Standard Operating Procedure for:

Biochemical Oxygen Demand (BOD)

DEPARTMENT OF ENVIRONMENTAL PROTECTION
LABORATORY
BIOLOGY PROGRAM
TALLAHASSEE FLORIDA

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

TABLE OF CONTENTS

1. SCOPE AND APPLICATION
2. SUMMARY OF METHOD
    2.1. Interferences
    2.2. Definitions
3. EQUIPMENT AND SUPPLIES
4. REAGENTS AND STANDARDS
5. CALIBRATION AND STANDARDIZATION
6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING
7. SAMPLE PREPARATION
8. SAMPLE ANALYSIS
9. DATA ARCHIVAL
10. QUALITY CONTROL
11. SAFETY/HAZARDOUS WASTE MANAGEMENT
12. REFERENCES

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

1. SCOPE AND APPLICATION

1.1. This method is based on Standard Methods 5210B, Online Edition. The test measures the oxygen depletion potential of ambient, surface, or effluent waters during a 5-day incubation period. It can also help determine the BOD removal efficiency of wastewater treatment plants. During the 5-day incubation, oxygen is essentially consumed by bacteria for biochemical degradation of organic materials (carbonaceous) and oxidation of inorganic materials (sulfides, ferrous iron, and reduced forms of nitrogen). The BOD test measures both carbonaceous and nitrogenous demand for oxygen. In the CBOD test, nitrification is inhibited and therefore measures only carbonaceous demand.

1.2. This procedure is used for samples with LIMS test IDs of BOD-INHIB and BOD-UNINHB.

1.3. Method Detection Limits (MDL)

1.3.1. A true MDL cannot be determined for the BOD test. Therefore, MDL is set at 0.20 mg/L, since this is the maximum allowable depletion for a dilution water blank. The PQL is set at 2.0 mg/L because of the 2.0 minimum depletion method requirement.

2. SUMMARY OF METHOD

2.1. A sample is poured into an airtight 300 mL BOD bottle. It is seeded to ensure a sufficient bacterial population for the test. Nutrients and buffers may also be added to the bottle. The initial dissolved oxygen of the sample bottle is measured. It is then incubated at 20 ± 1 ºC for five days. After this, a final DO measurement is taken. The BOD is calculated using the difference between initial and final DO measurements and corrected for seed depletion.

2.2. Interferences

2.2.1. During incubation, photosynthetic production of DO can occur if samples are not kept away from light.

2.2.2. Sodium sulfite solution, which is used for dechlorination of sample, can also exert an oxygen demand. A titration for each chlorinated sample should be run, to ensure only the minimum amount of sodium sulfite is used to dechlorinate the sample.

2.2.3. Some metals and other substances in the water sample may be toxic to bacteria and can therefore cause matrix interference. Using larger dilutions can help minimize the effect of a toxic sample.

2.3. Definitions

2.3.1. IDO – Initial Dissolved Oxygen is the DO measurement taken on the first day of the test.

2.3.2. FDO – Final Dissolved Oxygen is the DO measurement taken on the final day (Day 5) of the test.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

2.3.3. 2-1 Rule - The 2-1 Rule refers to the method requirement that for a test bottle to be considered valid, it should have a minimum depletion of 2.0 mg/L after a 5-day incubation period and at least 1.0 mg/L residual DO for the final reading.

3. EQUIPMENT AND SUPPLIES – Record any equipment repairs or maintenance in LIMS Laboratory Logbook *Maintenance_Log*

3.1. 300 mL Wheaton BOD bottles

3.1.1. Wash bottles in laboratory dishwasher. When cycle is complete, immediately remove bottles from dishwasher and place in drying oven until completely dry. If method blanks exceed method limits, wash bottles according to normal lab washing procedure and autoclave. See SOP BG-05 Biology Wash Party Procedures.

3.2. Aeration manifold with Tygon tubing designated for seed, blanks and samples.

3.3. Multi-lab 4010-1 Meter SN 20061905 and ProOBOD probe.

3.3.1. Calibrate per SOP BB-25

3.3.2. Multi-Lab Importer software resides in X:\Labs\Lab5\Biology\Bench Common\BOD\Multi-Lab Importer software

3.3.3. EZ_BODReaderVSa.exe resides in \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\BOD\autoez\New AutoEZ\All Versions. To run the program from the BOD computer, create a shortcut of the most current version of the software on the BOD computer desktop.

