pH adjustment of Samples: Algal Assay and Toxicity Bioassay

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-600/9-78-018, The *Selenastrum capricornutum* Printz Algal Assay Bottle Test and EPA, 1974, Marine Algal Assay Bottle Test and EPA/821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. It is used to adjust the pH of samples to the appropriate range for testing. Tests conducted by this procedure are: AA-AGP, AA-AGP-SW, BSY-AS-FCD, BSY-AS-FFS, BSY-AS-MMY, BSY-AS-MFS, BSY-AD-FCD, BSY-AD-FFS, BSY-AD-MMY, BSY-AD-MFS, BSY-CS-FCD, BSY-CS-FFS, BSY-CS-MMY, BSY-CS-MFS, BSY-CD-FCD, BSY-CD-FFS, BSY-CD-MMY, BSY-CD-MFS, BSY-CS-FSC and BSY-CD-FSC.

1.2. *Selenastrum capricornutum* is now known as *Pseudokirchneriella subcapitata* but the name in the EPA method manuals has not changed.

2. SUMMARY OF THE METHOD

2.1. The pH of Algal Growth Potential (AGP) samples having initial pH values of < 6.0 or > 10.0 SU and the pH of bioassay samples having initial pH values of < 6.0 or > 9.0 SU are adjusted, by the addition of either hydrochloric acid or sodium hydroxide, to the specified test pH.

3. EQUIPMENT AND SUPPLIES

3.1. 1 mL and 2 mL graduated glass pipet

3.1.1. For AGP samples (because of small volume), a clean graduated transfer pipette may be used if preferred. The volume of the adjusting solution is then recorded in drops.

3.2. Pipette pump to fit 2 mL pipet

3.3. Magnetic stirrer

3.4. 12 mm Magnetic stir bar, 1 per AGP sample

3.5. ~100 mm Magnetic stir bar, 1 per bioassay sample

3.6. Magnetic stir bar retriever

3.7. 500 mL Beakers, 1 per AGP sample

3.8. 250 mL Graduated cylinder, 1 per sample

3.9. 1000 mL Graduated cylinder, 1 per limiting nutrient sample

3.10. 500 mL Erlenmeyer flask, 1 per AGP sample

3.11. 2000 mL Erlenmeyer flask, 1 per limiting nutrient sample

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3.12. 4000 mL Erlenmeyer flask, 1 per screening bioassay sample

3.13. 6000 mL Erlenmeyer flask, 1 per definitive bioassay sample

3.14. Squeeze bottle with DI water

3.15. 30 mL Specimen cup

3.16. Waste container

3.17. Fisherbrand Accumet AB315 pH/mV Meter

3.17.1. Calibrated per SOP TA-06.43.

3.18. Digital thermometer

3.18.1. Calibrated per SOP TA-06.13, used to measure the temperature of the sample. The pH meter is adjusted to the temperature of the sample.

3.19. Fume hood

3.20. Latex or non-latex gloves

3.21. Safety glasses or face shield

3.22. Lab coat

3.23. *AGP Sample Log-in Record* logbook

3.24. *Bioassay Log-in* logbook

3.25. *Bioassay Bench Sheet* logbook

3.26. Computer with access to the Laboratory Information Management System (LIMS)

4. REAGENTS AND STANDARDS

4.1. Sodium Hydroxide, NaOH, 1.0 N, prepared per SOP TA-10.10

4.2. Hydrochloric acid, HCl, 1.2 N, prepared per SOP TA-10.10

4.3. Deionized (DI) water

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

6. METHOD: ADJUSTING pH IN ALGAL GROWTH POTENTIAL AND LIMITING NUTRIENT SAMPLES

Note: AGP and limiting nutrient sample pH is adjusted prior to pouring the samples into the test flasks rather than when the sample is prepared per SOP TA-08.01. It is not uncommon for the pH of a sample to shift from outside of the test range to within the test range over the course of sample preparation.

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6.1. Refer to the *AGP Sample Log-in Record* to determine which samples had initial values of pH < 6.0 or > 10.0 SU and therefore may need to have their pH adjusted.

6.2. Measure the pH of these samples per SOP TA-06.43. Adjust per the following:

6.2.1. If the sample is now within the range of 6.0 to 10.0 SU, proceed with the test set-up procedures per SOP TA-08.02 (as stated in Step 6.8).

6.2.2. If the sample pH is < 6.0 SU, **NaOH** will be used to raise the pH. If the sample pH is > 10.0 SU, **HCl** will be used to lower the pH. Select the proper reagent and proceed with Step 6.3.

