OPS LABORATORY PROCEDURE 2016

Version 2016.1

Goal

Analyzing and determining alkalinity and ammonia values as process control tools for operation of aerobic wastewater treatment plant.

Procedure

Please note that these procedures are NOT approved for EPA and/or NPDES reporting. They are *process control tools only* to help operators maintain plant operations, identify potential problems, and react in a timely manner to resolve operational issues. The equipment recommended is multi-functional, and meets all regulatory requirements, as well as having numerous applications for regulatory reporting

Operators will be required to run straight and diluted samples of ammonia and alkalinity to determine basic alkalinity needs of treatment plant based on influent ammonia and alkalinity values. Once these values are identified, calculations will be performed to identify if adequate alkalinity is available to provide complete nitrification. If not, the amount of additional alkalinity needed will be calculated.

In addition, profiling of aeration basins and final clarifiers will be part of this equation. Influent and effluent values will be determined by analysis, and appropriate remediation identified.

Finally, penalties referenced on the Lab Event Judges Sheets are incorporated by reference into this document. Any discrepancies in language will be resolved by the judging team.

Equipment Check List and Supplies

	USA BLUE BOOK
67868	10 ml Disposable Plastic serological pipets (10)
40676	Safety Pipet Filler Bulbs (2)
205152	Corning Low Form Polypropolene 250 ml beakers (13)
37950	100 ml graduated cylinders, Nalgene, Class B (6)
33301	100 ml volumetric flasks (3)
35493	500 ml Wide Mouth Sample Bottles (12)
35480	DI Carboys with spigots and inserts
30729	1 quart Wash Bottles
39828	2000 ml PP Beaker Container for Used Pipets (1)
36989	Kim Wipes
72522	Clorox Disinfecting Wipes
45632	Wypall wipes
40947	Bottle Carrier (2) For samples
39829	5000 ml Waste Beakers (2)
37960	250 ml Graduated Cylinder (1) For Alkalinity Reagent
63050	Multipurpose Trays (4) For Dirty Beakers, Graduated Cylinders and Carboy overflow
40853	500 ml Polypropylene Beakers (2) Wash Water Beakers for pH and Ammonia
59609	XL Level 2 Cut Resistant Gloves (1) for Breaking Cuvettes
59608	L Level 2 Cut Resistant Gloves (1) for Breaking Cuvettes
59607	M Level 2 Cut Resistant Gloves (1) for Breaking Cuvettes
59606	S Level 2 Cut Resistant Gloves (1) for Breaking Cuvettes
72109	10 Quart plastic buckets for trash
60688	Yellow Polyethylene Pails for disposal of Cuvettes
41122	Timers
	S, M, L, XL surgical gloves
	Thermo-Orion
STARA2110	Orion Star A211 pH Benchtop Meter with Electrode Stand
AC4010	!AQUAfast IV ammonia (ultra low range 0.1 to 3.0) AC4010 with activator and catalyst solutions
AQ4000	Orion Aquafast AQ 4000 Colorimeter
AQ4ZER	Orion Aquafast Zero Kit for AQ4000 Colorimeter
8107BNUMD	Ross Gel Triode for A Series Meters, 1 M Cable
96019	Orion Star Stirrer Probe
700011-WA	Total Alkalinity Test
951007	1000 ppm Ammonia as Nitrogen (N) Standard, 475 mls

General NOTES

1. Team Captain tells the Lead judge they are ready to begin and the Lead judge says "START" to signal the beginning of the event. The Lead judge and one other judge will be the timekeepers.
2. Event is complete when all tasks have been completed and Team Captain hands in the work sheets to the Lead judge and says the team is finished.
3. To ensure a fair contest and to avoid challenges, judges will not speak to contestants while the event is being performed.
4. The Event Coordinator will settle disputes with input from the event judges.
5. All team members must participate in the event, but are not limited to performing only one task.
6. After the event, the Event Coordinator with the assistance of the Lead Table Judge may explain to the Team Captain what was done incorrectly, but will NOT reveal penalty points or total score.
7. Team members may ask judges questions before the beginning of the event, but the judge may choose not to answer the question, depending on the question asked. Questions related to specific steps in the procedure will not be answered.

NOTE: ALL STEPS OF THE PROCEDURE MUST BE PERFORMED FROM MEMORY. NO BOOKS OR PRINTED MATERIALS ARE ALLOWED IN THE LABORATORY COMPETITION AREA.