3.3.4. Prior to first using any new version of the BOD program, make sure the program parameters are set as listed below by choosing **File/Setup/General** under the "General" tab.

- Company Name – FL DEP
- Bottle Volume 60 or 300 mL – 300
- Default Seed Volume m./Btl Volume – 1
- Seed Method Number: 1) Direct; 2) Seeded Dil Water; 3) Both – 1
- Both Seed Method Option – Max Vol ml – 0
- Both Seed Method Option – Vol Seed Added ml – 0
- IDO/FDO Requirement: 1)2-1 Rule; 2)25-75% Rule – 1
- Exclude Seed Controls from above Rule – FALSE
- MDL/Reporting Limit Option # 1) None; 2)Depltn Rule; 3)Seed Corrected Depltn – 1
- GGA Upper Control Limits – 228.5
- GGA Lower Control Limits – 167.5
- Outlier Rejection Criteria – Relative % i.e. 25 or (0 to turn OFF) – 30 Flag Only
- Outlier Rejection Criteria – Absolute Conc mg/L i.e. 1 or (0 to turn OFF) - 0
- Outlier Rejection Criteria – Cutoff Conc for Absolute Criteria mg/L i.e. 30 or 0 (to turn OFF)
- Slope (%)used to flag a sliding BOD or (0 to turn OFF) – 20
- Significant figures – 3
- Import path - \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Import Files
- Worksheet path - \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Worksheet

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

- Export Path - \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Worksheet
- Archive Path - \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Archive
- Site Elevation (Feet) – 876
- Theoretical D.O. Allowable Exceedance +/-(mg/L) – 0.4
- Default Super Saturation Warning Limit 9.1 (Set to Zero to use TDO+Exceedance Above – 9.1
- Default Temperature Warning Limit (20 +/-) – 3
- Date Format i.e. MM-DD-YY – MM-DD-YY
- External Import Path\Program (i.e.:'C:\EZ_BODReader1\Support\jnkProj1.exe' or leave blank) – (leave blank)
- External Data Transfer (Path\Program or leave blank) – (leave blank)
- Include Calibration History with Final Report – TRUE
- Test IDs (separate with semicolons) and Display Order i.e. BOD5; CBOD5 – BOD5; CBOD5;SolBOD5;SolCBOD
- Seed Option Display Order i.e. S1;S2;DS1;DS2;S3;S4….. – "S1;S2;S3;S4;S5;S6;DS1;DS2;DS3;DS4;DS5;DS6)"
- Set IDs and Display Order i.e. PLT;QA;IW – PLT;QA;IW
- Comm Port BODAutoEZ i.e. COM1 – COM3
- External Meter Present TRUE/FALSE –TRUE
- Maximum Bottle Number (before wrapping back to 1) – 490
- Maximum Allowable Blank Depletion Range +/- (i.e. 0.24) – 0.24
- Export Entire Worksheet – FALSE
- Track Setup Date_Time of each sample – FALSE

3.4. BOD AutoEZ Trays

3.5. Serological disposable pipettes (10mL) sterile and individually wrapped. Volumes of each new lot are checked per SOP MB-03.3

3.6. Graduated cylinders, 10-250 mL

3.7. Chlorine Test Strips

3.8. Accumet Excel XL 25 Dual Channel pH/Ion meter (S/N XL94005930) – calibrate per SOP MB-03.4

4. REAGENTS AND STANDARDS

4.1. Deionized water

4.2. Nitrification Inhibitor, Formula 2533 (2% TCMP)

4.3. Phosphate-buffer solution, commercially prepared

4.4. Magnesium sulfate solution, commercially prepared

4.5. Calcium chloride solution, commercially prepared

4.6. Ferric chloride solution, commercially prepared

4.7. Sodium sulfite solution

4.7.1. In a 1 L volumetric flask, add 1.575g Na_2SO_3 to a small volume of DI water. Bring volume up to 1 L with DI water. Prepare on day of use.

