calibrating DO probe:

10.7.1 The DO probe should be stored in a BOD bottle containing approximately 75 ml of filtered tap water. The probe should not be touching the water.

10.7.1.1 The water protects the probe membrane.

10.7.2 Press the power button to turn the instrument on.

10.7.3 Allow the probe to polarize and the temperature to stabilize for at least 15 minutes. If calibration is performed prematurely the values will drift and may be out of specification.

10.7.4 To calibrate DO probe, place probe into one of the two BOD bottles that were collected the day before(Section 10.2.3) and stored in the BOD incubator overnight.

10.7.5 Start the stirrer by pressing the red switch at the top of the probe.

10.7.6 Press the calibrate soft-key to change to Calibration mode.

10.7.7 Press the next soft-key to select mmHg reading. Using the up and down key to enter the current pressure reading from barometer. Press Enter to save reading.

10.7.8 Next, press the DO Cal soft-key to enter the manual DO calibration menu. Make sure that the display readings are stable, then enter the calibration value in mg per liter, using the up, down and digit soft-keys.

10.7.9 Press Enter to confirm your calibration. The screen will momentarily display “D.O. Calibration Saved.”

10.7.10 Press MODE to return to the Main mode. The instrument is now calibrated and ready to measure dissolved oxygen.

10.7.10.1 Replace membrane of DO probe every three to four weeks or earlier if there is a drift in the DO measurement, a significant change in DO from the previous day, or the readings will not stabilize after 30 seconds. Follow instructions in the YSI 5906 Membrane Kit.

10.8 Procedure for Setup and Initial DO reading of BOD Samples:

10.8.1 The BOD bottle is first used for the DO reading and then capped and sealed. After 5 days, the bottle is removed from the incubator for the final DO

measure. The difference between the two values times the appropriate dilution factor equals the BOD result.

10.8.1.1 Cleanliness of BOD bottles is critical to the validity of the test. Do not use bottles that have a film on the inside. Bottles may be cleaned with a mixture of Clorox and detergent or cleaned in the automatic dishwasher.

10.8.2 Set up one buffered water blank per batch (i.e. 300 ml of nutrient buffered water in BOD bottle).

10.8.3 Set up two standard bottles

10.8.3.1 LCS/LCSD (3.96 mg/L): Using a clean 300 ml BOD bottle, pipette 5 ml of prepared Polyseed into approximately 50 ml of prepared nutrient buffer water followed by 3 ml of Hach BOD standard Solution (Cat. 14865-10) or equivalent. Dilute with prepared nutrient buffer water half way up the neck of the bottle. Prepare both the LCS and LCSD using this procedure.

10.8.4 Set up three bottles for Polyseed samples. Pipette 15, 20 and 25 ml's of polyseed into each of three 300 ml BOD bottles and dilute with prepared dilution water half way up the neck of the bottles.

10.8.4.1 The resulting DO uptake should fall between 0.60 -1.00 but it is not a requirement. When averaging seed controls, only average the seed controls that drop ≥ 2.0 mg/L.

10.8.5 Make sure pH has been analyzed for all samples and adjusted as needed per Section 10.6.

10.8.6 For samples not needing dilutions or pH adjustment, shake the sample thoroughly before pouring the 20 ± 3°C sample into a BOD bottle and proceed to step 10.8.9.

10.8.6.1 Care must be taken in handling samples to keep the solid content consistent throughout the analysis.

10.8.6.2 Be careful not to entrap air in the BOD bottle.

10.8.7 For samples that were pH adjusted, mix the sample thoroughly before pouring a small amount of the 20 ± 3°C sample into a BOD bottle. Next pipette 5.0 ml of Polyseed solution into the bottle. Fill the bottle up to half way up neck with remaining sample and proceed to step 10.8.9.

10.8.8 For samples containing chlorine, refer to Section 10.9 for de-chlorination procedure.

10.8.8.1 After samples have been dechlorinated, pipette 5.0 ml of Polyseed solution into the bottle containing at least 50 ml of dechlorinated sample. Fill the bottle up to halfway up neck with remaining sample and proceed to step 10.8.9. For samples needing dilutions, refer to the BOD Dilution Table 10.8.9.8.

10.8.9 Nutrient buffer water is to be used to make dilutions.

10.8.9.1 To prepare a dilution, use a class A graduated cylinder to volumetrically measure the appropriate amount of sample and the appropriate amount of dilution water into a 500 ml beaker. Mix well. Make sure to make 400 ml of the dilution so there is sufficient volume to fill the 300 ml BOD bottle. Care must be taken to include a representative amount of solids when measuring out the sample into the BOD bottle.

10.8.9.2 When performing dilutions on a sample, always make at least three dilutions estimated to produce at least one reportable result.

