LABORATORY SKILLS

FOR

TREATMENT PLANT OPERATORS

Solids

BOD

pH

Ammonia

Chlorine Residual

Fecal Coliform

Phosphorus, Total and Reactive

Prepared and Presented by the IWEA Laboratory Committee Members.

LABORATORY SKILLS WASTEWATER PLANT OPERATORS

CONTENTS

ACKNOWLEDGEMENT

This manual, *Laboratory Skills for Treatment Plant Operators*, was written by members of the laboratory committee of Illinois Water Environment Association.

The authors have attempted to site references for all procedures and information found in this manual.

The contents of this manual are intended for operating personnel only and are not intended to be a standard of the Water Environment Federation. Users of this manual who need further background should refer to *Standard Methods for the Examination of Water and Wastewater*.

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LAB LANGUAGE

It sometimes seems as if laboratory professionals use a complex language full of acronyms and technical jargon. Below are definitions for some commonly used terms.

Accuracy	A measure of the agreement between the observed value and the true value.
Analyte	The element or compound for which you are measuring.
Analytical Batch	One to twenty samples of the same matrix that are prepared together with the same process and personnel and using the same lot of reagents and analyzed on the same day.
Calibration Curve	A method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.
Calibration Curve Verification, CCV	A solution of known concentration containing the analyte(s) of interest. This solution is used to verify the calibration curve accuracy.
Chain of Custody, COC	An unbroken trail of accountability that ensures the physical security of samples, data, and records.
Laboratory Control Sample, LCS	A solution containing a known quantity of the analyte(s) of interest. The LCS is a measure of accuracy.
Matrix	The main material of which a sample is composed.
Matrix Spike Duplicate, MSD	Samples to which a known amount of the analyte(s) of interest have been added. The MSD is a measure of precision.
Method Blank, MB	A sample which does not contain the analyte of interest and which is carried through all sample preparation and analysis steps.
Precision	A measure of the agreement between replicate analyses.
Standard Methods	A comprehensive reference covering all aspects of water and wastewater analysis techniques. *Standard Methods* is a joint publication of the American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF).
Standard Operating Procedure, SOP	Written laboratory specific document which details the method of an operation or analysis with procedures thoroughly described. The SOP is the accepted method for performing certain routine and repetitive tasks.

RECOGNIZING THE "STANDARD" IN *STANDARD METHODS*

It is important to be consistent when performing tests as prescribed in *Standard Methods.* The use of specific materials, conditions, and equipment are important to maintain uniformity between analysts and laboratories. By doing tests in the same manner, a technician in Yakima, Washington, can interpret and relate to a test performed in Savannah, Georgia.

The wastewater plant operator or technician needs to be aware of conditions, equipment, and procedures which make the "standard" in *Standard Methods*. Below is a brief listing of common things to watch and some examples which can be applied to any test:

1. Specific materials: glass micro-fiber filters, distilled water

2. Specific apparatus: analytical balance, drying oven, muffle furnace, steam table, desiccator, suction apparatus

3. Specific chemicals and reagents in specified concentrations, normalities, or molarities.

4. Specific time: 45 minutes, 1 hour, etc.

5. Specific Temperature: 103-105^{o}C, 180^{o}C or 550^{o}C

6. Technique and procedure: cycle of drying, cooling, desiccating and weighing to obtain constant weight, filtering, supernating, settling, mixing, timing, rinsing, distilling, evaporating, washing, and measuring, as well as proper sequence of steps.

7. Calculations: proper use of units of volume and weight with proper conversions from liters to milliliters or grams to milligrams.

QUALITY CONTROL

"How do I know that my results are valid?"

Performing analyses according to EPA approved procedures, such as those found in *Standard Methods*, is a good way to produce reliable results. However, a simple "I performed the analysis correctly" may not convince someone to trust your analytical results.

A strong quality assurance program is the best way to assure and document analysis validity. Quality assurance programs include all elements that can affect analyses. Some of these elements are: housekeeping, safety, appropriate standard operating procedures, training, reagent quality, instrument monitoring, and control charts.

The EPA approves methods for measuring pollutants in wastewater. You can find a list of the approved methods in 40 CFR 136. In April 2012, the EPA issued a Methods Update Rule. This rule includes quality control requirements for laboratory analyses. These requirements specify what quality control solutions need to be run with each analysis batch. When an analyst runs qc samples along with the analytical batch, and achieves acceptable results for those qc samples, it indicates that the sample results are also acceptable. If quality control solutions do not have acceptable results, it indicates the analyst may need to trouble shoot an instrument or procedure before continuing to use it to analyze samples.

Some commonly used quality control solutions are:

MB, Method Blanks – Method blanks are used to determine if the preparation and analysis procedures contaminate samples. Typically method blanks are deionized water. The method blank must go through sample preparation steps. If you run deionized water on an instrument without going through preparation steps, it is considered an instrument blank, not a method blank.

LCS, Laboratory Control Samples – Laboratory Control Samples measure analysis accuracy. You can purchase the LCS from a laboratory supply vendor. Many labs use a certified reference solution as a LCS. Certified reference standards should come with a certificate of analysis that lists the analytes present in the solution and the acceptable recovery range.

MSD, Matrix Spike Duplicates – Matrix Spike Duplicates measure analysis precision. The MSD can indicate whether the sample matrix interferes with the analysis. The MSD must go through all the sample preparation and analysis steps.

When thinking about quality control, the key question to ask is: "WHY?" Why is this analysis result accurate? Or, why is an analysis result erroneous or not representative? It serves as a way to examine technique, procedure, equipment, and ultimately, yourself.

SAMPLING TECHNIQUES

Before laboratory analysts do any testing, they must be confident of the integrity of their sample.

Samples are collected for many reasons. Inside the treatment plant, these reasons include such things as calculating plant loadings, monitoring treatment processes, determining compliance with NPDES permit limits, providing test data for future design work, and identifying shock loads and possible illegal discharges.

Samples can also be taken from the public sewer system or from discharge pipes from private industry. Many POTWs sample industry to monitor the discharge of non-compatible pollutants such as heavy metals, cyanide, FOG, etc.

There are several modes of sampling. These include grab, composite and split samples.

1. Grab samples are much as their name implies. Samples are taken to give an "instant in time" view of a discharge stream's characteristics. Grab samples are best for monitoring shock loads or sudden unusual changes (such as color or pH) in the waste stream.

2. Composite samples give a truer picture of a waste stream's overall (or average) characteristics. Composites samples are good for providing data to fulfill permit requirements. Many NPDES permits require that analyses be made on flow proportional composite samples in all cases except pH, chlorine residual, fecal coliform, and volatile organics.

   Composite samples can be either flow or time proportional. In flow proportional sampling, a measured amount of the stream is drawn into the sampling vessel for specified units of flow; for example, 100 mLs of sample may be drawn into the vessel for every 1000 gallons of flow.

   In time proportional sampling, a measured amount of the stream is drawn into the sample vessel for specified units of time; for example, 100 mLs of sample may be drawn into the vessel every hour. Time proportional composite sampling is most effective for discharges of predictable, continuous flow.

3. Split samples can be either grabs or composites. Split samples consist of a well mixed sample distributed into two containers. The two "splits" can then be sent to the laboratory for duplicate analysis or sent to different laboratories to compare their results.

Regardless of discharge source or sampling mode, there are routine procedures which should be followed when taking any sample.

1. Proper containers should be used for all samples. The type of container depends on the parameters the lab will be checking. Table I lists the proper containers for various sample types.

2. Proper preservatives should be used. The type of preservative depends on the parameters the lab will be checking. Table I also lists the proper preservatives for various sample types. At a minimum, all samples which are not immediately analyzed should be stored in a 40 F refrigerator.