4.7.2. Proper dechlorination of the sample serves as verification of the concentration of the sodium sulfite solution.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

4.8. Potassium iodide solution

4.8.1. In a 100 mL volumetric flask, add 10 g of potassium iodide to a small volume of DI water. Bring volume up to 100 mL with DI water.

4.9. Sulfuric acid, H_2SO_4, 1N

4.9.1. Slowly add 28 mL of concentrated sulfuric acid to deionized water and dilute to 1 L in a class A volumetric flask.

4.10. Sodium hydroxide, NaOH, 1N

4.10.1. Dissolve 40 g NaOH in deionized water and dilute to 1 L in a class A volumetric flask.

4.11. Glucose glutamic acid solution (GGA)

4.11.1. In a Class A volumetric flask add 0.15g of dried reagent grade glucose and 0.15g dried reagent grade glutamic acid to a small volume of DI water and bring volume up to 1L with deionized water.

4.11.2. A commercially prepared GGA standard may also be used.

4.11.3. Prepare on the day of use. Log preparation into LIMS Standard Preparation Tracker with a holding time of 24 hours.

4.12. Seed solution

4.12.1. Pour the entire contents of a PolySeed® capsule into a beaker containing 250 mL of dilution water. Aerate using tubing labeled for seed and stir the solution for one hour. Allow the bran to settle for 5 to 15 minutes. Decant into a flask through a mesh screen to remove the bran from the solution. Alternatively, a separatory funnel or pipette can be used to separate the solution from the bran. Place a stir bar in the beaker containing the decanted seed and spin for the remainder of the test. Use within 6 hours of hydration. Record preparation in LIMS Standard Preparation Tracker.

Note: The weight of the contents of one PolySeed® capsule is 0.187 g as determined by DEP staff on 5/3/2018. This weight should be used when entering PolySeed® preparation into Standard Preparation Tracker.

Note: Store Polyseed capsules in the original packaging in the BOD room.

5. CALIBRATION AND STANDARDIZATION

5.1. See SOP BB-25 for calibration and maintenance of DO meters.

6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1. See SOP FS 1000 for sample collection and preservation.

6.2. Store samples at 2 – 4 ºC. Analysis should start within 48 hours of collection. The temperature of samples in coolers upon receipt to lab should be no greater than 6 ºC. Samples should be Y-qualified if outside of this temperature range.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7. SAMPLE PREPARATION

7.1. Refer to SOP BB-030 for sample custody and label printing.

7.2. BOD Batch Preparation

7.2.1. Separate samples into batches, which contain a maximum of 20 samples by adding them to a *BOD prep worksheet.* BOD-UNINHB samples and BOD-INHIB samples will be in different batches. Each batch should include 3 seed controls- 10, 12, 15 mL, 2 dilution water blanks, 3 GGA standards and a lab duplicate.

NOTE: If another batch of seed is made, add the QC to the prep worksheet and analyze another set of controls using the new seed solution. Analyze another duplicate per 20 samples.

7.3. BOD Worksheet Setup

7.3.1. Open LIMS. Select **General -> LIMS Browser -> Search**

7.3.1.1 Check the following:

Search By: Job ID
Additional Selections: Analyses
Job Status: Reported/A/C/V
Filter By Date: Received

7.3.1.2 Click *Build List* for Job ID, select jobs, then click *Build List* for Analyses. Select all BOD analyses. Click OK. Click *Report/Files*. Click *Select All Events* and *Select All Jobs*.

7.3.1.3 Create a BOD Worksheet template. From Available Fields, add to Selected Fields/Column Order: Field ID, Sample ID, Analysis. Sort by Sample ID. Select Save Template and save as BOD Worksheet.

7.3.1.4 Create Delimited File and save to Desktop as "BOD*date*".

7.3.2 Open the template named SampleDEP.csv (\\fldep1\DEAR\Labs\Lab3\Biology\BOD\Import Files)

7.3.2.1 AutoEZ software: Copy and Paste the Desktop file ("BOD*date*") into the SampleDEP.csv template (only the Sample IDs and Field IDs). Change dilution volumes in the "Vol" column and change 0 to 4 in "Qcode" column for the duplicate sample. Duplicate samples should have "_dup" added after the sample ID. Delete all unused cells.

