INTRODUCTION

Thanks to a generous grant obtained by the Vermont Department of Environmental Conservation Wastewater Management Program and administered through the Vermont Rural Water Association we are able to bring you an update of the State Wastewater Laboratory Manual last updated in 1996. It is hoped that this manual will assist Vermont wastewater operators in understanding and properly performing NPDES required analyses at their facilities. There have been many changes in the analytes of interest as well as the technology available for analysis of those parameters.

We have attempted to maintain the basic style of the original manual to minimize confusion. One major difference you will notice is the addition of links to some methods, equipment, and procedures. These links are specific to those methods and are subject to change in the future. New links can be established by searching the topic or company included in the link if necessary.

The Department of Environmental Conservation would like to express its great appreciation for the valuable help and support given by the Vermont Rural Water Association, specifically Elizabeth Walker, who was extremely helpful from start to finish in this manual update. Thanks also to the Green Mountain Water Associations Laboratory Standards Committee and especially the chair of that committee, Jennifer Garrison, for her much-appreciated editing prowess and to committee members (and WWTF operators) Lucas Harrington and Josh Kemp for reading the drafts and adding their valuable comments. Another major contributor to the success of this update was Dr. Amy Polaczyk of the Wastewater Management Program. Her insightful edits and useful additions to the manual were extremely valuable.

We also thank all of the sources of information who allowed us to use their material. Those contributors include American Public Health Association®, American Water Works Association®, and Water Environment Federation for use of the 23rd Edition "Standard Methods For The Examination of Water and Wastewater", California State University, Sacramento Foundation for permission to use information from "Operation of Wastewater Treatment Plants" 7th Edition, and The Hanna® Company, Hach® Company, YSI®, and Idexx® for their valuable assistance and for allowing us to include links to their informational sites.

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Common Laboratory Glassware

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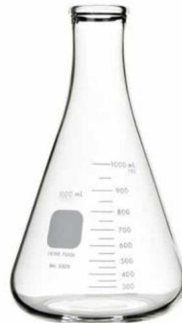
Common Laboratory Glassware

BURETS. Burets are used to deliver accurate volumes. They are especially useful in a procedure called titration.

FLASKS. Flasks are used for containing and mixing chemicals. There are

Support, Burette and Burette Clamp



Erlenmeyer Flask

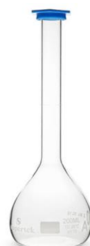
Flat Bottom Flask

Burette

Boiling Round Bottom Flask

Filtering Flask

Automatic Burette



Volumetric Flask

Kjeldahl Flask

Distilling Flask

BOTTLES. Bottles are used to store chemicals, to collect samples for testing purposes, and to dispense liquids.

Reagent Bottle

BOD Bottles

Separatory funnels are used to separate one chemical mixture from another. The separated chemical usually is dissolved in one of two layers of liquid.

Separatory Funnel

FUNNELS. A funnel is used for pouring solutions or transferring solid chemicals. This funnel also can be used with filter paper to remove solids from a solution.

Funnels

TUBES. Test tubes are used for mixing small quantities of chemicals. They are also used as containers for bacterial testing (culture tubes).

Test Tube

Culture Tube

Without Lip

A Buchner funnel is used to separate solids from a mixture. It is used with a filter flask and a vacuum.

Buchner Funnel

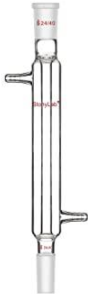
Wash Bottles

IMHOFF CONE. The Imhoff cone is used for the settleable solids testing of wastewater.

BEAKERS. Beakers are common pieces of laboratory equipment. They come in sizes from 1 ml to 4000 ml. They are used mainly for mixing chemicals and to measure approximate volumes.

GRADUATED CYLINDERS. Graduated cylinders also are basic to any laboratory and come in sizes from 5 ml to 4000 ml. They are used to measure volumes more accurately than beakers.

OTHER LABWARE AND EQUIPMENT



Desiccator **Condenser**

Dial Thermometer **NIST Traceable Lab Thermometer**

Petri Dish **Volumetric Pipettes** **Adjustable Volumetric Pipette**

OTHER LABWARE AND EQUIPMENT (continued....)

Volumetric Flask

Serological Pipettes

Common Laboratory Equipment

VT WSMD Wastewater Program Lab Manual Section #4

COMMON LABORATORY EQUIPMENT

BALANCES

Different types of balances are available for various weighing tasks. It is important to use the correct type of balance for each task. Among the simplest, but also the least accurate type of balance, is the **beam balance**. This type usually has one or two pans and one to three beams on which weights are moved to reach the balance point. Some beam balances have a dial rather than a slide which "fine tunes" to the final balance point. A much more sensitive balance is the **four-place analytical balance**. These balances are capable of weighing to the nearest 0.0001 gram or 0.1 mg. Four-place analytical balances are available in many styles, with one or two pans and a variety of systems for adjusting the weights. The **electronic balances** offer features that can simplify things a great deal while maintaining an extraordinary degree of accuracy.

Beam Balances

Electronic Balances

As with liquid measurements, the degree of precision required is usually given by the number of decimal places in the weighing instructions. Crude weighings are done on the triple beam pan balance (e.g., 150 grams or 10 grams, etc.). Somewhat more precise weighings can be done on the **torsion balance** (e.g., 3.1 g or 11.5 g., etc.). For weighings to two or more decimal places, such as 11.06 grams or 600 milligrams (0.600 g), the **analytical balance** must be used. **Total suspended solids analyses must be done on an analytical balance capable of weighing to the nearest 0.0001 gram (four-place analytical balance)**.

Balance Care

Balances must be kept clean, free of chemical residues, dust, etc. The actual moving parts - the pans, beams, and weights - should not be handled unless you are wearing gloves. Oils from your hands will be transferred to these parts, changing the weight.

These balances are very sensitive; it is important to locate them in the least disturbed area possible. All balances must be set up on a clean, hard, level surface. It is especially important that four-place analytical balances be set up on a separate, very heavy table and isolated from the vibration of pumps, hydraulic comminutors, blowers, etc.

Balances should ideally be in a temperature and humidity-controlled environment; however, this is usually not possible in the treatment plant. Avoid setting up the four-place balance near doorways or other sources of drafts, or in windows where sunlight will heat them up, or near ovens, furnaces, or other heat-producing appliances.

Follow the manufacturers' instructions for use.

Balance Servicing

Four-place analytical balances must be periodically serviced to maintain their accuracy. The absolute minimum recommended interval between servicing is one year, with six-month intervals being preferred. If the balance receives rough use or is in a location where it is subject to excessive dirt and fumes, it may require servicing more often. This servicing will include a complete cleaning of the actual moving parts, inspection and servicing of the knife edges, and calibration of the weights to be sure of the accuracy of the balance. This servicing should only be done by qualified professionals. A number of companies offer this service, and the prices vary depending on your location, the company doing the servicing, and what repairs, if any, are required. Contact service companies for rates and services provided. It is important to get on a schedule so that the servicing company can put you on the technician's route. Servicing can be obtained from the following companies, among others:

TMDE Calibration Labs, Inc. 839 River Road Richmond ME 04357 (877) 863-3522 TMDE.com	Alert Scientific, Inc. 469 School Street East Hartford, CT 06108-1138 1-800-872-2028 alertscientific.com	MaineCal 42 Main Street Harrrison, ME 04040 (207) 583-2500 mainecal.com

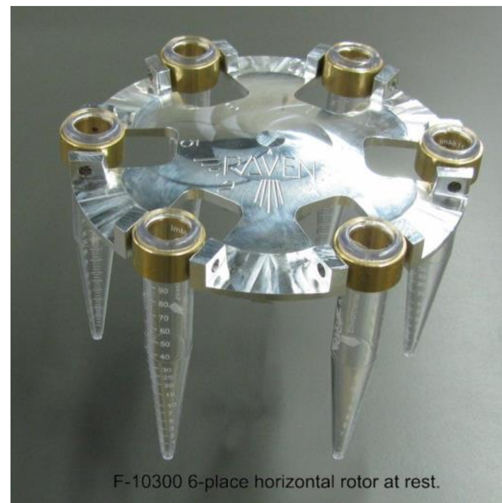
pH Meters

Vermont regulations for wastewater analysis require the use of pH meters capable of delivering pH accuracy of plus or minus 0.1 unit. The meter must also be capable of TWO-POINT CALIBRATION and temperature compensation. A variety of pH probes are available for many different applications. Care should be taken when ordering probes to assure that the probe matches the application.

Dissolved Oxygen Meters

The dissolved oxygen meter is extremely useful in measuring dissolved oxygen levels in sludge, aeration tanks, and when equipped with a stirring probe, for BOD measurements.

Centrifuges



Interior Mechanism

Centrifuges are commonly used in wastewater treatment facilities to estimate the suspended solids concentration in the aeration tanks. It is important to treat the centrifuge with respect. For example, it is extremely important to always balance the load in the centrifuge. Failure to do so can cause serious difficulties resulting in broken test tubes or worse.

Drying Ovens

Drying ovens can serve many purposes in the laboratory. (One purpose they are **NOT** intended for is reheating food items!) A common use is in the drying of filtered samples for the analysis of Total Suspended Solids. Drying ovens used for this purpose must be capable of maintaining a constant temperature of 104°C, plus or minus only one (1) degree.

Vacuum Pumps

Most wastewater laboratories are equipped with vacuum pumps used for drawing samples through some filtration apparatus. Filtration is necessary when performing analyses such as Total Suspended Solids and Fecal Coliform.

Incubators

BOD INCUBATOR

The incubator used for five-day incubation of BOD samples must be capable of maintaining a constant temperature of 20° C plus or minus only one (1) degree. The number of BOD
analyses to be performed must be carefully considered before determining the size of the incubator to be used. Incubators are not to be used as refrigerators to hold food!

DRY INCUBATOR

The Escherichia coli Bacteria analysis requires a two-hour "pre-incubation" period at 35°C. The incubator used for this purpose must be capable of maintaining a constant temperature of 35°C plus or minus 0.2 degrees.

Water Bath

After pre-incubation at 35°C, the E Coli sample must be transferred to a water bath capable of maintaining a constant temperature of 44.5°C plus or minus 0.2 degrees, for an additional 22 hours. The water bath is also used for the Fecal Coliform analysis and other bacteriological analyses.

Sampling

VT WSMD Wastewater Program Lab Manual Section #5

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Sampling

The Importance of Good Sampling

The collection of good, representative samples is probably the most important part of laboratory work and is often the most overlooked part. If the samples are not representative, no amount of care in analysis will produce accurate and precise values. If you do not collect samples properly, the time spent in analysis is wasted. Since the sample is a very small portion of the entire wastestream or basin, it is extremely important to select the sampling point, method, equipment, and time very carefully.

Quality samples are:

- Collected at a representative sampling point
- Stored and/or shipped at the appropriate temperatures (See Table 1)
- Analyzed within the maximum recommended holding times

It cannot be stressed enough - without care in sampling, any values generated are not accurate or precise, leading to the data not being an accurate representation of the plant conditions at time of sampling.

Choosing A Sampling Location

General

Again, the choice of a sampling location is an important decision that should be carefully considered. Samples should be taken in the middle of the flow in a well-mixed area. The sample should be taken from the middle depth (do not just skim the surface) to avoid floating wastes, etc. Eddies and backwaters should be avoided because of the settling that takes place there.

Influent

Influent samples should be taken after the comminutor or bar screen so as to avoid large solids and other such materials. Also avoid areas of settled grit and floating solids or grease because these are not truly representative of the whole stream. Influent samples must be collected at a point above any return flow such as RAS, etc. Leachate and septage must be included in the influent sample. In some cases due to facility design etc. this may be difficult or impossible. Under these conditions the sample must be taken at the source (septage truck, landfill leachate sampling point etc.) Required analyses must be performed on the leachate/septage sample. Using the best means of establishing the flow, the loading from the leachate/septage must be calculated in pounds. This result must be added to the influent results for reporting purposes (an example is included below). Samples must be collected before aerated grit chambers. If the facility has a fine screen grit removal system, the sample may be collected before or after the screen with the understanding that: **if collected downstream of the fine screen the sample collection point can NOT be used as an excuse for failing to meet the 85% removal requirement.**

Example of leachate/septage loading calculation (when it can't be collected in influent sample):

Leachate from a Hazardous Materials site is piped directly to the wastewater facility but enters the system downstream of the influent sampling site. A composite sample is collected at the sample port located at the HazMat site. Flow is determined by the number of times the tank discharges. (500-gallon leachate tank is emptied 5 times over the composite period). The BOD result from the leachate sample yields a result of 250 mg/L

Loading from the leachate is calculated using the pounds formula:

$$Flow\ (MGD) * BOD\ \left(\frac{mg}{L}\right) * 8.34\ = Pounds\ (lbs)\ BOD$$

In our example:

$$0.0025\ MGD * 250\ \left(\frac{mg}{L}\right) * 8.34\ = 5.2125\ lbs\ BOD$$

This result is then added to the calculated influent loading to establish the “total” influent loading.

For example, if the influent loading was calculated to be 1000 lbs, the Total influent loading including the leachate would be 1005.2 lbs.

Effluent

BOD, pH and Solids Samples

Generally, it is best to collect effluent samples at the "final" discharge point, after all treatment processes including chlorination and if applicable dechlorination.

Chlorine Residual Samples

The sample to be analyzed for total chlorine residual must be collected at a point beyond chlorine contact, at the end of the contact tank. If the effluent is dechlorinated before final discharge, the sample must be collected post dechlorination as well as post chlorination to establish the effectiveness of the dechlorinating agent. **Read your permit carefully.** Note that the permit may specify the days the *Escherichia coli* (*E. coli*) samples are to be collected, and that the sample for chlorine residual analysis must be collected at the same time and location as the *E. coli* sample.

E. coli

Effluent samples collected for the analysis of fecal coliform or *E. coli* must be collected at a point beyond chlorination. If some form of dechlorination is used at the facility, these samples must be collected beyond the point where dechlorination takes place. **

** If a large discrepancy is observed in results between samples collected after chlorine addition and those collected after the addition of the dechlorinating agent, it may be wise to determine what source of feed water is being used to deliver the dechlorinating agent. On more than one occasion the feed water used to deliver the dechlorinating agent has been found to be the source of coliform contamination.

Types of Samples

Grab Samples

A grab sample is defined in Vermont NPDES permits as an individual sample collected in a period of less than 15 minutes. A grab sample represents the condition of the effluent, influent, or other matrix being sampled, for that specific time only.

Examples of parameters that must be collected as grab samples include pH, chlorine residual, dissolved oxygen, *Escherichia coli* bacteria, and FOG.

Be sure to use the appropriate sample collection device/bottle for the parameter being analyzed. For example, coliform samples MUST be collected directly into properly treated sterilized bottles and cannot be transferred from one bottle to another. The effluent sample collection bottles/devices should be kept separate from, the influent sampling bottles/devices. It's a good idea to always collect samples from "cleanest to dirtiest" to help avoid contamination. (i.e., final effluent is sampled prior to influent)

Composite Sample

A composite sample as defined in Vermont NPDES permits is a sample consisting of a minimum of one (1) grab sample per hour collected during a 24-hour period (or lesser period as specified in the section of the permit on Monitoring and Reporting) and combined proportionally to flow over that same time period. Collection of a 24-hour composite is required unless stated otherwise in the NPDES/Pretreatment permit, as it allows for representation of discharges from industries/workplaces with 24-hour shifts, variations in loading etc. Composite samples collected at industries should be representative of the entire working day. If the industry is running a single shift, an eight-hour composite is sufficient. If however, two or three shifts are operating, the composite should be representative of sixteen or twenty-four hours, respectively. For municipal wastewater treatment facilities in Vermont the minimum compositing frequency and duration is eight samples, one per hour for eight (8) hours. Eight-hour composite samples must be collected between the hours of 6:00 a.m. and 6:00 p.m. This eight-hour frequency is mainly reserved for lagoon systems.

Examples of parameters that are generally collected as composite samples include: Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), the nitrogen series, and most metals.

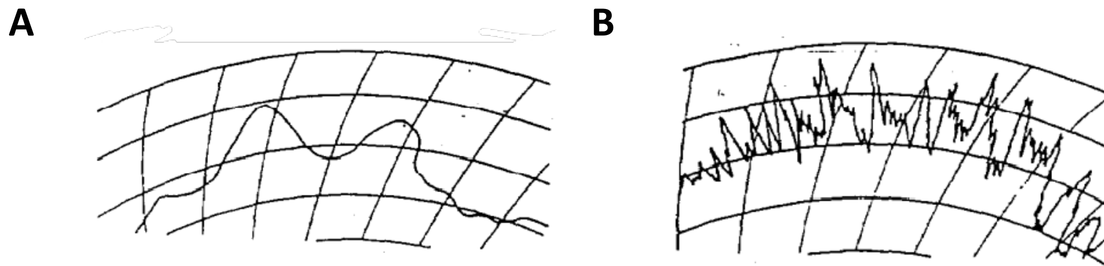
List of Metals Usually Collected with Composite Samples

Aluminum	Boron	Copper	Manganese	Selenium	Tin
Antimony	Cadmium	Iron	Mercury	Silver	Titanium
Arsenic	Calcium	Lead	Molybdenum	Sodium	Vanadium
Barium	Chromium	Lithium	Nickel	Strontium	Zinc
Beryllium	Cobalt	Magnesium	Phosphorus	Thallium	

There are several different methods of compositing: straight or time compositing, flow compositing and time-flow compositing. If the flow during the day is very constant, i.e., it varies less than 10% during the day (for example, the effluent from an aerated lagoon), the samples may be time composited. In a time composite, the same volume is taken every hour and put together for the composite. If the flow is not

constant (for example, the effluent for a sequencing batch reactor), a flow-proportional compositing method must be used. In flow compositing, the same volume is taken at certain flow intervals. For example, 100 mL of sample might be taken every time 1,000 gallons passes over the weir for the duration of the sampling period. This type of compositing is used in automatic sampling but would be inconvenient for manual sampling. Time-flow compositing is used for manual sampling of flows that vary by more than 10% during the sampling period. In this method of sampling, samples and flow measurements are taken at specific time intervals, most often hourly, and the composite made by adding together amounts of each hourly sample varying with the flow passing through during that hour. Depending on the flow pattern in the plant, either the chart or the integrator readings will be used to flow proportion the sample.

If the flow pattern is fairly even, as in Chart A below, the instantaneous flow readings can be taken directly from the chart at the time of sampling. If the flow varies considerably during the sampling period due to pumping patterns, etc., and the chart looks like B below, the flow reading must be taken from the integrator or totalizer. In order to get a flow for a specific sample, for example the 9:00 a.m. sample, the flow reading at 8:30 a.m. must be subtracted from the 9:30 a.m. reading. This is because we have chosen the 9:00 a.m. sample to describe the flow for an hour period centered on 9:00 a.m.



Once the sampling period is over, the flow proportioning scheme can be calculated. If the integrator readings were used, flows must be generated by subtracting each reading from the one preceding it.

Time-Flow Compositing

1. Add flows together.
2. Divide the size of the composite jug (remember to allow yourself a little head room) by the total of the flows.
3. Multiply the factor obtained above by each hourly flow for the number of milliliters (mL) to use from each hourly sample.

In the following example, the integrator readings are given, and a flow proportioning scheme is then calculated.

TIME	INTEGRATOR	DIFFERENCE	FACTOR	mL
7:30	77340	16		
8:30	77356	20		432
9:30	77376	24		540
10:30	77400	18		648
11:30	77418	16	x 27 =	486
12:30	77434	16		432
1:30	77450	12		432
2:30	77462	18		324
3:30	77480	___		486
		140		

Factor determination: For a 4 L jug, use the equation 3800 mL divided by 140 (Total Difference) = 27.14

(We use 3800 mL in this equation in order to leave headroom in the jug for shaking the sample.)

Another method, probably more representative of measuring flows from a flow chart, is to get an hourly flow. Some flow charts even have breaks within the lines at every quarter hour. This would be more accurate than the hourly flows taken off the chart when a flow is erratic.

Sample Containers

The type of sample containers used is dependent upon the parameters or tests the samples are being analyzed for. Some samples such as those for pH, DO, and chlorine residual analysis are affected by exposure to air. Gases in the samples and in the atmosphere may exchange and alter the levels in samples before they can be measured. For this reason, the samples must be collected carefully, with very little agitation into air-tight bottles, such as 300 mL DO bottles, and analyzed immediately. Chlorine residual samples are also affected by sunlight which dissipates the chlorine residual. These samples should be shielded from sunlight and analyzed quickly after collection.

BOD and TSS samples can be taken from the same four-liter (gallon) composite jug. This jug should be thoroughly washed with a chelating detergent, such a 2% Micro solution or with Alconox, to remove any trace of organic matter; rinse the jugs thoroughly after washing. Distilled H_2O should be used for the final rinse(s).

Most metals samples are collected in bottles that have been washed with 20% nitric acid solution (HNO_3). Five hundred (500) mL should be sufficient sample size unless such metals as hexavalent chromium (Cr+6) or mercury (Hg) are being analyzed. Your lab will then direct you as to special sample collection procedures. Be sure to check preservation requirements for individual parameters.

Coliform sample bottles must be washed and sterilized. A 250 mL bottle should be of sufficient size. For chlorinated samples, the bottle must contain a chlorine neutralizing agent, usually sodium thiosulfate. As the effluent is discharged to the river, its chlorine residual is diluted by the receiving water and is no

longer an effective disinfectant. The presence of thiosulfate in the sample bottle simulated this effect, giving a better picture of the coliform count in the effluent when it reaches the receiving water.

Containers for Total Kjeldahl Nitrogen (TKN) testing should be 500 mL plastic or glass bottles for everything but river samples which should be taken in one-liter containers. The samples can be poured from the composite or grabbed.

Total phosphorus is best taken in a 250 mL glass bottle. Again, samples are either poured off the composite or grabbed.

Table I gives a summary of the above, along with recommended preservation methods and the maximum recommended holding times from the Federal Register. For valid data, these preservation methods and holding times must be adhered to.

TABLE I: Summary Chart with Required Containers, Preservation and Holding Times

Parameter	**Sample Type**	**Container***	**Preservation**	**Maximum Hold Time**	**Note**
METALS					
PRIORITY POLLUTANTS					
Group 1: (arsenic, selenium, mercury	Water	P, 500 mL	HNO_3 to pH <2	6 months 28 days	b
Group 2: (cadmium, chromium, copper, lead, nickel, zinc)	Water	P, 500 mL	HNO_3 to pH <2	6 months	b
Group 3: (antimony, beryllium, silver, thallium)	Water	P, 500 mL	HNO_3 to pH <2	6 months	b
MISCELLANEOUS					
Group 4: (aluminum, calcium, magnesium, potassium, sodium)	Water	P, 250 mL	HNO_3 to pH <2	6 months	b
*****Group 5: (molybdenum, tin, strontium, vanadium)	Water	P, 500 mL	HNO_3 to pH <2	6 months	b
†Group 6: (cobalt, iron, manganese)	Water	P, 500 mL	HNO_3 to pH <2	6 months	b
Group 7: (hexavalent chromium)	Water	P, 500 mL	**DO NOT ACIDIFY** Cool, 6°C	24 hours	b
Group 8: (titanium)	Water	P, 500 mL	HNO_3 to pH <2	6 months	b
*****Group 5 can be combined with Group 3, total volume of 500 mL †Group 6 can be combined with Group 2, total volume of 500 mL					
SOLIDS					
Groups 1 through 8 (from above)	Soil	P, 500 mL	0 - 6°C	6 months	
Groups 1 through 8 (from above)	Liquid, Sludge	Two - P, 500 mL	0 - 6°C	6 months	
MICROBIOLOGY					
Coliform, Fecal	Water	P, 250 mL sterile	0 - 6°C	6 hours	c, d
Coliform, Total	Water	P, 250 mL sterile	0 - 6°C	6 hours	c, d
E Coli	Water	P, 250 mL sterile	0 - 6°C	6 hours	c, d
NUTRIENTS					
Chloride	Water	P, 50 mL	none required	28 days	

Parameter	Sample Type	Container	Preservation	Maximum Hold Time	Note
NUTRIENTS					
Chloride-[Ion Chromatography] (chloride, nitrate, nitrite, sulfate in 1 container)	Water	P, 50 mL	0 - 6°C	28 days	
Nitrogen (Nitrate + Nitrite)	Water	P, 250 mL	0 - 6°C, H_2SO_4 to pH < 2	28 days	c
Nitrogen (Nitrate)	Water	P, 50 mL	0 - 6°C, Filtered	48 hours	
Nitrogen (Nitrite)	Water	P, 50 mL	0 - 6°C, Filtered	48 hours	c
Nitrogen (Ammonia)	Water	P, 250 mL	0 - 4°C, H_2SO_4 to pH <2	28 days	e
Total Phosphorus	Water	G, 250 mL	none required	28 days	
Phosphorus-Ortho	Water	G, 75 mL tubes	Filter immediately, 0 - 4°C	48 hours	c
Silica	Water	P, 50 mL	Filter immediately for dissolved, 0 - 4°C	28 days	
Sulfate [Ion Chromatography]	Air	P, 50 mL	0 - 4°C after extraction	28 days	
Sulfate [Ion Chromatography] (chloride, nitrate, nitrite, sulfate in 1 container)	Water	P, 50 mL	0 - 4°C, filter	28 days	
ORGANICS					
Pesticides & PCBs & Semi-Volatiles	Water	G, 2.6 L amber, Teflon lined cap	0 - 4°C, Pesticides and PCBs need to be pH 5-9	7 days to extraction, 40 days after	d
Pesticides & PCBs & Semi-Volatiles	Solids	G, 500 mL amber, Teflon lined cap	0 - 4°C	7 days to extraction, 40 days after	
Volatile Organics	Water	G, 2-40 mL vials	0 - 4°C, HCl to pH <2	14 days	
Volatile Organics	Solids	G, 2-40 mL vials	0 - 4°C	14 days	

Parameter	Sample Type	Container	Preservation	Maximum Hold Time	Note
WET LAB					
Alkalinity	Water	P, 250 mL	Cool, 4°C	14 days	
BOD Carbonaceous 20 days	Water	P, 2 L	Cool, 4°C	48 hours	e, f
BOD 5-Day	Water	P, 2 L	Cool, 4°C	48 hours	c, f
Chlorophyll-a	Water	Glass fiber filter, Whatman GF/A, stored in black jar	Freeze filter, dark	21 days	
COD	Water	P, 250 mL	Cool, 4°C, H_2SO_4 to pH <2	28 days	c
Conductance	Water	P, 250 mL	Cool, 4°C, filtered	28 days	
Cyanide, Total	Water	P, 2 L, amber	Cool, 4°C, NaOH to pH > 12	14 days	g
Dissolved Oxygen, Probe	Water	G, 300 mL DO bottle	none required	analyze immediately	
Dissolved Oxygen, Winkler	Water	G, 300 mL DO bottle	$MnSO_4$, I-/Azide, dark	8 hours	h
Ignitability	Solid Liquid	G, 250 mL	Cool, 4°C		
Nitrogen, Total Kjeldahl	Solid	P, 250 mL	Cool, 4°C	28 days	
Nitrogen, Total Kjeldahl	Water	P, 250 mL	Cool, 4°C, H_2SO_4 to pH <2	28 days	e
Oil & Grease	Soil	G, 250 mL freon rinse, Teflon lined caps	Cool, 4°C	28 days	
Oil & Grease	Water	G, 1 L freon rinsed, Teflon lined caps	Cool, 4°C, HCl to pH <2	28 days	i
pH	Water	P, 250 mL	none required	analyze immediately	
Phenols	Water	G, 1 L, amber	Cool, 4°C, H_2SO_4 to pH <2	28 days	
Solids - Total Dissolved	Water	P, 250 mL	Cool, 4°C	7 days	
Solids - Total Suspended	Water	P, 1 L	Cool, 4°C	7 days	
Solids - Total Volatile	Water	P, 250 mL	Cool, 4°C	7 days	
TCLP	Solid	G, 250 mL, amber	Cool, 4°C	Extract ASAP	
Turbidity	Water	P, 250 mL	Cool, 4°C	48 hours	

*G = Glass; P = High Density Polyethylene (HDPE)

NOTES:

a. Collect with minimum agitation. Leave no air space. Insert ground glass stopper into DO bottle.
b. Approximately 0.5 mL concentrated HNO_3 per 250 mL.
c. Lab needs prior notice for this parameter.
d. Chlorinated samples need to be collected in sample bottle containing a dechlorinating chemical, such as $Na_2S_2O_3$.
e. Approximately 0.5 mL concentrated H_2SO_4 per 250 mL.
f. Hold time is from end of sample collection period.
g. Test for chlorine and sulfides in the field **PRIOR** to preservation.
h. Samples must be fixed in field, stored in the dark and analyzed within eight (8) hours.
i. Approximately five (5) mL 1:1 HCl.
j. Add five (5) drops 1:1 HCl to each vial

Temperature

VT WSMD Wastewater Program Lab Manual Section #6

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Temperature

Background

Temperature is perhaps the easiest of all analyses but one that may be very inaccurate without the operator knowing it. Many thermometers as they come from the factory are not accurately calibrated. It is safe to assume that the least expensive thermometers will be more often out of calibration than the more expensive. All thermometers should be checked against a NIST certified or NIST traceable thermometer at yearly intervals. It is especially important to calibrate thermometers which are used in incubators. The Vermont Agriculture and Environmental Laboratory (VAEL) has access to a certified thermometer and arrangements can be made with you to calibrate your thermometer. Other commercial laboratories also offer this service for a fee.

To better understand the requirements for thermometer calibrations:

Here is a link to the NIST Handbook 105 section 6 “Specifications and Tolerances for Thermometers.”

Specifications and Tolerances for Reference Standards and Field Standard Weights and Measures: Specifications and Tolerances for Field Standard Weights (nist.gov)

Equipment

It is necessary to match the thermometer to the application. For general laboratory measurements, the standard, liquid -filled thermometer capable of reading from -20℃ to 110℃, graduated in one degree increments, is sufficient. (Use of mercury -filled thermometers is no longer recommended.) For outside work, this same thermometer can be encased in a metal shield. Thermometers for the coliform water bath incubator must be graduated in 0.1℃ divisions as the maximum variation in temperature is only ±0.2℃.

Procedure

When taking temperatures, make sure that the thermometer has ample time to stabilize at the sample temperature. Also be sure that if the thermometer is a **partial immersion** type, it is immersed to the indicated line on the thermometer; if it’s a **total immersion** type, it is totally immersed. Temperature is reported to the nearest 1℃ or 0.1℃, depending on the thermometer and its function. It is imperative that temperatures be taken immediately after sampling. It is best to take the temperature in the waste stream rather than to collect a sample and take it back to the lab for a temperature reading.

There is a general method for checking the accuracy of the thermometers which can be done easily right in your lab. First, fill a beaker that is deep enough to cover the submersion line on thermometer in question with a mixture of ice and water. Stir the mixture so that the temperature becomes uniform. Put the thermometer in the mixture until it reads 0 °C or 32 °F. Remove the thermometer. Next heat the beaker until the water starts to boil. Insert the thermometer, it should read 100 °C or 212 °F. If it doesn't this may be due to differences in elevation/barometric pressure. Check the current barometric pressure, using the correlating boiling point from this chart:

Boiling Point of Water

Pressure mmHg	Temperature °C	Pressure mmHg	Temperature °C	Pressure mmHg	Temperature °C	Pressure mmHg	Temperature °C
700	97.714	725	98.686	750	99.630	775	100.548
701	97.753	726	98.724	751	99.667	776	100.584
702	97.792	727	98.762	752	99.704	777	100.620
703	97.832	728	98.800	753	99.741	778	100.656
704	97.871	729	98.838	754	99.778	779	100.692
705	97.910	730	98.877	755	99.815	780	100.728
706	97.949	731	98.915	756	99.852	781	100.764
707	97.989	732	98.953	757	99.889	782	100.800
708	98.028	733	98.991	758	99.926	783	100.836
709	98.067	734	99.029	759	99.963	784	100.872
710	98.106	735	99.067	760	100.000	785	100.908
711	98.145	736	99.104	761	100.037	786	100.944
712	98.184	737	99.142	762	100.074	787	100.979
713	98.223	738	99.180	763	100.110	788	101.015
714	98.261	739	99.218	764	100.147	789	101.015
715	98.300	740	99.255	765	100.184	790	101.087
716	98.339	741	99.293	766	100.220	791	101.122
717	98.378	742	99.331	767	100.257	792	101.158
718	98.416	743	99.368	768	100.293	793	101.193
719	98.455	744	99.406	769	100.330	794	101.229
720	98.493	745	99.443	770	100.366	795	101.264
721	98.532	746	99.481	771	100.403	796	101.300
722	98.570	747	99.518	772	100.439	797	101.335
723	98.609	748	99.555	773	100.475	798	101.370
724	98.647	749	99.592	774	100.511	799	101.406
						800	101.441

You should also carefully inspect the liquid column. It can separate and cause inaccuracies. This space may be eliminated by holding the thermometer in one hand and carefully tapping the palm of that hand against the open palm of the other hand to force the column together.