3. All samples should be labeled with the source, time and date of sample, the preservative, and the initials of the operator taking the sample.

4. Sample containers should not be filled more than three quarters full. This leaves sufficient headspace for laboratory personnel to adequately mix and pour the sample. The exception to this rule is volatile organics which must be sampled with no air in their vials.

5. If necessary clean the dipper or sampling vessel before using. When samples must be kept sterile, such as fecal coliform, use the sample container as the sampling vessel.

Many laboratories require that a chain of custody form accompany every sample. This form documents everyone who has handled the sample. Chain of custody becomes important when laboratory results are contested, used for enforcement, or used in court cases.

TABLE 1
Containers, Preservatives, and Hold Times

PARAMETER	CONTAINER	PRESERVATIVE	HOLD TIME
Ammonia	Plastic, glass	Cool, 4° C H_2SO_4 to pH 2	28 days
BOD & CBOD	Plastic, glass	Cool, 4° C	48 hours
COD	Plastic, glass	Cool, 4° C H_2SO_4 to pH 2	28 days
Cyanide	Plastic, glass	Cool, 4° C NaOH to pH 12	14 days
Fecal Coliform	Plastic, glass	Cool, 4° C	6 hours
Heavy Metals (except Hg)	Plastic, glass	HNO_3 to pH 2	6 months
Kjeldahl Nitrogen	Plastic, glass	Cool, 4° C H_2SO_4 to pH 2	28 days
Mercury	Plastic, glass	HNO_3 to pH 2	28 days
Nitrate	Plastic, glass	Cool, 4° C	48 hours
Oil and Grease	Glass	Cool, 4° C H_2SO_4 to pH 2	28 days
Phenols	Glass	Cool, 4° C H_2SO_4 to pH 2	28 days
Solids: TSS, %TS, %TVS	Plastic, glass	Cool, 4° C	7 days
Solids: Settleable	Plastic, glass	Cool, 4° C	48 hours
Sulfate	Plastic, glass	Cool, 4° C	7 days
VOC	40 mL vial with teflon septum	Cool, 4° C	14 days
BNA & Pesticides	Glass with teflon lined cap	Cool, 4° C	7 days before extraction; 40 days after extraction

Pipetting

Wastewater laboratories are filled with specialized instruments that must be used in a precise manner in order to obtain the desired accuracies. A pipette is a laboratory tool commonly used to transport a measured volume of liquid. Pipettes come in several designs for various purposes with differing levels of accuracy and precision, from single piece glass pipettes to more complex adjustable or electronic pipettes. Many pipette types work by creating a partial vacuum above the liquid-holding chamber and selectively releasing this vacuum to draw up and dispense liquid. The accuracy of a pipette is related to its type and the analyst's technique.

Common Types of Pipettes and Pipetting Equipment

Volumetric Pipette

Volumetric pipettes allow the user to measure a volume of solution extremely accurately. These pipettes have a large bulb with a long narrow portion above with a single graduation mark as it is calibrated for a single volume. Volumetric pipettes are commonly used to prepare solutions and standards.

Volumetric pipettes will deliver the specified or desired volume when drained and "tipped" to the edge of the receiving vessel. This type of pipette should not be "blown out".

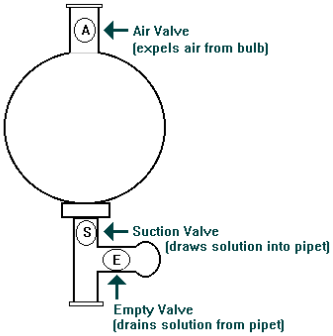
Measuring (graduated) Pipette

Measuring pipettes are a type of pipette consisting of a long tube with a series of graduations to indicate different calibrated volumes. Measuring pipettes can be used to measure various volumes from a single pipette due to their volume gradations. Measuring pipettes are often graduated in one of two ways:

Mohr pipettes are graduated, but not calibrated to the tip. If allowed to drain completely, too much liquid will be delivered. Because of this, Mohr pipets are never "blown out".

Serological pipettes are graduated, so they can deliver different measured volumes. Serological pipettes must be "blown out" to deliver the measured volume and are designated with a frosted band or double lines near the top.

Many pipettes are labeled TD (to deliver) or TC (to contain) Inspection of the graduations on the pipette should indicate which type of pipette is being used.



3-Way Bulb

A three-way pipette bulb is used to draw a liquid into any type of pipette. A three-way bulb is often chosen because it reduces the possibility of contact with the liquid, and any liquid drawn into a pipette

can easily be held in the pipette until delivery. The three valves provide a convenience not found in the standard bulb.

Using a 3-Way Bulb

1. Make sure there is enough liquid in a beaker or other container to completely fill the pipet. (NEVER pipette directly from a reagent bottle or stock container.)
2. Carefully place the attachment of the three-way bulb over the mouth of the pipette.
3. Squeeze the air valve (A) and the bulb simultaneously to empty the bulb of air. Then, release the air valve (A).
4. Place the tip of the pipette below the liquid's surface.
5. Gently squeeze the suction valve (S) to draw liquid into the pipette. When the liquid is above the desired volume, stop squeezing the suction valve. DO NOT remove the three-way bulb from the pipet. DO NOT ALLOW THE LIQUID TO ENTER THE BULB.
6. If the level of the liquid is not high enough squeeze the air valve (A) and the bulb again to expel air from the bulb. Draw more liquid by squeezing the suction valve (S).
7. Touch the tip of the pipette to the inside of the beaker to remove the drop hanging from the tip. Observe and record the initial volume.
8. To transfer the liquid to a receiving vessel, press the empty valve (E) until the desired volume is delivered. Remember to touch the tip of the pipette to the inside wall of the receiving vessel.

Automatic Micropipettes

These pipettes operate by piston-driven air displacement. A vacuum is generated by the vertical travel of a metal or ceramic piston within an airtight sleeve. As the piston moves upward, driven by the depression of the plunger, a vacuum is created in the space left vacant by the piston. The liquid around the tip moves into this vacuum (along with the air in the tip) and can then be transported and released as necessary. These pipettes are capable of being very precise and accurate. However, since they rely on air displacement, they are subject to inaccuracies caused by the changing environment, particularly temperature and user technique. For these reasons this equipment must be carefully maintained and calibrated, and users must be trained to exercise correct and consistent technique.

Pipette Calibration

To ensure accuracy it is necessary to perform pipette calibration at regular intervals. Calibration of equipment is desirable even in the most advanced equipment as overtime several factors can affect the readings of measuring equipment. Calibration process helps to check whether or not the equipment is offering correct reading so that it can be rectified in time and efficiency of processes or laboratory test is maintained.

Laboratory accreditation requires that the auto-pipettes and dispensers are checked and calibrated every 3 months. It is recommended that before calibration, the auto-pipettes are cleaned and visually inspected for damage. If an auto-pipette or dispenser requires cleaning or repair, check and recalibrate the unit before use.

Equipment Needed

A certified balance capable of reading gram weight to four decimal places.

A small beaker or flask as a weighing vessel.
A beaker containing deionized water.
Certified thermometer to measure water temperature.

Preparation for Testing and Examination
All items, including the water, used to perform the calibration must be allowed to equilibrate to room temperature. Record the temperature of the water on the form.

Cleaning
Follow the manufacturer's procedure for proper cleaning. Make sure the shaft of the pipettor is clean. If an O ring is present, be sure that it is in good condition. Replace if necessary. Lubricate as recommended by manufacturer. Clean the exterior surfaces. Check the uptake tube and the container for the presence of any dirt or biological growth and clean if necessary.

Visual Inspection
Check each unit for any breaks, cracks, and any major mechanical damage. Check for interior cracks that may occur in the glass cylinders of the auto-pipettors. If any damage is discovered, repair or replace the unit.