7.3.2.2 Save file as "BOD*date*" to Import Files.

7.3.3 Open the EZ_BOD Reader program.

7.3.3.1 Select **File-> New**.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7.3.3.2 Double click the blue header. Add dilution water serial #, GGA serial #, PolySeed® serial #, and Tray # to the worksheet.

7.3.3.3 Use **Worksheet-> Add Set/Batch of Samples** from the menu to add the seed control and sample sets to the worksheet. Use the file created and saved in Import Files.

7.3.3.4 Save the file with the file name "BODMM-DD-YYYY" (MM-DD-YYYY being the current month, date, and year) in the following directory: \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Worksheet in the appropriate year and month folder. Record the filename as "BODMM-DD" (MM-DD being the current month and day) in the *BOD Run Log*. Because of computer glitches in the program, saving the file may delete the last line from the header each time. To remedy this, use the enter key to add a few extra lines to the end of the header to prevent lost header info from multiple saves.

7.3.4 The BOD program keeps two temporary files. The first file is generated after the first automated reading. It is a copy of the original file and does not contain any data. The second file is generated after the next automated reading and should contain initial dissolved oxygen readings. Do not delete the temporary files.

7.3.5 The Multi-Lab importer file may be used if another software is not available. Copy the field IDs and sample IDs from the file created in step 7.3.1.4. Paste into the appropriate fields. Add rows for dilutions and duplicates.

7.4 Sample Pretreatment

7.4.1 Check pH and record in *BOD Run Log* for all samples. If pH is outside the range of 6.0 - 8.0, wait until the day of the analysis and warm the samples to 20 ± 3°C. Next, adjust the pH between 7.0 and 7.2 with sulfuric acid or sodium hydroxide. Do not dilute the sample by more than 0.5%. Record the serial # for sulfuric acid and/or sodium hydroxide used in the *BOD Run Log*.

7.4.2 If samples are suspected of containing chlorine or are of an unknown source, check for chlorine with chlorine test strips. Record lot number of chlorine test strips in the *BOD Run Log*. (This can be done the day before set-up. Samples do not need to be warmed first.) If residual chlorine is present, follow steps in section 7.4.4.

7.4.3 On day of set-up, place samples in a warm tap water bath for 3.5 minutes to warm to 20 ± 3°C. Check the temperature of the samples after warming using a clean temperature probe and aerate for 10 minutes using tubing labeled for samples. Document in the *BOD Preparation Worksheet* that samples were aerated. Record the date/time samples are placed into the water bath in the prep start Date/Time section in the *BOD Preparation Worksheet*. This is considered the start of analysis.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Note: Do not allow samples to sit between warming and aeration as this may affect the oxygen depletion of the sample and cause results to be inaccurate.

7.4.4 Dechlorination

Note: Samples should only be dechlorinated the day of the test. Too much sodium sulfite solution can create an oxygen demand and must be titrated to the exact amount.

7.4.4.1 Add 1 mL of concentrated sulfuric acid and 10 mL of potassium iodide (KI) solution to a 100 mL portion of sample

7.4.4.2 Add 5 drops of starch indicator to the sample solution.

7.4.4.3 Titrate with sodium sulfite solution until the sample is colorless. Record the volume of sodium sulfite used in the BOD Sample Dechlorination Log logbook.

7.4.4.4 Dechlorinate the original sample with the recorded amount per 100 mL volume of sample left and mix.

7.4.4.5 Recheck the sample with a chlorine strip, to ensure that chlorine has been neutralized. If residual chlorine is still present, repeat the titration process. Record the additional volume of sodium sulfite used in the BOD Sample Dechlorination Log logbook.

8. SAMPLE ANALYSIS

8.1 Dilution Water Preparation

8.1.1 Determine amount of deionized water needed. Multiply 300 mL by the number of GGA, blank, seed, and dilution bottles needed and round up to the nearest liter. Add this amount to clean, autoclaved large flasks or carboys and lightly aerate overnight using tubing labeled for dilution water. Dilution water can be stored overnight and shaken vigorously before set-up; however, best results are achieved when aerated.

