

STANDARD OPERATING PROCEDURES FOR THE LABORATORY

For complete directions for any analysis listed in this study guide please refer to “Basic Laboratory Procedures for the Operator-Analyst (Fifth Edition)” from the Water Environment Federation. Other references include “Standard Methods for the Examination of Water and Wastewater”, and US EPA approved methods.

Safety

Assure (current version of what used to be Safety Data Sheets (SDS) for all chemicals in use are readily available to personnel working on the laboratory. Require all laboratory personnel to read and understand the information included in these documents. Provide appropriate training for each laboratory procedure undertaken. Assure personal safety is the focus of all laboratory personnel. Identify Personal Protective Equipment (PPE) that is required for each procedure. Assure all PPE is available BEFORE conducting any analysis.

- Gloves appropriate to the tasks at hand
- Eyewear – glasses, goggles, shields
- Laboratory coat
- Closed toe shoes
- Long pants
- Long Sleeves preferred if no lab coat is available.
- Lab apron

It is important to wear your Personal Protective Equipment (PPE) in the laboratory. The PPE consists of safety glasses, goggles or a face shield, disposable gloves, and close toed shoes or boots. Other PPE may consist of insulated gloves when handling hot glassware, and an apron when dealing with strong acids or bases. The operator when working in the laboratory should wear at a minimum safety glasses and disposable gloves for PPE. A laboratory coat is also advisable as it will save you clothes.

WORKING WITH ACIDS

ALWAYS ADD ACID TO WATER. You want to dilute the acid when mixing it with water so add the acid to the larger quantity of water. When you add water to acid you may cause “bumping” – this is when the first drops of water instantly react and boil when they contact the concentrated acid and can violently expand out of the container. It is very dangerous and can cause severe injuries.

Choose your mixing container carefully, remember it may be hot to the touch due the mixing of water and acid. Be sure you use the necessary personal protective equipment.

Reagent Handling

When receiving new reagents (chemicals), a written (computer) record should be maintained. Items normally noted include:

- Check the expiration date on the reagent before accepting the reagent for use in the laboratory.
 - If it is expired, it is unsuitable for use in compliance analysis. Communicate with the supplier and replace the reagent. Or use only for in-house process control monitoring.
- Clearly write the reagent receipt date and the initials of the person receiving the chemical on the bottle, not the label, as it can be removed.
- Compatible chemical storage must be maintained. Do not store acids with or near bases or oxidizing agents with reducing agents. Consult (the new version of SDS) for chemical compatibility information.

it is not suitable for any he analysis for compliance reporting. Before each use check the expiration date of the reagents. The date of receipt and initials of the person receiving the product is written on the label. All systems that discharge use a pH meter and must have valid standards available for calibration. pH standards are probably the most often reagents found to be expired during a compliance inspection. pH standards are only good for about a year. A suggestion is to order new standards for pH meter calibration yearly.

GLASSWARE

There are many types of glassware used in the wastewater laboratory. The most common are beakers, Erlenmeyer flasks, graduated cylinders, and pipets. The glassware must be clean and dry to avoid contamination of the analysis. The glassware should be either stored upside down on a drying rack or stored in a closed cabinet. Check for cleanliness before each use. The exception to the rule of using dry glassware is Biochemical Oxygen Demand 5-day (BOD₅) bottles. The method allows the bottles to be clean and wet if necessary.

When washing glassware use a brush to scrub both the inside and outside of the glassware. All glassware should be washed with laboratory soap and tap water. The glassware should be rinsed three times with tap water and followed by three rinses with distilled/deionized water. The glassware should be inverted and placed on a drying rack. For stubborn deposits of material in the glassware an acid rinse/soak may be necessary. For glassware used in the total phosphorus analysis, rinse the glassware with muriatic acid. Pipets should be soaked in acid before washing. To wash pipets, use a pipet washer if possible. Pipets may be hand washed by soaking them in acid first then rinsing them with tap water three times and then a rinse of three times with distilled/deionized water. Check the cleanliness of glass pipets before each use. Many laboratories are now using disposable pipets for measuring wastewater samples. The disposable pipets are a one-time use and then thrown away. "Tensette" pipets are also commonly used, and the tips are one-time use.