Functionality Test
Check that the unit being tested operates properly. Check that the testing water is drawing into the unit smoothly and can be retained for approximately 10 seconds. If aspiration is not possible or very slow, clean the instrument. If a drop forms at the end of the tip of an auto-pipette during the 10 second testing interval, check again with a new tip. If no change then clean or replace the seal or O ring. If a dispenser or digital burette is being tested, bleed the unit so that no air bubbles are present.

Gravimetric Test
Set or adjust the unit to the nominal volume that will be dispensed. If an auto-pipette is being tested, pre-rinse the tip. Place the weighing beaker on the certified balance and then tare.
Take up testing liquid from the beaker containing deionized water and release several times. On the final take up of water, dispense into the tared beaker and record the weight. The weight of the water in mg should equal the mL setting of the unit. If the weight does not equal the volume, adjust the unit appropriately if possible. Remove beaker and discard water. Re-tare the beaker and repeat this process until the weight equals the volume dispensed. Once this has been achieved, repeat and record a minimum of five weighings (check with the owners manual as up to ten weighings may be required). Record the weights on a worksheet.

Calculate the precision and accuracy of all of your readings and compare it to the specifications cited in the operators manual.

If unsatisfactory results are obtained then clean or repair if possible, according to manufacturer's instructions, and retest.

Total Suspended Solids Dried at 103-105°C

Standard Methods, 22nd Ed., 2540D

General

Total suspended solids, TSS, is determined by filtering a well mixed sample through a glass fiber filter disk. Suspended solids are the portion of total solids retained by filter paper after filtration. Either a filter holder apparatus or a Gooch crucible may be used. Total suspended solids may be used to measure solids balance in unit processes that involve solids capture.

Apparatus

- Membrane filter apparatus or Gooch crucible;
- Vacuum pump or water aspirator;
- Vacuum flask, approximately 500-mL;
- Drying oven, 103°C to 105^0C
- Analytical balance
- Glass fiber filter disks, without organic binder, such as Whatman 934AH, Gelman type A/E, Milliport type AP40, or equivalent, to fit membrane filter apparatus or Gooch crucible.
- Desiccator
- Graduated cylinder(s)

Reagents

No reagents are required.

Procedure

1. Place a glass fiber filter disk rough side up in the filter holder apparatus or Gooch crucible.

2. Apply vacuum and wash the disk with three successive 20-mL portions of distilled water. Remove the filter disks from the filter holder apparatus and transfer to an aluminum or stainless steel container; or alternatively, remove the Gooch crucible and filter disk combination.

3. Dry the container and disk (or the crucible and filter disk combination if a Gooch crucible is used) at 103-105°C for 1 hour. Then cool in a dessicator. Weigh on a analytical balance . Repeat this process until a constant weight is attaned, or until the or until the weight loss is less than 4% of the previous weight or 0.5 mg, whichever is less. Record this weight as W_1, in grams.

   NOTE: Most labs determine a drying time that works for all their samples so they don't have to go through multiple dry/cool/weight cycles for each sample.

4. Shake the sample vigorously and transfer the desired volume to a graduated cylinder. Filter the measured volume of a sample through the filter disk with a vacuum pump or water aspirator, until all traces of water have passed through. With the suction on, rinse the graduated cylinder, filter funnel, wall and filter with three successive portions of distilled water, until all the water has passed through. Carefully remove the filter from the filter apparatus and transfer to the container. Alternatively remove the Gooch crucible and filter; dry at 103° to 105°C for at least 1 hour or until constant weight is attained; cool in a desiccator to room temperature; weigh and record weight as W_2, in grams.

Calculation

mg/L Total Suspended Solids

$$= \frac{(W_2 - W_1) \text{ X } 1000 \text{ mL/L X } 1000 \text{ mg/g}}{\text{mL of sample filtered}}$$

Where

W_2 and W_1 are measured in grams

W_1 = weight of the prepared crucible or filter

W_2 = weight of the crucible or filter after the filtration step

Fixed and Volatile Solids Ignited at 550°C

Standard Methods, 22nd Ed., 2540E

General

The fixed and volatile components in the total suspended solids may be determined by igniting the sample at 550° ± 50°C. The determination approximates roughly the amount of organic matter in the solid fraction of wastewater, activated sludge, industrial waste, or bottom sediments.

Interference

The glass fiber disk will fuse at slightly above 550°C, therefore, it is important not to exceed this temperature when the crucible or filter is ignited.

Reagents

No reagents are required.

Procedure

Place the previously dried and weighed sample residue from Step 4, Total Suspended Solids, in a muffle furnace at 550°C for 30 minutes. Allow the dish to partially cool in air until most of the heat has been dissipated, then cool in a desiccator to room temperature and weigh and record as W_3, in grams. If an aluminum dish was used to hold the filter pad in the TSS determination, the pad must be transferred to another container before ignition. Aluminum disks cannot be fired at 550°C.

Calculations

$$\text{mg/L Volatile Solids} = \frac{(W_2 - W_3)\ (1000)\ \text{mL/L}\ (1000)\ \text{mg/g}}{\text{mL sample}} \qquad (1)$$

$$\text{mg/L Fixed Solids} = \frac{(W_3 - W_1)\ (1000)\ \text{mL/L}\ (1000)\ \text{mg/g}}{\text{mL sample}} \qquad (2)$$

Where

W_1, W_2, and W_3 are expressed in grams

W_1 = weight of the prepared crucible or filter pad

W_2 = weight of crucible or filter pad after the sample filtration step, and

W_3 = weight of the crucible or filter pad after the ignition step

Total Solids, Volatile Solids, And Fixed Solids in Sludge

Standard Methods 22nd Ed., 2540 G

General

Volatile and fixed solids in sludge are determined by drying a weighed sample in an oven. Unlike total suspended solids in wastewater, which is expressed in milligrams per liter (ppm), solids in sludge are expressed in terms of percent by mass of the total amount of solids; this procedure makes the numbers more manageable.

Apparatus

- Drying oven
- Steam bath
- Evaporating dish
- Muffle furnace
- Desiccator
- Analytical balance (reading to 1 mg) or an analytical balance

Procedure

1. After cleaning an adequate size evaporating dish, place it in a muffle furnace at 550°C for 15 to 20 minutes. Allow the dish to partially cool in an oven until most of the heat has dissipated, then cool in a desiccator to room temperature. Weigh dish and record as W_1, in grams.

2. Pour a portion of the well-mixed sample (25 to 35 mL) into the dish, then weigh sample and dish together. Record as W_2, in grams.

3. Place dish on steam bath and evaporate to dryness. Then put dish in drying oven for 1 hour at 103°C – 105^{0}C. Cool in a desiccator, and weigh. Record the weight as W_3, in grams. Repeat drying and cooling step, then re-weigh sample until constant weight is achieved (<4% difference). Note: Alternately, perform all drying operations in oven, skipping the steam bath. Determine drying time necessary for largest sample "wet" weight to achieve constant weight. Dry all samples to this minimum time. Four hours to overnight might be required.

4. After weighing the dish and dry solids, compute the percent to total solids (Equation 1).

5. Place dish from previous step in muffle furnace at 550°C until sample is burned completely. Length of time complete burning depends on size of sample. If unsure that constant weight has been obtained, record mass from fist heating, then reheat and weigh again in the same manner until a constant mass reading is obtained.

   Allow the crucible to partially cool in air until most of the heat has dissipated; then cool in a desiccator to room temperature. Weigh the dish and record as W_4, in grams. The loss of weight ($W_3 - W_4$) is the volatile matter. The percent volatile matter may then be computed (Equation 2).