8.1.2 Immediately before use, add 1 mL of the following solutions per liter of DI water:

$MgSO_4$ solution

$CaCl_2$ solution

$FeCl_3$ solutionPhosphate buffer solution

8.1.3 Continue to aerate the water. For best results, remove aeration tubing at least 1 hr prior to set up.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

8.1.4 Record preparation into LIMS **Standard Preparation Tracker** with a holding time of 24 hours.

8.2 Sample Setup

8.2.1 Pull out number of trays needed for the run (12 bottles per tray) and record tray numbers in *BOD Prep Worksheet Manager* in order of use. Record bottle numbers in the *BOD Prep Worksheet Manager*. starting with the seed controls , blanks, GGAs, samples.

Note: Initial DO must be measured within 30 minutes after preparing dilutions. The analyst should be aware of the maximum number of controls/samples that he/she can prepare in order to read within 30 minutes.

8.2.2 Check for required dilutions in the historical data spreadsheet located in X:\Labs\Lab5\Biology\Bench Common\BOD labeled BOD dilutions.

8.2.3 If there is no historical data and the sample is wastewater or from a polluted water body, or pollution is suspected, make a minimum of 3 dilutions and use the following as a guide:

8.2.3.1 0.01 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater; 5 to25% for biologically treated effluent, and 25 to 100% for polluted river waters.

8.2.3.2 If a sample requires a dilution greater than 1:100, make a pre-dilution first before making final dilutions. A 1 L BOD bottle can be used for the primary dilution. Effluents should automatically get 3 dilutions regardless of appearance

8.2.4 Add dilution water to controls and samples with dilutions first. Samples with no dilutions (300 mL) can be poured directly into the BOD bottles. For dilutions, use a clean graduated cylinder to measure and pour the appropriate volume of sample. Vigorously shake sample container before pouring out sample to mix well and aid in removing excess oxygen.

8.2.5 Add 1 pump of nitrification inhibitor to all CBOD samples, seed, and inhibited GGA bottles. Add 6 mL of GGA solution to each GGA control bottle.

8.2.6 In the following order, add 10, 12, and 15 mL of seed to the seed bottles. Add 2 mL of seed to all GGA and sample bottles. Seed controls and amount of seed can be adjusted. See section 10.6: Troubleshooting.

8.2.7 Add 0.30 mL buffer /300 ml sample of the following solutions to any bottle that contains more than 67% of the sample after dilution:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

$MgSO_4$ solution

$CaCl_2$ solution

$FeCl_3$ solution

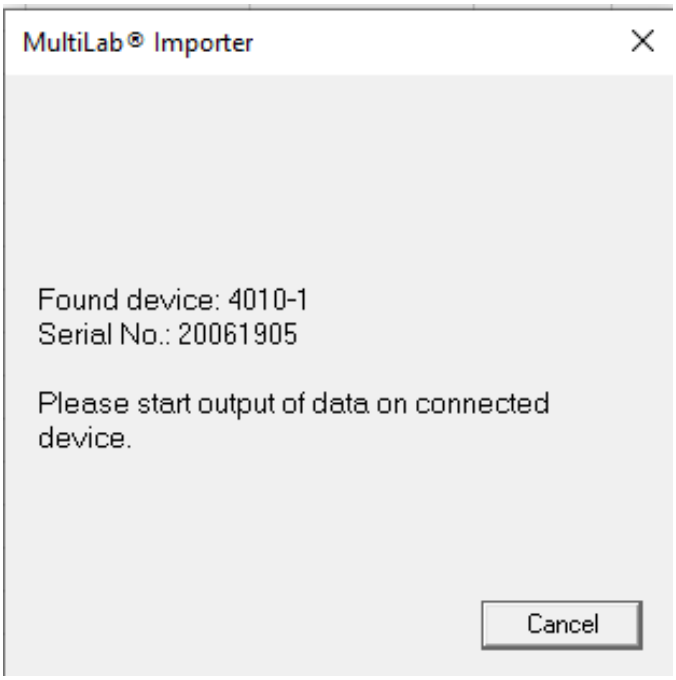
Phosphate buffer solution

8.2.8 Top off any unfilled bottles with dilution water. This should include, blank, seed control, GGA, and sample dilution bottles.