The below methods are abbreviated procedures of the most common analysis used in wastewater. The operator should refer to the Basic Laboratory Procedures for Water and Wastewater Examination 22 Edition from Water Environment Federation for a more detailed procedure. Additional information on laboratory equipment and reagents, refer to "Water and Wastewater Laboratory Techniques, Second Edition" from Water Environment Federation.

PH AND TEMPERATURE – STANDARD METHODS 4500 H⁺ AND STANDARD METHODS 2550 B

pH is an instantaneous sample that is usually measured in the field. The pH probe is calibrated daily or before each use. Follow the method for calibration as described by the manufacturer. The probe is usually calibrated using two standards either 4 and 7 or 7 and 10. The pH probe should be stored in a storage solution. The tip should be rinsed and blotted dry between

samples. Never store the pH probe in distilled/deionized water. The temperature probe should be checked and calibrated yearly to make sure the temperature probe is accurate. A certificate is usually provided with each pH probe stating it has been calibrated. The four things that are necessary for an EPA approved meter/probe combination are:

- The probe must be replaceable.
- The reading must be temperature compensated.
- The meter must have a range of 0-14 and read to two decimal places.
- Must be calibrated with a minimum of two standards.

Method of Analysis

1. Remove the pH probe from the storage solution, rinse, and blot dry.
2. Calibrate the pH probe and meter. Follow manufacturer's directions.
3. The probe is submerged in the solution to be measured, either in the flow or in a beaker.
4. The meter is set to read the solution.
5. When the reading is stable, the reading is recorded as pH in Standard Units (SU) on the Bench Sheet.
6. The temperature of the solution is also recorded at this time in either Fahrenheit or Celsius to one decimal place.
7. Rinse the pH probe after use and blot dry.
8. Place in storage solution.
9. Change storage solution weekly.
10. Use new buffer each time the probe is calibrated.

DISSOLVED OXYGEN (DO) – STANDARD METHODS 4500 O C OR 4500 O G

Dissolved Oxygen analysis can be used in the treatment facility for many things. It may be a required parameter to be monitored on the discharge and reported on the Discharge Monitoring Report (DMR) or it can be used as an operational parameter to help optimize the treatment of the wastewater. Another use for Dissolved Oxygen is in the Biochemical Oxygen Demand Test. Dissolved Oxygen can be measured two ways. The most common method is to use a probe and place it directly into the sample to be measured. If the sample has been collected and is to be analyzed in the lab, the operator has 15 minutes to have the measurement started from the time of collection. The sample is collected in a 300 mL BOD bottle that is stoppered and capped. The other method is to place the probe directly into the wastestream that is to be measured.

Method of Analysis

1. Calibrate the probe if necessary.
2. The probe is placed in the solution to be read, either in the BOD bottle (with a stir bar for mixing) or in the water in a tank, lagoon, or in the discharge.
3. The operator waits for the reading to be stabilized.
4. The reading is recorded on the Bench Sheet.
5. The probe is rinsed with distilled/deionized water and blotted dry.
6. Probe is stored per the manufacturer's instruction.

The second method of analyzing for Dissolved Oxygen is by titration.