Calculations

$$\text{Percent Total Solids} = \frac{\text{mass of dry solids } (W_3 - W_1) \times 100}{\text{Mass of wet sludge } (W_2 - W_1)} \quad (1)$$

$$\text{Percent Volatile Solids} = \frac{\text{mass of volatile solids } (W_3 - W_1) \times 100}{\text{Mass of dry solids } (W_3 - W_1)} \quad (2)$$

$$\text{Percent Fixed Solids} = \frac{\text{mass of ash } (W_4 - W_1) \times 100}{\text{Mass of dry solids } (W_3 - W_1)} \quad (3)$$

Where

All weights are expressed in grams

W_1 = mass of the prepared evaporating dish

W_2 = mass of the prepared dish and wet sample

W_3 = mass of the dish and sample after the drying step, and

W_4 = mass of the dish and sample after the ignition step

Reference

Water Pollution Control Federation, *Simplified Laboratory Procedures for Wastewater Examination,* Washington DC: Water Pollution Control Federation, 1985

BIOCHEMICAL OXYGEN DEMAND (BOD_5)

Biochemical oxygen demand, more commonly referred to as BOD_5, is a laboratory test which has its widest application in measuring waste loadings to treatment plants, and in evaluating the efficiency of waste removal. BOD is used to determine, or quantify, the relative oxygen requirements of wastewater, effluents, and polluted waters. The test measures the biochemical degradation of organic materials and, to some extent, the oxidation of some inorganic materials. This is accomplished indirectly by measuring the oxygen used by a sample during a specified incubation period. The 5-Day BOD test (BOD_5) is standard in nearly all laboratories and is specified in EPA permits for basic reporting.

The oxygen demand that the BOD test measures is the sum of carbonaceous and nitrogenous demands. Both types of demand are caused by the activity of bacteria present in a sample. The number and class of bacteria present influence the amount of each type of demand at work in a sample. Carbonaceous demand measures the oxygen demand of organic materials in the wastewater. Nitrogenous demand measures the oxygen demand of nitrogen containing materials such as ammonia in the wastewater.

A nitrification inhibiting chemical can be added to the BOD test so that only carbonaceous demand is measured. The chemical inhibition of nitrogenous demand provides a more direct and more reliable measure of BOD. Carbonaceous BOD (CBOD) is now commonly performed in wastewater treatment plants because it yields more accurate information on loadings and effluent quality.

The BOD method consists of filling an airtight bottle to overflowing with a sample and incubating at 20^o C for 5 days. Dissolved oxygen (DO) is measured initially and after the incubation period. The BOD is calculated from the difference between the initial and final DO.

The BOD concentration in most wastewaters exceeds the working range of the test due to oxygen saturation limits. It is necessary to dilute many samples to bring them into an acceptable concentration range. Dilutions are made with specially prepared dilution water, which contains nutrients and buffer. The dilution water contains additives because bacterial growth, essential to the test, requires nutrients and a stable pH environment. Seed is sometimes added to the sample as a source of additional bacteria. This is necessary when a sample, such as an industrial waste or a chlorinated effluent, does not have a sufficient population of bacteria to make the BOD test work.

BOD ANALYSIS

Standard Methods 5210 Biochemical Oxygen Demand
Luminescence Measurement of Dissolved Oxygen

Sample Preservative: Refrigerate at 4^0C.
Holding Time: 48 hrs at the time collection (for composite samples: time starts at the last time of the sample is collected)
Storage of Samples: If analysis is begun within two hours of collection, cold storage is unnecessary. If analysis is not started within two hours, keep at or below 4ºC from time of collection.

Equipment

Dissolved Oxygen Meter	DO Probe 1- Membrane Electrode probe 2- LDO - Lumicescent probe
BOD Bottles-300 ml and Stoppers	BOD Bottle Caps
Incubator (capable of maintaining 20+0.5^{0}C)	Thermometer for Incubator
Graduated Cylinders	Serological Pipets, Wide Tip: 2, 5, 10, 25 ml
Pipet filler or Pipet Bulb	Aspirator Bottle w/Hose Outlet
C-Flex tubing	Keck tubing Clamp
pH Meter	

Reagents

Dilution Water: - (Nutrients)	Phosphate Butter Solution
Magnesium Sulfate Solution, 2.25% (w/v)	Calcium Chloride Solution, 2.75% (w/v)
Ferric Chloride Solution, 0.025% (w/v)	

Note: *Vendors offer BOD dilution water reagents in pre-mixed, pre-measured amounts that can be added to specific water volumes.*

pH Adjusting

Sodium Hydroxide, 0.1N	Hydrochloric Acid, 0.1N

De-chlorinating

Sodium Sulfite Solution, 1.575 g/L	Orthotolidine Solution (OT) purchase from a lab supply vendor

Standard

Glucose/Glutamic Acid Standard (GGA)
Weigh exactly 0.150 g each of glucose and glutamic acid which have been previously dried in the oven for one hour. Dilute to 1 L in a volumetric flask with DI water. This solution is good for three months.
***Note**: Any of these reagents can be purchased commercially or be prepared as per the Standard Methods instructions.

Nitrification Inhibitor for C-BOD

Nitrification Inhibitor: Purchase from Hach Company, catalog number 2533-35.

10% Hydrochloric Acid (HCl)

This solution can be used to clean the BOD glassware, especially the BOD Dilution bottle (carboy) and rinse the glassware at least 3 times. This solution can be used for the tubing also which is used for the siphoning of the water for the BOD test.

Preparation of Dilution Water – IMPORTANT STEP

1. Source water may be distilled water or de-ionized water. It must be free of chlorine and any organic containment.

2. Determine volume of dilution water needed to run all samples and Quality Control. This is usually best determined by multiplying the number of bottles to be set up by 300ml and adding 500ml.

3. Add the calculated amount of source water to an aspirator bottle with tubing hose outlet, tubing attached and tubing clamped off.

4. Add 1 ml of each of the Dilution Water Reagents per liter of water.

5. Aerate the water for at least one hour. Age the water for a minimum of three days, preferably 5 days if possible. It is important to keep the dilution water out of direct sunlight. Before use, aerate the water again for at least one hour.

6. This water should be at $20+3^0$C when ready to set up BOD.

7. DO of aerated dilution water should be 7.5-9.3mg/L. Water containing >9.3mgDO/L is supersaturated and must be allowed to reduce DO by allowing the bottle to stand unshaken and un-aerated.

8. Dispose of excess dilution water daily.

   Note: Some labs ages dilution water, but does not add nutrients until the day of use. On the day of use, nutrients are added, aerated for one hour, and then are ready to go.

Do not underestimate the high quality of water and care the BOD test requires.

Seed Source

- Settled Primary Effluent: Mix primary effluent well, pour into Imhoff cone, allow 1 hour settling. Pour supernatant off into a beaker to be used as seed.
- Commercially prepared seed is available. An example is Polyseed. Be sure to follow manufacturer's instructions for preparation.

Sample Pretreatment

- Avoid samples containing residual chlorine, if possible, usually by sampling ahead of the chlorination process. However, if you think a sample is chlorinated you can test as follows:

- Pour a small amount of sample into a beaker. Add a few drops of OT solution. If solution turns yellow, chlorine is present and must be reduced with sodium sulfite solution before BOD is set up.
- Check pH of sample. If pH is > 10, adjust to 7 with 1+1 hydrochloric acid. If pH is < 4, adjust to 7 with 1N sodium hydroxide.

- Only if necessary, mix sample in blender until homogenized.

Remember the **2:1 Rule:** for an acceptable DO depletion; the sample is to **deplete > 2.0mg/l** and have **at least 1.0 mg/l of O_2 remaining**.