References

There is a short section on temperature in Standard Methods for the Examination of Water and Wastewater, 23rd Edition, Section 2550 B.

Specifications and Tolerances for Reference Standards and Field Standard Weights and Measures: Specifications and Tolerances for Field Standard Weights (nist.gov)

pH

VT WSMD Wastewater Program Lab Manual Section #7

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pH

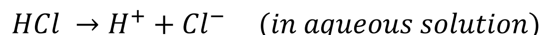
Background

pH is among the most important analyses performed at wastewater facilities. This is because so many processes are affected or dependent upon specific, sometimes narrow pH ranges. Important processes such as chemical coagulation, water softening, chlorination, corrosion, nitrification, BOD and many others occur efficiently only within certain pH ranges. Biological processes such as aerobic and anaerobic digestion, RBC treatment and reactions in aeration tanks are other examples of processes where operation outside of a narrow pH range can seriously reduce treatment efficiency.

The term pH is used to express how acidic or basic a material/solution is. pH is expressed as the negative logarithm of the hydrogen ion concentration. Therefore, a pH of 4 represents $1 * 10^{-4}$, or 0.0001, mol/L hydrogen ion concentration. We will explain this in more detail later in this section.

The Chemistry of pH

pH is actually a measurement of the hydrogen ions in water solution in moles/l. As previously mentioned, most common acids produce hydrogen ions (H^+) and most common bases produce hydroxyl ions (OH^-). An acid, HCL (Hydrochloric acid) for example, dissociates or breaks up to produce hydrogen ions and chlorine ions. This is written as follows:

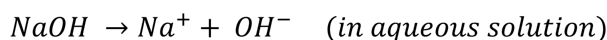


$$HCl \rightarrow H^+ + Cl^- \quad (in\ aqueous\ solution)$$

Sulfuric acid would dissociate as follows:

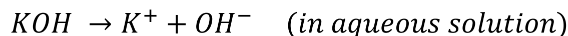
$$H_2SO_4 \rightarrow 2H^+ + SO_4^{2-} \quad (in\ aqueous\ solutions)$$

Since these materials have the free hydrogen ions in common, they are both acids. The same type of process applies to bases. For example, sodium hydroxide (NaOH) dissociates in water to produce Hydroxyl ions (OH^-) and sodium ions (Na^+):



$$NaOH \rightarrow Na^+ + OH^- \quad (in\ aqueous\ solution)$$

Potassium hydroxide (KOH) will dissociate to produce potassium ions (K^+) and hydroxyl ions (OH^-):



$$KOH \rightarrow K^+ + OH^- \quad (in\ aqueous\ solution)$$

These materials have the free hydroxyl ions in common and are both bases.

The degree to which a material dissociates depends on the amount of hydrogen ions or hydroxyl ions present, and therefore, determines the pH of the solution. Strong acids and strong bases dissociate to a greater extent than weak acids and bases.

Water, also weakly dissociates to form hydrogen ions and hydroxyl ions:

$$H_2O \rightarrow H^+ + OH^-$$

With the development of the pH electrode, chemists were able to measure the quantity of hydrogen ions present. In perfectly pure water $H^+ = 10^{-7}$ moles per liter and $OH^- = 10^{-7}$ moles per liter. The pH scale is derived by taking the negative of the log to the base 10 of the hydrogen ion concentration. In

other words, if the hydrogen ion concentration is 10^{-7} moles per liter, the pH will be 7, being the negative log to base 10 of the hydrogen ion concentration. A solution with the hydrogen ion concentration of 10^{-13} moles per liter would have a pH of 13.

Since pH is a logarithmic function, the most important thing to realize from a practical standpoint is that each single digit increase or decrease in pH represents a ten-fold change in concentration. For example: A solution with a pH of 2 is ten times more "acidic" than a solution with a pH of 3. This pH 2 solution would be one hundred times as acidic as solution of pH 4, and one thousand times as acidic as a solution of pH 5. The lower the pH number, the more acidic with seven being neutral.

Sometimes seeing pH expressed in scientific notation, as shown below, helps the reader appreciate the relationship between pH when expressed as a whole number and the actual concentration of hydrogen ions that this number represents.

pH		**H^+ in mol/L**
1	*	10^{-1} ------------- 0.1
2	*	10^{-2} ------------- 0.01
3	*	10^{-3} ------------- 0.001
4	*	10^{-4} ------------- 0.0001
5	*	10^{-5} ------------- 0.00001
6	*	10^{-6} ------------- 0.000001
7	*	10^{-7} ------------- 0.0000001
8	*	10^{-8} ------------- 0.00000001
9	*	10^{-9} ------------- 0.000000001
10	*	10^{-10} ------------ 0.0000000001
11	*	10^{-11} ------------ 0.00000000001
12	*	10^{-12} ------------ 0.000000000001
13	*	10^{-13} ------------ 0.0000000000001
14	*	10^{-14} ------------ 0.00000000000001

Whereas we could carry out this "more acidic than" analogy up to pH 14, stating that a pH of 1 is 10 trillion times as acidic as a pH 14, in solutions with higher than pH 7 it is really the basicity of the solution ($0H^-$) ions we are concerned with. The inverse is true for basicity. In other words; regarding basicity, the larger the number the stronger the basicity. For example, a pH of 8 is 10 times more alkaline than a pH of 7.

Methods of pH Measurement

Around 1925, the glass pH electrode was developed. Although it was a very satisfactory method of pH measurement under lab conditions, the fragile and cumbersome nature of this electrode made it unsuitable for field work. It also was adversely affected by high salinity and samples containing oil and grease.Modern electrodes are used in two-electrode systems or as a single combination electrode. The combination electrode with a plastic sleeve to protect the fragile tip is preferable.

Since there are many types of pH probes on the market, be sure to follow the manufacturer's instructions for your probe carefully regarding maintenance and pre-use preparation. Reference electrodes should be checked frequently for crystals in the probe body. A small amount of crystal might not hurt the electrode's performance but electrodes that have been allowed to dry out and have crystals well up the body might have to be discarded. By draining the filling solution, putting in distilled water, waiting 30 minutes, then repeating the process of draining and refilling with distilled water, it is possible to remove the crystals. Then flush with filling solution and refill. This process is laborious and time-consuming. This can be avoided by keeping the filling hole closed between uses. It must, however, always be open when the probe is in use! The glass electrode should be checked for an excessive air bubble. Small bubbles will not interfere with performance, but bubbles which occupy a large part of the tip will interfere.

Equipment

pH meter

The required specifications for pH meters include:

1) It must have a range of 0 to 14.
2) It must be capable of two-point standardization
3) It must have a pH accuracy rating of $\pm$ .01.
4) It must have slope display/control

Recommended specifications include:

1) Automatic Temperature Compensation (ATC)
2) Digital readout
3) "Low Battery" warning for portable units.

pH Probe

There are hundreds of pH probes available on the market designed for every conceivable use. When ordering a pH probe, be sure to choose one suited for your application. Generally, gel-filled probes are less expensive than the refillable type probes, but gel-filled probes must be replaced every six months to one year. This is because there is a limited amount of electrolyte solution in the probe and when it is used up, there is no way to rejuvenate the gel. The refillable type probes, on the other hand, if well maintained, could conceivably last much longer, justifying the initial expense.

Thermometer

Even if a pH meter is equipped with Automatic Temperature Compensation (ATC), it is still a good idea to check the temperature of the sample with a thermometer as a comparison for quality control. Both the meter's temperature probe and the thermometer used to verify the temp must be compared to a

NIST traceable thermometer annually. The ATC should be checked weekly with a NIST traceable thermometer.

Absorbent Papers
Kimwipes (or comparable lab tissue) are specially designed for laboratory use and are especially good for wiping down the pH probe and gently removing liquid from the glass tip.

Wash Bottle
A wash bottle, filled with distilled water, is used to rinse the pH probe.

Beakers or Plastic Bottle
Small (50 milliliter or less) beaker or clean plastic Dissolved Oxygen bottle caps can be used for buffers for standardization buffers. The buffers should be discarded after use.

Magnetic Stirrer (with stirring bar)
This device is extremely helpful in maintaining a constant movement of the sample during analysis. A slow, constant mixing produces a good homogenous sample and improves accuracy. Be careful not to break the electrode end. Mix rate should not create a vortex.

Reagents

- Distilled water
- pH buffer 7
- pH buffer 4
- pH buffer 10
- pH buffer 7 from a different lot (or pH buffer closer to typical expected pH.)

The pH buffers can be purchased in a variety of forms, pre-made, concentrated liquid, powder, tablet, etc. Use of the pre-made buffers is highly recommended as they are very reliable, generally have a shelf life of one to two years and are available in color-coded form for easy identification. If using the powder or tablets, be sure to follow the manufacturer's instructions carefully. Buffers should be NIST traceable. Prepared buffers should be purchased in 1 Liter volumes. It is not recommended to purchase larger volumes because of contamination and/or expiration issues. The date that a buffer is opened should be recorded on the container. Buffers have a shelf-life once opened. Check with the manufacturer to know when the buffer should be discarded and replaced.

Procedure

Standardization of the Meter
The meter must be standardized before each use. This should be done before the samples are collected.

1) **Turn on meter** and allow 10 – 15 minutes for stabilization. This allows the probe to polarize, necessary for accurate pH measurement.
2) Remove electrode(s) from storage and **rinse down with distilled water** using the wash bottle. Be sure to follow manufacturer's instruction for short-term or long-term storage of electrode. Concentrate the rinse to cover the reference junction and glass tip. It may be necessary to turn the

probe upside down to thoroughly rinse the area around the glass tip and remove contamination from /around and under the protective cap carefully.

3) **Blot dry with Kimwipes,** being careful not to touch the tip of the electrode. You can use the corner of the Kimwipe to pull a drop off the tip by touching it to the drop (not the electrode).

4) **Immerse the electrode(s) in a buffer no more than one or two pH units from the expected sample pH.** pH 7 is usually a good choice to set the response point. Be sure to immerse the probe so that both the glass tip and the reference junction are submerged, turn the magnetic stirrer on low or swirl the buffer. Record the pH of the buffer after the meter stabilizes.

5) Remove the electrode from the buffer, **rinse** down with distilled water and blot dry.

6) **Immerse the electrode in a second buffer** which should be (at least) three (3) units from the first, usually pH buffer 4 or 10. If your samples are on the acid side of pH 7, use a pH 4 buffer; if on the basic side, use a pH 10 buffer. Record pH reading after the meter stabilizes.

7) **Check the value of a third buffer. The third buffer should be between the standardization buffer values.** If you calibrated with pH 4 and 7, it is recommended to check the calibration with a buffer between 4.5 – 6.5. If you calibrated with pH 7 and 10, it is recommended to check the calibration with a buffer between 7.5 – 9.5. Checking a two-point calibration with another pH 7 buffer does not test the slope of the line, and therefore the calibration, of the pH meter.

    a. If the electrode(s) and meter are operating well the value shown on the meter should be less than 0.1 pH units from the stated buffer value.

Probe Maintenance

Applying air pressure to the fill hole of the probe is a simple probe maintenance procedure that, if performed on a regular basis, can lengthen the life of your re-fillable glass probe. This can be done by putting the nozzle of the empty filling solution bottle to the fill hole and squeezing. (Pressure should be maintained for 15 seconds.) When pressure is applied there should be a slight leaking of the filling solution for the junction area. If there is no leaking of solution, the probe has a clogged junction which can result in drifting, slow response, erratic readings, etc. To alleviate this problem, follow the manufacturer's instructions for cleaning the junction. Unfortunately, this procedure cannot be used on most gel-filled probes which usually have no fill hole.

Typically, cleaning refillable type probes consists of draining the fill solution (through the fill holes), filling the probe with distilled water, and draining until all traces of crystals and contaminants are removed. Then refill the probe with the appropriate filling solution (specific for the type of probe being used).

Probe Storage

Literature suggests that it is best to store the probe in pure potassium chloride solution when not in use. Ideally this KCL solution should be the same concentration as used in the filling solution on refillable probes or the gel in gel filled probes. Storage solutions can be purchased through any scientific instrument supplier, but it might be best to purchase storage solution for the specific type of probe being used.

NEVER STORE THE PROBE IN DISTILLED WATER!! This will damage the electrode by leaching ions from the glass membrane and reference electrode.

At the end of this section is a pH probe maintenance guide graciously supplied by the Hanna Instruments Company for your use. Although the information is somewhat specific to Hanna pH probes, much of it is excellent information that can be applied to most pH probe brands. **For specific information regarding the probe you are using, be sure to consult the user's manual for your specific probe.**

Sampling

pH samples have very short hold times and are subject to changes due to gases exchanging between the sample and the atmosphere. For this reason, the grab sample collected for pH analysis must be taken after the pH meter is standardized and then analyzed immediately. To prevent the exchange of gases the sample should be collected without agitation into a DO bottle, filled to the neck, and tightly stoppered. Samples MUST be analyzed within 15 minutes. The sooner the better! Alternatively, using a standardized meter, perform the analysis by placing the probe directly into the waste stream. There is no preservation method for pH samples.

Analysis

After meter standardization:

1) Raise the electrode(s) from the storage beakers, rinse down thoroughly with distilled water, and blot dry.
2) Be sure to include the glass bulb the area around it, the reference junction(s) and the probe body in the rinse.
3) Be sure to have the temperature probe in the sample.
4) Allow the meter time to equilibrate (to reach stable reading).
5) Record the pH and temperature at time of sample analysis.

pH values should be recorded to the nearest 0.1 pH units at the temperature of the sample. For example: pH= 7.3 at 21°C. This is because the temperature affects pH readings in two ways. Electrodes are sensitive to temperature changes and the potential, or voltage, they generate in response to the pH of solution changes with temperature. The potential pH unit change is accounted for by using a meter with temperature compensation. The second way in which temperature affects pH reading is that the ionization in the sample changes with temperature. This change is inherent in the sample and cannot be accounted for by the meter. It is important, therefore, to report the temperature at which the pH of the sample was read and analyze as soon as possible after sample collection. (Maximum hold time = 15 minutes).

Quality Control

Along with good sampling, the best method of assuring good quality data is the careful standardization of pH meters to two (2) buffers with a check of the third buffer to establish linearity. Standardization to

one (1) buffer is not sufficient. A malfunctioning pH meter or electrode may still read seven (7) but will not be able to accurately read pH 4 or pH 10.

Take a duplicate pH sample (in other words, collect samples in two (2) bottles at one of your sampling stations) at least once per week to be sure that the data is reproducible.

Most problems with pH analysis involve the pH probe, not the meter. A simple method to determine if the meter is malfunctioning is: Straighten a paperclip, place the end of the paperclip into the hole in the center of the BNC connector on the meter, then bend the paperclip to make contact with the metal ring of the BNC connector (see photo). The meter should display a reading of 7.00 +/- 0.1. (6.9-7.1). If it does not, there may indeed be a problem with the meter. If you are not comfortable with doing this yourself, contact the manufacturer to troubleshoot issues you are having.

A minimum 10% duplicate/replicate schedule must be established for the pH analysis; therefore, a duplicate/replicate analysis must be performed once every 10 pH analyses. Most operators choose to establish a once per week duplication/replication schedule.

Here is a link to the Hanna® pH meter and method. Be sure to use the instructions supplied with the meter in use at your facility. There are often subtle differences between brands. This link gives good information on use/care, calibration, and maintenance requirements for model 991001 portable pH meter.

[MAN991001_12_18.pdf (hannainst.com)]

VOLATILE ACIDS
and
ALKALINITY

VOLATILE ACIDS and ALKALINITY

Background

The volatile acids/alkalinity test is an important digester control test, much better than pH monitoring alone. Volatile acids/alkalinity tests will give more warning of impending digester problems, while pH testing may not indicate a problem until it is too late.

In a well-functioning digester, two groups of bacteria are working in harmony to break down organic matter. Saprophytic bacteria hydrolyze and convert complex organic compounds to low molecular weight (simple) compounds. These are essentially the waste products of the saprophytes. Among these waste products are short-chain fatty acids such as acetic, propionic and butyric acids. These are called volatile acids because they can be distilled at atmospheric pressure. Also working in the digester are the methane-formers. These bacteria convert the saprophytes' wastes to methane and carbon dioxide. In a stabilized system, the methane formers use the volatile acids as fast as the saprophytes produce them.

The alkalinity, or buffering capacity, of the system is the ability of the solution to resist massive changes in pH as acids are added and helps to stabilize the pH in the optimum range for the methane formers. As the saprophytes work on the wastes in the digester, volatile acids are produced. When these acids dissociate, hydrogen ions are released. The free hydrogen ions would gradually force the pH of the digester downward but the alkalinity of the system will pick up these ions and allow the methane-formers to "catch-up." If the system becomes out of balance, the alkalinity will gradually be exhausted and the pH in the digester will begin to fall. When the pH slips to below 6.5, the methane formers are inhibited and the digester goes "out of control," that is, the pH goes down faster because the methane formers are no longer effectively using the volatile acids. When the pH reaches 5, the saprophytes are inhibited and the digester has gone sour and no digestion is taking place.

Methane formers have a much narrower optimum range for pH and temperature and are slower in reproducing than the saprophytes. While the two groups of organisms do not compete with each other, any conditions which are favorable to the saprophytes and not to the methane formers result in a shift toward a sour digester. By monitoring the volatile acids/alkalinity relationship, this shift can be seen in an increase in volatile acids and a decrease in alkalinity.

The traditional method of digester control is monitoring pH. This method is not sufficient because a shift downward in pH has been preceded by the loss of the system's alkalinity. At this point, it may be too late to bring the digester back. A shift in the volatile acids/alkalinity relationship may occur days before the pH shift.

Strive to maintain a ratio between volatile acids and alkalinity of between 0.05 and 0.15. A shift over 0.15 warns of impending trouble and liming is indicated to bring the digester back into control.

Sampling

Samples for volatile acids/alkalinity analysis are taken from the primary digester. You can either sample from a tap off the recirculation line or from the sample taps at each level of the primary. The mixers should be on. Remember to let the sampling taps run a sufficient time to clear the lines. Samples should be run immediately.

Method

The following method is a combination of the potentiometric titration methods for acidity (Method 2310B) and alkalinity (Method 2320B), page 2-25 and 2-26 18th Edition of Standard Methods for the Examination of Water and Wastewater with sludge sample preparation techniques from the laboratory section of the EPA Operations Manual: Anaerobic Sludge Digestion.

Equipment

- pH meter with ATC
- 50 ml buret
- 100 ml beaker
- magnetic stirrer (sample may be agitated by hand but this is undesirable)
- 50 ml graduated cylinder
- hot plate

Reagents

1.0N STANDARD SULFURIC ACID SOLUTION ("STOCK")

1.0 n H_2SO_4 - Partially fill a one-liter volumetric flask with approximately 500 mls distilled water. Carefully, while stirring, add 28.0 mls concentrated (36N) H_2SO_4. Cool, then dilute to the one-liter mark with distilled water.

0.10N STANDARD SULFURIC ACID SOLUTION ("WORKING")

For expected alkalinities of greater than 20 mg/L:

> 0.10N H_2SO_4 - Partially fill a one-liter volumetric flask with approximately 500 mls distilled water. Carefully add 100 mls of the 1.0N H_2SO_4. Dilute to the one-liter mark with distilled water.

0.02N STANDARD SULFURIC ACID SOLUTION

For expected alkalinities of less than 20 mg/L:

> 0.02N H_2SO_4 - Partially fill a one-liter volumetric flask with approximately 500 mls distilled water. Carefully pipet 20 mls of the 1.0N H_2SO_4. Dilute to the one-liter mark with distilled water. This solution would be used in situations where the alkalinity is expected to be less than 20 mg/L.

1.0N STANDARD SODIUM HYDROXIDE SOLUTION ("STOCK")

1.0N NaOH - Partially fill a one-liter volumetric flask with approximately 500 mls distilled water. Carefully dissolve 40 grams of reagent grade sodium hydroxide in this. Dilute to the one-liter mark with distilled water.

0.1N SODIUM HYDROXIDE SOLUTION ("WORKING")

0.1N NaOH - Partially fill a one-liter volumetric flask with distilled water. Carefully add 100 mls of the 1.0N NaOH solution. Dilute to the one-liter mark with distilled water.

0.02N SODIUM HYDROXIDE SOLUTION

0.02N NaOH - Partially fill a one-liter volumetric flask with distilled water. Carefully pipet 20 mls of the 1.0N NaOH stock solution. Dilute to the one-liter mark with distilled water.

Procedure

1) Standardize the pH meter to buffer 7 and 4.

2) Filter, centrifuge, or decant off clear supernate from the sample.

> **NOTE:** There is literature that suggest that 30% or more of the alkalinity in anaerobic digesters may be contained in the sludge solids, and that by using only supernate in the analysis, much of the alkalinity is overlooked. There may be some validity to this argument and, in fact, either method (supernate or "whole sludge") is acceptable as long as the same method is used consistently.

If you decide to change from one method to the other, keep in mind that the ratio of alkalinity to volatile acids may change.

3) Add 50 mls of clear supernate to a 100 ml beaker.

4) Immerse pH electrodes into sample and change sample pH.

5) With meter on, titrate sample with standard H_2SO_4 to pH 4.0*. Note amount of acid used.

NOTE: If only interested in alkalinity - **STOP HERE** and perform CALCULATIONS.

6) Continue adding acid until sample reaches pH 3.5 to 3.3. Remove electrodes.

7) Check sample temperature.

8) Turn on hot plate and lightly boil the sample for at least three (3) minutes.

9) Standardize the pH meter to buffer 4.

10) Cool sample in cold water bath to the original temperature in Step 7.

11) Titrate sample with standard NaOH back to pH 4 (note buret reading).

12) Continue titration to pH 7 (note buret reading).

Calculations

The total alkalinity is figured first, using the amount of acid needed to titrate the sample from the starting pH to pH 4.0.

$$\text{Total Alkalinity, in mg/l} = \frac{\text{mls of acid used} \times \text{N(normality) of acid} \times 50{,}000}{\text{mls of sample used}}$$

$$\text{Total Alkalinity, in mg/l} = \frac{\text{mls of acid} \times 0.10 \times 50{,}000}{50 \text{ mls}}$$

$$= \text{mls of acid used} \times 100$$

The volatile acids alkalinity is next figured using the amount of hydroxide needed to titrate the sample from pH 4 back up to pH 7. From the volatile acids alkalinity is calculated the volatile acids.

$$\text{Volatile Acids, Alkalinity, in mg/l} = \frac{\text{mls of hydroxide used} \times \text{N of hydroxide} \times 50{,}000}{\text{mls of sample used}}$$

$$= \frac{\text{mls of hydroxide} \times 0.10 \times 50{,}000}{50 \text{ mls}}$$

$$= \text{mls of hydroxide} \times 100$$

To calculate the volatile acids, multiply the volatile acids alkalinity by:

If the volatile acids alkalinity is less than 180 mg/l by 1.0

If the volatile acids alkalinity is greater than 180 mg/l by 1.5

Example:

Using a 50 ml sample of digester supernate:
starting pH 7.4, mls acid 0.0
at pH 4.0, mls acid 23.5
temperature = 21 °C

After boiling and cooling to 21 °C:
starting pH 3.7, mls hydroxide 0.0
at pH 4.0, mls hydroxide 1.05
at pH 7.0, mls hydroxide 3.30

Total alkalinity = 23.5 x 100 = 2,350 mg/l
Volatile acids alkalinity = (3.30 - 1.05) x 100 = 225 mg/l
Volatile acids (alkalinity over 180 mg/l) = 225 x 1.5 = 338

The volatile acids/alkalinity ratio is now calculated:

$$\text{Volatile acids/alkalinity} = \frac{338}{2,350} = 0.14$$

Quality Control

Running samples in duplicate is the best means of checking your procedure. Run two 50 ml portions of the sample through the entire test. Question any tests that do not duplicate within a reasonable amount, say 10%.

Be sure to properly standardize the pH meter before beginning the analysis.

VOLATILE ACIDS and ALKALINITY TROUBLESHOOTING GUIDE

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Acidity, Results unreasonably high	Over titration due to missing end point	Add NaOH solution more slowly - allow time for sample to equilibrate before adding next drop.
	Improper calibration of pH meter	Recalibrate meter and rerun analysis
	Improper standardization of NaOH titrant	Restandardize titrant and rerun analysis
Acidity, Results unreasonably low	Using 0.1 or 0.2N NaOH as opposed to 0.02N	Dilute NaOH titrant to proper (0.02N) strength and rerun analysis
Alkalinity, Results unreasonably high	Over titration due to missing the end point	Add the H_2SO_4 solution very slowly; allow time for sample to equilibrate before adding next drop
	Improper standardization of H_2SO_4 titrant	Restandardize titrant and rerun analysis
Alkalinity, Results unreasonably low	Using higher concentration of H_2SO_4 than required	Restandardize H_2SO_4 titrant to 0.02N
	pH electrode has become fouled	Follow manufacturer's instructions for cleaning electrodes. If the sample contains soaps, oily substances or excessive suspended matter; dilute the sample to reduce this problem before reanalyzing.

Quality Control for Volatile Acids & Alkalinity

Document

Sample Collection

Grab - Exact time collected
Exact time analyzed

Location

Glassware Preparation - (Sampling Container)

Thoroughly rinse with distilled H_2O

Equipment

Meter

- Range 0 to 14
- Accuracy - 0.1 pH
- Repeatability - 0.1 pH
- Temperature Compensation
- Capable of 2 Point Calibration

pH Probe

- Document Age & Maintenance
- (Gel filled - refillable)
- Storage & Use Instruction
- **Stress Rinsing**

Temperature Probe

- Must Be Connected To Meter and In Analyte During Analysis
- Calibration of Probe Against NIST Traceable Thermometer

Buret

- Record all pertinent buret readings on bench sheets

Reagents

NIST Traceable (Fischer, etc.)
If using powder pillows or making your own buffers (from NIST Traceable materials)
Document quality of make up water
Preparation and Expiration Date
Standardization of Titrants Sulfuric Acid Sodium Hydroxide

Calibration

Document

- Date and Time
- Buffers Used & Order of Use
- Exact Instrument Results
- Analyst Performing Calibration
- Buffer Temperature

Sample

Type
Temperature
Duplicate Results
Special Conditions
High Ionic Strength
Dilute - poorly buffered

REPORTING VOLATILE ACID/ALKALINITY DATA

Volatile Acid/Alkalinity Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
METER STANDARDIZATION: (Date and Buffers Used)
NORMALITY OF ACID (H_2SO_4) TITRANT: (Date Standardized)
NORMALITY OF BASE (NaOH) TITRANT: (Date Standardized)

Volatile Acids	Volume (mls) of NaOH used to titrate sample from pH 4.0 to pH 7.0	Normality (concentration) of NaOH used	Sample Volume
Alkalinity	Volume (mls) of H_2SO_4 used to titrate sample from original sample pH to pH 4.0	Normality (concentration) of H_2SO_4 used	Sample Volume

References

A discussion of the volatile acid/alkalinity relationship can be found on page 163 of Operation of Wastewater Treatment Plants, 4th Edition, Volume 2. The actual analyses are described on pages 472 through 476 of that reference.

In the 18th Edition of Standard Methods for the Examination of Water and Wastewater, analytical methods for acidity (Method 2310B) and alkalinity (Method 2320B) can be found on pages 2-23 through 2-28.

Chlorine Residual

VT WSMD Wastewater Program Lab Manual Section #9

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Chlorine Residual

Background

Chlorine (Cl_2) has been used for many years as a disinfection agent to destroy disease-causing microorganisms (pathogens). It is a very powerful oxidizing agent making it an excellent disinfectant, particularly effective against many bacteria, viruses, and protozoa. It is also very toxic to organisms in the receiving water. The kill of the microorganisms is proportional to the contact time and the concentration of the chlorine. When chlorine is added to wastewater, either as chlorine gas or as hypochlorite, it hydrolyzes and produces free chlorine. Free chlorine readily combines with various substances such as ammonia, iron, manganese, and some organic components.

When ammonia is present (as is the case in treated wastewater) the free available chlorine combines with it to form chloramines which are combined available chlorine. Chloramines are also disinfectants but are some 100 times less effective than free chlorine. The relative amounts of free and combined chlorine preset will depend on the amount of nitrogen present, the pH, the temperature, and the amount of chlorine added. In most wastewater there is very little free chlorine left because of the small amounts of chlorine added relative to the amount of ammonia and other materials present.

Over-chlorination is to be avoided for a number of reasons; it is very expensive, it is harmful to aquatic life, and toxic compounds may form. It is important to measure the chlorine residual to maintain a balance between these disadvantages and the benefit of disinfection. The total chlorine residual should be kept as low as possible while maintaining a coliform/*E. coli* count within permitted conditions.

Methods of Measurement

EPA approved methods for the analysis of residual chlorine include the amperometric method, the iodometric method, the selective ion electrode method, and the DPD (N,N-diethyl-p-phenylenediamine) method. The high level iodometric methods are only good for measuring chlorine concentrations greater than 1 mg/L. Most Vermont wastewater permits require limits much less than 1 mg/L.

The low level amperometric method is actually more accurate than all other methods for measuring lower concentrations of TRC but it is more difficult than the colorimetric methods and requires greater skill and much greater maintenance. The electrodes must be kept very clean and free of any films or contaminants and the stirring of the sample must be kept slow enough so as not to allow loss of chlorine. For analysis of (TRC) Total Residual Chlorine concentrations of less than 0.2 mg/L the low-level amperometric or ultra-low level colorimetric methods should be used**. Links for HACH® low and ultra-low colorimetric methods are included at the end of the section**

Note: For industrial wastes you must choose a method that is not prone to interference by metals. The amperometric method is not effective in effluents containing certain analytes, such as silver, copper, cyanide, iron, nitrite and oxidized manganese. There are often complex anions in industrial wastes that can interfere with this method of analysis.

Instructions for the proper calibration of the amperometric meter are included with each specific probe.

Results using the DPD method may be obtained using either endpoint titration or by spectrophotometer. The DPD kits using powder pillows and a small “color wheel” comparator are EPA

approved for drinking water only. They are not approved for the analysis of chlorine in wastewater! Even though this may seem unfair, there is a very logical reason for it. Whereas drinking water is usually clear and colorless, this does not always hold true for wastewater effluent. It is the color and turbidity of the wastewater that interfere with the comparative method of chlorine analysis, causing the method to be deemed unacceptable for wastewater samples. The kit may be used for quick spot-checks but the reported daily tests must be performed using an approved method!

The good news is that there are now very "simple to use" kits for CL_2 analysis that include either a spectrophotometer or filter photometer. The use of these kits in CL_2 analysis is perfectly acceptable.

Procedure 4500-C1 G ("DPD Colorimetric Method") in 23rd Edition, Standard Methods for the Examination of Water and Wastewater states that the required apparatus includes either:

a. Spectrophotometer, for use at a wavelength of 515 nm and providing a light path of 1 cm or longer, or

b. Filter photometer, equipped with a filter having maximum transmission in the wavelength range of 490 to 530 and providing a light path of 1 cm or longer.