1. Collect the sample in a BOD bottle.
2. The dissolved oxygen sample is taken to the laboratory where two mL of manganese sulfate and two mL of Alkaline iodide-sodium azide solutions are added.
3. The bottle is capped and inverted several times to mix the chemicals with the sample to form a floc. If the floc is brown dissolved oxygen is present if the floc is white no dissolved oxygen.
4. After allowing the floc to settle, remove the stopper and 2 mL of sulfuric acid is slowly added along the neck of the bottle.
5. Replace the stopper and rinse the bottle thoroughly to prevent acid burns from acid that may have spilled on the outside of the bottle.
6. Mix by inverting several times.
7. Allow to stand approximately 5 minutes (the floc should be dissolved).
8. Remove 100 mL of the solution from the BOD bottle and pour into a 250 mL Erlenmeyer flask.
9. Add 1 mL Starch solution to the Erlenmeyer flask, the solution will turn blue.
10. Titrate with 0.0125 N Sodium Thiosulfate solution until the blue color disappears.
11. Record the end reading on the buret.
12. 1 mL of 0.0125 N Sodium Thiosulfate Solution used equals 1 mg/L dissolved oxygen.

BIOCHEMICAL OXYGEN DEMAND (BOD) – STANDARD METHODS 5210 B

The BOD test measures the amount of oxygen use by bacteria in the bottle over a 5-day period. The operator must remember that this result is 5 days old, and it may not accurately reflect what is currently happening in the treatment system. If dissolved oxygen is being analyzed by titration you will have to set up two identical sample bottles for each dilution. One bottle will be analyzed initially and will be recorded as the initial reading and the second bottle will be placed in the incubator and analyzed 5 days later. This will give you the final dissolved oxygen

reading. A better analysis to run for operational control of the treatment system would be Chemical Oxygen Demand (COD).

Equipment Needed

- One 300 mL bottle per dilution, with stopper and cap,
- Graduated cylinder for volumes over 25 mL,
- Measuring pipet for volumes under 25 mL,
- Prepared dilution water,
- Dissolved Oxygen meter with stirrer
- Bench sheet to record the dissolved oxygen in each bottle.

Method of Analysis

1. The required sample volume is delivered into the BOD bottle,
2. Record the volume of sample used on the bench sheet under sample volume.
3. Dilution water is added to the bottle by running the dilution water either down the inside of the bottle or putting the filler tube all the way to the bottom of the bottle.
 - a. The idea is to not allow any air to be added to the bottle that is not already in the dilution water and sample.
 - b. You want no air bubbles in the bottle.
 - c. Continue filling until the level in the bottle is halfway up the ground glass portion of the bottle neck.
4. DO probe is inserted into the BOD bottle, careful not to spill water out of the bottle.
5. Start the DO meter reading and wait for the unit to stabilize.
6. Record the DO meter reading on the bench sheet under Initial reading.
7. The stopper is added to the bottle and the cap snapped over the top of the bottle and stopper. After this has been completed tip the bottle upside down and check for air bubbles in the bottle.
8. Place the BOD bottle in the incubator at 20°C for 5 days.
9. After the 5 days remove the bottle from the incubator.
10. Remove the cap and stopper and insert the DO probe into the bottle being careful not to spill any of the solution in the bottle.
11. Start the meter and wait for the reading to stabilize.
12. Record the reading under final reading on the bench sheet.
 - a. There must be a loss of a minimum of 2 mg/L of Dissolved Oxygen from the initial Dissolved Oxygen reading for that bottle.
 - b. There must be a minimum of 1 mg/L of dissolved oxygen left in the bottle for the final reading. (Lose 2, keep 1)

Then subtract the final reading from the initial reading to obtain the dissolved oxygen used in the bottle.

$$\frac{\text{mg of DO used} \times 300 \text{ mL (BOD Bottle volume)}}{\text{mL of sample used}} = \text{mg/L BOD}$$

Link for BOD₅ - https://pubs.usgs.gov/twri/twri9a7/twri9a7_nfmchap7_2_bod.pdf

TOTAL SUSPENDED SOLIDS (TSS) – STANDARD METHODS 2540, US EPA METHOD 160.2

Total Suspended Solids (TSS) is a measure of the solids that are caught on a (Whatman AH45) filter. The analysis is usually run using a Gooch crucible. The analysis may also be run using a larger diameter filter that is placed on a membrane-filter apparatus. The test is a measure of weight (mg/L). A variation of this test may be used to determine the pounds of solids under aeration.