Warning!!! *Both NaOH and HCl can cause serious injury.* Follow laboratory safety procedures. Wear gloves, safety glasses or a face shield, and a lab coat.

6.3. For an **AGP** sample, pour 200 mL of AGP sample into a 500 mL flask. For an **AGP sample with a lab duplicate**, pour 400 mL of AGP sample into a 500 mL flask. For a **Limiting Nutrient** sample, pour 1300 mL of a Limiting Nutrient sample into a 2000 mL flask.

Note: These volumes are slightly more than the volume needed for each test.

6.4. Place the 12 mm stir bar in the flask and place the flask on the stirrer. Adjust stirrer speed to gently stir the sample.

6.5. Suspend the pH meter probe (pre-rinsed with DI water) in the flask ensuring that the tip of the probe is in the sample. Only the first 15 mm to 25 mm of the probe needs to be in the sample.

Note: The pH will be continuously monitored from this step until the pH adjustment is complete.

6.6. Use plastic transfer pipette to place one drop of the appropriate adjusting solution into the sample flask. Allow the sample to mix. Turn off the stirrer and allow the sample to become stationary. Measure the pH of the sample per SOP TA-06.43. Make note of the pH shift to estimate the number of drops it may take to raise or lower the pH to within the proper range of 6.0 to 10.0 SU.

6.6.1. Samples with pH < 6.0 should be adjusted to within proper range but remaining as close to pH 6.0 as possible (target 6.0 ± 0.2).

6.6.2. Samples with pH > 10.0 should be adjusted to within proper range but remaining as close to pH 10.0 as possible (target 9.8 ± 0.2).

6.6.3. If the adjusting solution appears to be too strong (i.e., one drop produces a large shift or a shift beyond the correct pH range), a weaker adjusting solution can be made. In a 50 mL flask, mix 1 mL of pH adjusting solution with 9 mL of unadjusted sample, swirl to mix, and continue the adjustment procedure using this weaker solution.

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Note: Sample is used as the diluent to keep the test solution as undiluted as possible.

Note: The pH reading is more accurate when the probe and the liquid are stationary (per manufacturer's instructions).

6.7. If the desired pH is exceeded by more than 0.2 S.U., repeat Step 6.6 until the pH of the sample is in the proper range of the target pH. Record the total number of drops of solution needed to adjust the pH and the adjusted pH value in the *AGP Sample Log-in Record*.

6.8. Continue test set-up procedures per SOP TA-08.02.

7. METHOD: ADJUSTING pH OF BIOASSAY SAMPLE

7.1. Refer to the *Bioassay Log-in* book to determine which samples had initial values of pH < 6.0 or > 9.0 SU and therefore may need to have their pH adjusted. All other sample adjustments such as initial aeration or salinity adjustment should have been completed.

7.1.1. Freshwater samples are adjusted to pH 7.0 ± 0.2 SU.

7.1.2. Saltwater samples are adjusted to pH 8.0 ± 0.2 SU.

7.2. Measure the pH of these samples per SOP TA-06.43. Adjust per the following:

7.2.1. If the sample is now within the range of 6.0 to 9.0 SU, the pH no longer needs adjusting. Proceed with the test set-up procedures in the SOP appropriate to the toxicity test.

7.2.2. If the sample pH is < 6.0 SU, **NaOH** will be used to raise the pH. If the sample pH is > 9.0 SU, **HCl** will be used to lower the pH. Select the proper pH adjusting solution and proceed with Step 7.3.

Warning!!! *Both NaOH and HCl can cause serious injury*. Follow laboratory safety procedures. Wear gloves, safety glasses or a splash shield, and a lab coat.

7.3. For a **screening bioassay**, pour 2200 mL of sample into a 4000 mL flask. For a **definitive (0.5 dilution) bioassay**, pour 4000 mL of sample into a 6000 mL flask.

Note: These volumes provide sufficient sample so that phys/chem parameters can be taken on the pH-adjusted sample.

7.4. Place the 50 mm stir bar in the flask and place the flask on the stirrer. Adjust stirrer speed to gently stir the sample.

7.5. Suspend the pH meter probe (pre-rinsed with DI water) in the flask ensuring that the tip of the probe is in the sample. Only the first 15 mm to 25 mm of the probe needs to be in the sample.

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Note: The pH will be continuously monitored from this step until the pH adjustment is complete.