This **colorimetric** method is based on the same principles as the DPD titrimetric method. That is, under acidic conditions chlorine reacts with DPD indicator to form a red colored complex. Then instead of titrating with Ferrous Ammonium Sulfate as in the titrimetric method, the sample is placed in a test tube or photometer cell and the chlorine content is measured with a spectrophotometer or photometer. The analytical procedure will be described later in this section.

The **amperometric** method for chlorine analysis is the standard against which all other methods are compared. However, the equipment necessary for this method is expensive. This method also requires more operator skills than any of the other methods. Basically, the procedure consists of titrating the sample with a standard phenylarsine oxide (PAO) solution while observing current changes on the titrator meter. As the current changes get smaller and smaller, less and less titrant is added until the needle ceases to move. The amount of titrant is then read and recorded. When 0.00564N PAO is used as titrant in a 200 mL sample, each mL of titrant is equal to 1 mg/Liter chlorine. Because each meter is somewhat different, it is important to follow the manufacturer's instructions for your specific meter. For further information you might refer to the Apparatus Section of the Amperometric Titration Method in 23rd Edition "Standard Methods". This analytical procedure will be described later in this section.

The **iodometric** method is suitable for the determination of chlorine residual in wastewater. However, for concentration less than one (1) mg/L the iodometric method might be less sensitive than required for permit compliance. This method uses back titration to arrive at the chlorine concentrations. First, a known excess volume of a standard thiosulfate solution is added to the sample. This thiosulfate reacts with the chlorine present. The excess thiosulfate is then titrated with a standard iodate solution to its endpoint. The chlorine residual can be calculated by subtracting the amount of iodate solution used from the amount of thiosulfate solution added originally. (This gives you the amount of thiosulfate solution that reacted with the chlorine. The ratio of reacted thiosulfate (mL) to chlorine in mg/L is 1:1. Example: If two mL iodate solutions are used, the chlorine residual would be recorded at two mg/L). Since there is no laboratory in Vermont known to be using this method the procedure will not be

included in this manual. The procedure may be found in the 23rd Edition, "Standard Methods" 4500-Cl G Iodometric Electrode Technique on pages 13 -14 (Section 4500-Cl^-:I)

The **selective ion electrode** method is a very simple method for chlorine residual analysis. However, the equipment necessary to perform the analysis can be very expensive and must be calibrated daily with freshly prepared standards.

Interferences

ALL methods for analysis of Total Chlorine depends on stochiometric production of iodine in waters containing reducing substances may not be analyzed accidently by these methods.

Color, turbidity, and certain metals like manganese interfere with all colorimetric analyses.

Sampling

Residual chlorine is subject to dissipation by exposure to sunlight, exchange of gasses with the atmosphere and reactions with compounds in the wastewater over time. For this reason, chlorine residual samples must be analyzed immediately. The sample should be taken gently into a 300 mL amber or opaque DO bottle filled to the top the stopper inserted and the bottle shielded from the sunlight.

There is a maximum hold time of 15 minutes for the Total Chlorine analysis but the closer to "IMMEDIATELY" the analysis is performed the better.

Samples for Cl_2 analysis cannot be stored or sent off to another lab for analysis!!!

DPD Colorimetric Method

Equipment

- Spectrophotometer with a light path of 1 cm or longer - capable of providing a wavelength of 515 nm.
  OR
- Filter Photometer - with a light path of **1** cm or longer and a filter with maximum transmission in the wavelength range of 490-530 nm
- Colorimeter/fixed photometer – same as for filter photometer

SAMPLE TYPE: Grab

CONTAINER: Glass or Plastic (Polyethylene or Equivalent) amber or opaque

PRESERVATIVE: None. Analyze immediately – absolute max hold time 15 minutes

SAMPLE VOLUME: As appropriate for instrument used

*If the Total Residual Chlorine exceeds 4mg/l, the sample should be diluted with chlorine demand-free water.

*For measurement of chlorine residual below 0.1 mg/L the light path length of whatever equipment is used must be between 5 – 10 cm.

Reagents

DPD Total Chlorine Reagent Powder Pillows (or DPD Total Chlorine Reagent Aluminum Packets, recommended):

Preparing DPD reagents yourself is not recommended due to time and safety considerations

Phosphate Buffer Solution: Dissolve *24* grams anhydrous disodium hydrogen phosphate, Na_2HPO_4 and 46 grams anhydrous potassium dihydrogen phosphate, KH_2PO_4, in distilled water. Combine this solution with I 00 mL distilled water in which 0.8 grams disodium ethylenediamine tetraacetate dihydrate, also called (ETDA), has been dissolved. Dilute to one liter with distilled water and add 0.02 g mercuric chloride ($HgCl_2$) to prevent mold growth, and to prevent interference in the free available chlorine test caused by any trace amounts of iodide in the reagents. ($HgCL_2$ is toxic.)

N,N-Diethyl-P-Phenylenediamine (DPD) Indicator Solution: Dissolve 1 g DPD Oxalate or 1.5 g DPD sulfate pentahydrate or 1.1 g anhydrous DPD sulfate in chlorine-free distilled water containing 8 mL of 1+3 sulfuric acid (25% solution) and 0.2 g disodium ethylenediamine tetraacetate dihydrate (EDTA). Make up to one liter, store in a brown glass-stoppered bottle, and discard when discolored. ·

Potassium Iodide (KI) Crystals

Purchase dry (anhydrous) crystals.

Procedure (DPD Colorimetric Method)

1) Add the contents of one DPD Total Residual Chlorine reagent powder pillow or aluminum packet - make sure to use the designated volume of sample for the pillow or packet used. (Some are designed for use with 10 or 25 mL samples.)
2) Wait at least three (3) minutes but not more than six minutes.
3) Follow instructions for the specific meter you are using for analysis.

With kind permission from the HACH company I have included the DPD colorimetric method from the Hach user manual for the DR 300 chlorine colorimeter. At the time this manual is being written the colorimetric method using some form of “pocket colorimeter” or spectrophotometer is by far the most common method for analysis of Total Residual Chlorine.

Both the low range and high range methods are included in the user manual. Most, if not all, Vermont WWTF operators/technicians may need to use the LOW Range method as the permit limits for chlorine residual are already as low as 0.02 mg/l in some cases. The high range method is only capable of reading results to 0.1 mg/l CL_2. Use of both is consistent with Standard Method 4500.CL-G

*There are a few simple steps that if followed religiously will help ensure good results.

1. ALWAYS rinse the sample cell AND THE CAP with distilled water after use – Before use rinse the sample cell to the cap with the sample!!

2. Wipe all fingerprints, moisture, condensation,and other debris off the outside of the sample cell with a KimWipe before placing it into the colorimeter.
3. Always place the sample cell into the colorimeter the same way. For example: Hach sample cells have a diamond imprinted on the sample cell – Make sure the diamond is facing the same direction every time. Most operators prefer to face the diamond toward the front.

The Low range method requires the use of a reagent blank. All reagents have color! That is, the reagent itself can account for a reading of 0 - 0.5 mg/L!! It is very important to determine the “amount” or intensity of the color in the DPD reagent. We find that by using a reagent blank *(the reagent blank consists of pure distilled water). The instrument is zeroed using distilled water just as if this were the sample. After zeroing, a reagent packet is added to the distilled water/sample, the cell is the inverted to mix, wait three to six minutes, and then read the result. This result can then be subtracted from the sample result.

For example:

Blank (distilled water) is zeroed

Reagent is added and the result is 0.03 mg/L

Sample is then analyzed yielding a result of 0.09 mg/L – The reagent blank result is subtracted from the sample result.

0.09 mg/L - 0.03 mg/L = .06 mg/L

This reagent blank can be subtracted from all sample results obtained from that batch of reagents. The analyst must re-establish the reagent blank every time a new batch /lot of reagents is used.

DPD Ferrous Titrimetric Method

Equipment

- 10 mL automatic burette and/or 50 mL burette 10 mL volumetric pipet
- 2 - 250 mL Erlenmeyer flasks 100 mL graduated pipet
- 2 - 5 mL graduated pipets 500 mL Erlenmeyer flask
- various glassware for reagent preparation

SAMPLE TYPE: Grab

CONTAINERS: Glass or plastic

PRESERVATIVE: None – Analyze immediately

Reagents

0.100N (Stock) Potassium Dichromate ($K_2Cr_2 0_7$) Solution

Dissolve 4.904 grams of anhydrous potassium dichromate ($K_2Cr_2 0_7$) into 600 mL distilled water. Dilute to 1,000 mL with distilled water**.**

0.025N (Working) Potassium Dichromate ($K_2Cr_2 0_7$) Solution

Pipette 25.00 mL of the 0.1N potassium dichromate solution into a 100 mL volumetric flask Dilute to 100 mL with distilled water. This solution is used to standardize the standard FAS solution.

0.0282N Standard Ferrous Ammonium Sulfate (FAS) Titrant

Dissolve 11.106 g ferrous ammonium sulfate, $Fe(NH_4)2(SO_4)2 \bullet 6H_2O$, in distilled water containing one mL of 1+3 sulfuric acid (25% solution) and make up to one liter with freshly boiled and cooled distilled water. This primary standard may be used for six months.

Instructions for the standardization of this FAS concentration to prepare the 0.00282N working solution are included at the end of "Reagents" section.

Phosphate Buffer Solution

Dissolve 24 grams anhydrous disodium hydrogen phosphate, $N82HPO_4$, and 46 grams anhydrous potassium dihydrogen phosphate, KH_2PO_4, in distilled water. Combine this solution with 100 mL distilled water in which 0.8 grams disodium ethylenediamine tetraacetate dihydrate, also called (ETDA), has been dissolved. Dilute to one liter with distilled water and add 0.02 g mercuric chloride ($HgCl_2$) to prevent mold growth, and to prevent interference in the free available chlorine test caused by any trace amounts of iodide in the reagents. ($HgCl_2$ is toxic.)

N,N-Diethyl-P-Phenylenediamine (DPD) Indicator Solution

Dissolve **l** g DPD Oxalate or 1.5 g DPD sulfate pentahydrate or 1.1 g anhydrous DPD sulfate in chlorine-free distilled water containing eight (8) mL of 1+3 sulfuric acid (25% solution) and 0.2 g disodium ethylenediamine tetraacetate dihydrate (EDTA). Make up to one liter, store in a brown glass-stoppered bottle, and discard when discolored. (The buffer and indicator are commercially available as a combined reagent in a stable powder form). It is advisable to purchase this reagent in ready-made form.

Concentrated Sulfuric Acid, H_2SO_4 (36N)

Ferroin Indicator in Dropping Bottle

Potassium Iodide (KI) Crystals

Chlorine Demand Free Water

Add sufficient chlorine to high quality distilled water to give five mg/L free chlorine. Let sit for two days. If the distilled **water** was of sufficient quality, there should be at least two mg/L free chlorine still present after standing for two days. At this **point,** remove the remaining chlorine by setting it in the sunlight or under an ultraviolet lamp until all traces of chlorine are gone. The 23rd Edition of Standard Method's for the Examination of Water and Wastewater offers suggestions on storage of this water.

FAS Standardization Procedure

Standardize the 0.0282 N FAS by the following procedure which should be run in duplicate:

1) Pipet 10.00 mL 0.025 N potassium dichromate ($K_2Cr_2O_7$) into a 250 mL Erlenmeyer flask. Use a volumetric pipet to measure out exactly 10.00 mL potassium dichromate.
2) Add approximately 90 mL distilled water.
3) Add approximately 25 mL concentrated sulfuric acid SLOWLY.
4) Cool to room temperature in a cool water bath.
5) Add three (3) drops ferroin indicator.
6) Titrate with 0.0282 N FAS from yellow, through green to aqua to the red-brown endpoint.
7) Record milliliters (mL) of FAS used.

The burette reading is then plugged into the equation:

$$Normality\ (N)\ FAS\ stock\ solution = \frac{0.25\ N\ K_2Cr_2O_7}{mL\ FAS\ used}$$

The resulting N of stock FAS is used in the following equation to yield the mL of stock FAS to be diluted in a 200 mL volumetric flask to make the working strength 0.00282 N:

$$Volume\ to\ use\ (mL) * N\ of\ FAS\ stock\ solution = 200\ mL * 0.00282\ N\ FAS\ working\ solution$$

$$Volume\ to\ use\ (mL) = \frac{0.564}{N\ of\ FAS\ stock\ solution}$$

The working strength solution is made up and used for one (1) week to a month at the longest.

Example: The standardization procedure is performed with the stock FAS solution and 8.8 mL are used in the titration.

$$N\ of\ FAS\ stock\ solution = \frac{0.25\ N\ K_2Cr_2O_7}{8.80\ mL} = 0.0284\ N\ FAS\ stock\ solution$$

The volume of the stock solution to be diluted to 200 mL is now calculated:

$$Volume\ to\ use\ (mL) = \frac{0.564}{0.0284\ N\ FAS\ stock\ solution} = 19.86\ mL$$

Using a burette, measure the volume of stock solution indicated (in this example, 19.86 mL or 19.85 mL) into a 200 mL volumetric flask. Add distilled water up to the 200 mL line, cap, and mix.

This is the 0.00282 N FAS solution to use for the daily chlorine residual measurements during the coming week.

DPD Titrimetric Procedure

1) Pipet *5* mL each of the phosphate buffer and the DPD indicator solutions into the 500 mL Erlenmeyer flask. Mix by swirling.

2) Add 100 mL of freshly collected sample.

   Perform STEP 3 ONLY IF free chlorine residual determination is desired. **If only total chlorine residual is desired, omit Step 3 and go from Step 2 to Step 4.**

3) Titrate immediately with 0.00282 N FAS until pink color disappears. Read burette and record as free chlorine residual. Do not re-zero (refill) burette.

4) Add approximately one gram potassium iodide, KI, crystals, mix and time for two minutes. The solution should turn pink again.

5) Titrate, with FAS, the new pink color until it disappears/is clear.

6) Read the burette and record this reading as total chlorine residual, in mg/L. NOTE: The total chlorine reading.

7) Should the sample return to a pink color, allow another two minutes and titrate to the colorless endpoint. This is the total chlorine residual.

If you get a high free chlorine residual, chances are that there was a small amount of KI left in the titration flask and what you actually have is some total residual showing up as free because of it. It is a good idea to rinse the flask several times with distilled water to help alleviate this problem.

Example: 1 mL of titrant is used to titrate for free chlorine. Then an additional 3 mL of titrant is needed to obtain the clear endpoint after the addition of the KI crystals the results would be recorded as:

Free CL2 = 1 mg/L

Total CL2 = 4 mg/L

Chlorine DPD Titrimetric Method Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Reagents turn pink before sample is added	Contaminated glassware	Rinse glassware thoroughly with distilled water. Rerun analysis.
	Reagents (DPD indicator) contaminated or has passed expiration date	Prepare fresh DPD indicator solution
Unreasonably low result (end point is reached with first drop of titrant even though you are sure the residual is quite high.)	Using "stock" 0.0282N FAS as opposed to proper "working" concentration of .00282N FAS	Use proper concentration of FAS-Retest
A strange orange color is produced instead of a pink color.	Possibly manganese contamination	Add 0.5mL sodium arsenite solution or 0.5 mL thioacetanide to sample before adding DPD indicator solution
To prepare sodium arsenite solution: Dissolve 5.0 g NaAs02 in distilled water and dilute to one liter.		
Unusually high Cl_2 result	pH of sample is too high causing dissolved oxygen to give color	Adjust sample pH to approximately 6.5
Pink color keeps coming back seconds after titrating to clear endpoint	You didn't wait the required two (2) minutes after adding the KI crystals before titrating the sample	Wait a full two (2) minutes
	High concentration of contaminants (ammonia) from ambient air are getting into the sample	Take precaution to avoid airborne contaminates

Quality Control for the DPD (FAS) Titrimetric Method

Document the Following (Included but not limited to):

Supply Water Quality

- Chlorine and Ammonia Free
- No Chlorine Demand
- Conductivity <10 micro siemens

Sampling

- Grab:
    - Exact time and Date Sampled
    - Exact time and Data Analyzed
- Volume – X mL
- Location – Be Exact

Glassware

- Washed (phosphate free detergent) & rinsed thoroughly (distilled water)
- Rinse with sample material, discard and refill

Reagents

- NIST Traceability (Hach, Fisher, etc)*
- Preparation, Standardization & Expiration Dates (documented in reagent log, on a bench sheet, or wherever QC data are kept)

Replication/Duplication

- once every 10 samples- once/week is typical

*SUGGESTED – may become mandatory in the future

Reporting Chlorine (DPD Titrimetric) Data

Total Chlorine (DPD Titrimetric) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME:
BURET READING START:
BURET READING AT ENDPOINT:
TOTAL CHLORINE RESULT: -Report result to one digit beyond the decimal point.

References

Hach® page with interesting general information regarding chlorine analysis

Chlorine - Water Quality Parameter Overview and Products | Hach

Hach's® colorimetric Low range Total Chlorine analysis. This analysis is for Total Chlorine in the range of 0.2-2.0 mg/L

DOC316.53.01450_4ed (1).pdf

Hach® ultra-low range total chlorine analysis. This method is for analysis of Total Chlorine in the range of 2 to 500 micrograms/liter or 0.02 to 0.5milligrams/liter . Note: special flow cells are required to use the DR300 colorimeter for this analysis.

DOC086.53.01000.book(DOC316.53.01032.fm) (azdhs.gov)

Solids

VT WSMD Wastewater Program Lab Manual Section #10

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Solids

Preface to 2021 Update

With some notable exceptions, the methods for “solids” analyses have remained somewhat the same since the 1990 version of the manual was prepared. Some significant differences include the permit limits imposed and the very low results being reported, especially regarding the Total Suspended Solids (TSS) analysis. Whereas it is possible to achieve accurate results down to 2.5 mg/L TSS using graduated glassware as described in Standard Methods For the Examination of Water and Wastewater, results less than 2.5 mg/L are not considered valid unless volumetric glassware/measuring device is used. Also, increased sample volume may be necessary to generate accurate results less than 2.5 mg/L. This will be described in detail at the end of the Total Suspended Solids section of this manual. One other notable change in the TSS section in the 23rd edition of Standard Methods is the formula used for calculation of TSS. NOTE that in the 23rd edition the formula is:

$$TSS\left(\frac{mg}{L}\right) = \frac{\left((Weight\ of\ Filter\ and\ residue\ \boldsymbol{in\ mg}) - (weight\ of\ filter\ \boldsymbol{in\ mg})\right) * 1000}{Volume\ of\ Sample\ (mL)}$$

Notice the difference between this and the formula from the original manual:

$$TSS\left(\frac{mg}{L}\right) = \frac{\left((weight\ of\ filter\ and\ residue\ \boldsymbol{in\ g}) - (weight\ of\ filter\ \boldsymbol{in\ g})\right) * 1{,}000{,}000}{Volume\ of\ Sample\ (mL)}$$

In order to use the “new” formula you must first convert the weights from grams to milligrams

Background

The term "solids" is generally used in referring to the material suspended or dissolved in wastewater. These solids fall into two general categories, organic and inorganic. Organic solids are those materials which originally come from living plants and animals. These will include body wastes, food scraps, cooking grease, potato peelings, and sometimes even old leather shoes. Inorganic solids are made of substances which were never living, such as gravel, salt, metal nuts and bolts, etc.

The concentration of solids in a wastewater is frequently used in describing the strength of the waste. The more solids present in a particular wastewater, the stronger that wastewater will be.

Normal domestic wastewater contains a very small concentration of solids when compared to the amount of water that carries it, usually less than 0.1%. This can be misleading, however, because it takes only a very small concentration of solids to create large pollution problems. The number and severity of pollution problems will depend on the type of solids that are involved.

As a general rule, large quantities of organic solids will create more pollution problems than will the same quantity of inorganic solids. Therefore, not only is it important to know how many solids are present in the system, but, also, the type of solids that are present. The test procedures for solids provide essential information about the types of solids coming into the treatment plant, the amount of (solids or strength of the influent), and whether the solids are actually being removed in the plant processes.

We perform "solids" analyses to determine the amount of solids material in wastewater. The solids test provides essential information about the types of solids coming into the treatment plant, the concentration of solids or strength of the influent and how well the plant or a given process is performing.

Definitions

Fixed Solids - those solids (total, suspended or dissolved) which remain after ignition for 15 to 20 minutes time at 550 degrees C +/- 50 degrees C. Those are also commonly referred to as ash. In general, fixed solids are thought to be made up of inorganic material, although some inorganic material can be lost during ignition.

Settleable Solids - the term applied to the material settling out of a sample within a one-hour period. Settleable solids may include floating material depending on the technique used in the test.

Total Dissolved Solids - this term refers to those solids which will pass through a standard glass fiber filter with a pore size of 2 microns or less.

Total Solids - the term applied to the material left in a dish after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids include "Total Suspended Solids" and "Total Dissolved Solids".

Total Suspended Solids - those solids which will not pass through a standard glass fiber filter with a pore size of 1.5- 2 microns. This will include both those solids that will settle or float in the clarifier and the lighter non-settleable solids (also referred to as "non-filterable residue").

Volatile Solids - those solids which are lost during ignition (in a sense, by burning) for 15-20 minutes at 550℃ +/- 50℃. In general, volatile solids are made up of organic material.

We will also discuss in this section:

Sampling - Samples must be taken from sample points that provide well-mixed, representative samples. For composite samples, individual sample volumes (should be) proportional to the flow rate at the time the sample is taken. When pouring a sample into a graduated cylinder, it should be mixed or stirred well and poured in such a manner that the solids will not settle out before pouring is completed. Large solids, such as pieces of wood, should be removed from the sample. It is highly recommended

that samples with large solids, such as the influent, be well-mixed before performing the analysis. Samples collected for the analysis of settled sludge volume must be fresh grab samples.

Settled sludge volume - a test that imitates but does not duplicate the action of the clarifier. Activated sludge from the aeration tank of a secondary plant is allowed to settle out in a graduated vessel. At specified time intervals, the level of the sludge is read.

Spin Testing - Using a centrifuge and a conversion factor, quick, process control-type measurements can be made on aeration tanks, return sludges and waste sludges.

Total Solids Methods

Equipment

- porcelain, platinum, or high-temperature glass evaporating dishes (9 cm)
- drying oven
- desiccator
- analytical (4-place) balance
- 25 mL graduated cylinder
- muffle furnace
- wide-bore pipets
- heat resistant gloves/tongs
- hot pad

Temperature

- 103°C to 105°C for water low in organic material such as streams and secondary effluents.
- 180°C for water high in organic matter such as influents and primary effluents.

Procedure

1) Prepare dishes--wash with soap and hot water, rinse with tap water, 10% HCL acid soak, tap water rinse, distilled water rinse two times.
2) Dry in oven at temperature to be used, overnight.
3) If doing fixed total solids (fired in furnace), the dishes must be fired for one hour then put in the oven for one hour.
4) Cool in desiccator.
5) Weigh.
6) Return to drying oven at 103°C to 105°C for at least one hour.
7) Return to desiccator to cool to room temperature.
8) Re-weigh. Weight should be within 0.5 mg of initial weight. Re-dry and re-weigh if not within 0.5 mg.
9) Pour measured sample into the dish (enough to yield a residue between 10 and 200 mg).

10) Place in oven and dry at approximately 98°C until all liquid has evaporated.
11) Bring oven to proper temperature of 103°C to 105°C and dry overnight.
12) Cool dishes in desiccator to room temperature.
13) Weigh.
14) Return to drying oven at 103°C to 105°C for approximately one hour.
15) Return to desiccator for one hour.
16) Re-weigh. The weights must be within 0.5 mg or the dish must be re-dried and re-weight until a constant weight is achieved.

- **STEPS 16-21 for Fixed Solids.** If fixed total solids are desired:

17) Place the dishes in muffle furnace at 550°C for 15 minutes to one hour.
18) Remove and cool in air until most of the heat has dissipated.
19) Transfer to desiccator, cool to room temperature.
20) Weigh.
21) Return to oven for at least one hour.
22) Re-desiccate for one hour and re-weigh to be sure of constant weight.

Calculations

$$Total\ solids\ \left(\frac{mg}{L}\right) = \frac{(A-B)*1000}{Sample\ Volume\ (mL)}$$

Where:

- A = weight of filter/dish and residue in mg
- B = weight of filter/dish in mg

$$Total\ Volatile\ Solids\ \left(\frac{mg}{L}\right) = \frac{(C-D)*1000}{Sample\ Volume\ (mL)}$$

$$Total\ Fixed\ Solids\ \left(\frac{mg}{L}\right) = \frac{(D-E)*1000}{Sample\ Volume\ (mL)}$$

Where:

- C = weight of residue and dish before ignition in mg
- D = weight of residue and dish or filter after ignition in mg
- E = weight of dish or filter in mg

The potential sources of errors are from temperature variations and from non-homogenous samples. It is important to monitor the drying oven and muffle furnace temperatures closely and be sure the samples are well-mixed before pouring out for analysis. A blank (distilled water only) should be run with each analysis. The blank should be treated in the same manner as the actual sample.

Quality Control for Total Solids Standard Method #2540-B

Document the Following (Including but not limited to):

Sampling (Grab or Composite)

- Grab - Exact Time and Date Sampled
- Composite - Flow Proportioning Date
- Grab or Composite -
    - Volume
    - Location

Glassware and Equipment

- Evaporating dish
    - Type:
    - Method of Cleaning:
        - 1 hour at 500℃ +/- 50℃ for volatile solids
        - 1 hour at 103℃ - 105℃ for only total solids

Oven Temperature

- Verify oven temperature at least twice per drying cycle

Duplication Schedule

- Analysis should be run in replicate (100%) - Replicates should be within 5% of their average.

Blank Result

- If not zero check for error.

The troubleshooting guide and references can be found at the end of the Solids Section.

Reporting Total Solids Results

TOTAL SOLIDS BENCH SHEET
ANALYST:
EXACT TIME AND DATE SAMPLE WAS COLLECTED:
EXACT TIME AND DATE SAMPLE WAS ANALYZED:
SAMPLE LOCATION:
SAMPLE VOLUME (mL):
WEIGHT OF EVAPORATED DISH and DRIED RESIDUE IN GRAMS (after evaporation):
WEIGHT OF EMPTY DISH ONLY (after evaporation):
WEIGHT OF DISH and DRIED RESIDUE minus (-) WEIGHT OF DISH ONLY - RESULT IN mg/L:
**** SHOW ALL CALCULATIONS AND RAW DATA:** Blank Data All weighings to establish constant weight Duplicate or Replicate Data

Total Suspended Solids: Standard Method #2540-D

The method for Total Suspended Solids has not changed significantly since the last version of this manual was written in 1990. However, many operators are apparently interested in determining just how “low they can go” regarding the reporting of Total Suspended Solids results. Results are often reported as <2 mg/L TSS. Results below 2.5 mg/L are obtainable but in order to achieve these results the use of volumetric glassware/equipment for measurement of sample volumes is required. AND since the method requires selection of a sample volume to produce between 2.5 and 200 mg dried residue the volume of sample may have to be increased substantially. The bottom line is this: Reporting TSS results of <2 mg/L is technically invalid because in order to obtain such results using one liter of sample the required minimum 2.5 mg of dried residue cannot have been satisfied. HOWEVER: By using volumes in excess of 1000 milliliters lower results can be reported. This will be explained in detail at the end of this section.

Equipment

- Gooch crucibles – sometimes used for influents, primary effluents
- Buchner funnel (or equivalent) - used for sludges, mixed liquors, influent and final effluent
- drying oven (103°C to 105°C)
- desiccator
- analytical (4-place balance)
- assorted graduated cylinders
- muffle furnace (if interested in "Fixed" Solids)
- glass fiber filters to fit Gooch crucibles or Buchner funnels - Whatman 934AH; Gelman type A/E; Millipore type AP4O or equivalent filters
- vacuum pump with water traps
- wash bottle with distilled water

Temperature

- 103°C to 105°C

Procedure

1) Prepare crucibles and/or funnels--wash with hot soapy water, rinse with tap water, 10% HCL acid soak, tap water rinse, distilled water rinse 2 or 3 times.
2) Seat the filters in the filtering apparatus, wrinkled side up.
3) Rinse the crucibles/funnels down three times with about 25 mL of distilled water.
4) Dry for at least 1 hour at 103°C to 105°C.
5) If crucibles are to be fired for "fixed" solids, place crucibles in muffle furnace for one hour at 550°C
6) Transfer to oven for at least one hour.
7) Cool in desiccator for 15 to 30 minutes.
8) Weigh.
9) Transfer to oven for at least one hour.
10) Re-desiccate for 15 to 30 minutes (the same time in desiccator should be used as for the first weighing).

11) Re-weigh to ensure a constant weight; the second weighing should be within 0.5 mg of the first.
12) Select sample volume, pour sample through filter.
13) Rinse graduated cylinder into the crucible and rinse the sides of the crucible/funnel with distilled water.
14) Dry in oven at 103°C to 105°C for at least one hour (longer time may be required for sludges).
15) Cool to room temperature in desiccator. (15-30 minutes, same each time)
16) Weigh.
17) Place back into drying oven for at least one hour.
18) Place in desiccator until cooled to room temperature. (15-30 minutes, same each time)
19) Re-weigh: if more than 0.5 mg difference from the previous weighing, put back in oven for 1 hour and repeat desiccation and weighing steps until constant weight is reached.
20) If "fixed" suspended solids are desired, the crucibles may be treated as the dishes for fixed total solids.

Calculations

$$TSS\ \left(\frac{mg}{L}\right) = \frac{\left(Initial\ Weighing\ (mg) - Final\ Weighing\ (mg)\right) * 1000}{Sample\ Volume\ (mL)}$$

$$Fixed\ Suspended\ Solids\ \left(\frac{mg}{L}\right) = \frac{(A - B) * 1000}{Sample\ Volume\ (mL)}$$

Where:

- A = Dry Weight in mg of the suspended solids and filter **after** ignition at 550°C for 15 minutes
- B = Dry Weight in mg of the suspended solids and filter **before** ignition at 550°C for 15 minutes

$$Volatile\ Suspended\ Solids\ (VSS)\left(\frac{mg}{L}\right) = TSS\ \left(\frac{mg}{L}\right) - Fixed\ Suspended\ Solids\ \left(\frac{mg}{L}\right)$$

It is very important that constant weights are achieved. If errors are allowed to accumulate in weighings, the large multiplication factors involved make for large errors in the final results. Temperatures are also very important for the accurate analysis of total suspended solids. Be sure to monitor the oven and furnace temperatures closely. Trying to filter too much sample through the filter will also lead to problems. Successive layers of solids on the filter will lead to smaller effective pore sizes because of the "mat" formed by the solids themselves. Avoid this problem by using smaller sample volumes. If the sample filters quickly, more can be measured out and added.

Total Suspended Solids Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Can't get constant weight (readings consistently vary by more than .5 mg)	Insufficient drying of filter and/or crucible.	Allow more time in drying oven before transferring to desiccator.
	Filter and/or crucible not at ambient (room) temperature when weighed.	Allow more time in desiccator before weighing.
	Desiccant is bad.	Replace or rejuvenate (by drying in oven) the desiccant. It is smart to use an indicator drying agent in the desiccator that changes color when the desiccant is going bad.
	Balance not properly zeroed before each weighing.	Always level and zero the balance before weighing anything.
Filter clogs before entire sample volume is poured through.	Too much sample used.	Reduce sample volume.
Filter develops holes when vacuum is applied.	Too much vacuum pressure.	Reduce vacuum pressure/add an air trap.
Replicate results are not within 10% of each other.	Sample not thoroughly shaken before being poured off.	Always shake the sample just before pouring off to assure a good homogeneous mix. Also rinse all measuring devices into filter apparatus and rinse funnel crucible thoroughly.
Filter weighs less after sample is filtered than it did before sample had been filtered through it.	Insufficient drying time initially. Constant weight not achieved.	Do not proceed with analysis until constant weight has been achieved.