Method of Analysis

Prepared Gooch crucible procedure

1. Place the glass-fiber filter disk, rough side up in the crucible.
2. Place crucible on the vacuum and turn vacuum on
3. Rinse the filter three times with 20 mL of distilled/deionized water.
4. Turn off vacuum and remove and place in drying oven for 1 hour at 105°C (if you are going to measure for volatiles place in the muffle furnace for 20 minutes at 550°C. Remove and place in the drying oven for 20 minutes)
5. Move the crucible to the desiccator to cool to room temperature and for storage.

The Gooch Crucible is only handled using tongs. The test starts by weighing a prepared gooch crucible. This is the initial weight. The crucible plus the filter is weighed and the weight of the sample is recorded as the initial weight.

1. The crucible is placed on a vacuum flask and the vacuum is started.
2. The crucible is rinsed with 20mL distilled/deionized water three times. The operator will then measure a volume of sample to be analyzed. The sample will be slowly poured through the crucible and the graduated cylinder will be rinsed three times and the water from each rinse will be poured through the filter.
3. After all the water has been filtered through the crucible the vacuum is turned off and the crucible and filter are removed.

4. Placed in the drying oven at 103°C for one hour.
5. The crucible and filter are moved to the desiccator to cool to room temperature.
6. After cooling the crucible is weighed and the weight is recorded under weight of crucible and sample.

Using a large filter on the Membrane-filter apparatus.

Place a large filter on the Membrane-filter Apparatus and place the funnel over the filter.

1. A large filter is placed in a weighing boat and the filter and weighing boat are weighed.
2. The weight is recorded to 4 decimals. This weight is record on the bench sheet under initial weight.
3. Place the filter on the vacuum apparatus and turn on the vacuum.
4. Pour the required volume of sample onto the filter.
5. Rinse the filter three times with 20 mLs of distilled/deionized water.
6. Remove the filter using tweezers and place in an aluminum weighing boat.
7. Place the weighing boat containing the filter in the drying oven as above.
8. Move to the desiccator to cool
9. When cool weigh on balance to four decimals and record on the bench sheet under final weight.

Calculating the Total Suspended Solids in the sample

$$\frac{\text{Weight of sample plus crucible} - \text{weight of the crucible}}{\text{mL of sample used}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \times \frac{1000 \text{ mL}}{1 \text{ L}} = \text{mg/L of solids}$$

If the sample is to be analyzed for volatile solids (VSS) the crucible and sample are then placed in the muffle furnace at 550°C for 20 minutes.

1. The sample is removed to the drying oven to cool.
2. The sample is removed from the drying oven and placed in the desiccator to cool to room temperature.
3. When cool to room temperature, the crucible and sample are weighed. This weight will be recorded as the ash weight.

$$\frac{\text{Weight of sample plus crucible} - \text{Ash weight of the crucible}}{\text{mL of sample used}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \times \frac{1000 \text{ mL}}{1 \text{ L}} = \text{mg/L of VSS}$$

[Understanding TSS Method and Procedure from Cole-Parmer \(coleparmer.com\)](http://coleparmer.com)

HACH TNT Methods

The following analysis are written using the HACH method of analysis. The methods list is a summary of the HACH TNT procedures. The exact procedure for the method used for analyzing the wastewater should be looked up and used. The operator does not have to use HACH equipment, but if HACH procedures are not used the operator will have look up the procedure. The following procedures are listed to show the operator the ease at which analysis may be completed in house.

CHEMICAL OXYGEN DEMAND (COD) HACH TNT METHODS 8000 AND 10299

COD is an indicative measure of the amount of oxygen that can be consumed by reactions in a measured solution. It is commonly expressed in mass of oxygen consumed over volume of solution which the units are milligrams per liter (mg/L). A COD test can be used to easily quantify the amount of organics in water. COD is used in quantifying the amount of oxidizable pollutants found in wastewater.