10.8.9.3 If you must dilute sample, do not use less than 1.0 mL of sample to make the dilution. Instead, make a pre-dilution first. See table 10.8.9.8

10.8.9.4 WPCP, Parks and Hatchery samples always require at least 3 dilutions.

10.8.9.5 As a general rule, most Municipal Influents should be diluted 1%, 2%, and 5%. Effluents should be diluted 10%, 20%, and 50%. Perform a 4th dilution if unsure of what dilution to make.

10.8.9.6 If sample seems to be clean, do a 20%, 50% and a 100% dilution.

10.8.9.7 For unknown samples, physical properties of sample such as appearance (dirty or clean) and smell can be used to guess at appropriate dilution. For WP samples it is helpful to check the history of the BOD sample results to determine if dilutions may be necessary.

Table 10.8.9.8 BOD DILUTION CHART (based on 400 ml final volume)

BOD RANGE	% DILUTION	ML'S OF SAMPLE
20,000 – 70,000	0.01	4.0(using 1:100 pre-diluted sample)
10,000 -35,000	0.02	4.0(using 1:100 pre-diluted sample)
4,000 - 14,000	0.03	12(using 1:100 pre-diluted sample)
1:100 Pre-dilution	Make a 1:100 dilution prior to making dilutions for the estimated BOD Ranges listed above.	
2,000 – 7,000	0.1	4.0(using 1:10 pre-diluted sample)
1,000 – 3,500	0.2	4.0(using 1:10 pre-diluted sample)
1:10 Pre-dilution	Make a 1:10 dilution prior to making dilutions for the estimated BOD Ranges listed above.	
400 – 11,400	0.5	2.0
200 – 700	1	4.0
100 – 350	2	8.0
40 – 140	5	20
20 – 70	10	40
10 – 35	20	80
8 – 28	25	100
5 – 17.5	40	160
4 - 14	50	200

10.8.10 Rinse probe and place DO probe into each BOD bottle and press red button to start stirrer. When reading stabilizes, record the measurement on the lab bench sheet along with the bottle #, dilution data, seed data, and time and probe check when required. Make sure to rinse probe with filtered tap water between each sample.

10.8.10.1 Note: Samples that are supersaturated (DO > 9.2 mg/L) with oxygen should be corrected by agitation or aeration if possible.

10.8.11 Make sure there are no air bubbles in the bottles. To remove air bubbles stuck on inside of BOD bottle, gently tap the sides of the BOD bottle with a glass stopper to release them.

10.8.12 Next, place a stopper in the each bottle to make a tight water seal. Then cover with plastic cup to prevent water seal from evaporating.

10.8.13 Place samples and QC bottles in the Dark BOD incubator set at 20ºC ± 1ºC for 5 days.

10.9 Treatment of Samples Containing Chlorine:

10.9.1 Measure 450 ml of sample into a 1L beaker and allow it to sit on counter for 1 hour for chlorine to dissipate. Check chlorine residual again. If chlorine is still present, the sample must be de-chlorinated. The pH must be between 6.0 and 8.0.

10.9.1.1 If the sample is going to be analyzed in duplicate, then measure 700 ml of sample.

10.9.2 Before dechlorinating the sample, first check the pH. The pH must be between 6.0 and 8.0. If the pH is outside this range, follow the procedure in step 10.6.6 to adjust pH to between 6.5 and 7.5.

10.9.3 Prepare Sodium Sulfite Solution according section 6.5.

10.9.4 To determine chlorine concentration, measure 100 ml of the pH adjusted sample and pour into a 150 ml beaker containing a small stir bar. Next add 2 ml of 1:100 H_2SO_4 solution and 0.15 grams of Potassium Iodide granular and gently stir on stir plate to mix.

10.9.5 Add 5 drops of starch indicator to the sample solution and using a burette, start titrating with Sodium Sulfite solution (See Section 6.5) to the starch-iodine endpoint for residual (until the sample is colorless). Record the volume of Sodium Sulfite solution used for the titration.

10.9.6 Determine how much sample is needed for analysis of sample and sample duplicate if sample is a QC sample. Measure this amount of sample into a clean Erlenmeyer flask and add the appropriate amount of Sodium Sulfite to neutralize the chlorine. Stir gently on stir plate and wait 15 minutes.

10.9.6.1 Ex: If it took 3 mL to neutralize 100 mL of sample, then you would need 9 mL to neutralize 300 mL of sample.

10.9.7 Recheck the sample with a chlorine strip to ensure the chlorine has been neutralized. If residual chlorine is still present, repeat the titration process.