For high strength wastewater, especially industrial sources, plant re-cycle streams and landfill leachates, pre-dilution of the sample may be necessary in order for the BOD to be in the acceptable range and not completely depleted.

Preparation of Equipment

- Suggested Sample Bottles: 2 Blanks + 2GGA + 3 seed +3 x (samples)
- i.e. 1 sample = 10 bottles, 2 samples = 13 bottles
- Tubing used to siphon and deliver the dilution water must be clean and free of algae.
- Set-up and calibrate DO meter for reading Initial DO's. Refer to manufacturer's instructions in regards to calibration of the DO meter.

Calibrate the DO Meter Daily

- You may use either air or Winkler method to calibrate the meter for the traditional membrane probe. If doing the air calibration, follow the manufacturer's instruction. If the DO meter readings drift or become erratic, it is time to replace the membrane.
- When the DO probe is not in use, store the DO probe in a BOD bottle containing approximately one inch of water. This is important to maintain the probe in a humid environment.
- Regarding the LDO probe calibration, it is slightly different from the membrane calibration – be sure to refer to manufacturer's manual.

Setting Up Samples

1. A minimum of 3 dilutions are suggested for each sample. The final DO reading must produce a 0_2 uptake 2.0mg/L and a residual DO 1.0 mg/L after 5 day incubation (2:1 Rule). Five dilutions are recommended if sample BOD is unknown as 3 bottles may not be sufficient to produce a minimum of 2 bottles with final DO within the acceptable range.

2. Fill each BOD bottle to 1/3 to 1/2 full with the buffered dilution water.

3. Then transfer a known amount of sample into each sample bottle. If necessary prepare sample dilutions with pipets or graduated cylinders and then transfer to BOD bottles. It is important to record the dilutions of the samples. Add samples according to determined wastewater strength (usually based on COD).

   Usually wastewater plant samples (influent and effluent strength do not vary widely) so the sample amounts will be similar from day to day.

4. Mix the sample well **immediately** before pipetting to avoid loss of solids by settling

5. Add a range of seed to the "SEED" bottles. Add 2-4mL seed (depending on concentration of seed being used) to the bottles requiring seed.

6. Add 6mL GGA Standard to each of the Standard BOD bottles. GGA requires seed addition.
7. If doing a C-BOD, add nitrification inhibitor (2 shots) to the BOD bottle. Do this before you add the final portion of the buffered dilution water.

8. Fill all bottles 1/3 of the way up the neck of the bottle with dilution water and stopper immediately. Deliver dilution water below the surface so no air is added to the sample. Important – DO NOT OVERFILL the BOD bottle with buffered water.

9. Read and record initial Dissolved Oxygen (DO) of each BOD bottle.

10. Insert stopper into BOD bottle. Make sure there are no air bubbles trapped under the stopper. It is important to have water surrounding the stopper forming a water seal that prevents oxygen from entering or escaping the BOD bottle.

11. Cover the BOD bottle with the BOD bottle "over cap". This helps prevent water from evaporating and helps maintain the water seal.

12. Incubate bottles in an incubator that maintains $20+0.5^{0}$C for 5 days.

13. After 5 days remove bottles from incubator and read Final DO on calibrated DO meter and perform the calculations to determine the BOD.

14. Record the necessary QA data in the Quality Control logs. For example the pH and temperature of the Buffered dilution water, the result of the Glucose- Glutamic Acid Standard and any other standard performed.

BOD Calculations

Unseeded Samples

$$\text{mg/L BOD} = \frac{D_1 - D_2}{P}$$

D_1 = initial D.O.
D_2 = final D.O.
P = sample percent (decimal form)

Seeded Samples

$$\text{mg/L BOD} = \frac{D_1 - D_2 - S}{P}$$

D_1 = initial D.O.
D_2 = final D.O.
P = sample percent (decimal form)
S = seed correction

Seed Correction

$$S = \frac{D_1 - D_2}{P} \times \frac{\text{2 (ml seed added to samples)}}{300}$$

D_1 = initial D.O.
D_2 = final D.O.
P = percent seed in SEED sample bottle (decimal form)

For example, when a 5% (15 mL) "SEED" is used and 2 mL seed is added to samples

$$S = \frac{D_1 - D_2}{7.5}$$

Reference

Standard Methods, 18th Edition, Method 5210, pages 5-1 to 5-5.

BOD TROUBLESHOOTING

Dilution Water – Unseeded Blank

SYMPTOM	POSSIBLE CAUSE	REMEDY
Depletion in Blanks bottles is more than .0.2 mg/l after incubation	Contaminated distilled water	Clean distilled water storage containers Reduce the stills' cooling water flow to increase removal of volatiles If deionizer is used, change resin cylinders and micro-filters
	Contaminated buffers	Buffers should be clear and show no visible precipitate. Phosphate buffers can become moldy or grow bacteria.
	D.O. meter improperly calibrated or malfunctioning	Check membrane, calibration technique and re-standardize sodium thiosulfate with bi-iodate
	Dirty Glassware (if only one bottle shows .0.2 mg depletion, dirty glassware is the likely cause)	Clean bottles, aerator tubing and equipment and develop a standard operating procedure (S.O.P.) for cleaning all equipment
	Dilution water is supersaturated	Allow the water to sit without aeration at room temperature for one hour before use. Insure that no bubbles are trapped in the bottles and the bottle has a water seal.

BOD TROUBLESHOOTING, CONTINUED

SYMPTOM	POSSIBLE CAUSE	REMEDY
Samples depletion is less than 0.1 mg/l	Insufficient seed	Increase amount of seed
	Soap Contamination	Re-wash using 10% HCl, rinse and re-rinse the glassware and tubing eliminate any soap residue
	Weak seed	Collect seed from a different source or a source of greater strength such as primary effluent. Buy a commercial seed.
	Seed contains a toxic material	Change seed source
	Distilled water contains a toxic material	Double distill or deionize water. Purchase a quantity of bottled water to eliminate this possibility
	Incubator temperature was too low	Check incubator temperature daily and insure constant operation
	DO meter improperly calibrated	Check membrane calibration technique and restandardize sodium thiosulfate with bi-iodate
D.O. of seeded blank after 5 days is >2.0 mg/l	Too much seed	Reduce amount of seed
	Incubator temperature too high	Check temperature and operation of the incubator
	D.O. meter improperly calibrated	See above

BOD TROUBLESHOOTING, CONTINUED

Quality Control Check Samples

Symptom	Possible Cause	Remedy
GGA Std sample results are too high	Contaminated distilled water	Drain, clean and replace water in storage tank
		Reduce stills' cooling water flow to increase removal of contaminants
	Contaminated buffers	Replace if they appear cloudy or if a precipitate is present
	D.O. meter improperly calibrated or is malfunctioning	Check membrane calibration technique and re-standardize sodium thiosulfate with bi-iodate
	Math error	Check calculations and verify any dilution factors
	Dirty glassware	Wash glassware and develop a standard operating procedure to insure cleanliness
	Improperly prepared standard	Prepare new glucose-glutamic acid or make new dilution prepared standard on a more frequent basis.
	Incubator malfunction	Check temperature and thermostat control

pH

pH is a term used to express the intensity of the acid or alkaline condition of a solution. It is important in wastewater treatment because the biological processes taking place only occur in a pH range favorable to the organisms involved. Also chemical processes used to coagulate wastewaters, dewater sludges, or oxidize certain substances, such as cyanide ions, require that pH be controlled within rather narrow limits. Further, the 503 sludge regulations have pH controls for the various sludge disposal options.