Quality Control for Total Suspended Solids Standard Method #2540-D

Document the Following (Including but not limited to):

Sample Collection

- Duration of Composite
- Flow Proportioning
- Include proportioning factor (i.e., 100 mL/1000 gallons)
- Flow measuring device calibration
- Collection device
- Refrigeration of Sample During and After Compositing Period
- Location
- Representative Nature
- Volume - sufficient to produce > 2.5 mg residue
- Hold Time - max 7 days at 4℃

Equipment

- Size and Type of Filtering Apparatus
- Size and Type Filter
- Calibration of 4 Place Analytical Balance
- Constant Temperature of 103 to 105℃ in Drying Oven

Glassware

- Class A measuring device or auto pipettor
- Clean and Rinse with distilled H2O

Procedure

- Establish constant weight of filter (& crucible) before and after sample filtration
- Thorough rinse of measuring glassware and funnel onto filter
- Desiccant condition

Blank and Duplicate Analysis Schedule

- 10 % replicate required
- 5% duplicate (minimum)
- 100 % Blank (distilled water) suggested
- Report Value to appropriate significant figure.

Reporting Total Suspended Solids Results

TOTAL SUSPENDED SOLIDS BENCH SHEET
ANALYST:
EXACT TIME AND DATE SAMPLE WAS COLLECTED:
EXACT TIME AND DATE SAMPLE WAS ANALYZED:
SAMPLE LOCATION:
SAMPLE VOLUME (mL):
AVERAGE INITIAL WEIGHT OF CRUCIBLE AND/OR FILTER (before filtering sample):
AVERAGE FINAL WEIGHT OF CRUCIBLE AND/OR FILTER (after filtering sample):
AVERAGE FINAL WEIGHT MINUS (-) AVERAGE INITIAL WEIGHT:
RESULT IN mg/L:
**** SHOW ALL CALCULATIONS AND RAW DATA:** Blank Data All weighings to establish constant weight Duplicate or Replicate Data NOTE: For Volatile or "Fixed" Suspended Solids record the weight of the container and dried residue prior to ignitions, the weight of the container and dried residue after ignition in addition to all the TSS data. Show all calculations.

Settled Sludge Volume

Equipment

- Mallory settleometer, a glass or plastic cylinder approximately 7 inches high x 5 inches diameter, a graduated cylinder will not suffice because of the great friction of the cylinder walls.
- Paddle
- Timer

Procedure

1) Take at least a two-liter sample with as little agitation as possible. Keep out of direct sunlight and run immediately.
2) Pour well-mixed sample into the settleometer.
3) Using the paddle, mix well by turning back and forth. then still the solution by holding the paddle motionless and remove the paddle.
4) Immediately set the timer for five minutes and observe the way in which the sludge floc settles. It is important to note the quality of the supernate, turbid or clear, the manner in which the sludge particles floc together, etc.
5) At five-minute intervals, read the level of the top of the floc blanket and record. After 30 minutes, the levels can be read every 10 minutes until 60 minutes.
6) Plot these readings on a graph of Settled Sludge Volume (SSV) versus time.

Calculation

From the SSV test, much can be learned. The SVI, or sludge volume index, can be calculated from the settled sludge volume at 30 minutes and the aeration tank suspended solids (MLS):

$$SVI = \frac{SSV_{30}}{MLSS\left(\frac{mg}{L}\right)} * 1000$$

An SVI of less than 80 is indicative of an old, fast settling sludge, between 80 and 150 is a normal sludge, and above 150 is a young, slower settling sludge.

More useful than the SVI is the information that can be gained by keeping daily graphs of SSV vs. time plotted on tracing paper which is laid over the master graph. By laying a week's graphs over one another, trends can be spotted in the settling characteristics of the sludges. If the lines move steadily lower, the sludge is settling faster, steadily higher indicates the sludge is settling more slowly.

If your sludge is settling very poorly, that is, if the 30-minute reading is greater than 800 mL or so, there are two possible causes. There may be excessive filamentous bacteria present or the concentration of solids in the mixed liquor may be too high. A microscopic examination of the sludge will reveal whether or not excessive filaments are the problem. If this is not the case, a variation on the settled sludge volume test will indicate if a high solids concentration is the problem.

<u>Unchlorinated</u> plant effluent should be added to the settleometer to the 500 mL mark. Mixed liquor is then added to the 1,000 mL mark and the test performed on this 50% dilution is described above. If the

sludge settles at a rate greater than twice that of the undiluted sample, excessive solids concentration in the mixed liquor is responsible for the poor settling.

For example, if the undiluted 30 minutes SSV is 900 mL and the 50% dilution 30-minute SSV is 375 mL, a high solids concentration in the mixed liquor is indicated.

Sources of Error

Samples for SSV testing must be very fresh, any delay in testing will affect the health of the micro-organisms and greatly affect the way the sludge flocs and settles. Samples must be stilled before starting the test. Even very slow circular currents can greatly accelerate the settling rate. Finally, the settleometer must be kept out of direct sunlight because of the effect on settling of convection currents set up by the sun's heat.

QC - Record % solids at 5-minute intervals. Use proper volumes.

Settleable Solids

Equipment

- Imhoff cones, 1 liter
- 2-foot glass or plastic stirring rod
- Timer

Procedure

1) Take at least a 1-liter sample, this can come from composites or grab samples (consult your permit).
2) Mix the sample well.
3) Pour into the Imhoff cone to the 1-liter mark.
4) Set timer for 45 minutes.
5) At the end of this period, run the glass rod gently around the edge of the cone to dislodge solids adhering there, or turn cone slowly, one revolution.
6) Set timer for 15 minutes.
7) Read volume after these 15 minutes (total settling time = 1 hour), allowing for any voids in the solids layer and subtracting them from the total reading.

Error may be introduced into this procedure as with the SSV test, by the action of currents due to sunlight or very cold samples in warm rooms. Failure to allow for the voids in the samples leads to inaccurate readings.

Quality Control For Settleable Solids: Standard Method #2540-F

Document the Following (Including by not limited to):

Sampling

- Grab –
  - Time and Date Collected
  - Time and Date Analyzed
  - **HOLD TIME - 48 hours (max)**
- Location

Glassware

- Wash and Rinse after each use

Procedure

- Settle 45 minutes
- Gently dislodge solids adhering to sides of cone
- Settle 15 minutes longer
- Account for voids
- Record results mL/L

Spin Testing

The centrifuge can be a helpful tool in solids testing. The solids content of any sample thick in solids such as mixed liquors, return sludges, and waste sludges can be approximated by centrifugation. It is necessary that the centrifuge be calibrated against the balance procedure weekly to use this method.

Equipment

- Centrifuge
- 15 mL graduated centrifuge tubes
- Timer

Procedure

1) Shake sample thoroughly before pouring off
2) Pour off duplicate tubes and load in centrifuge on opposite sides (the centrifuge must be loaded evenly).
3) Turn on and set timer for 15 minutes.
4) Shut down and read level of solids in test tubes.

Calculation

The tube readings are multiplied by the factor established from the weighing calibration procedure which should be done on a weekly basis. The same sample should be run both by the spin and the

suspended solids methods. The result obtained from the suspended solids test should be divided by the spin test result for the factor:

$$Factor = \frac{TSS\left(\frac{mg}{L}\right)}{Spin\ Test\ (mL\ )}$$

This factor should be used for all testing during that week. Because the sludge's composition varies so much, it is recommended that separate factors be established for each different type of sludge and be established every week or two weeks.

References

Total Solids:	Standard Methods for the Examination of Water and Wastewater 23rd Edition 2540-B
Fixed and Volatile Solids:	Standard Methods for the Examination of Water and Wastewater 23rd Edition, Method 2540-D
Total Suspended Solids:	Standard Methods for Examination of Water and Wastewater 23rd Edition, Method 2540-D
Settled Sludge Volume:	Operation of Wastewater Treatment Plants, A Field Study Training Program, Volume 2, Pages 377-379
Settleable Solids:	Standard Methods for the Examination of Water and Wastewater 23rd 23rd Edition 2540-F
Spin Testing:	Operations of Wastewater Treatment Plants, A Field Study Training Training Program, Volume 2, Page 367

Dissolved Oxygen

(DO)

VT WSMD Wastewater Program Lab Manual Section #11

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Dissolved Oxygen

Background

Oxygen is present in solution as both the dissolved form and in compounds, including sulfates (SO_4^{-2}), nitrates (NO_3^-), etc. When we measure dissolved oxygen in a solution, we are measuring the free oxygen gas dissolved in the solution. The dissolved oxygen is the oxygen that is available for use in respiration, or breathing, by fish, aquatic insects, plants, and aerobic bacteria. Without dissolved oxygen, the waters go septic, and the result can be unsightly and foul-smelling. Oxygen enters water from the air through the surface by diffusion and happens much more rapidly in waterfalls, rapids, and riffle areas than in quiet pools. Oxygen also enters the water from the daytime photosynthetic activities of aquatic plants. The levels of dissolved oxygen or DO in streams varies from a low of 0-3 mg/l for polluted waters to a high of 12-15 mg/l in clear, very cold streams. Seven to nine (7-9) mg/l is the average in natural waters. The levels are dependent on: 1) physical conditions such as rapids, dams, pools and temperature of the water; 2) chemical action such as rusting, etc.; and 3) biological activity going on in the water such as that of fish, insects, bacteria, and plants.

We test for DO in treatment plant effluents to determine the amount there, to aid in the stream's natural waste stabilization process; in secondary plant processes to determine if the amount of DO present is sufficient to maintain good aerobic biological treatment; and in influents to avoid septic conditions which may result in the formation of hydrogen sulfide and its corrosive results.

Methods of Measurement

The three methods of dissolved oxygen measurement described in this document are the Iodometric /Winkler titration method with azide modification, the membrane electrode method, and the Luminescent Dissolved Oxygen method. Since the acceptance of the Luminescent Dissolved Oxygen Method by EPA, it has become the most popular and commonly used method for dissolved oxygen and Biochemical Oxygen Demand measurement in Vermont (and with good reason)! The meter and probes are capable of reliable self-calibration, there are no membranes to change, and very little maintenance is required. Unfortunately, the equipment is more expensive than the alternative membrane versions. The method has proven to be very accurate and user friendly and will be described first.

Luminescent Dissolved Oxygen/Optical Probe Method

The Luminescent Dissolved Oxygen (LDO) probe measures the time it takes for a specific luminescent reaction to take place. Blue light is transmitted from light emitting diodes (LED) on the probe to a sensor. Luminescent material on the probe is “excited” by the blue light. As the material becomes less excited at a specific point it emits a red light. The probe measures the amount of time from when the material becomes excited by the blue light until it relaxes enough to emit the red light. The higher the oxygen concentration the shorter the time it takes for the red light to be emitted. (Oxygen “quenches” the luminescence). That amount of time is directly proportional to the concentration of oxygen. Between the blue light flashes, a separate LED emits red light onto the probe sensor. This serves as an internal standard.

These LDO probes are capable of very accurate measurements from 0.05 mg/Liter to 20.00 mg/Liter. After the initial setup the probes are self-calibrating and require very little maintenance besides thorough rinsing of the probe between readings.

There are some interferences with this type of probe that must be considered. High concentrations of Chlorine dioxide can have a detrimental effect. Bacterial or algal growth can cause problems, but this can be avoided by good rinsing practice. Oils are a problem with all DO probes. Again, rinsing minimizes this problem. Finally, alcohols and some organic solvents can damage the probe.

Equipment

- Luminescent Oxygen probe with stirrer and appropriate meter

Reagents

- Sodium Sulfite solution - 2M solution for blank
- Cobalt chloride 0.03 g/L

Add the 0.03 g/L Cobalt chloride to the 2M Sodium sulfite solution. This makes a blank solution of zero mg/L Dissolved Oxygen. * The 23rd edition of Standard Methods for the Examination of Water states "Add excess sodium sulfite and a trace of cobalt chloride to bring DO to zero."

Procedure

Calibrate the probe per manufacturer's instructions. Note: Many of the LDO probes have built in calibration. For daily or more frequent quality control purposes the instrument's automatic calibration is enough. Occasional checks with a blank and a control are recommended but not required.

Note: If analyzing for Biochemical Oxygen Demand, more frequent verification of calibration is required.

To prepare a water- saturated air sample, simply add a small amount (10 to 25 mL) of reagent grade water to a 300 mL BOD bottle. Place the glass stopper on the bottle and shake it vigorously for about 30 seconds. Let the bottle sit for approximately 30 minutes to let the water equilibrate to room temperature (which should be 20 °C +/- 2 °C) then place the DO probe into the BOD bottle and take the reading. You must note the barometric pressure and temperature at your laboratory at the time of analysis and use table 4500-O:II (from 23rd edition Standard Methods for the Examination of Water and Waste) to get the theoretical DO concentration. The meter reading must be within +/- 10% of the theoretical value. If not, the probe must be re-calibrated.

Example I: Assume you have completed the calibration procedure described above at a temperature of 20 °C with a barometric pressure reading (at your lab) of 750 mm of mercury. The meter reading is 9.1 mg/L. Check this reading against the theoretical value in Table 4500-O:II. You will see that the meter is reading exactly as it should. The calibration has verified the meter reading.

Example II: Assume you have completed the calibration at a temperature of 25 °C with a barometric pressure reading (at your lab) of 625 mm of mercury. The meter reading is 9.1 mg/L. Check the reading against the theoretical value in Table 4500-O:II. In this case you see that the theoretical value is 6.8 mg/L. The meter reading is NOT within +/-10% of the theoretical value. The meter must be recalibrated.

Once the calibration is completed, fill each BOD bottle to the top of the neck being very careful not to introduce oxygen. (This is best accomplished by letting the sample slowly flow down the inside of the bottle). Place the probe in the sample being careful not to entrap air, turn on the stirrer and take the reading.

Quality Control

Unless this analysis is required for reporting purposes in your permit, quality control procedures are not required. Recommended quality control procedures include the use of a blank, a control and 10% replication or duplication of sample analysis.

Reporting Dissolved Oxygen Results: Luminescent Electrode Method

Dissolved Oxygen (Luminescent Electrode Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE
SAMPLE VOLUME (Method):
SAMPLE TEMPERATURE: METER STANDARDIZATION METHOD: (atmospheric, Winkler, auto, etc.)
RESULT in mg/L:
Reporting is the same as described for the membrane electrode method

Membrane Electrode Method for Dissolved Oxygen Analysis

The membrane electrode method is based on the fact that the diffusion of dissolved oxygen across the electrode membrane produces a change in the potential of the electrode. This voltage change is measured by the DO meter. These electrodes, like pH electrodes, are sensitive to changes in temperature but most modern DO meters are equipped with automatic temperature compensation circuits. This method is especially useful for sludges, fast measurements in the plant, respiration rates and, when equipped with a stirring bottle probe, for BOD measurements.

Equipment and Reagent

- DO meter, YSI model 51, 54, 57, etc. or equivalent
- DO probe
- spare membranes
- thermometer
- probe filling solution
- razor blade (if using older style probe)

Calibration of DO Meter

DO meters can be calibrated by any of three methods. These are: atmospheric calibration, barometric calibration and calibration against the Winkler method. The atmospheric calibration method is fast and simple and is fine for in-plant process control testing but it is not generally a recommended method when calibrating for BOD readings. For BOD analysis, the DO meter should be calibrated using either the barometric or Winkler method. Each of these methods will be discussed below. *NOTE: Many of the newer Dissolved Oxygen meters have built in atmospheric calibration. The meter atmospheric pressure reading should be checked against a certified barometer at least annually (more often if there is reason to believe the reading is inaccurate).

Before going through the entire process of calibration it is always a good idea to check the membrane on the DO probe. Membranes must be replaced quite often. Some indications of the need for membrane replacement are: any sign of bubbles under the membrane, drifting of the meter and the inability to reach calibration. To replace the membrane, follow the manufacturer's instructions closely. Look for bubbles!

Once the membrane has been replaced:

1) Attach probe to meter.
2) Set master control to "red line" and adjust needle so that it lines up with the red line on the face with the "red line" knob.
3) Set the master control to "zero" and adjust the needle to zero with the zero knob.
4) Switch the master control to "calibrate" and allow the probe 15 minutes to polarize. Then proceed with one of the following methods of calibration.

NOTE: The probe should be stored in a wet environment; the sponge in the plastic cap provided, should be wetted.

Atmospheric Calibration

With the probe polarized, read the room temperature and consult the saturation value either in Standard Methods or printed on the back of the meter. This value should be corrected by the atmospheric correction factor given on the back of the meter and this corrected value set on the meter with the "calibration" knob. For example, if the thermometer reads 19 °C and the plant is located at about 500 feet above sea level:

- 19 °C saturation value is 9.3 mg/l
- 500 feet correction factor is 0.98
- 9.3 mg/l x .98 - 9.1 mg/l

The value of 9.1 mg/l should be set on the meter. The meter is now calibrated and ready to use.

Winkler Calibration

(Use with DO bottle probes with stirrer for BODs). With the probe polarized, fill a graduated cylinder with distilled water. Siphon this out carefully into 3 DO bottles. Put the probe into one bottle and perform a Winkler titration on the other two bottles as described previously. Calibrate the meter to the average of the two Winkler results.

Most DO probes need a minimum flow rate past the probe in order to read accurately. In moving water, this is not so much a problem but when taking DOs on clarifiers, lakes, or BOD bottles, etc., some means of agitation must be provided. In BOD bottles, magnetic stirrers can be used if your bottle probe is not equipped with its own stirrer. In large, quiet bodies of water, the lead to the probe can be moved up and down to provide an artificial flow.

Barometric Calibration

The third method for calibrating a DO meter is called barometric calibration. It is quicker to calibrate the meter by this method than by the Winkler calibration and it is permissible to utilize this method when using the meter for BOD analysis.

The meter should be warmed and polarized. The DO probe is put in a DO bottle partially filled (50 mL or so) with water and is allowed 15 minutes to equilibrate. The temperature of this water vapor is then taken and by using the chart below the solubility of oxygen at this temperature is determined. For example, assume the temperature is 21 °C. The chart below tells you that the solubility of saturated water vapor(s) at 21 °C is 8.9 mg/L. **However, most current DO probes automatically correct for temperature – check with the manufacturer to determine whether you need to manually perform this step.**

Solubility of Oxygen in Water Exposed to Water-Saturated Air

Temperature ° C	Oxygen Concentration in Water mg/L
10	11.27
11	11.01
12	10.76
13	10.52
14	10.29
15	10.07
16	9.85
17	9.64
18	9.45
19	9.26
20	9.07
21	8.90
22	8.72
23	8.56
24	8.40
25	8.24
26	8.09
27	7.95
28	7.81
29	7.67
30	7.54

If the barometer being used gives the barometric pressure reading in inches, use the following equation to calibrate the solubility at which the DO meter should be set:

$$S^1 = \frac{S * P}{29.92}$$

Where:

- S^1 = The solubility that you will set the meter at
- S = the solubility of saturated water vapor at 101.3 KPa (from the chart in column under “oxygen concentration in water”)
- P = Barometric pressure, in inches
- 29.92 = a constant

In the example, the calculation works out:

$$S^1 = \frac{8.90 * 29.75}{29.92}$$

$$S^1 = 8.849, rounds\ to\ 8.85\ considering\ significant\ digits$$

A different equation is used when the barometric pressure is measured in mm Hg. In this case the following equation should be used.

$$S^1 = \frac{S * P}{760}$$

Where:

- S^1 = The solubility that you will set the meter at
- S = the solubility of saturated water vapor at 101.3 KPa (from the chart in column under “oxygen concentration in water”)
- P = Barometric pressure, in mmHg
- 760 = a constant

At the same temperature (21 °C) and same barometric pressure (29.75" = 745 mm Hg), the example calculation becomes:

$$S^1 = \frac{8.90 * 756}{760}$$

$$S^1 = 8.853\ (rounds\ to\ 8.85)$$

These directions hold true for elevations less than 1,000 meters (3,281') and for temperatures below 25 °C.

Quality Control

For accurate data, it is important that all reagents are standardized correctly in the frequency required. The DO meter is standardized daily (each use) by the means outlined above and properly maintained to insure optimum performance.

Dissolved Oxygen Troubleshooting Guide: Membrane Electrode Method

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Air bubbles appear at the top of the BOD bottle after probe is inserted.	Membrane not cut around 'o' ring - (older type probes). Air is captured under membrane	Trim excess membrane just above 'o' ring.
Drifting, erratic reading **OR** Cannot get meter properly standardized.	Crease in membrane **OR** filling solution low.	Replace membrane; add fresh potassium chloride filling solution – Re-standardize meter and re-run analysis.
	Flow rate of sample past the probe membrane is insufficient.	Increase meter speed - If probe stirrer is malfunctioning use a magnetic stirrer.
	Probe is not allowed to polarize before analysis.	Turn meter on 15 minutes before standardization or analysis.

Quality Control for Dissolved Oxygen Analysis: Membrane Electrode Method

Document the Following (Including by not limited to):

Sampling

- Grab -
  - Exact Time and Date Sampled
  - Exact Time and Date Analyzed
- Location
- Temperature

Glassware

- Properly washed and rinsed

Instrument

- Calibration and Maintenance
- Method of Standardization
  - Date
  - Analyst's initials

Duplication/Replication Schedule

- Minimum of 5%, 10% is recommended

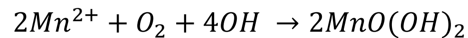
Reporting Dissolved Oxygen Results: Membrane Electrode Method

Dissolved Oxygen (Membrane Electrode Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME (method):
SAMPLE TEMPERATURE:
METER STANDARDIZATION METHOD: (Atmospheric, Winkler, etc.)
RESULT in mg/L:

Winkler Titration Method

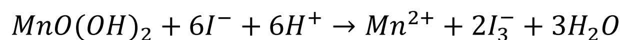
The Winkler procedure is based on the oxidizing property of DO or the tendency of free oxygen to attach to certain ions. The azide modification is used to eliminate the interference of nitrite, which is found in many biologically treated effluents, some streams at certain times of the year, and in the BOD test.

In the presence of dissolved oxygen, Mn2+ (the manganous ion) from DO solution #1 (manganous sulfate) reacts with the dissolved oxygen under alkaline conditions supplied by the addition of DO solution #2 (the alkali-azide-iodide solution) to form a brown (manganic hydroxide) floc.



$$2Mn^{2+} + O_2 + 4OH \rightarrow 2MnO(OH)_2$$

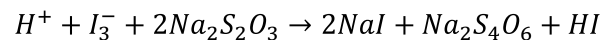
With the addition of DO solution #3 (sulfuric acid), the oxidized manganese is reduced back to the divalent form, and iodine is released (from the iodide ions supplied in DO solution #2.)



$$MnO(OH)_2 + 6I^- + 6H^+ \rightarrow Mn^{2+} + 2I_3^- + 3H_2O$$

The amount of iodine formed is proportional to the amount of dissolved oxygen originally present in the solution.

By titrating with a standard solution of Sodium Thiosulfate we can measure the amount of iodine present which as stated is directly proportional to the DO concentration.



$$H^+ + I_3^- + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6 + HI$$

The addition of starch indicator to the solution before titration makes endpoint determination easier by producing a dark blue color which contrasts the colorless endpoint.

Sampling Handling and Preservation

Samples for DO should be taken with a minimum of turbulence, to avoid air entrapment, into a 300 mL DO bottle. The bottle should be filled into the neck and stoppered tightly. * It is very important NOT to agitate the sample or allow it to remain in contact with the ambient air. Samples should be doped (the DO solutions added) and titrated immediately, although they can be held for up to eight (8) hours if fixed (Manganous sulfate and alkali-iodide solutions added) and stored in the dark (true for Winkler Method only). Then add the sulfuric acid and shake the sample. *Note: If there is an appreciable iodine demand in the sample (an iodine demand might be expected if there is a high concentration of alkali metals such as aluminum or mercury in the sample) preserve the sample with 0.7 mL of sulfuric acid and 1 milliliter of sodium azide solution. Place the glass stopper in the bottle, create a water seal and cover with the plastic cap. Perform the analysis as soon as possible!

When a Kemmerer sampler is used, the BOD sample bottle should be filled to overflowing. (Overflow for approximately 10 seconds.) The outlet tube of Kemmerer should be inserted to bottom of BOD bottle. Care must be taken to prevent turbulence and the formation of bubbles when filling the bottle.

At the time of sampling, the sample temperature should be recorded as precisely as required.

Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron.

Winkler-Iodometric Method (w/ azide modification)

300 mL (Full Bottle)

Equipment

- 3 2 mL automatic or graduated pipets
- 2 500 mL Erlenmeyer flasks
- burette, 50 mL or 10 or 25 mL automatic filling burette
- 2 dropping bottles, for starch and back titrant solution
- 10 mL volumetric pipet
- 100 mL graduated cylinder
- magnetic stirrer (optional)
- sample bottles - 300 mL (+ 3 mL) capacity BOD incubation bottles with tapered round glass pointed stoppers and flared mouths

Reagents

- Manganous Sulfate ($MnSO_4$) Solution DO Solution #1

  Dissolve 480 g MnSO4 - 4H2O in distilled water and dilute to 1 liter.

  Alternatively, use 400 g of MnSO4 2H2O or 364 g of MnSO4H2O per liter.

- Alkaline Iodide-Azide (OH-,I-,NaN_3) Solution DO Solution #2

  Gradually add and dissolve 500 grams of reagent grade Sodium Hydroxide (NaOH) OR 700 grams Potassium Hydroxide (KOH) in 600 mL distilled water in a 2 liter beaker. In another beaker add 135 grams Sodium Iodide (NaI) OR (NaN3) in 40 mL of distilled water. Carefully add this solution to the Sodium Hydroxide solution, cool to room temperature, pour into one-liter volumetric flask, and dilute to one-liter with distilled water.

- Sulfuric Acid (H_2SO_4) Concentrated, 36N DO Solution #3
- Sodium Thiosulfate ($Na_2S_2O_3$), Stock Solution, 0.75N:

  Dissolve 186.15 g $Na_2S_2O_3 \bullet 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 mL chloroform.

- Sodium Thiosulfate ($Na_2S_2O_3$), Standard Titrant, 0.0375N:

  Prepare by diluting 50.0 mL of stock solution to 1 liter. Preserve by adding 5 mL of chloroform. Standard sodium thiosulfate, exactly 0.0375N is equivalent to 1.0 mg of DO per 1.00 mL. Standardize with 0.0375N potassium bi-iodate. SEE STANDARDIZATION PROCESS IN APPENDIX I AT THE END OF THE DISSOLVED OXYGEN SECTION.

  ***NOTE**: 0.0375 N Phenylarsine Oxide can be used in place of the 0.0375 N Sodium Thiosulfate solution as a titrant. To prepare this solution, dissolve 6.30 grams of Phenylarsine Oxide crystals in 700 mL of freshly boiled and cooled distilled water. Dilute to 1 Liter with distilled water. Preserve with 2.5 mL Chloroform.

- Potassium Bi-Iodate ($KH(IO_3)_2$) Stock Solution, 0.15N

This stock solution of Potassium Bi-iodate if stored in an amber glass bottle with screw cap, and refrigerated, has a shelf life of approximately 6 months and can be used to make up the working solution of Potassium Bi-iodate every 2 weeks to 1 month.

After drying 5 grams of reagent grade Potassium Bi-iodate for 2 hours at 103 °C, weigh out 4.873 grams and dissolve this in 600 mL distilled water. Dilute to 1 liter with distilled water.

- Potassium Bi-Iodate ($KH(IO_3)_2$) Primary Standard, 0.0375N

  Dilute exactly 250 mL of the 0.15N Potassium Bi-iodate solution to 1 liter with distilled water. Store in an amber glass bottle with screw top. Discard after 1 month.

  *NOTE: The primary standard potassium bi-iodate KH(IO3)2 should be used in place of potassium dichromate, for standardization of the thiosulfate solution. Potassium bi-iodate seems to eliminate the return of the blue color that is titrated out.

- Starch Indicator Solution

  Prepare an emulsion of 10 grams soluble (potato) starch in a beaker with a small amount of distilled water. Pour this emulsion into 1 liter of boiling distilled water and allow to boil for a few minutes. Let the solution settle overnight and pour off the semi-clear portion. Discard the thick substance left in the bottom of the container. Preserve the starch solution by adding 1 g salicylic acid, or 5 mL Chloroform or a couple of drops of Toluene. This solution should be kept refrigerated.

Procedure: 300 mL Method

1) Remove the stopper and add 2 mL solution #1 (MnSO4). Hold the pipet just above the liquid surface and add 2 mL solution #2 (OH-, I-, N3-) the same way, slowly and just above the sample surface, to avoid adding any air bubbles.
2) Re-stopper and mix by inverting the bottle 10 to 15 times.
3) Allow the bottle to settle so that the brown floc occupies ½ the bottle volume or less.
4) Invert the bottle again 10 to 15 times.
5) Allow the floc layer to settle again to ½ the bottle volume or less.
6) Carefully, add 2 mL concentrated H2SO4, solution #3.
7) Stopper and shake until the solution is uniformly mixed.
8) Pour the entire contents into a 500 mL Erlenmeyer flask.
9) While swirling the flask, titrate with 0.0375N sodium thiosulfate solution from the starting orange-yellow until it becomes a pale-yellow color. (0.0375N phenylarsine oxide (PAO) may be substituted as a titrant.)
10) Add 1 mL starch solution, the sample will turn blue.
11) Continue titration until the blue goes to clear.
12) Check the endpoint by back-titration (as described on page 6 of this section).

    NOTE: Occasionally, a dark brown or black precipitate persists in the bottle after acidification. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few drops of H2SO4 will effect dissolution.

Calculation

When using a 300 mL sample (full DO bottle) and 0.0375N sodium thiosulfate, then 1 mL thiosulfate used = 1 mg/l DO present in sample.

The Winkler method with azide modification is not applicable under the following conditions: a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; b) samples high in suspended solids; c) samples containing organic substances which are readily oxidized in a highly alkaline solution or which are oxidized by free iodine in an acid solution; d) untreated domestic sewage; e) biological flocs; and f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

Dissolved Oxygen Troubleshooting Guide for Winkler Titration Method with Azide Modification

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Cannot get floc to dissolve completely after addition of sulfuric acid (DO #3)	High DO in sample. Insufficient quantity of acid added to dissolve the iodine formed. Insufficient mixing.	Add more sulfuric acid until the entire floc is dissolved. Mix by inversion. Continue to mix by inversion.
A white floc forms after the addition of manganous sulfate (DO #1) and the alkaline iodide - azide (DO #2) solutions. * Referred to as a "snowball" or "white-out".	Very little or no dissolve oxygen present in sample.	Add the sulfuric acid solution after allowing the floc to settle. If the sample turns clear (or milky) there is no dissolved oxygen in the sample.
Difficult to determine trace endpoint - "Fading" endpoint.	Background color interfering with reading. Starch solution weak. The starch indicator solution was added without first titrating the sample to a pale straw yellow color.	Use a white burette stand or place a white piece of paper under the flask to help differentiate clear from light blue sample appearance. Prepare fresh solution - Re-run analysis. Re-run the analysis. Before adding the starch indicator solution titrate the sample to a light - straw yellow color. Then add starch indicator and continue the titration to the clear endpoint.

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Result is unreasonably high.	Improper concentration of Sodium Thiosulfate titrant (too weak).	Re-standardize the titrant to 0.025N for the 203 mL version of the test or 0.0375N for the 300 mL version. Re-run analysis.
	Sample has a chlorine residual.	Neutralize any chlorine residual before performing the analysis.
Result is unreasonably low.	Improper concentration of sodium thiosulfate titrant (too strong).	Re-standardize the titrant. Re-run analysis.
	The sample contains >5 mg/l ferric iron salts.	Add potassium fluoride (approximately 1 milliliter) to the sample before adding the manganous sulfate (DO #1) solution **OR** Add an 80% to 85% solution of phosphoric acid in place of the sulfuric acid for acidification of the sample (approximately 1 milliliter).