SETUP

Teams will have two minutes before beginning the event to organize items on the tables. Any item, with the exception of meters and carboys, may be moved. The lead judge will time the setup. At the end of the setup time, the judge will say "TIME", team members must remove their hands from the table. Judges will then place bench sheets face down on the tables. See "General Notes #1" above for instructions. NOTE: The lids may be removed from the volumetric flasks during this time period. Lids MUST remain on sharpies.

LABELING OF GLASSWARE

Labeling must be completed before any rinsing of bottles, measuring or dilution of samples takes place and prior to placement of sample into container.

Please note that this does not mean the competitors must wait for all containers to be labeled before proceeding with the competition. As each individual bottle/container is labeled, the contestant may begin the next steps of the procedure.

All beakers, volumetric flasks, graduated cylinders and cuvettes must be properly (AND LEGIBLY) labeled ***according to the bench sheet provided***. (There will be a total of three (3) sharpies provided for labeling.

Label one set of six (6) 250 ml beakers AND graduated cylinders for **pH and alkalinity test** as 1A-6A as shown below (NOTE: written description in parenthesis not to be included on label, just information that has been underlined):

- **1A** (influent pH and alkalinity)
- **2A** (aeration basin 1 effluent pH and alkalinity)
- **3A** (aeration basin 2 effluent pH and alkalinity)
- **4A** (aeration basin 3 effluent pH and alkalinity)
- **5A** (Anaerobic Digester 1 return pH)
- **6A** (Belt Press 1 return pH)

1. Label one set of six (6) 250 ml beakers for **ammonia test** as 1B-6B as shown below AND a set of **3 - 100 ml volumetric flasks** to be used for sample dilutions as **1B, 5B and 6B** . All sample beakers must be used by contestants. You may not pour samples directly from containers or volumetric flasks into measuring container. They must be poured from labeled sample beakers. (NOTE: written description in parenthesis not to be included on label, only information that has been underlined)
    - **1B (influent ammonia)**
    - **2B (aeration basin 1 effluent ammonia)**
    - **3B (aeration basin 2 effluent ammonia)**
    - **4B (aeration basin 3 effluent ammonia)**
    - **5B (Anaerobic Digester 1 return ammonia)**
    - **6B (Belt Press 1 return ammonia)**
2. Competitors will be provided with a set of six (6) cuvettes that are color coded with dots on bottom of cuvettes (**Avery Round Color Coding Labels 0.25 diameter 06720 and 5795**) which will correspond to the colors/ID on the Ammonia Bench Sheet.

3. Cuvettes will only be identified by colored label. Colors must match the IDs of the samples as listed and shown on the Ammonia Analysis Bench Sheet.
    - **1B (influent ammonia) Red**
    - **2B (aeration basin 1 effluent ammonia) Dark Blue**
    - **3B (aeration basin 2 effluent ammonia) Yellow**
    - **4B (aeration basin 3 effluent ammonia) Green**
    - **5B (Anaerobic Digester 1 return ammonia) Orange**
    - **6B (Belt Press 1 return ammonia) Pink**

4. Label one (1) 250 ml Graduated Cylinder as **Alk R (alkalinity reagent)**
5. Mix all samples and Alkalinity Reagent by inverting 5 times [5 X] (NOTE: a single inversion consists of taking a bottle from an upright position (12 o'clock) and turning it to 90° (3 o'clock), then back to the upright position. NOTE: The intent of mixing the samples is to produce a homogenous sample for analysis. The competitor should be aware of this during the competition. It is our intent that the sample be mixed immediately prior to rinsing of beaker and measuring of sample. It is not acceptable to mix samples and allow them to sit on the counter for any period of time. Immediately = within 5 seconds.
6. Rinse all beakers and graduated cylinders with sample and/or Alkalinity Reagent
7. For pH/Alkalinity tests, using rinsed 100 ml graduated cylinders, measure 100 mls of sample into corresponding labeled beakers. Proceed with pH and Alkalinity testing.
8. For Alkalinity test, mix and pour 100 mls of Reagent solution into the 250 ml graduated cylinder. Use for alkalinity testing. NOTE: Pipette with bulb may be rested in the 250 ml cylinder between samples as long as the pipette is not contaminated between samples (tip or side does not come in contact with any surface)

pH and ALKALINITY PROCEDURE

For this procedure, there will be a total of six (6) pH samples, and four (4) alkalinity samples. pH and alkalinity will be run using the same sample beakers.