Definitions

pH - an indication of the intensity of the acidic or basic character of a solution at a given temperature. pH can range in value from 0 - 14. Official definition is pH = -log [H+]

pH meter - an instrument for measuring pH, essentially a voltmeter specially manufactured to measure the potential created when a glass electrode is dipped into solution and to convert that potential to pH units

pH electrode - a closed end glass tube containing a solution of constant pH and silver wire or foil (for electrical contact). When a pH electrode is attached to a voltmeter the potential created is directly proportional to the hydrogen concentration of the solution.

Nernst equation - the voltage across a cell varies with the temperature, pressure, and ion concentration. In the case of pH, usually we need only be concerned with temperature.

LABORATORY PROCEDURE
pH

Collection of Sample
Glass or plastic

Preservative
None

Hold time
None -Analyze samples immediately upon collection

Instruments
pH meter or ion selective meter with appropriate pH probe
temperature compensation probe

Reagents
pH buffers, two are recommended for precise measurement, with a check in the middle.

pH buffer 4.00, color coded red/pink, purchase from a laboratory vendor
pH buffer 7.00, color coded yellow, purchase from a laboratory vendor
pH buffer 10.00, color coded blue, purchase from a laboratory vendor

Test Procedure
Buffers and samples should be at the same temperature. If not, consult the Meter Instruction manual for temperature compensation.

Calibrate the meter as instructed by the manual.

Always use fresh buffers for calibration. Be sure to watch expiration date of buffers.

Rinse the probe between buffers and samples with deionized or distilled water.

Store probes in appropriate buffer or standard solution when not in use.

References
Standard Methods, 21st Edition, Method 4500-H+

AMMONIA NITROGEN BY SPECIFIC ION ELECTRODE

Nitrogen compounds exist in the atmosphere and in the life processes of all plants and animals. Nitrogen chemistry is complex because nitrogen can assume several oxidation states and changes in its oxidation state are brought about by living organisms.

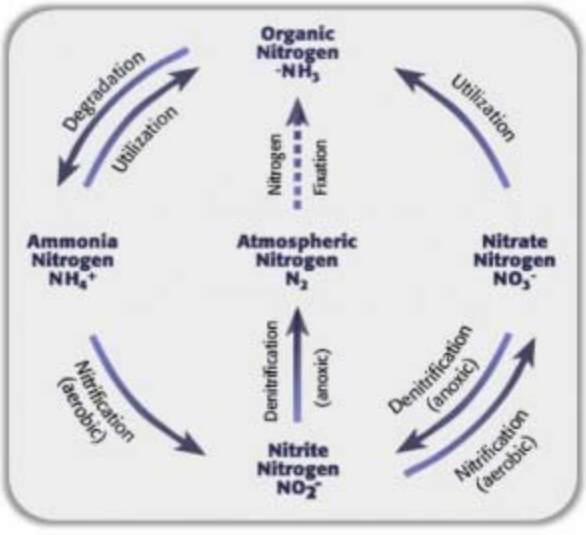
The relationship between the various nitrogen oxidation states is called the Nitrogen Cycle. Learning about the Nitrogen Cycle aids in understanding the reactions taking place in treatment processes.

Animal and human wastes contain appreciable amounts of protein and urea. When bacteria act on these proteins and urea, they convert it to ammonia and other nitrogen containing compounds. This is why ammonia usually increases as wastewater flows through a treatment plant. Under aerobic conditions, nitrosomonas bacteria act on ammonia to produce nitrites. These nitrites, in turn, can be acted on by nitrobacter bacteria to form nitrates.

Under anaerobic conditions, both nitrates and nitrites can be reduced by a process called denitrification. First nitrates are reduced to nitrites. Then the nitrites are further reduced to produce nitrogen gas which escapes to the atmosphere.

Many NPDES permits include ammonia monitoring requirements. A common way to test for ammonia is to use a specific ion electrode. This analysis is similar to using a pH meter. The analyst calibrates a specific ion meter by measuring the millivolts of known concentrations of ammonia standards and plotting them on a graph. When the analyst reads the millivolts of an unknown sample, he can use the graph to determine the ammonia concentration.

Modern specific ion meters do the graphing for you. When the analyst calibrates the meter with known standards, he enters their concentrations into the meter's software. The meter uses its internal microprocessor to make the graph. Then, when the analyst measures a sample, the meter displays its ammonia concentration.



AMMONIA LABORATORY PROCEDURE

Standard Methods 4500-NH_3 D - 1998

Two major factors that influence ammonia method selection are concentration and presence of interferences. In general, direct manual determination of ammonia using an ISE electrode is confined to drinking waters, clean surface or ground water, and good-quality nitrified wastewater effluent. The procedure is listed in manufacturers' instruction manuals and *Standard Methods.*

This method is applicable to the measurement of 0.03 to 1400 mg NH_3-N/L.

Interferences

Grease, oil, and solvents can interfere with ammonia specific ion electrode measurement. Most interference can be eliminated through use of a preliminary distillation step. You can find information on how to distill samples prior to ammonia analysis in *Standard Methods.'*

Residual chlorine reacts with ammonia. If a sample is likely to contain residual chlorine, treat with a decholorinating agent – such as sodium thiosulfate – prior to analysis.

Equipment

1. pH/Specific ion meter
2. Ammonia selective ion electrode
3. Magnetic stir plate and stir bars
4. Volumetric flasks, class A
5. Volumetric pipettes or pipetters
6. Graduated cylinders
7. Beakers, 150 mL

Reagents

1. Ammonia free water – Most labs use DI water as their source for ammonia free water.

2. pH Indicating Ionic Strength Adjuster (ISA) – This is a 5N sodium hydroxide solution with a blue indicator. You can purchase pH indicating ISA from a laboratory supply vendor.

3. 1000 mg/L stock ammonia solution – You can purchase stock ammonia solution from a laboratory supply vendor.

ISE Probe Preparation

Follow the manufacturer's directions on filling the probe with electrolyte and installing the membrane. This step must be done the day before analysis if the probe is stored dry. Place the prepared ISE probe in DI water.

Standards

Prepare two or three standards that bracket the range you want to analyze. The instructions below are for 0.2 mg/L, 2 mg/L and 20 mg/L calibration standards:

1. 20 mg/L calibration standard: Pipet 10 mL of the 1000 ppm standard into a 500 mL volumetric flask. Dilute to volume with ammonia free water. Mix well.

2. 2 mg/L calibration standard: Pipet 10 mL of the 20 mg/L calibration standard into a 100 mL volumetric flask. Dilute to volume with ammonia free water. Mix well.

3. 0.2 mg/L calibration standard: Pipet 1.0 mL of the 20 mg/L calibration standard into a 100 mL volumetric flask. Dilute to volume with ammonia free water. Mix well.

Calibration

Follow your specific ion meter's calibration instructions. General procedure is below.

1. Calibrate meter with low level standard (0.2 mg/L standard)
    - Pour the contents of the 0.2 mg/L standard into a beaker. Put a stir bar in the beaker.
    - Place beaker on magnetic stirrer and stir gently.
    - Immerse the ammonia electrode in sample.
    - Add 2 mL of the ISA. Be sure blue color persists. If not, add more ISA in 1 mL increments.
    - Wait for the mV reading to stabilize. This may take several minutes. Enter the calibration standard's concentration.

2. Calibrate the meter with increasingly higher concentration standards (2 mg/L)
    - Repeat step 1 above for calibrating with the 2 mg/L standard.

3. Calibrate the meter with the highest concentration standard (20 mg/L)
    - Repeat step 1 above for calibrating with the 20 mg/L standard

The meter's reading of the standards should change about 59 mV with each standard.