Quality Control for Dissolved Oxygen: Winkler Titration w/ azide modification

Document the following:

<u>Sampling</u>

- Grab
    - Exact time and Date sampled
    - Exact time and Date analyzed
- Volume
- Location
- Temperature

<u>Glassware</u>

- 300 mL version
- Glassware properly washed and rinsed

<u>Reagents</u>

- NIST traceable
- Preparation and Expiration dates

Reporting Dissolved Oxygen: Winkler Titrant Method

Dissolved Oxygen (Winkler Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME (method):
SAMPLE TEMPERATURE:
ANY MODIFICATION TO NEGATE INTERFERENCES:
BURET READING: Start ______________ Endpoint _______________
AMOUNT OF BACK TITRANT USED TO PRODUCE BLUE COLOR:
CALCULATIONS (Back Titration):
RESULTS IN mg/L: Reagent lot #s and expiration dates:

Dissolved Oxygen on Activated Sludge Using Winkler Method

Since activated sludge is a biological floc that will continue to use oxygen after taking a sample, it is necessary to stop the oxygen uptake as soon as possible to get a valid result. This is done by adding copper sulfate-sulfamic acid to the flocculate and settling out the activated sludge providing a clear supernatant on which to perform the Winkler method.

Equipment

In addition to the equipment and reagents required for the Winkler Method, you will need:

- 1 liter glass stoppered bottle

Reagents

- Copper Sulfate-Sulfamic Acid ($CuSO_4$-NH_2SO_2OH) Inhibitor Solution

  Dissolve 32 g of technical-grade NH2SO2OH in 475 mL distilled water. (DO NOT use heat to help dissolve). Dissolve 50 g CuSO4 - 5H2O in 500 mL distilled water. Mix these two solutions and carefully add 25 mL concentrated acetic acid.

Procedure

1) Add 10 mL CuSO4-NH2SO2OH inhibitor to the 1 liter glass stoppered bottle.
2) Take a sample of the activated sludge by placing the 1 liter bottle in a special sampler that fills the bottle via a tube near the bottom.
3) Fill the bottle to the very top and stopper the bottle immediately and mix by inverting several times.
4) After suspended solids have settled, siphon off the clear layer (supernatant) into a 300 mL DO bottle being careful not to introduce any air by turbulence. Stopper the bottle tightly.
5) Continue Winkler method as described in Section 11 on Page 3 or Page 7 of this manual.

It is important to be aware of the difficulty of avoiding air entrapment into the bottle. It is recommended that a DO probe be used in the actual tank when DOs less than 1 mg/l are present.

Troubleshooting, quality control and reporting are the same as described for in the Winkler Titration method with azide modification here.

References

Winkler Method (with Azide Modification): Standard Methods 23rd Edition 4500-OC (General Reference)

The Membrane Electrode Method for the analysis of dissolved oxygen can be found in pages 4-149 through 4-152 in the 23rd Edition of Standard Methods for the Examination of Water and Wastewater.

The Copper Sulfate-Sulfamic Acid method used for analysis of DO in activated sludge is described on page 4-149 of Standard Methods for the Examination of Water and Wastewater – 23rd Edition.

APPENDIX I

Standardization of 0.0375N Sodium Thiosulfate

Run in duplicate:

1. Dissolve 2 g potassium iodide, KI, in 100 mL distilled water in a 500 mL Erlenmeyer flask.
2. Slowly, while stirring, add 10 mL 10% sulfuric acid, H2SO4.
3. Add 20.0 mL (use a volumetric pipet) of 0.0375N potassium bi-iodate (KH(IO3)2 and mix.
4. Place flasks in dark for 5 minutes
5. Dilute to approximately 400 mL with distilled water.
6. Titrate with approximately 0.0375N sodium thiosulfate, to a pale straw color, add starch and titrate until blue color disappears.
7. Should use exactly 20 mL of thiosulfate if the normality is exactly 0.0375.

If you use more than 20 mL, the thiosulfate is weaker than 0.0375N, if you use less than 20 mL, it is stronger. If the thiosulfate is too weak, adjust the normality to 0.0375N by adding thiosulfate crystals; if it is too strong, adjust by diluting with distilled water. Two examples follow:

Example 1

For 0.0375N Sodium Thiosulfate:

$$N_1V_1 = N_2V_2$$

$$0.0375N * 20\ mL = N_2 * 21.40\ mL$$

$$0.75 = N_2 * 21.40\ mL$$

$$N_2 = \frac{0.75}{21.40}$$

$$N_2 = 0.350N$$

The thiosulfate solution is too weak and must be adjusted upward by adding thiosulfate crystals. For every increase of 0.0001N you wish to make in a liter of thiosulfate, add 0.0248g of crystals. In this example, 0.0375N - 0.035N is an increase of .0025N desired. Therefore, you want to add 25 x .0288g of crystals or 0.62g to a liter.

Example 2

For 0.0375N Sodium Thiosulfate: 19.35 mL thiosulfate used in the standard titration. Again, using the equation $N_1V_1 = N_2V_2$, the normality of the thiosulfate is calculated:

$$N_1V_1 = N_2V_2$$

$$0.0375N * 20\ mL = N_2 * 19.35\ mL$$

$$0.75 = N_2 * 19.35\ mL$$

$$N_2 = \frac{0.75}{19.35}$$

$$N_2 = 0.388N$$

The thiosulfate solution is too strong, and the solution must be diluted to the proper strength. To find out show much distilled water to add, again use the formula $N_1V_1 – N_2V_2$

$$N_1V_1 = N_2V_2$$

$$0.0388N * 961\ mL = 0.0375N * V_2$$

$$V_2 = \frac{37.29}{0.0375}$$

$$V_2 = 994.4\ mL$$

We need to add 994.4 - 961 mL of distilled water = 33.4 mL.

That is, to the 961 mL of sodium thiosulfate remaining we add 33.4 mL of distilled water.

Biochemical Oxygen Demand

(BOD)

VT WSMD Wastewater Program Lab Manual Section #12

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Biochemical Oxygen Demand

Background

We perform the BOD analysis to determine the amount of oxygen required to biochemically break down the organic material in wastewater and the oxygen used to oxidize inorganic materials such as sulfides and ferrous iron. Unless an inhibitor is used, the BOD test may also measure the amount of oxygen required to convert ammonia to nitrates. This is referred to as the nitrogenous BOD.

In the BOD analysis proper dilutions of samples are prepared. Initial dissolved oxygen readings are then taken. After a five-day incubation period at 20℃ +/- 1℃, Dissolved Oxygen readings are again taken, and calculations are performed based on the difference between these readings and readings taken before incubation.

NOTE: Some operators use the Chemical Oxygen Demand (COD) analysis to estimate the BOD. The ratio of COD to BOD varies greatly between facilities and even within a facility, especially at facilities with varying industries discharging to them. Whereas a BOD analysis depends upon the microbiology (bugs) to break down the organic matter, the COD “burns” organic matter using strong acid(s) and high temperature. The great advantage of this analysis (COD) is that it can be completed in a matter of hours as opposed to the BOD which takes 5 days. What might be considered a disadvantage is that the COD, by nature of “burning” all organics in a short period of time, includes organic materials (such as many solvents) that would not be metabolized (broken down) in the 5-day period allowed for in the BOD analysis. Further, by “burning” all organics in such a short period, the analysis does not allow for the nitrogenous demand that would be observed in a 5-day BOD. Even accounting for these inherent differences, the COD results can be obtained in an hour and can be a good indicator of BOD results that aren’t available for 5 days. Further information regarding the analysis of Chemical Oxygen Demand can be found in section 5220 of the 23rd edition of Standard Methods for Analysis of Water and Wastewater

Or at this link from the Hach® company: DOC312.53.94012_1Ed_LCK314 (1).pdf

BOD Sampling and Preservation

Samples for analysis of BOD are generally required to be collected as 24-hour composites (a sample composed of a number of discrete grab samples collected over the entire sampling period). The composite samples should be properly flow proportioned (representative of the volume and nature of the discharge over the sampling period). Some Vermont facilities such as lagoons and industries that operate only during a single shift, are allowed by permit to collect 8-hour composites. The same rules apply to this sample (i.e. must be properly flow proportioned based on an 8-hour sampling period.) Flow proportioning is required if the hourly flow varies by more than 10% over the sampling period.

If, for process control purposes, analysis of a grab sample is desired, this sample does not need to be refrigerated if analyzed within 2 hours. Samples collected for analysis per the facility’s NPDES permit. MUST be maintained at a temperature at or below 6 °C, but not freezing, for the entire sampling period and until the analysis is begun.

If using an automatic sampler for collection of a composite sample the unit must be maintained at 6 °C during the entire composite period.

If manually taking the discrete samples for a composite, each discrete sample must be maintained at 6 °C until compositing takes place. The composite must then be kept at 6 °C until analysis takes place.

The BOD analysis must begin no more than 48 hours from the time the last discrete sample is collected. It is preferable to perform the analysis within six hours from the time the last sample is collected.

Just before analysis, the sample must be warmed to 20 °C. This can be accomplished by placing the refrigerated sample container into a sink or other basin partially filled with warm water to bring the temperature up fairly quickly but not so fast as to adversely affect the nature of the sample (approximately 15 minutes). The sample **MUST** be brought to 20 °C +/- 2 °C before beginning the analysis.

Equipment

- **Air Pump**. Used as a means of aerating the dilution water. Shaking also works depending on the volume of dilution water required. (It's difficult to shake 10 gallons of dilution water!)
- **Beakers, Assorted Sizes**. Always pour reagents into small beakers before pipetting to avoid contamination of the entire reagent bottle.
- **Carboy**. To contain the dilution water. The size needed depends on the number of samples you expect to run. We attach a rubber hose to this for delivery of the dilution water into the cylinders.
- **Dissolved Oxygen Bottles**. These bottles must have a flared mouth with ground glass stoppers and are generally 300 mL in volume.
- **Dissolved Oxygen Meter**. With a stirrer type probe, specially designed to fit into D.O. bottles. Alternatively, a magnetic stirrer can be used but this necessitates the removal of the magnetic bar before incubation or disposal.
- **Filter**. Used as a means of aerating the dilution water.
- **Graduated or volumetric Cylinders, Assorted Sizes**. For measuring the sample, etc.
- **Incubator**. The incubator must be capable of maintaining a constant temperature of 20 °C (plus or minus just 1 °C).
- **Mixer**. The Propeller type mixer can be used to mix the sample just before delivering into the D.O. bottles.
- **Overcaps**. Placed over the flared mouth of the D.O. bottles to reduce evaporation of the water seal during incubation.
- **pH Meter**. The pH of the sample must be 6.5 to 7.5.
- **Pipette**. For measuring small volumes of sample and reagents.
- **Pipette Bulb**. For drawing reagents or sample into pipette. Never mouth pipette.
- **Refrigerator**. Capable of maintaining 6 °C. For storage of reagents and sample.
- **Stand**. A stand or shelf used to set carboy on for elevation, which is helpful in delivering the dilution water into the BOD bottles.
- **Thermometer**. Thermometers must be NIST traceable or annually calibrated against either an NIST or NIST traceable thermometer.
- **Tubing**. Used as a means of aerating the dilution water.

NOTE: The following four equipment pieces are necessary if using the Iodometric Method for either D.O. measurement or meter standardization.

- **Burette and Burette Stand**. A 25 mL burette with 0.1 mL graduations is used for titration of the sodium thiosulfate solution.
- **Dissolved Oxygen Bottles With Auto Pipettes**. These are used to store the reagents for D.O. analysis. The auto pipettes can be set to consistently deliver 1 mL of each of the reagents.
- **Dropping Bottles**. These bottles with dropper caps are handy for storing the starch and back titrant solutions.
- **Erlenmeyer Flasks**. 500 mL wide mouth flasks are used during the titration process.

Reagents

NOTE: For accuracy, Class A volumetric flasks/pipettes must be used when making reagents.

The phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solutions listed below can be purchased in premade/combined powder pillows or reagent packets. Use of these pre-made reagents is highly recommended.

- Phosphate Buffer Solution:
  Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.
- Magnesium Sulfate Solution:
  Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.
- Calcium Chloride Solution:
  Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.
- Ferric Chloride Solution:
  Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.
- Acid and Alkali Solutions, 1N:
  For neutralization of caustic or acidic water samples.
  - Acid - Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.
  - Alkali - Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.
- Sodium Sulfite Solution, 0.025N:
  Dissolve 1.575 g Na_2SO_3 in distilled water and dilute to 1 liter (or 0.788 g to 500 mL). This solution is not stable; prepare daily.
- Nitrification Inhibitor, 2-Chloro-6-(Trichloro methyl) pyridine:
  This can be purchased from the Hach Chemical Company or other laboratory supply vendors.
- Glucose-Glutamic Acid Solution:
  Dry reagent-grade glucose and reagent-grade glutamic acid at 103 °C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use. This can be purchased pre-made. Glucose-Glutamic Acid (GGA) may be purchased in ampules.

- Ammonium Chloride Solution:
  Dissolve 1.15 g NH_4Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

If performing the Winkler Titration Method for determination of initial and final Dissolved Oxygen readings or for standardizing the D.O. meter, all reagents listed under the heading in Section 11 on Pages 14 - 15 are needed.

Dilution Water Preparation

Dilution water consists of high-quality distilled water to which we add nutrients and a buffering solution (to optimize the conditions for bacteriological growth). The use of deionized water is not recommended as organic compounds can leach through from the resins. It is extremely important that the oxygen depletion of the dilution water is 0.2 mg/L or less. Several suggestions for producing dilution water of this quality are offered in the 23rd Edition of Standard Methods.

To the carboy containing distilled water add one milliliter each of calcium chloride, magnesium sulfate, ferric chloride, and phosphate buffer solutions per liter of distilled water, then aerate using the air pump, filter, and tubing. Alternatively, powder pillows/packets prepared for specific dilution water volumes can be used.

Take the samples out of the refrigerator and begin the process of bringing the sample temperature up to 20°C. Place the carboy in an elevated location to facilitate draining.

While the sample is warming up, turn on the D.O. meter, prepare the G.G.A. and sodium sulfite solutions, decide what dilutions of sample to use, and set up and label the glassware to be used.

Dissolved Oxygen Meter Standardization

Please refer to the Dissolved Oxygen Section of this manual for instructions describing meter standardization methods

Sample Pretreatment

pH

The pH of the sample must be between 6.5 and 7.5. Samples can be neutralized using either sulfuric acid or sodium hydroxide.

Temperature

The sample temperature must be brought up to 20 °C before beginning the analysis.

DO NOT BEGIN THE ANALYSIS UNTIL THE SAMPLE HAS WARMED TO 20 °C.

Dechlorination

The dechlorination procedure consists of adding Sodium Sulfite in sufficient quantity to destroy the residual chlorine. NOTE: If the chlorine residual is less than 0.5 mg/Liter it might be possible to remove

the residual by shaking the sample, then removing the cover to allow the gas to escape. Repeat until the residual is removed. **Addition of Sodium Sulfite solution should be avoided if possible because this solution acts as an oxygen scavenger and if too much is added to the sample it can significantly increase the oxygen demand in the sample!**

> **NOTE:** Untreated dairy effluent samples often contain high levels of chlorine from equipment cleaning practices. Always check these samples for chlorine residual and pH before proceeding. These samples should also be seeded.

The method of dechlorination is as follows:

To a 100 mL volume of sample add 1 mL of 1:1 acetic acid or 1 mL of 1:50 sulfuric acid (1:50 means 1 part sulfuric acid to 50 parts distilled water). Add 1 mL of potassium iodide solution (10 g/100 mL) and 2 to 3 drops of starch indicator solution. Titrate with 0.025N Sodium Sulfite solution until the colorless end point is reached. Record this amount of solution used in the titration. Then add this amount of sodium sulfite solution per 100 mL of sample remaining. Let it set for 10 to 20 minutes and recheck for chlorine residual.

Review

At this point the following tasks have been performed:

1) Sample warmed to 20 °C.
2) pH adjusted to 6.5 to 7.5.
3) Sodium sulfite solution prepared (if dechlorination is necessary).
4) Sample dechlorinated if necessary.
5) GGA solution prepared. (powder pillow/packet can be used)
6) Glassware labeled.
7) D.O. meter standardized.

Sample Dilution

This chart can be used as a rough guide to proper dilutions. Once you become familiar with the procedure and the quality of samples analyzed, the dilutions used can be adjusted to more closely correspond to your sample requirements.

Choose the proper dilutions for your samples. As a guide:

SAMPLE TYPE		DILUTION	BOD mg/L
Dilution Water	=	100%	
Good Secondary Effluent	=	10%, 20%, 50%	4 - 75
Poor Secondary Effluent	=	5%, 10%, 20%	10 - 150
Very Poor Secondary or Primary Effluent	=	2%, 5%, 10%	20 - 375
Influents	=	1%, 2%, 5%	40 - 750
GGA	=	2%	198 +/- 35
Dairy Wastes	=	.01, .03, .1, .3, 1% (depending on the strength)	200 - 75,000
Meat Packing Wastes	=	.2, .7, 2%	100 - 750
Paper Wastes, Untreated	=	1%, 2%, 5%	40 - 750
Paper Wastes, Treated	=	2%, 5%, 10%	20 - 275

We strongly suggest that you always use three dilutions for each sample. By doing this you reduce the likelihood of not producing the required oxygen **depletion** of at 2.0 mg/L (the oxygen depletion of the sample after the five-day incubation period MUST be at least 2.0 mg/L or the results are considered invalid. Also the oxygen **residual** at the end of the five-day incubation period MUST be at least 1 mg/L). This is referred to as the **R1-D2 rule**. It is very important that this requirement be met. The terms depletion and residual are defined below.

Depletion

Refers to the amount of oxygen "used up" during the incubation period. To determine the depletion, we simply subtract the average of the two final D.O. readings from the initial D.O. reading.

Example:
Initial D.O. = 8.5 mg/L
Average Final D.O. = 5.5 mg/L
Depletion = 8.5 - 5.5 mg/L
Depletion = 3.0 mg/L

Residual

Refers to the amount of dissolved oxygen remaining in the sample at the end of the incubation period. In other words, Final D.O. = Residual.

Sample Dilution

Assume a fairly good quality secondary effluent will be used as a sample with sample concentrations of 20, 60 and 90%.

Using 300 milliliters as the total volume 60, 180 and 270 milliliters of sample are required respectively.

The **blank** consists of dilution water only. Simply fill a 300 milliliter BOD bottle with dilution water. Be careful not to entrain air. Dispense the dilution water slowly along the inside of the glass.

Next, measure the 60 mL sample using a 100 mL graduated pipette or graduated cylinder. **Remember to shake the sample first.** Slowly dispense the sample into the BOD bottle by touching the pipette to the inside of the bottle and allowing the sample to run down the inside of the glass to avoid air entrainment. If using a graduated cylinder pour the sample volume slowly down the inside of the glass.

NOTE: When using small quantities of sample it's a good idea to add dilution water to each of the cylinders before adding sample.

Measure and dispense the 180 and 270 milliliter samples in the same manner. It is always best to use the measuring device that is the closest in volume to the desired volume of sample. For the 180 milliliter sample a 200 milliliter graduated cylinder would be preferable while it might be necessary to use a 500 milliliter cylinder for the 270 milliliter sample. Ideally, an auto pipette capable of delivering the exact volume desired should be used.

Seeded Samples

The SEED is added next. The purpose of the seed is to ensure that a sufficient number of microorganisms is present to oxidize the biodegradable organic matter in the sample. The seed must be used if the sample has been disinfected or is comprised of dairy wastewater.

Primary effluent, or settled influent, is commonly used as seed. A commercially prepared seed can also be used. The same volume of seed is added to each of the sample dilutions and the 2% GGA solution.

The seed volume is determined by using this formula for 300 milliliter BOD bottle.

0.8 divided by the expected seed BOD multiplied by the total bottle volume (300 mL) = mL of seed

0.8 is used because it is the average of the acceptable range of seed contribution (0.6 – 1.0).

EXAMPLE:

If the influent being used as a seed usually produces a BOD of 250 mg/L we would divide 0.8 by 250 and then multiply this number by the total bottle volume (300 mL).

$$\frac{0.8}{250\frac{mg}{L}} * 300\ mL = 0.96$$

We would round this number off to 1 and use 1 mL of seed.

Add the amount of seed calculated as in the example above, to each of the dilutions of the effluent sample and to the 2% GGA solution.

Next, prepare the seed sample dilutions. If we are using settled influent as seed, we might use the typical influent dilutions of 1%, 2% and 5%. To prepare these dilutions we would measure out 3, 6 and 15 milliliters of settled influent respectively and add these volumes to the appropriate bottles. Then fill each bottle into the neck with dilution water.

Replace the ground glass stoppers.

> **NOTE:** If nitrification inhibition of the effluent sample is desired, for consistency, the inhibitor is added to each of the "blank" bottles as well as to each of the effluent sample bottles. Or if desired a separate blank containing trichloromethyl pyridine (TCMP) (may be set up along with the "uninhibited" blanks. The U.S. EPA allows use of nitrification inhibitor only if the facility's NPDES permit specifically requires Carbonaceous Biochemical Oxygen Demand (CBOD).

Determination of the Initial D.O.

1) Before beginning to read initial D.O.'s, we should once again check the meter reading of the distilled water bottle set aside. It should be very close to the original reading.
2) Now after dumping the excess liquid in the well, we simply remove the cap of the first of the two "blank" bottles. Place the D.O. probe into the bottle, being careful not to entrap air in doing so. Turn on the stirrer.
3) Check for air bubbles in the sample. If there are no air bubbles record the reading If there are air bubbles, reinsert the probe carefully eliminating all air.
4) Place the plastic cover over each of the two bottles and place them into the box to be incubated.
5) Repeat this procedure for the remaining dilutions of effluent, seed and GGA.
6) Always move from the least concentrated to most concentrated solutions to reduce the chances for contamination.
7) If necessary, fill the bottle “well” (the portion between the glass stopper and the flared mouth) with dilution water. This helps ensure the airtight seal. Then place the plastic cap over the bottle
8) Bottles are to be incubated at 20 °C plus or minus 1 °C, for five days.

Determination of the Final D.O.

After five days (±3 hours) of incubation, Dissolved Oxygen remaining in the bottles is determined. Again, the meter is standardized. Remove the bottles from the incubator. Check each bottle for air bubbles. Discard those bottles which contain bubbles. Determine the residual D.O.'s of the remaining bottles. D.O. residuals of each dilution are read and recorded on the bench sheet. The D.O. results of the two bottles used for averaging the final D.O. should not vary by more than 0.5 mg/L. Large variations in D.O. readings between these bottles containing the same concentration of sample are indicative of either contamination or use of a non-homogenous sample.

Blank Depletion

The D.O. depletion of the blank after the five-day incubation period should not exceed 0.2 mg/L. There is no "Blank Correction" calculation. If the Blank demonstrates a depletion of greater than 0.2 mg/L, the cause of the problem must be identified and corrected. Only the highest quality distilled water should be used when making up the dilution water. Sources of contamination must be eliminated.

Calculation of Unseeded BOD

When no seed has been used, the BOD calculation is simply:

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(Initial\ DO\ \left(\frac{mg}{L}\right) - Final\ DO\ \left(\frac{mg}{L}\right)\right) * BOD\ Bottle\ Volume\ (mL)}{Sample\ Volume\ (mL)}$$

EXAMPLE: A 30 milliliter concentration of sample diluted in a 300 mL BOD Bottle yields these results:

- Initial D.O. = 8.5 mg/L
- Average Final D.O. = 6.0 mg/L

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(Initial\ DO\ \left(\frac{mg}{L}\right) - Final\ DO\ \left(\frac{mg}{L}\right)\right) * 300\ mL}{Sample\ Volume\ (mL)}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(8.5\frac{mg}{L} - 6.0\frac{mg}{L}\right) * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{2.5\frac{mg}{L} * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = 25\frac{mg}{L}$$

Don't forget the R1-D2 rule!! Let's try another example where the rule applies.

A BOD sample yields the following results:

Sample volume (mL)	Initial D.O. mg/L	Average Final D.O. mg/L	Depletion mg/L
30	8.0	7.0	1.0
60	8.0	6.0	2.0
150	8.0	3.0	5.0

In this example we must first calculate the results for each concentration that fits the rule and then average these results.

The 30 mL. sample is not included in the calculation because the DEPLETION is less than 2.0.

The result of the 60 mL. sample is (8-6) * 300 / 60 = 2mg/L x 300 mL / 60mL = 10 mg/L

The result of the 150 mL. sample is (8-3) * 300 / 150 = 5 x 300 / 150 = 10 mg/L

The average of the two is 10 mg/L so we would report the BOD as 10 mg/L.

Calculation of Seeded BOD

The calculation of seeded BOD's is a bit tougher. In Vermont, we require that the BOD of the seed be established in the same manner as the sample (recommended three dilutions). Therefore, we calculate the BOD of the seed material using the calculation we just described. We use these results to calculate the seed correction factor which is then applied to the overall BOD calculation of the effluent sample.

The actual BOD calculation for a seeded sample is:

$$BOD\left(\frac{mg}{L}\right) = \frac{\left(\left(I.D.O.\left(\frac{mg}{L}\right) - F.D.O.\left(\frac{mg}{L}\right)\right) - Seed\ Correction\ Factor\right) * BOD\ Bottle\ (mL)}{Sample\ Volume\ (mL)}$$

Where:

- I.D.O. = Initial Dissolved Oxygen reading before incubation.
- F.D.O. = Final Dissolved Oxygen after incubation
- Seed Correction Factor = $\frac{Actual\ Seed\ BOD\left(\frac{mg}{L}\right) * Seed\ Used\ (mL)}{Total\ Volume\ of\ Sample\ After\ Dilution\ (mL)}$
- Since dilutions are being made in 300 mL BOD bottles we would use 300 mL as a total volume.

The BOD calculation for a seeded BOD can be performed in four steps.

1) Calculate the depletion (Initial minus the average final D.O.'s.)

EXAMPLE: If we had an average initial D.O. reading of 8.6 mg/L and an average final D.O. reading of 5.6 our answer would be 3.0 mg/L. We get this answer by subtracting 5.6mg/L, from the initial D.O. reading of 8.6mg/L.

2) Calculate the Seed BOD. Since the seed is essentially an unchlorinated influent sample we use the calculation for unseeded BOD's:

NOTE: We must actually physically determine the seed BOD by running a BOD analysis of the seed material itself along with the whatever sample we are analyzing. This number cannot just be estimated or represent a BOD run on the seed material a month ago!!

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{\left(I.D.O.\left(\frac{mg}{L}\right) - F.D.O.\left(\frac{mg}{L}\right)\right) * 300\ mL}{Sample\ Volume\ (mL)}$$

EXAMPLE: Using a 3 mL volume of influent sample (this influent will serve as our seed material) if our average Initial D.O. reading was 8.5mg/L and our average final D.O. reading was 5.5 mg/L our Seed BOD would be 300mg/L. We arrive at this number by applying the formula:

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{\left(8.5\left(\frac{mg}{L}\right) - 5.5\ \left(\frac{mg}{L}\right)\right) * 300\ mL}{3\ mL}$$

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{3\frac{mg}{L} * 300\ mL}{3\ mL}$$

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{900\ \frac{mg * mL}{L}}{3\ mL}$$

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = 300\frac{mg}{L}$$

***Note:** The (mL) cancel out in the equation leaving us with only mg/L in the final answer

Seed BOD = 300 mg/L

3) Perform the calculation for the Seed Contribution. For our example assume that we used 1 mL of seed in each dilution of sample:

$$Seed\ Contribution\ Factor = \frac{\left(Seed\ BOD\ \left(\frac{mg}{L}\right) * Seed\ Used\ (mL)\right)}{Total\ Volume\ of\ Sample\ Dilution\ (mL)}$$

$$Seed\ Contribution\ Factor = \frac{\left(300\frac{mg}{L} * 1\ mL\right)}{300\ mL} = 1.0\frac{mg}{L}$$

***Important Note:** The seed contribution should be between 0.6 and 1.0 mg/L

4) Plug these numbers into the equation.
Following our example above, we have a 30 mL sample, with an initial D.O. of 8.6 and an average final D.O. of 4.6. Our seed correction factor as calculated in Step 4 above is 1.0.

$$BOD\left(\frac{mg}{L}\right) = \frac{\left(\left(I.D.O.\left(\frac{mg}{L}\right) - F.D.O.\left(\frac{mg}{L}\right)\right) - Seed\ Correction\ Factor\right) * 300\ mL}{Sample\ Volume\ (mL)}$$

$$BOD\left(\frac{mg}{L}\right) = \frac{\left(\left(8.6\frac{mg}{L} - 4.6\frac{mg}{L}\right) - 1.0\frac{mg}{L}\right) * 300\ mL}{30\ mL}$$

$$BOD\left(\frac{mg}{L}\right) = \frac{\left(4.0\frac{mg}{L} - 1.0\frac{mg}{L}\right) * 300\ mL}{30\ mL}$$

$$BOD\left(\frac{mg}{L}\right) = \frac{3.0\frac{mg}{L} * 300\ mL}{30\ mL}$$

$$BOD\left(\frac{mg}{L}\right) = \frac{900\ \frac{mg * mL}{L}}{30\ mL}$$

$$BOD\left(\frac{mg}{L}\right) = 30\frac{mg}{L}$$

The other effluent dilutions which meet the R1-D2 rule would then be calculated using the procedure shown above and the results averaged to determine the final BOD result to be reported.

***Note**: BODs should be reported only to the nearest whole number 30mg/L NOT 30.02mg/L or 29.98mg/L

Perform a BOD of the Glucose Glutamic Acid (GGA) and Calculate the BOD of the solution

The GGA is used to check on possible dilution water toxicants and seed source reliability. For example, distilled water could be contaminated by copper causing the seed to be relatively inactive. These factors can often yield inaccurate lower BOD results. Therefore, dilution water quality, seed reliability and analytical technique needs to be checked by measuring BOD on pure organic compounds.

A 2% dilution (6 mL) of GGA is prepared the same as any sample. The 2% GGA solution is seeded with the same volume of seed used in all the samples dilutions.

This seeded 2% glucose-glutamic acid solution should yield a BOD in the range of 198+/- 30 mg/L.

Note: If the BOD of the GGA is slightly below the desired range this suggests that perhaps insufficient seed was used. Consider increasing the volume of seed used in future analyses. Conversely, if the GGA reading is slightly higher than the desired range consider using less seed in future analyses. If the BOD of the GGA is significantly outside the desired range, it is important to find the possible sources of error, correct the problems and perform the analysis again. Remember to calculate the GGA BOD using the “seeded BOD” formula.

Remember the R1-D2 rule!! Remember that the seed contribution should be 0.6-1.0 mg/L

Good Luck!