The DEQ Technical Assistance Section recommends using the HACH TNT method 821 and 822 of analysis for COD as it is the easiest method for the operator to use. For the complete laboratory procedure refer to the HACH COD 821 and 822 Method for analysis. Other methods are available but are not covered in this document.

Sample Collection

- Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples that contain solids to get a representative sample.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at 2–6 °C (36–43 °F) for a maximum of 28 days.
- Correct the test result for the dilution caused by the volume additions.

Sample Analysis

1. Set the DRB200 reactor power to on. Set the temperature to 150 °C.

2. Measure 100 mL of sample in a blender. Blend for 30 seconds or until homogenized. If the sample does not have suspended solids, ignore this step.
3. Pour the homogenized sample into a 250-mL beaker and stir slowly with a magnetic stir plate. If the sample does not have suspended solids, ignore this step.
4. Invert a test vial several times to mix.
5. Use a pipet to add 2.0 mL of sample to the test vial. Hold the vial by the cap, over a sink. Invert gently several times to mix. **The vial gets very hot during mixing.**
6. Insert the vial in the preheated DRB200 reactor. Close the lid.
7. Keep the vial in the reactor for 2 hours.
8. When the timer expires, set the reactor power to off.
9. Let the temperature decrease for about 20 minutes to 120 °C or less.
10. Hold the vial by the cap and invert gently several times while the vial is still hot.
11. Put the vial in a test tube rack. Let the temperature of the vial decrease to room temperature.
12. Clean the outside of the vial.
13. Set the spectrophotometer to the correct wavelength.
14. Insert the vial into the cell holder.
15. Press Read
16. Results show in mg/L COD.

AMMONIA HACH TNT METHODS 831 & 832

For the complete method of Ammonia Analysis see HACH Ammonia Methods 831 and 832. Other methods are available but are not covered in this document.

Sample collection

- Collect samples in clean glass or plastic bottles.
- Analyze the samples as soon as possible for best results.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated hydrochloric acid. No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for a maximum of 28 days.
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5 N sodium hydroxide solution.
- If completing the analysis inhouse continue to Method of Analysis

Method of Analysis

1. Remove the lid from the Dosi™ Cap Zip on the Ammonia vial, then remove the cap from the vial.

2. Add 0.2 mL of sample to the vial.
3. Immediately turn the Dosi™ Cap over so the reagent side goes into the vial and screw the cap onto the vial.
4. Shake the vial 2–3 times to dissolve the reagent in the cap. Look through the open end of the Dosi™ Cap to make sure that the reagent has dissolved.
5. Start Reaction time of 15 minutes.
6. After 15 minutes clean the outside of the vial
7. Place in Spectrophotometer at correct setting
8. Press read.
9. Report on benchsheet.

ALKALINITY CaCO_3 (HACH TNT METHOD 10239)

For the complete method of Alkalinity Analysis see HACH Alkalinity Method 10239. Other methods are available but are not covered in this document.

Sample Collection

- Collect samples in clean glass or plastic bottles with tight-fitting caps. Completely fill the bottle and immediately tighten the cap.
- Prevent agitation of the sample or exposure to air.
- Analyze the samples as soon as possible for best results.
- If immediate analysis is not possible, keep the samples at or below 6 °C (43 °F) for a maximum of 24 hours.
- Let the sample temperature increase to room temperature before analysis.

Method of Analysis

1. Use a pipet to add 2.0 mL of Solution A to the test vial.
2. Use a pipet to add 0.5 mL of sample to the test vial.
3. Tighten the cap on the vial and invert until completely mixed. Make sure that the contents are well mixed.
4. Start the reaction time of 5 minutes.
5. When the timer expires, clean the outside of the vial.
6. Insert the vial into the cell holder.
7. Press READ.
8. Results show in mg/L CaCO_3 .

NITRATE NO₃-N (HACH TNT METHODS 835 & 836)

For the complete method of Ammonia Analysis see HACH Nitrate Methods 835 & 836 . Other methods are available but are not covered in this document.