8.3 IDO readings

8.3.1 Calibrate the meter according to SOP BB-25.

8.3.2 Multi-Lab Importer: In the Multi-Lab importer file, select **Add ins** in the header section. Then, select **import data**. If the connection is successful, the following screen will populate:



8.3.3 Click in the cell labeled **Click here to begin data output** in the Multi-Lab importer file. Seal the probe into the BOD bottle and turn on the stir paddle. Make sure this is no air (bubbles) in the bottle. Once "O2" has stopped flashing on the meter, and the reading is stable, press **F2** to export the data to the file. Check to make sure the readings are going into the appropriate cells in the file. Rinse the probe well with DI water. Continue to the next BOD bottle and follow the same procedure. With each new reading, press **F2** to export the data to the file. Select **Cancel** when all readings are complete. If a sample must be re-read, do not overwrite the original reading. Select **Cancel** in the Multi-lab importer file. Add **two** new rows below the sample row that needs to be re-read and fill in the sample information. The header information will re-populate above the readings. Make a note in the cell row that was re-read

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

and provide the reason. Also, make a note in *BOD Prep. Worksheet Manager*. The new reading will be used for data upload.

8.3.4 AutoEZ software: Open the appropriate worksheet in EZ_BODReaderVS. Use the EZ_BODReaderVS menu bar and select **Read**, **External Meter**, and click the **Cal Air** Button. The software will then tell you to perform a manual calibration. Press the meter's **Cal** key, and then the meter's **Enter** key'. When the calibration is completed, the meter will beep. Review the meter's display, and if acceptable, press the meter's **F2** key. Lastly, Click the EZ_BODReaderVS **Get Calibr** button. Review the displayed information and press the **Save Calibr** Button. Close the External Reader form. Record the calibration D.O. value in the DEP calibration excel file.

8.3.5 Capture D.O. Readings: Use the EZ_BODReaderVS menu bar, open the desired worksheet and select **Read**, **External Meter** and select I.D.O. The program will display the meter's values. To record the displayed values on a worksheet, left click on the desired sample line's D.O. Cell. Use the program's Undo button if you accidentally clicked on the wrong cell.

8.3.6 IDO readings should be between 7.5 and 9.5 mg/L. See section 10.4.1 if readings are outside this range. Check all readings to ensure they are consistent. Controls and duplicate dilutions should have similar IDO readings. Re-read any bottles that seem inconsistent with expectations.

8.3.7 Check the calibration of the probe according to SOP BB25 after the run has completed. Enter the DO readings and standard deviations in the calibration excel workbook. If the probe is failing calibration, determine the cause according to SOP BB25 Troubleshooting section. Re-calibrate, and immediately re-read any samples that were read with the failing probe. If the problem cannot be fixed, contact a senior analyst or supervisor. IDO measurements must be read within 30 minutes of the start time.

8.3.8 Select **File-> Save As** "BOD_MMDDYY" Save in \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Worksheet in the appropriate year and month folder.

8.3.9 Use a glass stopper to make a tight water seal on each bottle. Make sure there is no air (bubbles) in the bottles. If necessary, add dilution water to eliminate any air space. Add a plastic cover to prevent evaporation of the water seal during incubation. Place the trays on the shelf and cover to prevent exposure to light during incubation which can cause photosynthetic production of oxygen.

8.4 Before the samples are discarded, the analyst should verify that the controls and sample IDs on the worksheet match the order in the *BOD Preparation Worksheet*

8.5 FDO readings

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

8.5.1 Calibrate the instrument per SOP BB-25.

8.5.2 Read FDOs 5 days +/- 6 hours from the time incubation. The documented time of IDO measurement can be used for reference.

8.5.3 Remove plastic covers and glass stoppers from bottles.

8.5.4 Open the appropriate file from \\fldep1\DEAR\Labs\Lab3\Biology\BOD\Worksheet.

8.5.5 Multi-Lab importer: Click on the **FDO** tab in the file. Follow steps from 8.4.2.

8.5.6 AutoEZ software: Open the appropriate worksheet in EZ_BODReaderVS. Follow steps from 8.4 and 8.5. Select F.D.O. to read samples.

8.5.7 Check the calibration of the probe after the run has completed and enter the DO readings and standard deviations in the calibration excel workbook.