- **Remember, if samples are sent to another lab for BOD analysis it is still the responsibility of the permittee to ensure that the results are acceptable.**
  - **Be sure to read the lab results carefully and make sure all the rules have been satisfied. (all temps, initial DO, R1-D2, blank depletion, seed contribution 0.6-1, etc.)**
  - **The Permittee needs to resolve the situation with the lab and, if there is uncertainty about permit compliance, resampling may be needed. Coordinate with the permitting analyst for your facility to determine the next step forward.**
  - **Applicable NODI codes should be used if the facility does not have reliable data to report.**

SAMPLE BENCH SHEET #1

FACILITY: Maple City
SAMPLING: **DATE:** 02/15/2021 **COLLECTED BY:** AB
CHLORINATED: No **Other Disinfection:** None
Composite: 1/hour **Start Time:** 7 a.m. **Duration:** 8 hours **Grab Sample**__________ **Time**________

In to incubator: Date: 02/16/2021 **Time** 11 a.m. **Temp.** 20.5C **Analyst** CD **Seed Used** None

Out of incubator: Date: 02/21/2021 **Time** 1 p.m. **Temp.** 20C **Analyst** AB

D.O. VALUES

Sample	1 sample volume	2 Bottles Numbers	3 Initial	4 Residual	5 Average Residual	6 Depletion	7 Seed Depl.	8 Corrected Depletion	9 BOD mg/L	10 Final BOD
Blank	300 mL	1,2,3	8.50/8.50	8.40/8.40	8.40	0.10			----	
GGA	6 mL	4,5,6	8.55/8.55	4.30/4.40	4.35	4.20			210	
Inf	3 mL	10,15,18	8.50/8.50	5.90/6.10	6.00	2.50			250	
Inf	6 mL	19,20,21	8.45/8.45	2.40/2.50	2.45	6.00			300	
Inf	15 mL	25,28,30	8.50/8.50	0.80/0.50	0.65 (R<1)					
Eff	30 mL	31,32,33	8.45/8.45	8.40/8.40	8.40	0.05 (D<2)				
Eff	60 mL	34,35,36	8.55/8.55	7.10/7.00	7.05	1.50 (D<2)				
Eff	150 mL	37,38,39	8.55/8.55	4.55/5.45	5.50	3.05			6.1	
Eff Dup	30 mL	40,43,44	8.50/8.50	8.45/8.50	8.48	0.02 (D<2)				
Eff Dup	60 mL	46,48,50	8.55/8.55	7.20/7.10	7.15	1.40 (D<2)				
Eff Dup	150 mL	100,98,96	8.55/8.55	4.60/4.60	4.60	3.95			7.9	

(INCLUDE ACTUAL CALCULATIONS ON REVERSE SIDE)

CALCULATIONS: (To accompany bench sheet)

- Influent 3 mL (8.5mg/L – 6mg/L) *300mL / 3 mL = 250 mg/L
- Influent 6 mL (8.45mg/L – 2.45mg/L) *300 mL /6mL = 300 mg/L
- **Average BOD** = 250mg/L + 300 mg/L /2 **= 275 mg/L**
- Influent 15 mL ---> Average residual less than 1
- Effluent 30 mL ---> Depletion less than 2
- Effluent 60 mL ---> Depletion less than 2
- Effluent 150 mL ---> (8.55mg/L – 3.50 mg/L) * 300mL / 150 mL = 6.1mg/L
- Effluent Duplicate 30 mL ---> Depletion less than 2
- Effluent Duplicate 60 mL ---> Depletion less than 2
- Effluent Duplicate 150 mL ---> (8.55mg/L – 4.60mg/L) * 300mL /150 mL = 7.9 mg/L
- **Average BOD** = (6.1mg/L + 7.9mg/L) / 2 = **7 mg/L**

SAMPLE BENCH SHEET #2

FACILITY: Maple City
SAMPLING: **DATE:** 02/15/2021 **COLLECTED BY:** AB
CHLORINATED: No **Other Disinfection:** Ultraviolet
Composite: 1/hour **Start Time:** 7 a.m. **Duration:** 8 hours **Grab Sample**__________ **Time**________
Into incubator: Date: 02/16/2021 **Time** 11 a.m. **Temp.** 20.5C **Analyst** CD **Seed Used** 1 mL Primary Effluent

Out of incubator: Date: 02/21/2021 **Time** 1 p.m. **Temp.** 20C **Analyst** AB

D.O. VALUES

Sample	1 Dilution/ sample volume	2 Bottles Numbers	3 Initial	4 Residual	5 Average Residual	6 Depletion	7 Seed Depl.	8 Corrected Depletion	9 BOD mg/L	10 Final BOD
Blank	100%/300 mL	1,2,3	8.50/8.50	8.40/8.40	8.40	0.10			---	
GGA	2%/6 mL	4,5,6	8.55/8.55	4.00/4.00	4.00	4.55	0.825	3.725	186.25	186
Seed	1%/3 mL	10,15,18	8.50/8.50	5.90/6.10	6.00	2.50			250	
Seed	2%/6 mL	19,20,21	8.45/8.45	2.40/2.50	2.45	6.00			300	
Seed	5%/15 mL	25,28,30	8.50/8.50	0.80/0.50	0.65(R<1)					
Eff	10%/30 mL	31,32,33	8.45/8.45	8.40/8.40	8.40	0.05(D<2)				
Eff	20%/60 mL	34,35,36	8.55/8.55	7.10/7.00	7.05	1.50(D<2)				
Eff	50%/150 mL	37,38,39	8.55/8.55	5.55/5.45	5.50	3.05	0.825	2.225	4.45	
Eff Dup	10%/30 mL	40,43,44	8.50/8.50	8.45/8.50	8.48	0.02(D<2)				
Eff Dup	20%/60 mL	46,48,50	8.55/8.55	7.20/7.10	7.15	1.40(D<2)				
Eff Dup	50%/150 mL	100,98,96	8.55/8.55	4.60/4.60	4.60	3.95	0.825	3.125	6.25	

(INCLUDE ACTUAL CALCULATIONS ON REVERSE SIDE)

CALCULATIONS FOR SEEDED BOD

Seed 3mL (8.5mg/L – 6.0mg/L) * 300mL / 3 mL = 250 mg/L

Seed BOD = 250 + 300 = 275 mg/L
2

Seed 6 mL 8.45mg/L - 2.45mg/L * 300mL / 6mL = 300mg/L

Seed 15 mL ---> Average residual less than 1

Seed Correction/Contribution = 275mg/L x 1mL = .9 mg/L

300mL

Effluent 30 mL ---> Depletion less than 2

Effluent 60 mL ---> Depletion less than 2

Effluent 150 mL---> ((8.55 mg/L – 5.5 mg/L) –(0.9mg/L)) *300mL /150 mL = 4.3 mg/L

Effluent Duplicate 30 mL ---> Depletion less than 2

Effluent Duplicate 60 mL ---> Depletion less than 2

Effluent Duplicate 150 mL ---> ((8.55mg/L - 4.6mg/L) – (0.9mg/L)) *300mL /150 mL = 6.1mg/L

Avg Effluent and Effluent duplicate = 4.3mg/L + 6.1mg/L / 2 = **5.2mg/L**

Biochemical Oxygen Demand Troubleshooting Guide

PROBLEM	LIKELY CAUSE	SOLUTION
BLANK DEPLETION (unseeded dilution water) is greater than the allowable .2 mg/L.	Contamination of BOD bottles, carboy, or syphon.	Clean bottles, carboy and syphon thoroughly with hot 2% Micro solution. Rinse thoroughly with hot tap water followed by distilled water rinses. If the problem persists use chromic acid to clean the bottles, followed by several rinses of hot tap water and finally several distilled water rinses. Use an appropriately sized brush to aid in thorough cleaning.
	Contaminated distilled water.	Thoroughly clean the distilled water storage tank. Place a 0.4-micron organic filter at the end of the system, especially if deionizing resins are used. Check the conductivity of the distilled water. If >1.0. Find another source of distilled water until the problem can be eliminated <u>or</u> as a last resort an attempt can be made to oxidize the contaminating materials by adding a small amount of seed material (.5 mL) to the distilled water 5-7 days before the dilution water is prepared.
	Contaminated nutrients used to make up the dilution water.	If the nutrients, especially the phosphate buffer, are over 1 year old or show any sign of contamination discard and prepare new solutions.

PROBLEM	LIKELY CAUSE	SOLUTION
BLANK DEPLETION (unseeded dilution water) is greater than the allowable .2 mg/L.	Initial D.O. of dilution water as >9.2 mg/L.	Never begin the BOD analysis if the D.O. of the dilution water or samples is greater than 9.2 mg/L. The ideal range if 7.5-8.5 mg/L. The D.O. can be reduced by shaking or aerating the dilution water then letting it set for 1/2 hour before use. Recheck the D.O. at this point. Be sure the dilution water is 20 °C before beginning the analysis.
	D.O. meter improperly calibrated.	Check calibration method. Check D.O. probe. Make sure membrane is in good shape--no bubbles under membrane and fresh filling solution.
All dilution of sample produce less than the required D.O. depletion of 2 mg/L.	Sample concentrations too low. Toxicity.	Increase sample concentration. Check sample for chlorine residual and pH extreme. Remove chlorine and/or adjust pH if necessary and properly seed the sample.
All dilution of sample product less than the required residual D.O. of 1 mg/L.	Sample concentration too high.	Decrease sample concentration.
Bubbles appear in BOD bottles after incubation.	Supersaturated samples -- Initial D.O. was >9.2 mg/L. Temperature of sample was less than 20 °C.	D.O. not begin analysis until D.O. sample is <9.2. Ideal range = 7.5-8.5 mg/L.

PROBLEM	LIKELY CAUSE	SOLUTION
Reverse depletion. The depletion of the more concentrated sample is less than the depletion of the least concentrated sample.	Toxicity.	Check sample or seed for pH (should be between 6-8) adjust if necessary. Check sample or seed for chlorine residual and dechlorinate as necessary using sodium sulfite solution.

Quality Control for Biochemical Oxygen Demand: Standard Method #5210-B

Document the Following (Including by not limited to):

<u>Sample Collection</u>

- Grab - Exact Time
- Composite - Duration - sampling start and stop times
- Flow Proportioning -
    - Include:
        - Proportioning Factor
            - Example: 100 mL/1000 gallons
        - Flow Measuring Device
        - Calibration
- Collection Device
- Refrigeration of Sample - during and after sampling
- Location
- Representative Nature - Volume required

<u>Hold Time</u>

- Preferably 6 hours (at 4 °C) - max 48 hours after last discrete sample is collected
    - Sample line and container **<u>clean</u>** - no residual organics
    - Mix sample thoroughly before pouring off for composite **<u>BUT</u>** don't agitate excessively.

<u>Glassware Preparation/Sampling Equipment</u>

- Must be <u>scrupulously clean</u>
    - No residue
    - No dust
    - <u>Well</u> rinsed (5-10 times) with distilled H_2O.
- BOD Bottles - airtight, ground glass stoppers.

<u>Reagents</u>

- Distilled water for dilution water preparation: **<u>Deionized water is not recommended</u>**
    - No copper
    - Conductivity <1
- Preparation and expiration dates for <u>all</u> reagents.
- NIST Traceability
- <u>GGA</u> - <u>MUST</u> be made the day the analysis is performed
- <u>Sodium Sulfite</u> solution for neutralization of chlorine <u>must</u> be made the day the analysis is performed.

Equipment

- Properly calibrated D.O. meter with stirrer equipped probe (or magnetic stirrer) Incubator 20 °C ±1 °C - **DARK!** - checked every 4 hours

Sample Preparation - Before beginning analysis!

- Sample and Dilution water must be 20 °C or slightly higher
- Sample and Dilution water DO must be ≤ 9.2 mg/L (preferably less than 9.0 mg/L)

Validity of Results

- Blank - Depletion ≤0.2 mg/L
- Sample - Residual D.O. ≥ 1 mg/L
- Sample - D.O. Depletion ≥ 2 mg/L
- GGA - = 200 ± 37 mg/L
- Seed BOD - must be analyzed not calculated
- Seed correction factor - calculation must be recorded

Duplication Schedule

- Minimum 5% duplication 10% replication
- GGA - minimum 10%
- Seeded Sample - GGA minimum 10%

Recommended Control Limits

- Perform minimum of 20 GGA checks over 6 to 12 month period.
- Calculate mean and standard deviation
- Perform minimum of 10% GGA checks after that with results remaining within one standard deviation of mean.

References

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Annual Book of ASTM Standards, Part 31 Water. Published by the American Society of Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

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Coliform

VT WSMD Wastewater Program Lab Manual Section #13

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Escherichia coli Membrane Filtration Method

Background

Some facts about *E. coli* that help us to identify and enumerate include:

1) It is thermotolerant at 44°C. Some bacteria cannot survive at this temperature, but *E. coli* can function well at 44°C.
2) It is a Gram-Negative Bacteria. The inclusion of sodium laurel sulfate and sodium deoxycholate in the media offer selectivity against gram-positive bacteria. It is easily differentiated from gram-positive bacteria.
3) It ferments lactose. This is an important point as fermentation of lactose results in the production of acid and gas at both 35°C and 44.5°C. Many bacteria cannot ferment lactose.
4) It can hydrolyze tryptophan to indole. The indole test is one method of identifying *E. coli*.
5) It hydrolyzes methyl umbellyferyl beta D glucuronide to glucuronic acid umbellyferyl. This causes fluorescence which is easily detected in the MUG test.

Just as important for identification purposes is what *E. coli* does not do:

1) It does not hydrolyze urea. This makes it easily identified when transferred to the urea substrate.
2) It does not utilize sodium citrate. This makes confirmation of colonies easy. By transferring questionable colonies to Simmon's citrate agar and incubating for 24 hours at 35°C we can easily confirm *E. coli* as other coliform will grow colonies but *E. coli* will not.

Equipment

- Autoclave, dry oven or other type of sterilizer
- Kraft paper
- Aluminum foil
- Autoclave indicator tape
- Masking tape
- Funnel assembly - 47 mm, autoclavable
- Membrane filters - made specifically for microbiology
  - cellulose nitrate or cellulose nitrate/cellulose acetate
  - 47 mm, sterile, white, gridded on one side
  - 0.45 um ± 0.02 um pore size
- Absorbent pads - 47 mm, sterile
- Petri dishes - 50 mm x 12 mm, plastic, sterile, tight-fitting halves
- Forceps, straight or curved, with smooth tips to handle filters without damage
  - Beakers - 50 mL, 250 mL
- Vacuum pump
- Vacuum flask 500 mL
  - Fitted with rubber stopper and glass tubing or a filter manifold for holding a number of filter bases
- Vacuum flask 1000 mL

  - For safety trap-placed between the filter flask and the vacuum source.
- Tubing - thick walled tygon or rubber
- Plastic bags - 18 oz. Whirl-pak or similar waterproof bag, e.g. Ziploc
- Water bath - must be able to maintain a temperature of 44.5℃ ±0.2℃.
- Incubator - air, must be able to maintain a temperature of 35.0℃ ±0.5℃.
- Thermometer† - range of 20℃ to 50℃ with no larger than 0.2℃ graduations.
- Sample bottles - glass or plastic (polypropylene), autoclavable
- Fluorescent lamp - top lighting only
- Microscope or magnifying glass to give at least 5x magnification
- Colony counter - hand held
- Graduated cylinders - 100 mL, 1000 mL
- Volumetric flasks - 250 mL
- Erlenmeyer flasks - 250 mL, 250 mL screw top, 2000 mL screw top
- Graduated pipet - 5 mL, 25 mL
- Bacteriological pipets - 10 mL, 1 mL
- Refrigerator - capable of maintaining 4℃
- Stirrer magnet - bar
- Magnetic stirrer
- Burner - alcohol or Bunsen
- Parafilm or volumetric flask covers
- 0.45 um filters (Millipore HA, Gelman GN 6 Metric, MFS (Micro Filtration Systems), or similar)

† Thermometers should be checked at least once a year against a certifiable NIST (National Institute of Standards Technology) thermometer or one traceable to an NIST thermometer. Any deviation from the NIST thermometer should be noted and taped to the thermometer, e.g. reads 0.1℃ high at 44.5℃. Readings taken from this thermometer should always be adjusted according to the deviation.

Cleaning and Sterilization of Equipment

As discussed earlier in this manual, glassware used for bacteria testing should not be acid washed. This also applies to any equipment such as spatulas, stirrer bars, etc. with which reagents are made for coliform tests. Any acid residue on the glassware will kill bacteria. Wash bacteria glassware in hot, soapy water with a detergent that doesn't inhibit growth, e.g. Micro. Rinse glassware thoroughly in hot tap water and then with distilled or deionized water at least 3 times.

Sterilization of bacterial glassware such as sample bottles, dilution bottles, funnel setups and pipets can be accomplished using an autoclave set at 15 psi and 121℃ or a dry oven set at 170℃. Keep in mind that reusable plasticware or liquids should never be sterilized in a dry oven.

Items such as funnels and pipets should be wrapped in Kraft paper and secured with autoclave indicator tape before being placed in the autoclave to allow the steam to penetrate. (Glassware sterilized in a dry oven may be wrapped in aluminum foil.) Also, always loosen the caps of sample and dilution bottles before autoclaving them.

The 10% sodium thiosulfate solution should be added to the sample bottles BEFORE they are sterilized. 0.1 mL (2 drops) will neutralize the chlorine in 100 mL of effluent. For effluents disinfected by ultra-violet light, the addition of sodium thiosulfate isn't necessary.

It is advisable to place a piece of autoclave indicator tape on equipment to be sterilized so that it can easily be identified later as having been processed. Autoclave indicator tape has invisible writing which appears after 15 minutes in the autoclave.

The following chart lists several items commonly used in Fecal Coliform analysis along with the required sterilization time for each. If your autoclave includes instructions indicating longer sterilization periods, follow those instructions except in the case of media, which should never be autoclaved for more than 15 minutes.

Item	**Autoclave**	**Dry Oven**
Sample Bottles	30 min	2 hours
Pipets	30 min	2 hours
Funnels	30 min	2 hours
1 L Dilution H_2O	35 min	
Used Petri Dishes	30 min	2 hours

Sample Collection, Preservation and Holding Time

E. coli samples should be taken at the end of the outfall pipe or at the weir in the chlorine contact chamber, or at the outfall from the de-chlorination tank if your facility is required to dechlorinate. These samples must be taken as grabs into clean, sterile, sodium thiosulfate treated bottles. It is important to take the sample directly into the sterile bottle. Do not transfer an *E. coli* sample from a dipper to the sterile sample bottle because of contamination problems. Allow about 1 inch of headspace for efficient mixing and shake the sample to mix it with sodium thiosulfate. It is usually required that you take the chlorine residual at the same time to establish a correlation between the two. Process the sample as soon as possible, but always within 8 hours. If immediate processing isn't possible, refrigerate the sample at 4°C. If a sample is sent to another lab for analysis, it **must** be kept in a cooler with ice and arrive at the other lab within 6 hours.

Reagents

Order only reagent grade chemicals. Store chemicals and media as indicated on the container label. **IT IS HIGHLY ADVISABLE TO USE ONLY COMMERCIALLY PREPARED MEDIA.** Refrigerate media. Discard media if the expiration date has passed. Also use only high-grade distilled water for reagent preparation.

- 70% Ethanol, methanol, or isopropanol in a small wide mouth container for flame-sterilizing forceps
- Phosphate Buffered Dilution Water:

NaH2PO4	-	Sodium Dihydrogen Phosphate	0.58 g
NaHPO4	-	Sodium Monohydrogen Phosphate	2.50 g
NaCl	-	Sodium Chloride	8.50 g

Preparation: Dissolve the ingredients in 1 L of high-grade distilled water in a flask and dispense in appropriate amounts in screw-cap bottles or culture tubes, and/or into containers for use as rinse water. Autoclave after preparation at 121℃ (15 lbs pressure) for 15 minutes. Final pH should be 7.4 ± 0.2.

- mTEC Agar (Difco 0334-15-0)

Composition:

Proteose Peptone	5.0 g
Yeast Extract	3.0 g
Lactose	10.0 g
NaCl	7.5 g
Dipotassium Phosphate	3.3 g
Monopotassium Phosphate	1.0 g
Sodium Laurel Sulfate	0.2 g
Sodium Deoxycholate	0.1 g
Brom Cresol Purple	0.08 g
Brom Phenol Red	0.08 g
Agar	15.0 g

mTEC prepared Agar plates and urea substrate can be purchased from multiple sources, including:

Northeast Laboratory
PO Box 788
Waterville, Maine 04901
Telephone: 207-873-7711.

Preparation: Use commercially prepared media if possible. If you have no other option prepare the media by adding 45.26 g of dehydrated mTEC medium to 1 L of reagent grade water in flask and heat to boiling, until ingredients dissolve. Autoclave at 121℃ (15 lb pressure) for 15 minutes and cool in a 44-46 ℃ water bath. Pour the medium into each 30 x 10 mm culture dish to a 4-5 mm depth (approximately 4-6 mL) and allow to solidify. Final pH should be 7.3 ± 0.2. Store in refrigerator. Hold time 1 month.

- Urea Substrate Medium

Composition:

Urea	2.0 g
Phenol Red	0.01 g

Preparation: Use commercially prepared urea, substrate medium if possible. If commercially prepared reagent is not available. Add dry ingredients to 100 mL reagent grade water in a flask. Stir to dissolve to pH 5.0 with a few drops of 1N HCl. The substrate solution should be a straw yellow color at pH 5.0 ± 0.2. Store at 6°C - 8°C for 1 week.

Calibration and Standardization

Check temperatures in incubators daily to insure operation within stated limits.

Check thermometers at least annually against an NIST certified thermometer or one traceable to NIST.

Procedure

Prepare the mTEC agar and urea substrate. Mark the petri dishes and report forms with sample identification and sample volumes. It is best to label the bottom and top portions of the petri dishes. Pour about 1" of ethanol into a 50 mL beaker. Keep the forceps in it all the time except when handling a filter. This ethanol keeps the forceps sterile. Unwrap a funnel and place it aseptically on the vacuum flask. Open the filter by the edge with the forceps. Place the filter on the funnel base grid side up and the funnel head on the filter. Pour 100 mL sterile dilution water onto the filter. This is the blank. Turn on the vacuum pump and draw the blank through the filter. Rinse the sides of the funnel twice with 20-30 mL of dilution water. Turn off the vacuum pump and loosen the funnel head. Open the petri dish labeled "Blank 100 mL," and place the cover inside up on the work surface. Loosen the funnel head. Flame the forceps. Take the funnel head off with one hand and pick up the filter with the other. Always grasp the filter by the edge. Place the funnel head back on the base. Place the filter mTEC on the media. Pull back the leading edge of the filter and roll it forward to exclude air as described below.

Try to place the filter into the plate without dragging it across the edge of the plate. Repeat this procedure at the other 3 "corners" of the filter. Bubbles prevent media from soaking into the filter, and any bacteria over a bubble won't grow. Although it may be tempting to poke at bubbles to get rid of them, the rolling back procedure works better. Cover the plate, invert, and incubate at 35°C for 2 hours. Shake the sample bottle vigorously to distribute the bacteria uniformly and measure the desired volume of sample into the funnel. Filter the sample just as you did the "blank" beginning with the smallest volume. For 10 mL or less, pour about 20 mL of dilution water onto the filter before adding the sample. This allows the bacteria to disperse and not grow in one clump. Always filter 3 different volumes of sample, e.g. 0.1 mL, 1 mL and 10 mL, to hopefully produce a readable filter. Shake the sample vigorously 25 times each time before pipetting off a volume. After the sample has been filtered, run a "positive". A positive is generally a drop of influent, or effluent collected before disinfection, filtered the same as the blank and the sample. Growth on the positive filter assures that the media will grow *E. coli* colonies.

As described above for the "blank" use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the mTEC agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane if bubbles occur. Close the dish, invert, and incubate at 35°C for 2 hours.

After 2 hours incubation at 35°C, transfer the plates to Whirl-Pak bags, seal and place inverted in a 44.5°C water bath for 22-24 hours.

After 22-24 hours, remove the dishes from the water bath. Place absorbent pads in new petri dishes or the lids of the same petri dishes and saturate with urea broth. Aseptically transfer the membranes to absorbent pads saturated with urea substrate and hold at room temperature.

After 15-20 minutes incubation on the urea substrate at room temperature, count and record the number of yellow or yellow-brown colonies on those membrane filters ideally containing 20-80 colonies.

Verification Procedure

Questionable colonies from the urease test can be verified by many means. One fairly simple and effective method of verification includes the transfer of questionable colonies to a slant or plate containing Simmon's citrate agar.

Simmon's Citrate Agar (BBL 11619, Difco 0091-02)

Composition:

Magnesium Sulfate	0.2 g
Monoammonium Phosphate	1.0 g
Dipotassium Phosphate	3.3 g
Sodium Citrate	2.0 g
Sodium Chloride	5.0 g
Brom Thymol Blue	0.08 g
Agar	15.0 g

Preparation: Add 24.28 g of Simmon's citrate agar to 1 L of reagent water. Heat in boiling water bath with mixing for complete solution. Dispense in screw-cap tubes and sterilize at 121°C (15 lb pressure) for 15 minutes. Cool tubes and slant. The final pH should be 6.9 ± 0.2.

Transfer questionable colonies onto Simmon's Citrate Agar slant (or plate). Incubate at 35°C for 24 hours. It there is any growth the colonies are NOT *E. coli*.

Other verification procedures are described in "Test Methods for Escherichia Coli and Enterococci in Water by the Membrane Filter Procedure". Copies of this publication can be obtained from:

National Technical Information Services
5285 Port Royal Road
Springfield, Virginia 22161
Order #PB 86-158-052

The following section describing counting colonies and calculating results is edited from EPA Microbiological Methods for Monitoring the Environment.

Calculations of Results

Escherichia Coli results are reported as colony forming units (CFUs) per 100 mL.

The equation for calculating results is:

$$\frac{E.coli\ CFUs}{100\ mL} = \frac{\#\ of\ E.coli\ Colonies}{Sample\ Volume\ (mL)} * 100$$

The desired number of colonies to be counted on a plate is 20-80. Plates containing more than 80 colonies should be avoided as there is a great likelihood of false positive.

Therefore, if all plates produce more than 80 *E. coli* colonies the plate with the least # of countable colonies is used in calculating the results. But be sure to note that the result is an estimate based on a plate containing >80 colonies. For example:

If sample volumes of 10, 30, and 50 mL produced results of 86, 120, 150 colonies respectively the result would be calculated as:

$$\frac{86\ CFUs}{10\ mL} * 100 = Estimated\ \frac{860\ CFUs}{100\ mL}$$

with a note in the "comments" section of the report stating that the result was based on a 10 mL sample which produced >80 colonies.

If only one plate produces 20-80 colonies, then only that plate would be used in calculating the result. For example:

If sample volumes of 10, 30, and 50 mL produce results of 5, 15 and 25 colonies respectively, the result would be calculated as:

$$\frac{25\ CFUs}{50\ mL} * 100 = \frac{50\ CFUs}{100\ mL}$$

If more than one plate produces colonies in the 20-80 range,an average must be calculated. That is: All values within the acceptable range of 20-80, are added together and their sum is divided by the total of their volumes. For example:

If sample volumes of 10, 30, and 50 mL produce results of 20, 50 and 95 colonies respectively, the result would be calculated as:

$$\frac{(20 + 50)\ CFUs}{(10 + 30)\ mL} * 100 = \frac{175\ CFUs}{100\ mL}$$

If none of the plates produce at least 20 colonies, the result is calculated using all the colonies counted divided by the total volume used. For example:

If sample volumes of 10, 30, and 50 mL produce results of 1, 3 and 5 mL respectively, the result is calculated:

$$\frac{(1 + 3 + 5)\ CFUs}{(10 + 30 + 50)\ mL} * 100 = \frac{10\ CFUs}{100\ mL}$$

If no colonies are produced on any of the plates (except of course the positive control) the results would be calculated using the largest volume and assuming 1 colony was produced on that plate. A < sign would be included with that result. For example:

> If sample volumes of 10, 30, and 50 mL all produce results of zero then the result would be calculated as:
>
> $$\frac{1\ CFU}{50\ mL} * 100 =< \frac{2\ CFUs}{100\ mL}$$

Escherichia coli Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Colonies are observed on the blank.	Autoclave/sterilizer not performing properly/non-sterile technique.	Verify that all equipment, glassware is sterile, forceps are dipped in alcohol and flamed before each use and good sterile technique is used throughout the procedure.
	Dilution water not sterile.	Watch dilution water carefully for growth or discoloration. Discard when either is observed.
No colonies observed in positive control.	Bacterial inhibitors in sample, sample container, or equipment.	Use sodium thiosulfate in sample collection bottle. Addition of EDTA to the collection bottle (before sterilization) can eliminate inhibition by several heavy metals.
All colonies are bundled together in center of filter.	Poor dilution technique.	Add dilution water to funnel first then add sample to allow dispersion of bacteria.
None of the dilutions used produced the desired 20-80 colonies (<20 on all plates).	Insufficient sample volume.	Increase sample volume - up to 100 mL (undiluted).
All dilutions used produce more than 60 colonies.	Too much sample used.	Dilute sample.
White areas with no colony growth appear on filter.	Air bubbles under filter inhibit media transfer.	Be sure there are no bubbles under filter before placing plates in incubator.
	Condensation from top of petri dish has precipitated onto filter.	Place plates into water bath in inverted position.

Quality Control for *Escherichia coli* Membrane Filter Procedure

Document the Following (Including but not limited to):

Supply Water Quality

- Chlorine and Ammonia Free
- No Chlorine Demand
- Conductivity
- Copper, Lead, Zinc, Nickel, Cadmium, Chromium, Iron, Silver levels (annual) <.01 mg/l*.

Sample Collection

- GRAB - Exact time collected
  - Exact time analyzed
  - Exact location
- Must be collected directly into sterilized sample container.
- DO NOT overfill container - Lose sodium thiosulfate
- **HOLD TIME: 8 hours at 10℃ (max)**
- *EDTA addition eliminates metal toxicity problem.

Sampling Container & Analytical Glassware

- Sterility! Sterility! Sterility!
- Container must be sterilized. Sodium Thiosulfate solution must be placed in container before sterilization.
- All glassware must be sterilized and remain wrapped until analysis is performed.

***NOTE**: Supply water quality verification is recommended at this time but may soon be required.

Equipment

- Autoclave
  - Use indicator tape each use
  - Sterility indicator/monthly
  - Annual calibration/professional
  - Record date, time, and temp of each use
  - Water bath incubator
  - Constant temp 44.5℃ ± 0.2℃
  - Thermometer:
    - must have 0.1℃ graduations
    - must be calibrated (NIST)
  - Second incubator
    - constant temp of 35℃ ± 1℃

Reagents

- Media
- NIST Traceability
- Preparation (received) date and expiration date

- Verify pH of prepared media 1 per lot
- Dilution water - sterility
- Blank - dilution water only - 0 colonies
- Positive control - 1 mL primary effluent (etc.) - should produce a large number of colonies or colonies Too Numerous To Count TNTC

Duplication Schedule

- 100% replication of single dilution
- 5% minimum duplication

Validity

- Count plates with 20-80 CFU's
- Use weighted average of plates which were in 20-80 range

Reporting Escherichia Coliform Results- Membrane Filtration Method

Escherichia Coliform Bench Sheet- MF Method
Analyst:
Exact time and date sampled:
Exact time and date analyzed:
Exact time into and out of 35℃ incubator:
Exact time into and out of water bath:
Incubation Temperature:
Blank - colonies on plate:
Sample Dilution #1 - # of CFU's on plate: Sample Dilution #2 - # of CFU's on plate: Sample Dilution #3 - # of CFU's on plate:
Replicate Dilution - # of CFU's on plate:
Show calculations to determine CFU's/100 mL: NOTE: "TNTC" is not a valid result. An actual number must be calculated.

Escherichia Coli Analysis Enzyme Substrate Coliform Test

This method, just as in the modified membrane filtration method, depends upon the fact that the 4-methyl-umberlifery-B-D-glucuronide (MUG), included in the fluorogenic substrate, will be hydrolyzed by B-D-glucuronidase enzyme produced in most *E. coli*. This reaction produces a bluish fluorescence that can be seen under an ultraviolet light at 365-366nm. In this method, other chromogenic (develops a color reaction) substrates are included. These chromogenic substrates are hydrolyzed by the enzyme B-D-galactosidase (produced by total coliform) to cause a color change which indicates the presence of total coliform. A member of the Total Coliform group (yellow in color) that fluoresces can be identified as an *E. coli*.

Use of these methods is perfectly acceptable. However, keep in mind that Quality Control requirements must be met. These requirements might exceed those described in the method. Check with your assigned Wastewater Program analyst to determine what additional quality control procedures might be required. These QC requirements may include a blank and positive control to be run with each analysis performed **and** the establishment of a minimum 10% duplication schedule.

All NPDES reporting requires methods be sufficiently sensitive to assure compliance with permit limits. Some facility permits specifically require that the membrane filtration method be used - Be sure to check your permit.

Here is a link to the IDEXX Colilert® method for simultaneous detection of Total and Escherichia Coliforms.

https://www.idexx.com/files/colilert-procedure-en.pdf

Colilert 18 - IDEXX US This link includes an excellent description of the chemistry involved as well as an instructional demonstration of the method.

Thermotolerant (Fecal) Coliform Membrane Filtration Method

Background

Coliform bacteria occur in a variety of environments. Soil, decaying vegetation and natural waters all contain coliform bacteria, but fecal coliforms originate in the intestinal tracts of warm-blooded animals.

The coliform bacteria tests were developed to give a measure of the presence of pathogenic (disease causing) organisms. Although most coliforms are not pathogenic, their numbers are related to the numbers of pathogenic organisms. A good kill of coliforms will indicate a good kill of pathogens, and therefore coliforms are called indicator organisms.