1. pH Meter will be in the "on" position
2. Remove pH probe/stirrer from soaking solution, rinse thoroughly with DI water and **blot** dry using a kimwipe
3. Place sample beaker under electrode, and submerge electrode/stirrer in sample
4. Press "STIRRER" key to turn Stirrer on
5. Press "MEASURE" key
6. Press "START" on the timer provided to begin a 20-second stabilization time.
7. After 20 seconds, record the pH reading (report to two decimal places after the decimal point)
8. Rinse 10 ml serological pipet with Reagent solution for use in the alkalinity test. Since this is a Serological or 'blow-out' pipette, it is calibrated so that the last drop of liquid needs to be **blown out** of the pipet. For this procedure, you may use the pipette safety bulb to dispense (blow out) all of the volume in the pipette into sample.

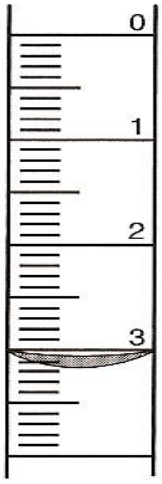


Illustration of Proper Pipetting Technique

Liquid was drawn up to exactly the zero mark and was then dispensed. Reading the value at the bottom of the meniscus shows that 3.19 mL of liquid was dispensed

9. Without moving the sample beaker, pipet 10 mls ORION Total Alkalinity Reagent into the sample beaker. Be sure to wipe the pipette with a kim wipe to remove liquid on the outside prior to dispensing in beaker. Avoid contamination of pipette between samples.
10. Press "STIRRER" key
11. Press "MEASURE" key
12. Press "START" on the timer provided to begin a 20-second stabilization time.
13. After 20 seconds, record the Total Alkalinity from the chart provided - pH as Total Alkalinity mg/L

14. Press “STIRRER” key to turn Stirrer off
15. Remove the pH probe/stirrer from sample, rinse it off with distilled water into waste beaker, blot dry, and proceed to next sample
16. When all analyses are complete, with the Stirrer in the off position, rinse pH probe/stirrer with distilled water, blot dry and return to soaking solution beaker.
17. Complete bench worksheet by indicating unit of measure su (pH) or mg/L or ppm (alkalinity), date, time and analyst performing the test, on the worksheet.

AMMONIA PROCEDURE

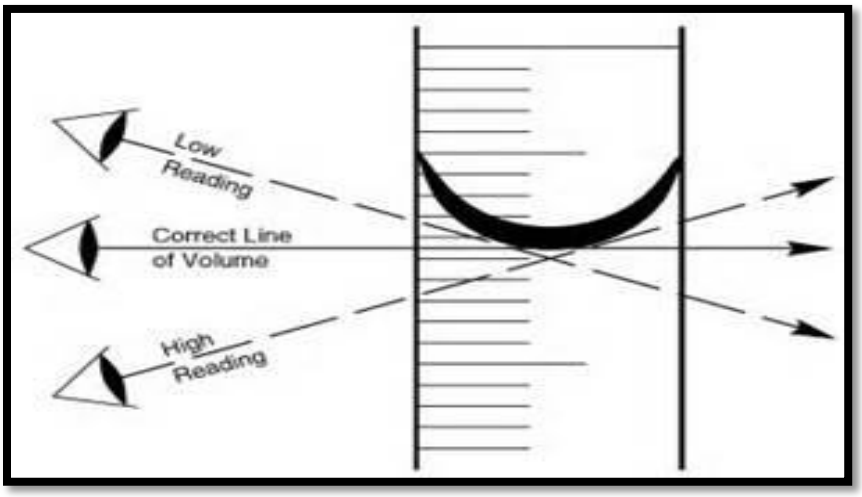
Using Thermo Scientific Orion AQUAfast AQ4000 Colorimeter and Ammonia (as N) Ultra Low Range (0.1 – 3.0 mg/L) Auto Test Ampoules (4010):

There are a total of six (6) samples for analysis (see above), of which three (3) will need to be diluted prior to analysis. The dilution information is below. The remaining three (3) samples will be run directly without dilution. ***All samples must be transferred to a beaker prior to measuring***. Use beakers that have been properly labeled and rinsed as described in the Labeling section above. In addition, you will need to label and rinse volumetric flasks according to labeling instructions

Sample Preparation

1. Label three (3)- 100 ml ***volumetric flasks*** as:
    a. **1B** (influent ammonia)
    b. **5B** (Anaerobic Digester 1 return ammonia)
    c. **6B** (Belt Press 1 influent ammonia)
2. For Ammonia tests, pour 150 mls of sample into corresponding beaker (+/- 5%)
3. Rinse each sample flask with distilled water
4. Rinse the 10 ml serologic pipets with the samples
5. Using a 10 ml serologic pipet, transfer 10 mls of sample from the beaker to the flask labeled **1B**, wiping the outside of the pipet with kim wipes prior to dispensing sample into flask. Blowing out of the pipette is required for full delivery. Dilute samples in flask to 100 mls with distilled water from the carboy or wash bottles, paying close attention to the meniscus. The bottom of the meniscus curve must touch the 100 ml mark on the flask. This will make a 1:10 dilution, which means it

will have 1 part of sample to 9 parts of distilled water.