Sample Collection

- Collect samples in clean glass or plastic bottles.
- Analyze the samples as soon as possible for best results.
- If immediate analysis is not possible, immediately filter and keep the samples at or below 6 °C (43 °F) for a maximum of 48 hours.
- To preserve samples for a maximum of 14 days, adjust the sample pH to 2 or less. with concentrated sulfuric acid (approximately 2 mL per liter) and keep at or below 6 °C (43 °F). The test results then include nitrate and nitrite.
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5 N sodium hydroxide solution.
- Correct the test result for the dilution caused by the volume additions.

Method of Analysis

1. Use a pipet to add 0.2 mL of sample to the test vial.
2. Use a pipet to add 1.0 mL of Solution A to the test vial.
3. Tighten the cap on the vial and invert until completely mixed.
4. Start the reaction time of 15 minutes.
5. When the timer expires, clean the outside of the vial.
6. Insert the vial into the cell holder.
7. Results show in mg/L NO₃—N.

NITRITE NO₂—N (HACH TNT METHOD 839, 840 & 841)

For the complete method of Ammonia Analysis see HACH Nitrite Methods 839, 840 & 841. Other methods are available but are not covered in this document.

Sample Collection

- Collect samples in clean glass or plastic bottles.
- To preserve samples for later analysis, keep the samples at or below 6 °C (43 °F) for up to 48 hours.
- Let the sample temperature increase to room temperature before analysis.

Method of Analysis

1. Collect samples in clean glass or plastic bottles.
2. To preserve samples for later analysis, keep the samples at or below 6 °C (43 °F) for up to 48 hours.
3. Let the sample temperature increase to room temperature before analysis.
4. Start the reaction time of 10 minutes.
5. When the timer expires, clean the outside of the vial.
6. Insert the vial into the cell holder.
7. Press Read
8. Results show in mg/L NO₂—N.

TOTAL PHOSPHORUS PO₄³⁻ (HACH TNT METHODS 843, 844, & 845)

For the complete method of Phosphorus Analysis see HACH Phosphorus Methods 843, 844 & 845. Other methods are available but are not covered in this document.

Sample Collection

- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- Analyze the samples as soon as possible for best results.
- Do not use a detergent that contains phosphate to clean the sample bottles. The phosphate in the detergent will contaminate the sample.
- To preserve samples for later analysis, adjust the sample pH to 2 or less with concentrated sulfuric acid (approximately 2 mL per liter). Do not acidify samples to be analyzed only for reactive phosphorus. No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for a maximum of 28 days (Reactive phosphorus only: 48 hours).
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5 N sodium hydroxide solution.
- Correct the test result for the dilution caused by the volume additions.

Method of Analysis

1. Set the DRB200 reactor power to on.
2. Set the temperature to 100 °C.
3. Carefully remove the lid from the DosiCap™ Zip cap.
4. Remove the cap from the test vial.
5. Use a pipet to add 2.0 mL of sample to the test vial.
6. Turn the DosiCap™ Zip over so that the reagent side goes on the test vial.
7. Tighten the cap on the vial. Shake the vial 2–3 times to dissolve the reagent in the cap.

8. Look through the open end of the DosiCap™ to make sure that the reagent has dissolved.
9. Insert the vial in the preheated DRB200 reactor. Close the lid.
10. Keep the vial in the reactor for 1 hour.
11. When the timer expires, carefully remove the vial from the reactor.
12. Set the vial in a test tube rack to cool.
13. Let the temperature of the vial decrease to room temperature.
14. Shake the vial 2–3 times.
15. Use a pipet to add 0.2 mL of Solution B to the test vial. Immediately tighten the cap on the solution B container.
16. Put a grey DosiCap™ C on the vial. Tighten the cap on the vial and invert the vial 2–3 times.
17. Start the reaction time of 10 minutes.
18. When the timer expires, invert the vial 2–3 times.
19. Clean the outside of the vial.
20. Insert the vial into the cell holder.
21. Press Read
22. Results show in mg/L PO₄³⁻.