In past years, wastewater treatment operators were permitted to test their effluent for total or fecal coliforms. Coliform results are always expressed as colonies or MPN (**m**ost **p**robable **n**umber) per 100 mL of sample water. The maximum allowable number of coliforms was 500/100 mL for total coliforms and 200/100 mL for fecal coliforms. Next only the fecal coliform test (Now referred to as “Thermotolerant Coliform Bacteria test) was acceptable because these bacteria are more closely associated with sewage. Now most Vermont National Pollutant Discharge Elimination Systems (NPDES)

permits require analysis of *E. coli* bacteria specifically, while a few still require fecal coliform analysis, so we will describe it here.

There are two approved methods for the analysis of fecal coliform. These methods are the **m**embrane **f**ilter (MF) procedure and the multiple-tube fermentation procedure. (Also referred to as the most probable number (MPN) method.) The MPN test may be used for primary or secondary effluents, but the membrane filter test may be used only for secondary or tertiary effluents. Since primary effluent is often turbid, it may be difficult to filter a sufficient amount to adequately test this effluent. Also, colonies growing on a filter with many solids present, tend to run together, making it quite difficult to count these colonies.

The membrane filter method involves filtration of three different volumes of sample. The filters are placed in petri dishes, containing agar or liquid broth absorbed onto a pad, and incubated for 24 hours.

Equipment

- Autoclave, dry oven or other type of sterilizer
- Kraft paper
- Aluminum foil
- Autoclave indicator tape
- Masking tape
- Funnel assembly - 47 mm, autoclavable
- Membrane filters - made specifically for microbiology
- cellulose nitrate or cellulose nitrate/cellulose acetate
    - 47 mm, sterile, white, gridded on one side
    - 0.45 um or 0.7 um pore size
- Absorbent pads - 47 mm, sterile
- Petri dishes - 50 mm x 9 mm, plastic, sterile, tight-fitting halves
- Forceps - flat tipped
- Beakers - 50 mL, 250 mL
- Vacuum pump
- Vacuum flask - 500 mL, fitted with rubber stopper and glass tubing
- Vacuum flask - 1000 mL
- Tubing - thick walled tygon or rubber
- Plastic bags - 18 oz. Whirl-pak or similar waterproof bag, e.g., Ziploc
- Water bath - must be able to maintain a temperature of 44.5℃ ± 0.2℃.
- Incubator - air, must be able to maintain a temperature of 35.0℃ ± 0.5℃
- Thermometer† - range of 20℃ to 50℃ with no larger than 0.2℃ graduations.
- Sample bottles - glass or plastic (polypropylene), autoclaveable
- Fluorescent lamp
- Microscope or magnifying glass to give 10x - 15x magnification
- Colony counter - hand held
- Graduated cylinders - 100 mL, 1000 mL
- Volumetric flasks - 250 mL
- Erlenmeyer flasks - 250 mL, 250 mL screw top, 2000 mL screw top
- Graduated pipet - 5 mL, 25 mL
- Bacteriological pipets - 10 mL, 1 mL
- Refrigerator
- Glass stirring rod
- Stirrer magnet - bar
- Magnetic stirrer
- Burner - alcohol or Bunsen
- Parafilm or volumetric flask covers
- 0.45 um filters (Millipore HA, Gelman GN 6 Metric, MFS (Micro Filtration Systems), or similar)

† Thermometers should be checked at least once a year against a certifiable NIST (National Institute of Science and Technology) thermometer or one traceable to an NIST thermometer. Any deviation from the NIST thermometer should be noted and taped to the thermometer, e.g. reads 0.1°C high at 44.5°C. Readings taken from this thermometer should always be adjusted according to the deviation.

Cleaning and Sterilization of Equipment

Glassware used for bacteria testing should not be acid washed. This also applies to any equipment such as spatulas, stirrer bars, etc. with which reagents are made for coliform tests. Any acid residue on the glassware will kill bacteria. Wash bacteria glassware in hot, soapy water with a detergent that doesn't inhibit growth, e.g., Micro. Rinse glassware thoroughly in hot tap water and then with distilled or deionized water at least 3 times.

Sterilization of bacterial glassware such as sample bottles, dilution bottles, funnel setups and pipets can be accomplished using an autoclave set at 15 psi and 121°C or a dry oven set at 170°C. Keep in mind that reusable plasticware or liquids should never be sterilized in a dry oven.

Items such as funnels and pipets should be wrapped in kraft paper and secured with autoclave indicator tape before being placed in the autoclave, so as to allow the steam to penetrate. (Glassware sterilized in a dry oven may be wrapped in aluminum foil.) Also, always loosen the caps of sample and dilution bottles before autoclaving them.

The 10% sodium thiosulfate solution should be added to the sample bottles BEFORE they are sterilized. 0.1 mL (2 drops) will neutralize the chlorine in 100 mL of effluent. For effluents disinfected by ultra-violet light, the addition of sodium thiosulfate isn't necessary.

It is advisable to place a piece of autoclave indicator tape on equipment to be sterilized so that it can easily be identified later as having been processed. Autoclave indicator tape has invisible writing which appears after 15 minutes in the autoclave.

The following chart lists several items commonly used in Fecal Coliform analysis along with the required sterilization time for each. If your autoclave includes instructions indicating longer sterilization periods, follow those instructions except in the case of media such as lauryl tryptose broth, which should never be autoclaved for more than 15 minutes.

Item	Autoclave	Dry Oven
Sample Bottles	30 min	2 hours
Dilution Bottles	30 min	2 hours
Pipets	30 min	2 hours
Funnels	30 min	2 hours
1 L Dilution H_2O	35 min	
100 mL Lauryl Tryptose Broth	15 min	
Used Petri Dishes	30 min	2 hours

Reagents

NOTE: It is highly recommended that dilution water and prepared media (in petri dishes) be purchased rather than prepared on site. Therefore, we have eliminated the reagent list for preparation of the agar plates and those needed to prepare dilution water.

Sample Collection, Preservation and Holding Time

Coliform samples should be taken at the end of the outfall pipe after all treatment including de-chlorination if applicable, or at the weir at the end of the chlorine contact chamber. These samples must be taken as grabs into clean, sterile, sodium thiosulfate treated bottles. It is important to take the sample directly into the sterile bottle. Do not transfer a coliform sample from a dipper to the sterile sample bottle because of contamination problems. Allow about 1 inch of headspace for efficient mixing, and shake the sample to mix it with the sodium thiosulfate. It is usually required that you take the chlorine residual at the same time to establish a correlation between the two. Process the sample as soon as possible, refrigerate the sample at 10℃ or less. If a sample is sent to another lab for analysis, it **must** be kept in a cooler with ice and arrive at the other lab for analysis to be analyzed within 8 hours. Never mail a bacteria sample.

Filtration

1) Fill out the paperwork, e.g. dilutions you will use.
2) Set up the filtration equipment on a clean surface. Always use a trap flask to protect the vacuum pump from any water that may overflow from the vacuum flask.
3) Label the bottom half of petri dishes with the volume (e.g.,0.1 mL, 1 mL, 10 mL, etc.) of sample or blank they will contain.
4) If you are using agar plates, they are ready to be used with a filter. If you are using liquid media and absorbent pads, aseptically transfer an absorbent pad to the bottom half of each petri dish. Flame the top of the ampule before breaking it off and pour the entire contents onto the pad.
5) Pour 70% ethanol to the 40 mL line of a 50 mL beaker. Keep the forceps in it all the time except when handling a filter. This ethanol keeps the forceps sterile.
6) Unwrap a funnel and place it aseptically on the vacuum flask.
7) Open the filter package, pass the forceps through a flame to burn off the ethanol, and grasp the filter by the edge with the forceps.
8) Place the filter on the funnel base and the funnel head on the filter.
9) Pour 100 mL sterile dilution water onto the filter. This is the blank.
10) Turn on the vacuum pump and draw the blank through the filter. Rinse the sides of the funnel twice with 20-30 mL of dilution water.
11) Turn off the vacuum pump and loosen the funnel head.
12) Open the petri dish labeled "Blank 100 mL," and place the cover inside up on the work surface. Pour off any excess media. Loosen the funnel head.
13) Flame the forceps. Take the funnel head off with one hand and using the forceps pick up the filter with the other. Always grasp the filter by the edge. Place the funnel head back on the base.

14) Place the filter on the agar or absorbent pad. Pull back the leading edge of the filter and roll it forward to exclude air as described below.
    - Try to place the filter into the plate without dragging it across the edge of the plate. Repeat this procedure at the other 3 "corners" of the filter. If you are using agar plates, any air bubbles will show up on the underside of the plate. Bubbles prevent media from soaking into the filter, and any bacteria over a bubble won't grow. Although it may be tempting to poke at bubbles at get rid of them, the "rolling" procedure works better.

15) Cover the plate.

16) Filter the sample similarly beginning with the smallest volume. For 10 mL or less, pour about 20 mL of dilution water onto the filter before adding the sample. This allows the bacteria to disperse and not grow in one clump. Shake the sample about 25 times before pipetting off a volume. Always filter 3 different volumes of sample, e.g. 1 mL,10 mL and 50 mL to hopefully produce a readable filter.

17) After the sample has been filtered, run a "positive" control. A positive control is generally a drop of influent, or primary effluent collected before disinfection, filtered the same as the blank and sample. Growth on the positive filter assures that the media will grow fecal coliforms.

Incubation

The prepared Petri dishes should be placed into waterproof “whirl-pack” bags. Remove as much air as possible from the bags before sealing. Then invert the bags so that the dishes are upside down in the water bath. Make sure the dishes are completely submerged with anchors or by putting weights on them. This will help ensure that the important constant temperature of 44.5 ℃ +/- 0.2℃ is maintained for the entire 24 hour +/- 2 hours incubation time.

NOTE: Be sure that the water bath is at the required temperature before putting the samples in for incubation.

Incubation within a very strict temperature range of only 0.2 ℃ is very important for accurate measurement of Fecal Coliform. Although there are dry incubators that might be capable of maintaining the required temperature range if left unopened for the entire incubation period, water baths are preferred.

Calculations

Fecal Coliforms (Thermotolerant Coliform) will appear blue. Only blue colonies should be counted. Disregard any gray or cream-colored colonies. These are NOT Fecal Coliform.

Fecal Coliform (Thermotolerant Coliforms) results are reported as #Colony Forming Units (CFU) per 100 mL. The equation for calculating the results is:

$$\frac{\#\ of\ CFUs}{Volume\ of\ Sample\ (mL)} * 100$$

The desired number of colonies to be counted on a plate is 20-60.

If only one plate produces 20-60 colonies, then only that plate would be used in calculating the result. For example:

If sample volumes of 10, 30, and 50 mL produce results of 5, 15 and 25 colonies respectfully, the result would be calculated as:

$$\frac{25\ CFUs}{50\ mL} * 100 = \frac{50\ CFUs}{100\ mL}$$

If more than one plate produces colonies in the 20-60 range. A "weighted" average must be calculated. That is: All volumes within the acceptable range of 20-60, are added together and their sum is divided by the total of their volumes. For example, if 10 and 30 mL samples produce 20 and 50 CFUs:

$$\frac{(20\ CFUs + 50\ CFUs)}{(10\ mL + 30\ mL)} * 100 = \frac{175\ CFUs}{100\ mL}$$

If none of the plates produce at least 20 colonies the result is calculated using all the colonies counted divided by the total volume used. For example, if 10, 30, and 50 mL samples produce 1, 3, and 5 CFUs:

$$\frac{(1\ CFU + 3\ CFUs + 5\ CFUs)}{(10\ mL + 30\ mL + 50\ mL)} * 100 = \frac{10\ CFUs}{100\ mL}$$

If all plates produce more than 60 colonies the plate with the least number of countable colonies is used in calculating the result. For example:

If sample volume of 10, 30, and 50 mL produce results of 90, 150, and 200 colonies respectively, the result would be calculated as:

$$\frac{90\ CFUs}{10\ mL} * 100 = \frac{900\ CFUs}{100\ mL}$$

If no colonies are produced on any of the plates (except of course the positive control) the result would be calculated using the largest volume and assuming 1 colony was produced on that plate. A < sign would be included with that result. For example:

If sample volumes of 10, 30 and 50 mL all produce results of zero then the result would be calculated as:

$$\frac{1\ CFU}{50\ mL} * 100 = < \frac{2\ CFUs}{100\ mL}$$

When calculating monthly averages for Fecal Coliforms you must use the geometric mean. (*Note: At the time this manual was printed monthly averages for Fecal Coliform are not required in Vermont NPDES permits but are in some other states.)

To calculate geometric mean:

1) Find the log of the results
2) Find the sum of the logs
3) Divide the sum of the logs by the number of results
4) Find the antilog of the number

For example, if the results of 3 weekly Fecal Coliform analyses are:

50 CFUs/100 mL

30 CFUs/100 mL

20 CFUs/100 mL

The monthly average would be calculated like this:

1) Find the log of the results:

$$\log(50) = 1.69897$$

$$\log(30) = 1.47712$$

$$\log(20) = 1.30103$$

2) Find the sum of the logs:

$$1.69897 + 1.47712 + 1.30103 = 4.47712$$

3) Divide the sum of the logs by the number of the result:

$$\frac{4.47712}{3} = 1.49237$$

4) Find the antilog of the number:

$$10^{1.49237} = 31.07229$$

5) **Answer:** 31 CFUs/100 mL

Fecal Coliform Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Colonies are observed on the blank.	Autoclave/sterilizer not performing properly/non-sterile technique. Dilution water not sterile.	Verify that all equipment, glassware is sterile, forceps are dipped in alcohol and flamed before each use and good sterile technique is used throughout the procedure. Watch dilution water carefully for growth or discoloration. Discard when either is observed.
No colonies observed in positive control.	Bacterial inhibitors in sample, sample container, or equipment.	Use sodium thiosulfate in sample collection bottle. Addition of EDTA to the collection bottle (before sterilization) can eliminate inhibition by several heavy metals.
All colonies are bundled together in center of filter.	Poor dilution technique.	Add dilution water to funnel first then add sample to allow dispersion of bacteria.
None of the dilutions used produced the desired 20-60 CFUs (<20 on all plates).	Insufficient sample volume.	Increase sample volume - up to 100 mL (undiluted).
All dilutions used produce more than 60 CFUs.	Too much sample used.	Dilute sample.
White areas with no colony growth appear on filter.	Air bubbles under filter inhibit media transfer. Condensation from top of petri dish has precipitated onto filter.	Be sure there are no bubbles under filter before placing plates in incubator. Place plates into water bath in inverted position.

Quality Control for Fecal Coliform Membrane Filter Procedure Standard Method 9222-D

Document the Following (Including but not limited to):

Supply Water Quality

- Chlorine and Ammonia Free
- No Chlorine Demand
- Conductivity
- Copper, Lead, Zinc, Nickel, Cadmium, Chromium, Iron, Silver levels (annual) <0.01 mg/l*.

Sample Collection

- GRAB - Exact time collected
    - Exact time analyzed
    - Exact location
- Must be collected directly into sterilized sample container.
- DO NOT overfill container - Lose sodium thiosulfate
- **HOLD TIME: 8 hours at 10℃(max)**
- *EDTA addition eliminates metal toxicity problem.

Sampling Container & Analytical Glassware

- Sterility! Sterility! Sterility!
- Container must be sterilized. Sodium Thiosulfate solution must be placed in container before sterilization.
- All glassware must be sterilized and remain wrapped until analysis is performed.

NOTE: Supply water quality verification is recommended at this time but may soon be required.

Equipment

- Autoclave
    - Use indicator tape each use
    - Sterility indicator (use monthly)
    - Annual calibration/professional
    - Record date, time and temp of each use
    - Water bath incubator
        - Constant temp 44.5℃ ± 0.2℃
        - Thermometer:
            - must have 0.1℃ graduations
            - must be calibrated (NIST)

Reagents

- Media
- NIST Traceability
- Preparation, date received and expiration date
- Verify pH of prepared media 1/lot

- Dilution water - sterility
- Blank - dilution water only - 0 CFU's
- Positive control -1 mL primary effluent (etc.) – should produce very high number of colonies or colonies Too Numerous To Count (TNTC)

Duplication Schedule

- 100% replication of single dilution
- 5% minimum duplication

Validity

- Count plates with 20-60 CFU's
- Use weighted average of plates which were in 20-60 range

Reporting Fecal Coliform Results

Fecal Coliform Bench Sheet
Analyst:
Exact time and date sampled:
Exact time and date analyzed:
Exact time into and out of incubator:
Incubation Temperature:
Blank – CFU's on plate:
Sample Dilution #1 - # of CFU's on plate: Sample Dilution #2 - # of CFU's on plate: Sample Dilution #3 - # of CFU's on plate:
Replicate Dilution - # of CFU's on plate:
Show calculations to determine CFU's/100 mL: NOTE: "TNTC" is not a valid result. An actual number must be calculated.

Thermotolerant (Fecal) Coliform Enzyme Substrate Method (MPN)

Enzyme substrate methods use chromogenic (cause a color reaction) and fluorogenic (cause a light reaction).

The 23rd edition of Standard Methods has 28 pages of quality control procedures related to Coliform analysis. Section 9223B2 "Quality Control" refers to required quality control methods for use of enzyme substrate tests such as Colilert® and Colisure®. This Section specifically states that "Method users must adhere to the quality assurance (QA)/QC guidelines in Section 9020, including, but not limited to, analytical QC (Section 9020B.9), instrumentation/equipment (Sections 9020 B.4 and 9030B) and supplies (Section9020B.5) Refer to Table 9020: I for key QC procedures." Whereas the State has the right to require more stringent requirements than EPA, less stringent requirements are prohibited. Therefore, for analyses performed at or for NPDES regulated wastewater facilities quality control procedures beyond those described in IDEXX methods may be required. At a minimum reagent blanks and positive controls may be required for each analysis.

Here is a link to the IDEXX Colilert 18 method for Fecal Coliform:

Colilert 18 - IDEXX US

This link gives an excellent description of the method and the chemistry involved. There is also a very handy instructional video. The video demonstrates the method for Total Coliform and Escherichia Coliform method first. To view the Fecal Coliform method demonstration simply fast forward to that section of the video.

References

Fecal Coliform: Standard Methods for the Examination of Water and Wastewater, 23rd Edition, Method 9222D, Pages 9-60 through 9-61.

Escherichia coliform: EPA Method #1103.1, 2010, Test Methods for Escherichia Coli and Enterococci in Water by the Membrane Filter Procedure

Standard Methods for the Examination of Water and Wastewater, 23rd Edition, Method #9213 D 3.

Nitrogen

VT WSMD Wastewater Program Lab Manual Section #14

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Nitrogen

Nitrogen limits are included in most Vermont NPDES permits, particularly those in the Long Island Sound Watershed. Whereas Phosphorus is often the “limiting factor” considered responsible for eutrophication and algal blooms in fresh waterbodies such as Lake Champlain, nitrogen is considered the limiting factor responsible for these problems in salt water. Both nutrients play a role in creating eutrophication and algal blooms.

Under “normal conditions” in nature Phosphorus and Nitrogen are not available in concentrations sufficient to allow widespread algal blooms. Nutrient concentrations rise significantly due to runoff from impervious surfaces, dirt roads, farms, fertilized lawns, and other sources that enter the water body.

In wastewater we are primarily concerned with four forms of nitrogen: ammonia, organic nitrogen, nitrites, and nitrates. The Total Kjeldahl Nitrogen (TKN) analysis measures ammonia and organic nitrogen. The nitrite and nitrate (NO_x) analysis convert all nitrites to nitrates and the result includes the sum of both forms. In order to “speciate” or measure each of the two forms the nitrite and nitrate analyses must be performed individually.

NOTE: Total Nitrogen is the sum of Total Kjeldahl Nitrogen, Nitrites and Nitrates. **If ammonia is analyzed separately be sure NOT to include the ammonia result in the Total Nitrogen result as it is already included in the TKN**!!

Remember; $TN = TKN + NO_2 + NO_3$

TKN = Ammonia + organic nitrogen

Ammonia Nitrogen

Ammonia, (NH3) is a very important parameter to be analyzed at wastewater facilities. It is extremely toxic to aquatic organisms, and analysis can be very informative regarding the effectiveness of treatment. Finding excessive ammonia in facility effluent suggests the facility is failing in the breakdown of ammonia to nitrites and nitrates. The efficiency of nitrification can further be established by measuring concentrations of nitrite (NO2) and nitrate (NO3). High nitrites in effluent can indicate a lack of complete nitrification which is a serious problem. This will be discussed further in the appropriate section of this manual.

The concentration and presence of interferences in the sample determine the method used for analysis. Whereas the selective ammonia ion electrode method is acceptable and has been used by some Vermont wastewater facilities, many operators have found it to require excessive maintenance and affected by many interferences. Likewise, the titrimetric method has been determined by most operators to be too time consuming, cumbersome and requires distillation of the sample before analysis. **Note:** For all of these methods it is important to <u>use ammonia free distilled water.</u> **Deionized water is not recommended as it often contains ammonia. Also samples from <u>each individual facility</u> must be proven to NOT contain interferences in such quantity as to adversely affect analytical results.** That can be accomplished by performing the analysis on distilled vs. non- distilled samples. If results do not vary significantly (<10% is ok) analyses may be performed without distillation.

For instructions regarding distillation: refer to Standard Methods for Examination of Water and Wastewater 23rd edition section 4500 NH3 B

Interferences: There are many substances that can interfere with the ammonia analysis. These include urea, glycine, glutamic acid, cyanates, acetamide, chlorine and others depending upon the method used. It is very important to dechlorinate the sample immediately upon collection if a residual chlorine is expected.

Hold Time: Best results are obtained from samples that are analyzed immediately. If this isn't possible the unacidified sample should be refrigerated at 6 ℃ and analyzed within 24 hours.

Procedures: At this time Vermont facility operators seem to prefer the Hach® Test 'n Tube methods for ammonia analysis. A link to that method is provided here. Keep in mind that this method uses deionized water as a blank with a blank correction. **It is preferred to use ammonia free distilled water for all ammonia analyses. ALSO keep in mind that this method is an "equivalent method". That means that it is generally accepted IF quality control practices are equivalent to the original method. Check with your assigned Wastewater Program analyst to assure the method is accepted for NPDES reporting purposes.**

Since the Hach TNT methods for many parameters have become so widely used by Vermont wastewater operators it seems prudent to attempt to clear up some of the confusion regarding acceptability of these methods. Several Hach methods are EPA approved and acceptable for compliance reporting according to 10 CFR part 136. However, many are not. Whereas Hach methods are generally very good, simplified methods designed to be easy to use while generating valuable results, the nomenclature used in describing the acceptability is somewhat confusing.

For example: The Hach TNT Plus (tm) 830/831/832 Ammonia Method 10205

The three different numbers refer simply to different concentration ranges.

"Method" 830 range = 0.015-2.00mg/L NH3-N

"Method" 831 range = 1 – 12 mg/L NH3-N

"Method" 832 range = 2 – 47 mg/L NH3-N

Method 10205 is considered by Hach to be equivalent to EPA methods 350.1, 351.1, and 351.2 for the purposes of regulatory reporting of Ammonia (as nitrogen) and Total Kjeldahl Nitrogen to the original EPA method.

In a 2007 EPA memo the EPA stated that the chemistry is equivalent to those methods. Hach method 10205 does not appear in 40 CFR 136. BUT in using this method it is acceptable to cite EPA method 350.1, 351.1, or 351.2 **ONLY IF** the quality control procedures from the original EPA methods are used.

Bottom line: This Hach method and associated procedures are acceptable as long as quality control requirements from the original EPA methods are met.

The link to the Hach® TNT method 830 for low range ammonia is:

https://www.hach.com/asset-get.download-en.jsa?id=19556239154

For higher ranges download Hach methods 831 or 832

Here's a link to Hach® method 832 for high range ammonia:

DOC312.53.94127_1Ed_TNT832 (3).pdf

Nitrites/Nitrates

The analysis for nitrite **and** nitrate (NO_x) converts all nitrites to nitrates and the result includes the sum of both forms. In order to "speciate" or measure each of the two forms the nitrite and nitrate analyses must be performed individually. At this point in time most NPDES permits do not require "speciation". Therefore, the combined Nitrite/Nitrate analysis is often the method chosen by operators.

Actually "speciation", the measurement of nitrites and nitrates separately, can be quite useful to operators as measurement of nitrites can help identify problems with the process (such as incomplete or lack of nitrification). In at least one case at a Vermont wastewater facility this knowledge could have saved the municipality a great deal of time, money, and frustration. In that case, more and more chlorine was needed to produce the required chlorine residual to provide an acceptable coliform "kill". The facility dug up the chlorine line, suspecting a leak underground. As it turned out there was no leak. In fact, the problem was a loss of nitrification at the facility. Incomplete nitrification occurs when the oxygen concentration is insufficient to convert nitrites to nitrates. The problem is compounded because nitrites act as a "chlorine sponge." In fact, it requires at least 6 times as much chlorine to produce a residual in "partial nitrification" situations.

Partial nitrification can be caused by a number of factors including cold temperature, nutrient deficiencies, high influent ammonium, toxicity, pH changes, insufficient retention time in the aeration tank, very high soluble BOD or low Dissolved oxygen. Also, nitrites can be toxic to aquatic organisms **AND** wastewater organisms including floc-forming bacteria* (from Climate Policy Watcher article, link below). Obviously, analysis of nitrites can be a very useful process control tool.

*For more information regarding nitrification there is an excellent article in "Climate Policy Watcher" entitled Nitrite Ion Accumulation. The link is:

- Nitrite Ion Accumulation - Nitrification - Climate Policy Watcher (climate-policy-watcher.org)

The most commonly used method at Vermont wastewater facilities for Nitrite/Nitrate analysis is the Hach TNT 835/836 method 10206. This method converts the nitrite to nitrate to give a combined nitrite/nitrate result. Nitrite levels in exceedance of 2 mg/L interfere with the analysis. A procedure for elimination this interference is provided in the method description. But how would you know there was a nitrite interference without performing the separate nitrite analysis? The good news is that under "normal conditions" nitrite levels would be far less than 2 mg/L. Keep in mind that this analysis is

delegated as “equivalent” by the Hach company. Therefore, be sure to check with the assigned Wastewater Program analyst for acceptability regarding NPDES reporting purposes.

Here is a link to Hach TNT 835/836 method 10206 for determination of nitrite/nitrate:

Hach Nitrate Method 10206 Final 01102013 (1).pdf

Nitrite (NO_2^-)

As mentioned earlier, the nitrite analysis can be very useful. There is a colorimetric method described in section 4500 NO2 B in Standard Methods For Analysis of Water and Wastewater The method requires the use of nitrite free distilled water which is very difficult to prepare in most wastewater facility situations. For that and other reasons most Vermont wastewater technician prefer to use the Hach TNT 839 method. The Hach methods make use of “reagent blanks” to negate color, turbidity and other interferences that might be found in the reagents themselves. The result of the reagent blank is then subtracted from the sample result. This allows for the use of deionized water as a “reagent”.

This method is described as “equivalent” to EPA method 10207. Be sure to check with the assigned Wastewater Program analyst for acceptability regarding NPDES reporting purposes. Quality control requirements are described in the method. Be aware that Hach® recommends that standards for this analysis be prepared from directions in section 4500-NO2 B of Standard Methods for the Examination of Water and Wastewater

https://www.hach.com/asset-get.download-en.jsa?id=7639982523

Preservation

Note: Never use acid to preserve a sample for nitrite analysis. This could cause conversion of nitrites to nitrates.

Analyze samples for nitrite as soon as possible to prevent bacterial conversion of nitrites to nitrates.

Nitrate (NO_3^-)

Most of the methods described in 4500-NO3 of Standard Methods For the Analysis of Water and Wastewater are for one reason or another not very practical for in-house analysis at wastewater facilities. The Ultraviolet Spectrophotometric method is for samples with very low organic material (i.e. drinking water or uncontaminated natural water). The Nitrate Electrode method requires that temperature variations between sample and standards be kept within +/- 1℃, sample pH must be held constant, and the ionic strength of the sample and standards must be constant.

The most commonly used method for nitrate analysis performed at Vermont wastewater facilities is Hach TNT method 10206. The acceptability information noted below is taken from the Hach® website.

Method 10206 using the Nitrate TNTplus Vial Test, LR (0.2-13.5 mg/L NO3-N) and Nitrate TNT plus Vial Test, HR (5-35 mg/L NO3--N); (Product # TNT835 and TNT836) is **EPA approved** for drinking water cited

in the 40 Code of Federal Regulations (CFR) 141 and is approved for wastewater cited under 40 CFR 136 (Clean water Act).

A link to this method is provided here:

https://www.hach.com/asset-get.download-en.jsa?id=7639983742

Preservation:

- It is important that the sample be analyzed within 3 hours for this analysis (method).
- It is very important that the sample be between 20℃ and 23℃ before the analysis is performed for this method.
- Nitrite concentrations >2 mg/L will interfere with this method.

  *These preservation requirements apply specifically to the Hach TNT plus method .

Total Nitrogen

Total nitrogen is the sum of Total Kjeldahl Nitrogen, NO2 and NO3. It can be calculated from the results of those analyses performed by any of the methods described in 40 CFR 136 **OR** A Total Nitrogen analysis can be performed using a method such as Hach TNT for Total Nitrogen Persulfate Digestion Low range method 10071. The low range method has a range of 0.5 to 25mg/L N.

Be aware that at this time Hach method 10071 is not approved by EPA. It has not received "Hach's®" rating of equivalent and therefore its acceptability for NPDES reporting is not approved by the VT DEC. This could change. Be sure to check with the VT DEC before reporting results of this analysis for NPDES report purposes.

The link to the Hach TNT Persulfate Digestion method 10071is:

https://www.hach.com/asset-get.download-en.jsa?id=7639983804https://www.hach.com/asset-

A method description offered by the Hach® Company describes this method as using" an alkaline persulfate digestion under heat to convert all forms of nitrogen to nitrate. Chromotrophic acid is then added to react with nitrate and form a yellow complex with an absorption of 420nm."

Method 4500 N-C in Standard Methods For Examination of Water and Wastewater does describe a persulfate digestion method in which "total nitrogen is determined oxidation of all nitrogenous compounds to nitrate by alkaline oxidation at 100 to 110℃.

Kjeldahl Nitrogen: Semi-Micro Kjeldahl Method and Ion Selective Electrode Method

Background

Nitrogen is often the limiting factor in biological systems, especially systems that depend on bacteria for the breakdown of substances. Knowing how much nitrogen is present in a system can be very useful because a deficient supply of nitrogen can result in the inability of bacteria to grow.

Total Kjeldahl Nitrogen includes organic nitrogen compounds as well as ammonia nitrogen. This procedure allows for the measurement of nitrogen in amino acids, proteins, peptides and ammonia.

Many Vermont wastewater facilities performing in house nitrogen analysis at this time are using the Hach® TNT method for nitrogen analysis. For Total Kjeldahl Nitrogen, Hach® TNT 880 Method 10242 is often used. Here is a link to that method:

http://www.hach.com/simplified-tkn-s-tkn-tntplus-vial-test-0-16-mg-l-n-25-tests/produc…

After clicking on that link download the "Methods and Procedures" to see instructions for the analysis.

The ion selective electrode method described here is another method used at Vermont wastewater facilities for the determination of Total Kjeldahl Nitrogen. Digestion and distillation are required in all TKN methods for NPDES testing. After digestion and distillation, the following methods of TKN determination are also acceptable Titration, automated phenate, semi-automated block digester or potentiometric

Interferences

1. Metals: The interference from metals can be eliminated with the addition of the NaI EDTA solution.
2. *High Nitrate Concentrations: Nitrate more than 10 mg/L can cause a negative interference. This interference can be eliminated by running the sample through an anion exchange resin (chloride form) to remove the nitrate prior to TKN analysis.
    a. Unfortunately, this technique is far less successful when analyzing wastewater samples when the suspended solids concentration is high.
3. Organic Matter: Large amounts of organic matter can cause a positive interference. To negate this interference, add 10 mL concentrated H_2SO_4 to the digestion flask per gram of organic matter. (The organic matter can be estimated from COD results by assuming that 3 grams COD equals 1 gram of organic matter.)
4. Inorganic salts and solids cause boiling temperature to rise causing pyrolytic loss of nitrogen - addition of more H_2SO_4 gives reasonable results (1 mL additional H_2SO_4 per gram of salt).