Sample Analysis

For each sample you are analyzing:

- Pour 100 mL of sample into a beaker. Put a stir bar in the beaker.
- Place beaker on magnetic stirrer and stir gently.
- Immerse the ammonia electrode in sample.
- Add 2 mL of the ISA. Be sure blue color persists. If not, add more ISA in 1 mL increments. *Note: if more than 2 mL of ISA is required to adjust the pH of the sample, then the same volume of ISA should be uses in the preparation of the standards.*
- Wait for meter to stabilize. This may take several minutes. Record the sample's ammonia concentration after the meter has stabilized.
- Make sure to rinse electrode between samples.
- If the sample's ammonia concentration exceeds the range of the calibration curve, dilute the sample and run again. Use your own judgment as to whether to make a 50:50 or 10:100 dilution.

Quality Control

It is good laboratory practice to verify you are performing the procedure correctly by including a quality control samples in each batch.

1. After you calibrate the meter, check your electrode slope. It should be within the range of -54 to -60.
    - If it is not in this range, determine the cause of the error and correct. Then re-calibrate the meter.
    - Some meters can display the calibration curve's coefficient of calibration. It should be 0.995 or greater.

2. Analyze a blank. If the blank is greater than 0.05 mg/L, determine cause of error and correct.

3. Analyze a LCS, laboratory control sample. The LCS can be a certified reference solution or a standard prepared from a 1000 mg/L solution.
    - If you choose to prepare the LCS from a 1000 mg/L ammonia solution, use a solution with a different lot number or manufacturer than that used to prepare calibration standards.
    - The LCS must be within $\pm$ 10% of its true value. If it is not, fix any problems and re-run the LCS. It may be necessary to recalibrate the meter.

4. Analyze a standard at the end of each sample batch. If the standard is within $\pm$ 10% of its true value, this demonstrates that your calibration remained stable throughout the batch.

5. Analyze matrix spike duplicates to track whether various matrices effect the measured ammonia concentration.

Tips

- Make sure all samples and standards are at room temperature before beginning analysis.
- After immersing electrode, make sure there are no air bubbles present on the electrode.
- It takes time for an ammonia electrode to stabilize, especially when measuring low concentrations. Make sure to wait until the meter is stable before taking measurements.
- If samples have a particularly difficult matrix, you may want to use a preliminary distillation step. You can find the distillation procedure in *Standard Methods, Method 4500-NH_3-B.*
- If a sample's ammonia concentration is greater than the highest calibration standard, dilute the sample and re-run. Make sure to multiply the result displayed on the meter by the appropriate dilution factor.
- If more than 2 mL of ISA is required to adjust the pH of the sample, then the same volume of ISA should be uses in the preparation of the standards.

AMMONIA NITROGEN TROUBLESHOOTING

Symptom	Possible Cause	Remedy
Slow response time	Ion electrode has fouled membrane	Change membrane
	Low ammonia concentration in sample	Wait at least 5 minutes for a stable reading. The electrode response time is slower for low concentrations
Quality control check is unacceptable	Calibration failed	Recalibrate with fresh standards. Check mV readings of both standards. The difference should be between 54 and 60mV. If this slope is outside the acceptance range, change membrane.
	QC check made incorrectly	Try a fresh QC. Remake if necessary.
	QC solution is a different temperature than standards	Some magnetic stirrers generate enough heat to change temperature. Place a piece of styrofoam beneath beaker.
	Calibration curve no longer valid	Verify calibration every two hours by reading the 1 mg/l standard. If value had changed, recalibrate.
	Ammonia lost to atmosphere	Do not add ISA until immediately prior to sample analysis. Ammonia is lost at high pH.
	Air bubbles on probe	After immersing electrodes in solution, check for any bubbles on membrane surface and remove by shaking.

AMMONIA NITROGEN TROUBLESHOOTING, CONTINUED

Symptom	Possible Cause	Remedy
Low ammonia recovery in sample	Sample pH low	Verify the pH of the sample is greater than 11. Additional ISA may be necessary, especially for preserved samples.
	Calibration curve has shifted	Verify calibration every two hours by reading the calibration check standard. If the value has changed, recalibrate.
	Ammonia has been lost to atmosphere	Do not add ISA until just prior to analysis. Ammonia is lost at high pH.
Sample result is above the calibration range.	Ammonia concentration of sample is high.	Dilute sample to bring the reading within range. Remember to take dilution into account when reporting the ammonia result by multiplying by a volume factor.

Analytical Method: Total Chlorine

Introduction

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can coexist in the same solution and can be determined together as total chlorine. Free chlorine is present as hypochlorous acid or hypochlorite ion. Combined chlorine represents a combination of chlorine-containing compounds including but not limited to monochloramine, dichloramine, nitrogen trichloride,and other chloro derivatives. The combined chlorine oxidizes triiodide ion (I_3^-) to iodine (I_2). The iodine and free chlorine reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a red solution. The color intensity is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test and a total chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration.

Equipment

Hach Pocket Colorimeter II
10 mL samples cells with caps - catalog # 24276-06
50 mL beakers

Reagents

DPD total chlorine reagent AccuVac ampoules - catalog # 25030-25

Sample Collection, Preservation and Storage

Total chlorine analysis is performed on grab samples and must be run within 15 minutes after collection. Samples may be collected in either polyethylene or equivalent plastic or glass containers.

Quality Control

Standards may be purchased to verify accuracy. 20% of samples should be run in duplicate to verify precision.

Procedure

1. Fill a 10 mL sample cell with sample (this is a sample blank). Collect at least 40 mL of sample in a 50 mL beaker.

2. Press the power key to turn on the meter. The arrow in the display should indicate the low range channel (LR).

3. Remove the instrument cap. Place the blank in the cell holder, with the diamond mark facing the keypad. Cover the cell with the instrument cap.

4. Press ZERO/SCROLL. The display will show "----"then "0.00". Remove the blank.

5. Fill a DPD total chlorine reagent AccuVac ampoule with sample. Note: Keep the tip immersed until the ampoule fills completely.

6. Quickly invert the ampoule several times to mix. Wipe off any liquid or fingerprints. Note: A pink color will form if chlorine is present. Accuracy is not affected by undissolved powder.

7. Insert the ampoule into the cell holder, and then cover with the instrument cap. Wait three to six minutes after filling the AccuVac ampoule.

8. Press READ/ENTER. The instrument will show ---- followed by the results in mg/L chlorine.

9. Record result.

References

Standard Methods for the Analysis of Water and Wastewater, 20th Edition

Code of Federal Regulations (CFR) 40 Part 136, Guidelines establishing test procedures for the analysis of pollutants.

Hach Pocket Colorimeter II Instruction Manual, 4th Edition

Safety – Reference Laboratory Chemical Hygiene Plan for safety procedures.

Use of personal protective equipment (PPE) such as gloves, safety glasses is recommended.

Definitions – Laboratory Glossary of Definitions and Purposes

Analytical Method: Fecal Coliform

Introduction

Analysis is performed to determine the amount of fecal coliform bacteria present in effluent. Fecal coliform bacteria are present in warm-blooded mammals and can be associated with disease. There can be high densities of fecal coliform in the influent wastewater, therefore the effluent maybe disinfected prior to discharge.

Equipment

Sterile Filter Funnel Assembly
44.5^{o} C Water Bath
Bunsen burner or alcohol burner
Membrane filters - 0.45 micron pore size
150 mm sterile petri dish
Vacuum source
Forceps
m-FC agar plates
Igniter
Sterile Whirl Pak Bags

Reagents

m-FC agar
Sterile rinse water
Ethyl alcohol

Sample Collection, Preservation and Storage

Fecal coliform analysis is performed on grab samples and must be run within two hours, (max 6hrs) after collection. Samples should be kept at <8^{o} C until analysis. Chain of Custody forms must be kept for samples and completed by sampler and receiving party.