6. Repeat steps 3 and 4 for all three (3) samples
7. Screw lids on flasks
8. Mix samples by inverting 5 times [5 X] (NOTE: a single inversion consists of taking a bottle from an upright position (12 o'clock) and turning it a full 90° (3 o'clock), then back to the upright position
9. Place all pipets in the pipet washer
10. Measure samples using the procedure outlined below

Sample Measurement

1. Rinse sample cup with sample (for samples 1B, 5B and 6B, use the diluted samples prepared in volumetric flasks)
2. Fill sample cup to the 25 ml mark with the sample
3. Add 2 drops of ***Catalyst Solution*** to the sample cup. Place the Auto-Text cuvette in the sample cup. Stir the tip of the cuvette three (3) revolutions around the sample cup to mix the contents of the sample cup.
4. Add 2 drops of ***Activator Solution*** to the sample cup.
5. Stir the tip of the cuvette three (3) revolutions around the sample cup to mix the contents of the sample cup.
6. Slip on Level 2 Cut Resistant Glove over latex glove to snap off the tip of the cuvette. NOTE: The Level 2 glove only has to be in place for the breaking of the cuvettes. It can be put on and taken off for each cuvette, or left on for the full procedure. The glove may be placed over the latex glove, or the latex glove can be removed and then replaced.
7. Snap the tip of the cuvette off by pressing the cuvette against the side of the cup. The cuvette will fill leaving a small bubble to facilitate mixing

8. Mix the contents of the cuvette by inverting it 3 times, allowing the bubble to travel from end to end each time. Wipe all liquid from the exterior of the cuvette with kim wipes
9. Insert the cuvette into the AQ4000 colorimeter. Align the ▼ on the Auto-Test cuvette with the ◆ on the adapter to obtain a continuous beeping and you can view ***** across the display.
10. If the ***** and beeping are not observed, rotate the cuvette to the right or left to initiate the measurement.
11. Immediately cover the cuvette with the cuvette cover
12. Press "Measure" key
13. Press "timer" key ***immediately*** (NOTE: Due to time constraints, the two minute color development time normally associated with the procedure will be by-passed by pushing the "timer" key).
14. Results may be recorded on worksheet as soon as the unit displays them. Diluted samples will need to be reported as calculated values based on dilution factors (results X 10).
15. Discard all used cuvettes in special container provided
16. When all analysis are completed, place cover on cuvette holder
17. Perform calculations on worksheet, and answer questions.

SAFETY PROCEDURES

Safety is of major concern during laboratory operations. As such, "good housekeeping" practices for laboratory operations have been incorporated into the procedure.

1. Safety glasses must be worn at all times. Prescription glasses may be worn in lieu of safety glasses.

2. Latex gloves must be worn at all times. You may bring your own gloves or wear gloves that we provide. Please note that if you have a latex allergy, we encourage you to provide your own gloves. If a glove tears during the competition, *it must be replaced prior to that contestant continuing with the competition*. The competition will not stop, but that competitor is required to change gloves before continuing with their participation or penalties are assessed. No penalties will be assessed for tearing a glove, the penalty will be for not replacing a torn glove.

3. A Level 2 Cut Resistant glove must be worn when breaking cuvette tips. You will only need to wear a glove on the hand that is breaking the tip of the cuvette. The glove will not be required to be worn for the full procedure, just while breaking off the tip. The glove MUST be worn, and not just held in the hand while breaking the tip. All sizes of gloves will be available for the competition.

4. Good housekeeping will be required. Competitors will be required to wipe counters, instruments, and work areas ***dry*** with paper towels where they have spilled or splashed any liquids during the competition. This must be done prior to disinfecting counters

5. Competitors must place used graduated cylinders and used beakers in trays provided (note: beakers and flasks and graduated cylinders must be emptied in waste receptacle prior to going into tray)

6. Sample bottles must have lids in place (screwed on), and placed back in bottle carriers

7. When counters are completely dry, contestants will then be required to wipe counters thoroughly with disinfectant wipes. The counters do not need to be wiped dry after applying disinfectant.

8. All trash must be disposed in waste receptacles along with used latex gloves. Latex gloves are considered “contaminated”, and must be removed and disposed in waste receptacles prior to turning in paperwork.

9. Cuvettes must be disposed in designated container (not trash)

10. Penalties for Safety related issues discussed here are found on the Judges penalty sheet labeled SAFETY.