10.9.8 Make appropriate BOD dilutions using the neutralized sample and prepared nutrient buffer water. Add 5 ml of Polyseed solution(Section 6.4)

10.9.9 If it takes more than 15 mL of sodium sulfite to neutralize chlorine, then the sample should be J flagged as estimated due to high chlorine residual. Comment: BOD-SM5210B- <J> - Value estimated. Sample result estimated due to high chlorine content. CA#

10.9.10 Comment on sample if Chlorine is present. BOD-SM5210B – Sample contained chlorine. No corrective action is needed.

10.10 Procedure to set of CBOD Samples:

10.10.1 The CBOD bottle is first used for the DO reading and then capped and sealed. After 5 days, the bottle is removed from the incubator for the final DO measure. The difference between the two values times the appropriate dilution factor equals the CBOD result.

10.10.2 Set up one buffered water blank by adding 0.16g of the HACH Nitrification Inhibitor into a 300 ml BOD bottle. Add prepared nutrient buffer water all the way to the rim of the bottle. Do not shake or stir the sample as the DO probe stirrer will provide enough mixing to dissolve the reagent. There should be one blank per batch.

10.10.3 Set up two standard bottles both containing HACH Nitrification Inhibitor.

10.10.4 LCS/LCSD (3.28 mg/L): Add 0.16g of the HACH Nitrification Inhibitor into a clean 300 ml BOD bottle. Next add 50 ml of prepared nutrient buffer. Pipette 5 ml of prepared Polyseed followed by 3 ml of Hach BOD standard Solution (Cat. 14865-10). Dilute with prepared nutrient buffer water all the way to the rim of the bottle. Prepare both the LCS and LCSD using this procedure. Do not shake or stir the sample as the DO probe stirrer will provide enough mixing to dissolve the reagent.

10.10.5 For CBOD samples, fill BOD bottle 2/3 full with sample and then add 0.16g of the HACH Nitrification Inhibitor. Swirl to dissolve and then fill the BOD bottle to half way up the neck of the bottle with remaining sample. Do not shake or stir the sample as the DO probe stirrer will provide enough mixing to dissolve the reagent.

10.10.6 Read the DO as usual.

10.11 Procedure for Final DO reading of BOD Samples:

10.11.1 Set up BOD water. Refer to 10.2 for Procedure for setting up BOD water.

10.11.2 Perform Winkler Titration. Refer to 10.3 for Procedure for Dissolved Oxygen by Modified Azide Winkler (to be performed twice).

10.11.3 Calibrate DO probe. Refer to 10.7 Procedure for Calibrating DO probe.

10.11.4 Remove samples from BOD incubator after 5 days of incubation.

10.11.5 Remove plastic caps and glass stoppers from bottles.

10.11.6 Place DO probe into each BOD bottle and press red button to start stirrer. When reading stabilizes, record the measurement on the lab bench sheet along with the time and probe check when required. Make sure to rinse probe with filtered tap water between each sample.

10.11.7 Calculate the final DO results using the equations in Section 11.

11 Calculations

11.1 Seed Correction Factor (SCF):

11.1.1 To determine SCF calculate the average seed result using only the seed control bottles that result in residual DO of $\geq$ 1.0 mg/L and a DO uptake of $\geq$ 2.0 mg/L after a 5 day incubation.

11.1.2 Seed Result = $\frac{(IDO-FDO)}{V_{Control}}\ x\ V_{Sample}$

Where:

IDO = Initial Dissolved Oxygen reading on Day 1 of test
FDO = Final Dissolved Oxygen reading on Day 5 of test
$V_{Control}$ = Volume of seed added to the Seed control bottle
V_{Sample} = Volume of seed added to the sample bottles

11.2 BOD Sample Concentration:

11.2.1 When dilution water is not seeded:

BOD_5, mg/l = IDO – FDO

Where:

IDO = Initial Dissolved Oxygen reading on Day 1 of test
FDO = Final Dissolved Oxygen reading on Day 5 of test

11.2.2 When dilution water is seeded:

BOD_5, mg/l = $DF\ x\ [(IDO - FDO) - (SCF)]$

Where:

IDO = Initial Dissolved Oxygen reading on Day 1 of test
FDO = Final Dissolved Oxygen reading on Day 5 of test
DF = Dilution Factor is (100) / (% Percent Sample Volume)
SCF = Average Seed Result

11.2.3 If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/l and DO depletion of at least 2 mg/l and there is no evidence of toxicity at the higher sample concentration or the existence of an obvious anomaly, average the results in the acceptable range.