Quality Control

There are several measures of quality control performed with the analysis. Controls for the media, rinse water and technique are set-up with each series of samples.

Procedure

Disinfect lab bench with ethyl alcohol to disinfect area where analysis is performed.

1. Arrange the required amount of m-FC agar plates on bench for number of samples to be run plus controls. Label plates with sample information and volume.

   Note: Set-up two control plates with the samples, one is a sterile water control at the beginning of samples and a rinse control at the end.

2. Open up sterile 150 mm petri dish. Unwrap sterile filter assembly and place filter funnel on open petri dish. Place filter base in vacuum flask.

   Note: Handle filter assembly aseptically, not touching surfaces that come into contact with samples.

3. Pour a small portion of ethyl alcohol into small beaker or bottle and place forceps in it. Turn on gas and light Bunsen burner.

4. Remove forceps from alcohol and place them in flame, allow to cool. Using forceps grasp a sterile membrane filter and place on filter support. Attach filter funnel to support.

   Note: When handling filters with forceps make sure to grab only the edge of the filter, and remember to handle filter assembly aseptically.

5. Pour a 25 ml portion of sterile rinse water into funnel using a graduated cylinder.

6. Open valve on vacuum and filter sample. Once sample is filtered turn vacuum off.

7. Remove filter funnel, flame forceps and transfer filter to agar plate. When placing filter on agar, try to roll and press filter onto agar so that there are no air bubbles under filter.

   Note: Lift top off petri dish and hold it, do not put on bench. Do not let filter touch any surface other than the agar.

8. Record all information required on the fecal coliform bench sheet. This includes sample date and time, analysis date and time, analyst initials, sample volume, and sample collection data form completed by operations personnel.

9. Proceed with running samples.

   Note: When running samples always do the following:
   Start and end sample series with rinse water controls.
   Run a positive control for each batch of media prepared.

10. Flame forceps, allow to cool, grasp and place sterile filter on filter support. Place funnel on support.

11. Shake sample bottle vigorously and then measure 25 ml, put sample into funnel to be filtered.

    Note: Sample volumes may change due to type of sample, effluent conditions or plant process. Set-up two dilutions if sample is turbid or suspected of higher fecal counts. Typical dilutions are 1, 10 and 25 ml.

12. Apply vacuum until sample is filtered, with vacuum still on, rinse sides of filter funnel down three times with 20 to 30 ml of sterile rinse water.

13. Turn vacuum off, remove funnel, flame forceps, transfer filter to designated agar plate

14. Repeat steps 11 - 14 for each sample or dilution.

15. After sample or dilution series is done, run a second 25 ml of sterile water.

16. Invert agar plates, put in Whirl Pak bag and place in 44.5^{o} water bath for 24 $\pm$ 2 hrs.

17. After the 24 $\pm$ 2 hrs incubation time, remove plates from water bath and read using a microscope. Record the number of fecal colonies (typical) and non-fecal colonies (atypical) in the respective columns on the Fecal Coliform Report, along with date and time read. Calculate the count per 100 ml using the correct volume factor.

    Note: All plates are to be examined under the microscope with the lids off. Place plate under microscope with 10 to 40x magnification, illuminate with fluorescent light. Each colony is considered to have grown from one organism. Colonies are usually round to oval shaped and can be the size of a pinhead to larger. There are two types of colonies, typical and atypical. The differences for fecal coliform are as follows: colonies that have a blue color to them are considered fecal coliform or typical. Any blue tinge from light to dark blue signifies a fecal colony. Colonies that are yellow, green or clear are considered atypical colonies and not counted as fecal colonies.

18. Confirmation and calculation of fecal coliform counts.

    Note: If the fecal coliform count exceeds the limit then colonies need to go through a confirmation process.

References

Standard Methods for the Analysis of Water and Wastewater, 22nd Edition

Code of Federal Regulations (CFR) 40 Part 136, Guidelines establishing test procedures for the analysis of pollutants.

Phosphorus, Reactive (Orthophosphate)
PhosVer3 Method
Hach Test 'N Tube Method 8048

The Hach-Phosphorus, Reactive (Orthophosphate), is applicable to liquid wastewater.
It has range of 0.06-5.00 mg/L $P0_4^{3-}$ or 0.02 to 1.60 mg/L P.

Collection of Sample
Glass or plastic

Preservative
None, analyze immediately for best results.

Hold Time
Preserve samples up to 48 hours by filtering immediately and storing at 4 degrees C.
Warm to room temperature before analysis.

Instruments
Hach DR2800 or equivalent

Regents
Hach kit Reactive Phosphorus TNT (Test 'N Tube) reagent set, Catalog Number 27425-45
Pipet, volumetric 5 mL
Phosphate Standard Solution Catalog Number 2569-49
Test tube rack

Test Procedure

- On the appropriate Hach Spectrophotometer, select test program 535 P React. PV TNT
- Use a pipet to add 5.0 mL of the sample to a Reactive Phosphorus TNT Dilution Vial. If using less than 5 mL sample, add enough DI water to bring volume to 5 mL. Cap and mix. Wipe the outside of the vial with a damp towel, followed by a dry one, to remove finger prints or other marks.
- Insert the vial into the round cell holder.
- ***Prepare a Standard Vial tube.***
- Press Zero. The display will show: 0.00 mg/L PO_4^{3-}
- Add the contents of one PhosVer3 Phosphate Powder Pillow to each vial.
- Immediately cap the vial tightly and shake for at least 20 seconds. The powder will not dissolve
- completely.
- Press TIMER>OK, a two minute reaction period will begin.
- Wipe the vial for finger prints or other marks.
- When the timer has expired, insert the vial into the cell holder.
- Press READ, results are in mg/L PO_4^{3-}

On the Hach Spectrophotometer, to change between types of Phosphorus, after entering the program Number, press Options, press More, press Chemical Forms, press Return.

Phosphorus, Total
Hach Test 'N Tube Method 8190
PhosVer3 with Acid Persulfate Digestion

The Hach-Phosphorus, Total, is applicable to liquid wastewater.
It has range of 0.06-3.5 mg/L $P0_4^{3-}$ or 0.02-1.10 mg/L P.

Collection of Sample
Glass or Plastic

Preservative/Hold time
Sulfuric acid, 28 days at 4 degrees C

Instruments
Hach DR 2800 or equivalent

Reagents
Hach Total Phosphorus Test 'N Tube (TNT) Reagent Set, Catalog Number 27426-45
Hach Digestor Reactor,
Pipet, volumetric or TenSette
Phosphate Standard Solution
Test tube rack

Test Procedure

- Turn on the Digestor to preheat.
- Pipet 5 ml of sample into a TNT vial. If using less than 5 mL sample, add enough DI water to bring total volume to 5 mL.
- **Pipet 5 ml of Standard into a TNT vial.**
- Add the contents of one Potassium Persulfate Powder Pillow to each vial.
- Cap tightly and shake to dissolve.
- Insert the vials into the digestor and heat for 30:00 minutes.
- When time expires, allow to cool to room temperature.

Turn on the Hach Spectrophotometer. Choose program 536.

- Pipet 2 mL of 1.54 N Sodium Hydroxide Solution into each vial. Cap and Mix.
- Wipe the outside of the vial.
- Insert the **Standard** vial into the 16mm cell holder. Press Zero.
- Add the contents of one PhosVer 3 Powder Pillow to each vial.
- Cap tightly and shake to mix for 20-30 seconds. The powder will not dissolve completely.
- Press Timer, choose the 2 minute reaction period.
- When time has expired, insert the vial into the cell holder.
- Press READ, results are in mg/L PO_4^{3-}

On the Hach Spectrophotometer, to change between types of Phosphorus, after entering the program Number, press Options, press More, press Chemical Forms, press Return.