8.5.8 Look at all BOD values to ensure that they seem logical. The difference between each BOD for all dilutions of a sample should not be greater than 30%. Check all flagged BODs. Review the flag codes in the help file in the BOD program. Re-read any samples if necessary.

8.5.9 If the probe is failing calibration, determine the cause according to SOP BB25 Troubleshooting section... Re-calibrate the probe and re-read all bottles. If the probe cannot be fixed, contact a senior analyst or supervisor.

8.6 Before the samples are discarded, the analyst should verify that the controls and sample IDs on the worksheet match the order in the *BOD Preparation Worksheet* Enter the analyst date and time in the review section of the worksheet header to confirm that the controls and sample IDs have been verified.

9. DATA ARCHIVAL

9.1. Print the BOD Worksheet by selecting View-> Benchsheet, then **File-> Print** from the EZ_BOD Reader program menu.

9.2. Refer to SOP BB-031 for QC Manager data uploading and verification instructions, and SOP BB-032 for authorization instructions.

9.3. Calculations

9.3.1. Seed Correction Factor (SCF): To determine SCF calculate the average seed result using only the seed control bottles that follow the 2-1 Rule.

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

where:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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

9.3.2. Calculate the BOD_5 for each sample dilution.

$$BOD_5 = DF \times [(IDO - FDO) - (SCF)]$$

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 Bottle Volume/Sample Volume
SCF = Average Seed Result

9.3.3. The final BOD result is the average of all dilutions that follow the 2-1 rule. If no dilutions follow the 2-1 rule, use the dilution with the highest sample volume (lowest dilution) as the final result. If all the dilutions have an FDO 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 "Sample depleted to less than 1.0 mg/L for final DO measurement. Result is an estimate and may be higher than result reported". The BOD program should calculate the final BOD result accordingly; but always check the calculations to ensure that the correct BOD values are used in the calculation.

9.3.4. AutoEZ software: If any final DO readings are changed, the worksheet must be recalculated. If a flag is removed from a BOD, then the file has to be saved before recalculating "As Flagged".

10. QUALITY CONTROL

10.1 Check each new lot of buffers, seed, and GGA prior to first use to ensure acceptable results are achieved. Record results in lab logbook Lab_Supplies_QC log.

10.2 Negative Control

10.2.1 Run a dilution water quality check with every batch. The dilution water blank should contain only dilution water. Do not add seed or nitrification inhibitor.

10.2.2 The method acceptance limit for the blank is ≤ 0.20 mg/L. The laboratory historical limit for the blank is ≤ 0.50 mg/L.

10.2.3 It the blank exceeds the method limit but is within historic limits, do not qualify results. Report results with the following comment: "Dilution water blank is within laboratory historical limit of 0.50 mg/L."

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

10.2.4 If the blank exceeds both limits, report batch results with a V-qualifier and add the following comment: "Dilution water blank exceeds laboratory historical limit of 0.50 mg/L."

10.3 Positive Control

10.3.1 Run a glucose-glutamic acid check with every batch. Use 6 mL of GGA solution/300 mL of dilution water.

10.3.2 The method acceptance limit for the BOD GGA standard is 198 ± 30.5 mg/L, and for the CBOD GGA standard is 165 ± 26 mg/L.

10.4 If the GGA standard exceeds method limits, report batch results with a "J" qualifier and the following comment: "Refer to QA report for QC exceeding limits".

10.5 Sample Replicates

10.5.1 Analyze one sample duplicate per batch. The acceptance limit is ≤ 25% RPD.

10.6 Troubleshooting

10.6.1 Initial DO Readings should be within 7.5-9.5 mg/L.

10.6.1.1 If readings are too high, samples or dilution water may be supersaturated with oxygen. Make sure samples are within 20 ± 3°C and vigorously shake or aerate with appropriately labeled tubing to reduce DO level.

10.6.1.2 If readings are too low, samples or dilution water may need to be further aerated using appropriately labeled tubing. Also, check to make sure probe stirrers are working properly.

10.6.1.3 Check meter calibration.

10.6.2 Dilution water blanks are exceeding method and/or historical limits.

10.6.2.1 Check cleanliness of BOD incubation bottles.

10.6.2.2 Make sure deionized water is aerated using appropriately labeled tubing overnight in a clean flask before using for dilution water.