11.3 LCS/LCSD Recovery (GGA Recovery):

11.3.1 To calculate the true value of the LCS sample, calculate the amount of DO equivalent added to the amount of sample used.

$$\frac{(396\frac{\text{mg}}{\text{L}})\ x\ (0.003\ L)}{0.300\ \ L} = 3.96 \text{ mg/L (true value)}$$

11.3.2 An LCS result of 3.55 mg/L after Seed Correction Factor is

$$\text{LCS \%R} = \left(\frac{3.55\frac{\text{mg}}{\text{L}}}{3.96\frac{mg}{L}}\right) x\ 100 = 89.7\%$$

11.4 Percent Relative Standard Deviation (%RSD):

$$\%\text{RSD} = \frac{\sigma_{n-1}}{\bar{X}} * 100$$

11.4.1 Where:

σ_{n-1} = Sample Standard Deviation

$\bar{X}$ = Mean of the values

11.5 Relative Percent Difference (%RPD or RPD):

$$\%\text{RPD} = \frac{|X_1 - X_2|}{\frac{(X_1 + X_2)}{2}} * 100$$

11.5.1 Where:

$|X_1 - X_2|$ = Absolute difference between two values

$\frac{(X_1 + X_2)}{2}$ = Average of two values

11.6 Percent Drift, %Drift:

$$\%\text{Drift} = \frac{(\text{Concentration}_{\text{Calculated}} - \text{Concentration}_{\text{Expected}})}{\text{Concentration}_{\text{Expected}}} * 100$$

11.6.1 Where:

$\text{Concentration}_{\text{Calculated}}$ = Concentration calculated from result

$\text{Concentration}_{\text{Expected}}$ = Theoretical concentration of the standard

12 Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedure, reference 13.5.

13 References

13.1 Standard Methods for the Examination of Water and Wastewater, 2016. SM5210B 5-Day Biochemical Oxygen Demand Test.

13.2 EPD Laboratory Quality Assurance Plan, online revision.
13.3 GA EPD Laboratory SOP's – Initial Demonstration of Capability SOP 6-001, online revision or Continuing Demonstration of Capability SOP 6-002, online revision.
13.4 GA EPD Laboratory SOP-EPD Laboratory Procedures for Control Charts and Control Limits SOP, SOP 6-025, online revision.
13.5 GA EPD Laboratory SOP-EPD Laboratory Waste Management SOP, SOP 6-015, online revision.
13.6 GA EPD Laboratory SOP – Determination of Method Detection Limit, Method Detection Limit SOP, SOP 6-007, online revision.
13.7 GA EPD Laboratory Safety Plan – EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

14 Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

Table 14.1 RLs for SM5210B

Parameter/Method	Analyte	Matrix (aqueous)	
		RL	Unit
SM5210B	BOD	2.0	mg/l
SM5210B	CBOD	2.0	mg/l

Table 14.2 Acceptance Criteria for Method SM 5210B

Method	Analyte	Accuracy Water (%R)	Precision Water (%RPD)
SM5210B	BOD	85-115	30
SM5210B	CBOD	81-118	30

Table 14.3 Summary of Calibration and QC Procedures for Method SM 5210B

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
SM 5210 B	BOD/CBOD	Dilution Water Blank	Once per batch	< 0.2 mg/l	Initiate a corrective action, evaluate out of control event and comment on diluted samples in batch	Flag with a "B"
		Glucose/ Glutamic Acid Check	Once per batch	QC Acceptance Criteria Table, SOP 3-017 Appendix A	Initiate a corrective action, evaluate out of control event and comment on all samples in the batch.	Flag with a "J"

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
SM5210B	BOD/CBOD	Initial Demonstration: Demonstrate ability to generate acceptable accuracy and precision using four analysis of a QC check sample, a method blank and a blind sample. In addition, the analyst must prepare one standard.	Once per analyst	QC Acceptance Criteria Table A.1, SOP 3-017 Appendix A and Initial Demonstration of Capability SOP (Reference 13.3)	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria.	
		Continuing Demonstration: Demonstrate ability to generate acceptable accuracy and precision using a variety of analysis options of a QC sample(s)	Every 6 months	QC Acceptance Criteria Table A.1, SOP 3-017 Appendix A and Continuing Demonstration of Capability SOP (Reference 13.3)	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Sample Duplicate	Once per batch	QC Acceptance Criteria Table A.1, SOP 3-017 Appendix A	Initiate a corrective action, evaluate out of control event and comment on sample	

Appendix A – Quality Assurance Criteria for Method SM5210B

Table A.1 Quality Assurance Criteria for Method SM5210B			
QC Type	Analyte	Accuracy(%R) LCL - UCL	Precision (%RPD)
LCS/LCSD	BOD	85 - 115	30
LCS/LCSD	CBOD	81 - 118	30
Control Chart data generated from 01/01/2019 -01/01/2021			

Updates to Previous Version:
Appendix A added.
Updated for online revision.
Section 3
Section 5
Section 6
Section 10
Table A.1
Table 14.2