7.6. Use a glass pipette with a pipette pump to place one drop of the appropriate adjusting solution into the sample flask. Allow the sample to mix, then turn off the stirrer, and allow the sample to become stationary. Measure the pH of the sample per SOP TA-06.43. Make note of the pH shift in order to estimate the number of drops it may take to raise or lower the pH to the proper pH (**freshwater** pH 7.0 ± 0.2, **saltwater** pH 8.0 ± 0.2).

7.6.1. **Freshwater** samples of pH < 6.0 or pH > 9.0 should be adjusted to within the proper range but remaining as close to pH 7.0 as possible (target 7.0 ± 0.2).

7.6.2. **Saltwater** samples of pH < 6.0 or pH > 9.0 should be adjusted to within the proper range but remaining as close to pH 8.0 as possible (target 8.0 ± 0.2).

7.6.3. If the adjusting solution appears to be too strong, (i.e., one drop produces a large shift or a shift beyond the correct pH range), a weaker adjusting solution can be made. In a 50 mL flask, mix 1 mL of the pH-adjusting solution with 9 mL of unadjusted sample, swirl to mix, and continue the adjustment procedure substituting the weaker solution for the stronger solution.

Note: Sample is used as the diluent to keep the test solution as undiluted as possible.

Note: The pH reading is more accurate when the probe and the liquid are stationary (per manufacturer's instructions).

7.7. Repeat Step 7.6 until the pH of the sample is in the proper range. Record the total number of drops or milliliters of solution needed to adjust the pH and the adjusted pH value on the appropriate *Bioassay Bench Sheet*.

7.8. Continue test set-up per the appropriate toxicity test SOP.

8. DATA ARCHIVAL

8.1. DEP Laboratory Information Management System (LIMS)

8.1.1. LIMS Entry for Laboratory Parameters SOP TA-06.42

8.2. *AGP Sample Log-in Record*

8.3. *Bioassay Log-in* logbook

8.4. *Bioassay Bench Sheet*

8.5. Use a black, waterproof pen for all bench sheets and logbooks.

8.6. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

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8.6.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

8.6.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

8.6.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

9. QUALITY CONTROL

9.1. Refer to the DEP Laboratory Quality Manual for corrective action and handling out-of-control data.

9.2. If a problem occurs during sample processing, a nonconformance report (NCR) is submitted describing the problem and the corrective action.

9.2.1. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

10.2. Refer to the DEP Laboratory Quality Manual.

10.3. Refer to the Laboratory Health and Safety Plan.

10.4. Read MSDS information for reagent handling and disposal.

11. REFERENCES

11.1. DEP Laboratory Quality Manual, DEP Laboratory Quality Assurance Manual and SOPs | Florida Department of Environmental Protection

11.2. DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

11.3. DEP SOP FS 1000, *General Sampling*

11.4. DEP SOP TA-06.39, *Thermo Scientific Orion Star A213 Benchtop RDO/DO Meter Calibration and DO Measurement*

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11.5. DEP SOP TA-06.36, *Calibration and Use of the Fisherbrand Traceable Bench Conductivity Meter for Conductivity and Salinity*

11.6. DEP SOP TA-06.43, Fisherbrand Accumet AB315 pH/mV Meter

11.7. DEP SOP TA-08-01, *Initial Algal Assay and Limiting Nutrient Sample Handling*

11.8. DEP SOP TA-08-02, *Algal Growth Potential (AGP) and Limiting Nutrient (LN) Algal Assay Set-Up*

11.9. DEP SOP TA-10.10, *Preparation of Stock HCl (Acid) and NaOH (Base) Solutions*

APPENDIX OF CHANGES:

06/11/2008	Added Scope of Application, Summary of the Method, References and Appendix of Changes.
06/04/2009	Added comment for Materials #4. Corrected the spelling of Accumet in comment for Materials #22.
06/04/2009	Updated reference to Biology Quality Manual.
03/24/2011	Changed Biology Quality Manual to Bureau Quality Manual. Updated referenced to the Quality Manual.
02/21/2012	Updated reference to Bureau Quality Manual.
01/15/2014	Reformatted SOP.
01/28/2015	Formatted for 508 compliance.
02/10/2016	Updated links. Updated names of and moved the FDEP Laboratory Quality Manual and Laboratory Health and Safety Plan links to the reference section. In the reference section, listed SOP identifier before the SOP title. Italicized SOP titles.
03/10/2017	Updated SOPs and References.
02/28/2018	Updated links
02/22/2019	Updated links and references to meter SOPs
02/23/2021	Updated based on universal language.
02/22/2022	Updated links.
02/24/2023	Updated pH meter and unified font.
02/26/2024	Made sure all PH SOPs were the same. Added LIMS entry SOP. Updated reference title. Updated links.

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