10.6.2.3 Ensure that the probe is rinsed thoroughly between samples.

10.6.2.4 Check cleanliness of probe. See SOP BB25 Troubleshooting section.

10.6.2.5 Obtain clean calibration water if contamination is suspected.

10.6.3 GGA standard exceeding method limits.

10.6.3.1 Check the GGA standard as it may be expired or ineffective. Dry new aliquots and prepare fresh GGA.

10.6.3.2 High GGA values may indicate contamination of dilution water or high seed values or too much seed added.

10.6.3.3 Low GGA values may indicate poor seed quality, not enough seed being added, or toxicity.

10.6.3.4 The PolySeed solution can be prepared with various amounts of dilution water. If the GGA values are too high, the seed solution may be too

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

concentrated. Increase the amount of dilution water used to prepare the solution. If the GGA values are too low, decrease the amount of dilution water. The results achieved are linearly proportionate to the amount of dilution water added. For example, if the correction factor needs to increase by 30 %, add 30 % less dilution water. The best results are achieved with a correction factor of 0.6 to 1.0 mg/L; however, if the GGA is within passing limits, there is no need to adjust the seed solution. The amount of seed added to controls and bottles can also be adjusted. Preliminary testing is required for each new lot of Polyseed.

10.7 Refer to QA manual for corrective action and handling out-of-control data.

11. SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1 Refer to the Laboratory Health and Safety Plan.

11.2 Read MSDS information for reagent handling and disposal.

12. REFERENCES

12.1. Standard Methods Online Edition, 5210B

12.2. NELAC Quality Systems Manual

12.3. FDEP Laboratory Quality Manual
http://publicfiles.dep.state.fl.us/dear/labs/lab_qualitymanual_18.pdf

12.4. FDEP Laboratory Health and Safety Plan
http://publicfiles.dep.state.fl.us/DEAR/DEAR%20Safety/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202016.pdf

12.5. DEP SOP-001/01 FS 1000, General Sampling Procedures

12.6. DEP SOP BB-25, YSI Model 50B/AutoEZ DO Meter Calibration

12.7. DEP SOP BB-30, Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples

12.8. DEP SOP BB-31, LIMS QC Manager Data Uploading Instructions for Bench Biology and Microbiology Samples

12.9. DEP SOP BB-32, Test Level Authorization of Uploaded Results for Bench Biology Samples

APPENDIX OF CHANGES

05/18/2009	Updated SOP style and updated to online edition of SM5210B.
05/18/2010	Updated file locations and reference to updated safety manual.
05/04/2011	Updated reference to QA manual and file directories.
04/24/2013	Added instructions for preparing GGA.
04/23/2014	Changed Sample Preparation and Sample Analysis instructions to be compatible with new EZ_BOD Reader program.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

01/16/2015	Formatted for 508 compliance.
02/24/2015	updated to reflect new file locations and added software setup information.
05/20/2016	Edited Sample Preparation; Sample Analysis; Data Archival; Quality Control; Troubleshooting; References; and hyperlinks.
12/23/2016	Updated BOD AutoEZ program settings, added clarification for new procedures, removed “greater than 10x the blank” instructions.
12/19/2017	Added batch information regarding seed preparation in section 7.0. Added information for preparation of BOD water in section 8.0. Updated links to FDEP Laboratory Quality Manual and FDEP Laboratory Health and Safety Plan in References section.
12/27/2018	Edited Equipment and Supplies; Reagents and Standards; Sample Preparation; Sample Analysis and Quality Control.
1218/2019	Updated Equipment and Supplies; Seed Solution; Sample Collection, Preservation and Handling, BOD Batch Preparation, Sample Pre-Treatment, Sample Analysis; Quality Control.
12/19/2019	Second review of updates made on 12/18/2019 and wording edited throughout document to clarify instruction. Updated SOP format to conform to the Protocol for the Preparation and Document Control of Standard Operating Procedures (SOPs), Forms, and External Documents LB-001-1.12.
01/26/2021	Removed all references to the BOD AutoEZ robotic analyzer, polarographic style probes, and YSI model 50B meter. Updated sections 3 Equipment and Supplies, 8.4 IDO readings and 8.6 FDO readings to include instructions for the Multi-Lab Importer software and external reader

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.