    *NOTE: If acid addition is necessary for sample analysis the same volume of acid should be added to the blank.

Equipment

- pH Meter - capable of accepting ion selective electrodes and measuring in millivolt units.
- Ammonia probe - such as the Orion 95-10

- Magnetic Stirring Device - with TFE coated stirring bar
- Digestion apparatus - A Kjeldahl digestion apparatus with appropriately sized digestion flasks and suction to remove SO_3 fumes and water.
- Block digester (Technicon BD-40) or individual heating elements or hot plates
- Various graduated and volumetric pipets

Reagents

*NOTE: If samples are to be distilled see additional reagents list in Appendix A (as required for NPDES testing).

- Ammonia Free Distilled Water
  - Traces of ammonia in distilled water can be removed by adding 0.1 milliliter concentrated H_2SO_4 per liter to distilled water and redistilling. It's a good idea to throw out the first 100 milliliters of the distillate.
- Mercuric Sulfate Solution
  - Dissolve 8 grams mercuric oxide (HgO) into 100 milliliters *****6N H_2SO_4. This solution can be kept refrigerated at 4°C for up to one year.
  - *6N H_2SO_4 is prepared by pouring 167 milliliters concentrated (36N) H_2SO_4 into a one-liter volumetric flask containing about 500 milliliters distilled water. Then bring the total volume to one liter with distilled water.
- Digestion Reagent
  - To 650 milliliters distilled water add 200 milliliters concentrated H_2SO_4 **CAREFULLY!**
  - Dissolve 134 grams K_2SO_4 into the dilute H_2SO_4 solution
  - Then slowly add, while stirring, 25 milliliters of the mercuric sulfate solution (from #2 above). Dilute this solution to one liter with distilled water. This solution should be stored at room temperature to prevent crystallization. It can be kept for three months.
- 10N NaOH
  - Two liters of 10 N NaOH can be prepared by partially filling a two-liter wide mouth flask with distilled water. Adding 800 grams NaOH and diluting to two liters with distilled water. This solution can be stored at room temperature for up to six months.
- 10N NAOH, NaI, EDTA Solution
  - NOTE: This solution is needed only if interference from metals is suspected in the sample.
  - To approximately 500 milliliters distilled water add 800 grams NaOH, 600 grams NaI, and 4 grams EDTA. Dilute to 2 liters with distilled water. This solution can be stored for up to six months at room temperature.
- Stock $(NH_4)_2$ SO_4 Standard Solution 100 mg/liter TKN-N
  - Dry $(NH_4)_2SO_4$ for at least one hour at 105°C. Cool for one hour in a desiccator.
  - Weigh out 0.4706 grams $(NH_4)_2SO_4$ add this to approximately 500 milliliters distilled water and dilute to one liter with distilled water. This solution can be stored for 30 days refrigerated at 4°C.
- Working $(NH_4)SO_4$ Standard Solution 10 mg/liter TKN-N
  - Dilute 10 milliliters of the stock $(NH_4)_2$ SO_4 standard solution to 100 milliliters with distilled water. This solution must be used the day it is prepared.

- Calibration Standards
  - (select 5 from table which bracket analytical range.)

	Value of Std. in mg/l	**mg/50 mL**	**100 mg/l Std. per 50 mL**	**10 mg/l Std. per 50 mL**
	30 mg/l	1.5 mg	15 mL	
	20 mg/l	1.0 mg	10 mL	
******************	10 mg/l	0.5 mg	5 mL	
	8 mg/l	0.4 mg	4 mL	
	7 mg/l	0.35 mg	3.5 mL	
Normal range of wastewater	4 mg/l	0.2 mg	2 mL	
	2 mg/l	0.1 mg		10 mL
	1 mg/l	0.05 mg		5 mL
	0.6 mg/l	0.03 mg		3 mL
*******************	0.5 mg/l	0.025 mg		2.5 mL
	0.4 mg/l	0.02 mg		2 mL
	0.2 mg/l	0.01 mg		1 mL

Procedure

1) Digestion
   a. On the day the analysis will be performed, rinse boiling flasks with dilute acid (10% H_2SO_4 or 20% HCL). Rinse three times with distilled water.
   b. Using the table provided, prepare 5 calibration standards suitable to the expected range of samples to be analyzed.
   c. Shake samples vigorously before dispensing via a graduated cylinder. 50 mL is total volume. If dilution is needed, use less and record the dilution factor on the data sheet.
   d. To each flask, add boiling stones and 10 mL of digestion reagent. (Wide-mouth serological pipettes give the quickest delivery.)

e. Turn on vacuum for all digestion racks that are to be used - plug the unused manifold intakes with empty digestion flasks. Adjust the boiling rate and allow to boil until the solution clears or is pale straw color.

f. Heat the solution for 30 minutes after the dense white SO_3 fumes appear.

g. Allow flasks to cool.

h. If you are using the type of flask that the ammonia probe can fit into, simply dilute to 50 milliliters with distilled water (in the flask); cover and refrigerate.

If the probe will not fit into the flask: Measure out 44 milliliters distilled water into a graduated cylinder. Use this volume of distilled water to wash the contents of the digestion flask into a clean dry container capable of accommodating the ammonia probe. Refrigerate. This is a good stopping point if the procedure cannot be finished in one day. If distillation is required, go to Appendix A; if not, proceed to Section 2.

2) TKN Ammonia Probe: METER CALIBRATION (probe should be stored in filling solution when not in use).

a. Let the meter warm up for about 20 minutes before reading. Change the filling solution before each probe use (2.5 mL).

b. Place 100 mL distilled water into a 200 mL Berzelius beaker. Add a magnetic stirring bar.

c. Using a graduated pipette add 1 mL 10 N NaOH while stirring.

d. Place washed/dried probe into solution. Then set the meter to REL MV scale. Check for air bubbles on the probe membrane. If there are bubbles present remove them by gently tapping the probe against the side of the beaker or by removing and then reinserting the probe. Most ammonia probes are designed with a method for removing air bubbles from the probe body. Usually, this can be accomplished by gently pulling up on the probe lead wire and releasing.

e. Using a volumetric pipette add 1.0 mL of 100 mg/L $(NH_4)_2SO_4$ stock solution into the beaker.

f. Wait for the meter to stabilize then adjust the meter to read 000.0 by turning the calibration knob or pushing the zero button.

g. Using a 10.0 mL volumetric pipette transfer 10 mL of 100 mg/L $(NH_4)_2SO_4$ stock solution into the beaker. Once the meter stabilizes record the volume on a data sheet. The calibration is okay if the reading is -57.0 mv +/- 3 mv. If the response is slow the membrane should be changed according to instructions in the Ammonia Probe instruction pamphlet. If -57.0 ± 3 mv cannot be achieved, do an inner body check as outlined in the pamphlet. If that fails several times, a new probe will have to be purchased.

3) TKN Ammonia Probe: Sample Analysis (Treat standards exactly the same as samples.)

IMPORTANT NOTE: If samples are distilled use only 1 mL of NaOH. If samples are not distilled use a total of 10 mL NaOH as described below.

a. Add 6 mL 10 N NaOH to each Nalgene bottle containing digested sample.

b. Cool digested samples and standards in ice bath. Standards and samples should be at the same temperature.

c. Place a stir bar into the sample, place the rinsed and dried probe below the sample surface. Check for bubbles on the probe membrane - remove any bubbles by tapping the probe against the bottle (keeping the stir bar at the lowest possible RPM while providing adequate mixing will help cut down on bubble formation).

d. Allow the meter to recover to a value at least as positive as the value corresponding to the lowest concentration standard.

e. **If sample is suspected of containing interfering metals**:
Inject 4 mL 10 N NaOH, NaI, EDTA into sample using a graduated pipette. Check for air bubbles.

If sample does not contain interfering metals:
Inject 4 mL 10 N NaOH solution into sample using a graduated pipette. Check for air bubbles.

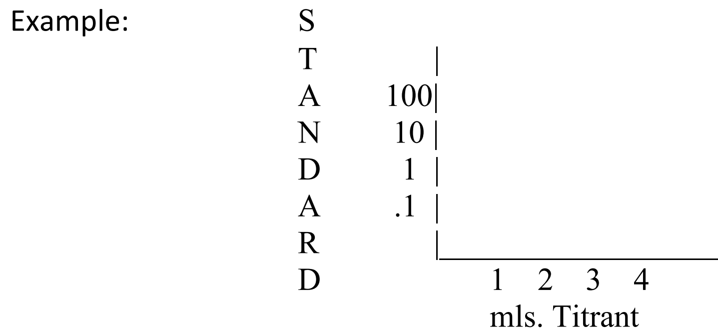
If you are unsure whether or not the sample contains metals:
Use the NaOH, NaI, EDTA solution.

f. Record the lowest (most negative) MV reading. Be sure to watch the meter carefully so as not to miss the "bottom".

g. After removing the probe and stir bar from the sample, rinse and dry.

Calculations

Plot a curve for your standards, using semilogarithmic graph paper or using Excel with a log scale on the Y axis.



Then from this curve obtain your sample results.

Then calculate your final result by applying this formula.

$$NH_3 - N\frac{mg}{L} = A * B * \left(\frac{101 + C}{101}\right)$$

Where:

A = dilution factor

B = concentration of NH_3-N mg/L from calibration curve

C = volume of 10 N NaOH added in excess of:

- 1 mL if the sample was distilled **OR**
- 10 mL if the sample was not distilled

Total Kjeldahl Nitrogen (Ion Selective Electrode Method)
Troubleshooting Guide

NOTE: This troubleshooting guide has been taken in its entirety from the Orion Model 95-12 Ammonia Electrode Instruction Manual.

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Off-scale or over-range reading	Defective Meter	Perform meter checkout procedure (see meter instruction manual)
	Defective inner body	Refer to Troubleshooting Guide (check inner body operation)
	Electrodes not plugged in properly	Unplug electrodes and reseat
	Internal filling solution not added	Fill outer body of electrode with proper amount of internal filling solution
	Air bubble on membrane	Remove bubble by redipping electrode
	Electrodes not in solution	Put electrodes in solution
Noisy or unstable readings (erratic - rapidly changing)	Insufficient internal filling solution	Fill outer body of electrode with proper amount of interal filling solution
	Defective Meter	Perform meter check out procedure (see meter instruction manual)
	Bottom cap loose	Ensure that bottom cap is screwed on tight enough to close gap between bottom cap and body
	Defective inner body	Check inner body operation
	ISA not used	Use recommended ISA, Orion Cat. No. 951211
	Meter or stirrer improperly grounded	Check meter and stirrer for grounding

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Drift (Reading slowly changing in one direction)	Internal filling solution leakage	Ensure that membrane is installed properly
	Incorrect internal filling solution	Refill outer body of electrode using filling solution shipped with electrode
	Total level of dissolved species above 1M	Dilute solution
	Electrode in sample too long; NH_3 loss	Reduce surface-area-to-volume ratio, slow rate of stirring, avoid high temperatures
	Membrane failure (wet, perforation, discoloration)	Replace membrane
	Solutions not at constant temperature	Allow solutions to come to room temperature before use
	Heat generated by Magnetic stirrer	Place insulating material between stirrer and beaker
	Defective inner body	Check inner body operation
	Electrode exposed to air for extended period	Hold electrode by outer body and pull up on electrode cable. Interal filling solution will flow under membrane and restore electrode response.
	Samples and standards at different temperatures	Allow solutions to come to room temperature before measurement

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Low slope or No slope	Standards contaminated or incorrectly made	Prepare fresh standards
	ISA not used	Used recommended ISA, Orion Cat. No 951211
	Standard used as ISA	Use ISA!
	Electrode exposed to air for extended period	Hold electrode by outer body and pull up on electrode cable. Interal filling solution will flow under membrane and restore electrode response.
	Membrane failure (wet, perforation, discoloration)	Replace membrane
	Defective inner body	Check inner body operation
"Wrong Answer" (But calibration curve is OK)	Incorrect scaling of semilog paper	Plot millivolts on the linear axis. On the log axis, be sure concentration numbers within each decade are increasing with increasing concentration
	Incorrect sign	Be sure to note sign of millivolt value correctly
	Incorrect standards	Prepare fresh standards
	Wrong units used	Apply correct conversion factor: 10^{-3}M = 17 ppm as NH_3 = 14 ppm as N
	Complexing agents in sample	Use known addition or titration techniques, or a decomplexing procedure
	ISA added to standards and not samples	Add same proportion of ISA to standards and samples

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Frequent build-up of deposits on ammonia probe membrane	Precipitation of hydroxides from sample	Add EDTA (Ethylene dramine tetraacetic acid) to the NaOH-NaI solution

Quality Control for Total Kjeldahl Nitrogen

Document the Following:

- Supply Water Quality Conductivity
  Ammonia Free
- **ALL** reagents <u>must</u> be made with ammonia free distilled water.

Sampling

- Sample Type
- Sample Time
- Duration of Composite
- Type of Composite
  - Time/Flow - include discrete volumes
  - Flow - include sample volume/discharge volume
  - Straight - document <10% flow rate change during sampling event
- Sample Location
- Preservation - H_2SO_4 <pH2 (2mL/Liter)
  - Volume of H_2SO_4 used
  - Refrigerate at 4°C
  - Hold Time - The maximum allowable hold time if properly preserved and refrigerated is 28 days. However, the sample should be analyzed as soon as possible due to conversion of organic nitrogen to ammonia.

Glassware

- Acid washed - Distilled water rinses - Class A

Equipment

- Distillation Apparatus: Properly "steamed out" before each use.
  Properly cleaned between samples

Analytical Results

- Blank - Treated just as sample result
- Standards - Treated just as sample. Number and concentrations used.
- Preparation Method results
- Standard Curve

Reporting Total Kjeldahl Nitrogen Data

TKN Bench Sheet
Sample Type:
Sampling Time & Date:
Sample Volume:
Sample Preservation:
Analyst:
Analysis Time and Date:
Method:
Blank Result:
Standards:
Concentrations Used:
Standard Curve:
Results of Individual Standards:
Sample Results:
Raw Data (millivolt readings):
Plotted Results on Curve:
Calculations:

Sample TKN Data Sheet

Date: 06-21-2021
Analyst: A D F
Probe Slope Check: -57.2 MV

TKN Report Sheet

	Lab ID	mL Sample	MV	Conc (mg/l)	COMMENTS
1	Blank	50 DI Water	-9.1	0.054	
2	Standard 0.2	1 mL 0.01 mg/l	-40.1	.186	Back Calculations
3	Standard 0.4	2 mL 0.01 mg/l	-62.9	.463	corr = .994
4	Standard 1.0	5 mL 0.01 mg/l	-80.5	.935	
5	Standard 4.0	20 mL 0.01 mg/l	-116.7	3.968	
6	QC TV 4.8	25	-105.0	4.92	$\% \text{Bias}= \frac{4.92-4.80}{4.80} = +2.5\%$
7	12345	50	-43.1	0.16 <DL	
8	12341	2	-83.7	26.5	$\%\text{RPD}= \frac{28.4-26.5}{27.5}= \frac{1.9}{27.5} =6.9\%$
9	12341 Dup	2	-85.4	28.4	
10	12342	25	-103.2	4.57	$\%\text{Rec}= \frac{(0.54)(53)-<DL}{3(10)}=95.4\%$
11	12343	50	-64.2	0.43	
12	12344	25	-111.3	6.34	
13	12346	50	-105.1	2.44	
14	Spike 12345	50 + 3	-70.5	0.54	3 mL 10 mg/l spike
15					

References

The selective ion electrode method for the determination of Total Kjeldahl Nitrogen can be found in section 4500-N in the 23rd Edition of Standard Methods for the Examination of Water and Wastewater - and in EPA's Methods for Chemical Analysis of Water and Wastes. Method 351.4 Storet No .00625

The troubleshooting section can be found in the Orion Model 95-12 Ammonia Electrode Instruction Manual

Appendix I: Total Kjeldahl Nitrogen Distillation

Reagents

- NaOH - THIOSULFATE SOLUTION: 500 g NaOH, 25 g $Na_2S_2O_3.5H_2O$ in 1 liter D.I. water.
- SULFURIC ACID: 0.1 mL concentrated H_2SO_4 to 10 mL D.I. water in each 50 mL volumetric used to collect distillate.

Glassware Preparation

- Steam out distillation apparatus before use.
- Label 50 mL volumetric receiving flasks for all blanks standard and samples.
- Add sulfuric acid described in reagent section to each flask.
- Condenser temp. must be below 29°C.

Procedure

1) Quantitatively transfer digested sample, standard, or blank into distilling unit. Use D.I. water to make the total volume about 30 mL.
2) Add 10 mL NaOH - thiosulfate reagent.
3) Close both stopcocks and turn rheostat up to 110 to do distillation. Be sure deliver tube is below surface of sulfuric acid in receiving flask.
4) At end of distillation raise delivery tube out of liquid, then turn rheostat down to 30.
5) Open both stopcocks and drain chamber.
6) Rinse several times with D.I. water.
7) With both stopcocks open and stream off (at 30) add sample and reagent.
8) Lower delivery tube below liquid, close stopcocks and turn up to 110 for next distillation.
9) Do 2 distillation blanks at beginning and one after each high sample.
10) Bring distillates up to 50 mL volume with D.I. water.
11) Cover with parafilm and continue with analysis by phenate method or by ammonia probe (see procedure section).

Reference

Standard Methods for the Examination of Water and Wastewater, 23rd Edition, Method 4500- NH_3B

Phosphorus

VT WSMD Wastewater Program Lab Manual Section #15

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Phosphorus

Background

Phosphorus in wastewater is almost always present in the form of phosphates. There are three major classifications of phosphates. They are: (1) Orthophosphates: Fertilizers are the major source of this class of phosphates; (2) Polyphosphates: (Pyro-, meta-, etc.). Detergents and commercial cleaning agents comprise the major source of these; and (3) Organic Phosphates: These are formed mainly by biological processes, their major source being body wastes and food residues. Organic phosphates may also be formed from orthophosphates during biological treatment. Analysis of Total Phosphorus includes all these forms of phosphates.

It is very useful to monitor the amount of phosphorus present in the waste stream because of its extreme importance in the growth of organisms. In fact, phosphorus can often be the limiting factor in the growth of organisms. Phosphorus plays an important part in "algal blooms", a common problem in Vermont lakes and streams.

Sampling and Preservation

Samples for phosphorus analysis should be taken from a composite sample into a glass or plastic bottle. If the sample will not be processed the same day, it should be preserved by adding a sufficient volume of concentrated sulfuric acid (H_2SO_4) to lower the sample to pH 2 or less. It should then be refrigerated at 6°C until analyzed.

Glassware Preparation

Phosphate contamination is common because of the tendency of phosphates to adsorb onto glass surfaces. It is extremely important, therefore, to clean all glassware used in the analysis of phosphorus very carefully. All glassware should be acid-washed with a **hot** 1:1 HC1 solution and then thoroughly rinsed with distilled water. **It is an extremely good idea to use this glassware for phosphorus determination only!** After use, the glassware should be washed rinsed with distilled water and covered until its next use. If the glassware is filled with distilled water until used again, the acid washing is needed less frequently.

Equipment

- Spectrophotometer capable of measuring at 880 nm with a light path of 2.5 cm or longer.
  OR
  Filter photometer with a red color filter and a light path of 0.5 cm or longer.
- 1 cm cell or cuvette (for phosphorus concentrations of 0.3 to 1.2 mg/L)

Glassware

This depends on the method of digestion, etc. See specific method descriptions.

Generally:

- Minimum of 9 50 mL graduated cylinders
- 1 mL pipet (for H_2SO_4)
- 4 mL pipet (for ammonium molybdate solution)
- 2 mL pipet (for ascorbic acid)

* **REMEMBER**: All glassware used in the analysis of Phosphorus must be acid washed and should be dedicated to this analysis only.

Methods

There are three colorimetric methods for analysis of Total Phosphorus listed in the 23rd edition of Standard Methods for The Examination of Water and Wastewater. They are:

1. The vanadomolbdophosphoric acid method. This method is good for phosphorus in the range of 1-20 mg/L P (4500 - P. c)
2. The stannous chloride method (4500 -P. d)
3. The ascorbic acid method   This is an excellent method for Phosphorus in the range of 0.01 to 6 mg/L as P.

The ascorbic acid method is the preferred method in the great majority of Vermont wastewater facilities. The preferred means of performing this method is with the use of Hach T ’n T method 8190 for analysis of Total Phosphorus.

HACH® TEST’N TUBE METHOD

The most common method used by Vermont wastewater analysts for the determination of Total Phosphorus is Hach Test ’n Tube method. This method has proven to yield good results, is relatively easy to perform, safe if performed according to HACH instructions and negates the need for preparing dangerous chemicals onsite.

We have provided a link to the Hach method 8190 Test ‘N Tube method 8190 for Total Phosphorus. The method is applicable for phosphorus concentrations of 0.02 to 1.10 mg/l P. (0.06 to 3.5 mg/L PO_4^{3-}
Note: The State does recommend the use of at least two standards to be run every time the analysis is performed. These standards should bracket the expected phosphorus concentration of samples being analyzed.

https://www.hach.com/asset-get.download.jsa?id=7639983838

Note: There are other kits available for the analysis of Total Phosphorus from companies other than Hach®. This link is provided because at this time most Vermont facilities have the Hach® equipment necessary to perform the analysis. There are also other digestive methods available from Hach® and other companies

WET CHEMISTRY ASCORBIC ACID METHOD

The wet chemistry method described here is EPA accepted but not commonly performed at Vermont laboratories. The method is cumbersome, requires the use of dangerous chemicals and is no longer recommended. There are more “automated” ascorbic acid methods including the Hach® Test ‘N tube ascorbic acid method that are safer and CWA acceptable. The method can detect phosphorus in the range of 0.01 to 6 mg/L as P.

Reagent

- Ascorbic Acid
  0.1M - Dissolve 1.76 g ascorbic acid in 100 mL distilled water. Refrigerate at 6 °C. This reagent must be prepared fresh weekly.
- Sulfuric Acid
  5N - Partially fill a 500 mL volumetric flask with approximately 400 mL distilled water. **Carefully** add 70 mL concentrated sulfuric acid (H_2SO_4). **Slowly and carefully** bring the volume to 500 mL with distilled water.
- Potassium Antimonyl Tartrate Solution
  Partially fill (400 mL) a 500 mL volumetric flask with distilled water. Dissolve 1.3715 g potassium antimonyl tartrate $K(SbO)\ C_4H_4O_6 \cdot ½H_2O$ in the distilled water and then dilute to 500 mL with distilled water. This reagent should be stored in a glass-stoppered bottle.
- Ammonium Molybdate Solution
  Dissolve 20 g Ammonium Molybdate $(NH_4)_6\ Mo_7O_{24} \bullet 4H_2O$ in 500 mL distilled water. This reagent should be stored in a glass-stoppered bottle.
- Combined Reagent
  Allow all the reagents listed above to reach room temperature. Then in a 1-liter beaker (or other large mouth container **ADD IN THIS ORDER:**
  - 50 mL 5N sulfuric acid (H_2SO_4) and 5 mL potassium antimonyl tartrate solution. Mix.
  - Then add 15 mL Ammonium Molybdate solution. Mix.
  - Add 30 mL of the 0.1M Ascorbic Acid Solution. Mix.
  - **This reagent must be used within 4 hours**.

ASCORBIC ACID METHOD

Procedure

Before colorimetric determination of phosphorus, all wastewater samples and standards used for calibration curve must be properly digested using one of the two methods described here.

NOTE: The perchloric acid digestion method, although acceptable is not mentioned here because of the inherent danger and special equipment associated with that method.

Digestion Method #1 - Sulfuric Acid - Nitric Acid Digestion

Equipment

- Fume Hood
- Digestion Rack
- Micro-Kjeldahl digestions flasks

Reagents

- Concentrated Sulfuric Acid (H2SO4)
- Concentrated Nitric Acid (HNO3)
- Phenolphthalein Indicator
- 1N Sodium Hydroxide Solution (NaOH)

Procedure

1) Pipet 50 mL of sample into a dry micro Kjeldahl flask or tube
2) Add 1 mL conc H2SO4 and 5 mL conc HNO3
3) Heat slowly on digestion rack until there is approximately 1 mL of solution left. Continue digestion carefully until the solution becomes colorless
4) Cool to room temperature
5) Add about 20 mL of distilled water to solution
6) Add 1 drop (0.05 mL) phenolphthalein
7) Add 1N NaOH one drop at a time until the solution develops a slight pink color
8) Dilute this solution to 100 mL with distilled water

Digestion Method #2 - Persulfate Digestion

Equipment

- Hot Plate
- Glass scoop

Reagents

- Phenolphthalein indicator
- Sulfuric Acid Solution:
  - Dilute concentrated sulfuric acid by slowly and carefully adding 300 mL of conc H_2SO_4 to 600 mL of distilled water. Then continue dilution with distilled water to 1 liter
- Ammonium persulfate $(NH_4)_2S_2O_8$ Solid
  OR Potassium persulfate $K_2S_2O_8$ Solid
- Sodium Hydroxide (NaOH) 1N.

Procedure

1) Pour 50 mL of well mixed sample into a suitable container (200 mL beaker)
2) Add 1 drop (.05mL) phenolphthalein indicator
3) If a red color develops, add sulfuric acid solution one drop at a time until red disappears
4) Add 1 mL of the sulfuric acid solution and 0.4 g solid $(NH_4)S_2O_8$ **or** 0.5 g solid $K_2S_2O_8$
5) Add one boiling chip (bead)
6) Gently boil the solution on the hot plate for 45 minutes or until a final volume of about 10 mL is reached (or heat for 30 minutes in autoclave at 98-137Kpa)
7) Cool to room temperature
8) Dilute to 30 mL with distilled water
9) Add 1 drop (.05mL) phenolphthalein indicator
10) Add sodium hydroxide solution (NaOH) until the sample develops a slight pink color
11) Dilute this sample to 100 mL with distilled water

Total Phosphorus Determination: Ascorbic Acid Method

Procedure

1) Pipet 50.0 mL of digested sample into a 125 mL Erlenmeyer flask.
2) Add 1 drop (.05mL) phenolphthalein indicator solution
3) If sample turns red add 5N sulfuric acid solution one drop at a time until red color disappears
4) Add 8.0 mL of the combined reagent and mix the solution thoroughly
5) After 10 minutes, but not more than 30 minutes, measure the absorbance at 880 nm

NOTE: The color should be stable for about an hour, but it is highly recommended that the absorbance be read within 30 minutes after the addition of the combined reagent.

Calculation

Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations on linear graph paper or a program with graphing ability. This standard curve must be prepared at least once a year using at least 6 standard concentrations.

Obtain concentration value of sample directly from prepared standard curve. Report results as P, mg/L.

Standards

EPA requires that at least one (preferably more) standard be run to calibrate the spectrophotometer each time. Below are instructions for making three stock solutions, and directions for making dilutions of the stock solutions for a set of six appropriate standards. The standards chosen here to encompass a wide range of likely results. They can be modified to suit specific situations. Use Class A volumetric glassware for all solutions.

Solution A: Stock phosphate solution of 100 mg/L. Dissolve 0.4393 g of predried (105 °C for 1 hour) KH2PO4 in distilled water and dilute to 1,000 mL. Make fresh each month.

$$1.0\ mL = 0.1\ mg\ P$$

Solution B: Stock phosphate solution of 10 mg/L. Dilute 100.0 mL of Solution A to 1,000 mL with distilled water.

$$1.0\ mL = 0.01\ mg\ P$$

Solution C: Stock phosphate solution of 1 mg/L. Dilute 100.0 mL of Solution B to 1,000 mL with distilled water.

$$1.0\ mL = 0.001\ mg\ P$$

The set of standards (0.1, 0.2, 0.4, 0.8, 1.0, 1.5 mg/L) is then made:

1. 0.10 mg/L: Dilute 1.0 mL of Solution B to 100 mL

$$\frac{0.01\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.10\frac{mg}{L}$$

2. 0.20 mg/L: Dilute 2.0 mL of Solution B to 100 mL

$$\frac{0.02\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.20\frac{mg}{L}$$

3. 0.40 mg/L: Dilute 4.0 mL of Solution B to 100 mL

$$\frac{0.04\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.40\frac{mg}{L}$$

4. 0.80 mg/L: Dilute 8.0 mL of Solution B to 100 mL

$$\frac{0.08\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.80\frac{mg}{L}$$

5. 1.00 mg/L: Dilute 10.0 mL of Solution B to 100 mL

$$\frac{0.10\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 1.0\frac{mg}{L}$$

6. 1.5 mg/L: Dilute 15.0 mL of Solution B to 100 mL

$$\frac{0.15\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 1.5\frac{mg}{L}$$

Total Phosphorus Ascorbic Acid Method Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Inconsistent or abnormally high phosphorus results	Contaminated Glassware	Acid wash all glassware used in the analysis and use only dedicated glassware
	Fingerprints on sample cell or Improper placement of cell	Make sure to handle cell so as to avoid fingerprints in light path - **Be careful** to place the cell into measuring device described in manufacturer's instructions. **Clean nitrile gloves should be worn when handling cells.**
	Sample phosphorus concentration too high	Dilute the sample prior to digestion
	Highly colored or turbid sample	Use a blank which consists of a sample to which all reagents except the ascorbic acid and antimonyl potassium tartrate have been added. The blank absorbance is then subtracted from the absorbance of each sample. Dilute sample before digestion

Quality Control for Total Phosphorus

Document the Following (Including but not limited to):

<u>Supply Water Quality</u>

- Conductivity < 10 micro siemens
- Phosphate Free

<u>Sampling</u>

- Sample Type - (usually a composite)
- Sample Time - time sample collection started and ended
- Duration of Composite - 8 hour, 24 hour
- Type of Composite
    - Time/Flow - include discrete sample volumes
    - Flow - include volume/sample per volume of discharge
    - Straight - document <10% change in flow rate during sampling event
- Sample Location

<u>Glassware</u>

- Acid washed - Distilled water rinses
- Dedicated to Phosphorus Analysis ONLY

<u>Equipment</u>

- Spectrophotometer
    - wavelength 880 nm
    - light path < or = 2.5 cm
- Photometer
    - filter Red
    - light path < or = 0.5 cm
- Service Records

<u>Analytical Results</u>

- Blank
    - What was used
    - How was it treated
    - Results
    - Frequency
- Standards
    - Lot #s, preparation and expiration dates
    - Number and concentrations used
    - Frequency of Use - at least one per set up
    - Results
    - Graph - Plotted standard results – annual

<u>Duplicate</u>

- Replicate schedule

Reporting Total Phosphorus Data

TOTAL PHOSPHORUS BENCH SHEET
SAMPLE TYPE:
SAMPLING TIME AND DATE:
SAMPLE VOLUME:
SAMPLE PRESERVATION:
ANALYST:
ANALYSIS TIME AND DATE:
METHOD:
BLANK RESULTS (include calculation if applicable):
STANDARDS
Concentrations Used:
Results (include calculations if applicable):
SAMPLE RESULTS
Raw Data and Calculations:

Orthophosphate Colorimetric Method Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Inconsistent or abnormally high orthophosphate results	Contaminated Glassware	Acid wash all glassware used in the analysis and use only dedicated glassware. Rinse the sample vial and **CAP** before analysis
	Fingerprints on sample cell or Improper placement of cell	Make sure to handle cell so as to avoid fingerprints in light path - **Be careful** to place the cell into measuring device described in manufacturer's instructions. **Nitrile gloves should be worn when handling cells**
	Sample orthophosphate concentration too high	Dilute the sample prior to digestion

References

Methods for the analysis of orthophosphate can be found on page 356.3-1 of The Manual of Methods for chemical analysis of Water and Wastes and in the 23rd Edition of Standard Methods for the Examination of Water and Wastewater section 4500-P

Hach® Methods can be found via links provided.

Orthophosphate/Reactive Phosphorus for Process Control

Phosphates that can be measured with colorimetric analyses **without** first being digested are referred to as reactive phosphorus. This portion of the phosphorus in wastewater usually consists of mostly orthophosphate but can contain other condensed phosphates. Most Vermont wastewater facilities are interested in orthophosphate concentrations as a quick indicator of Total Phosphorus concentrations for process control purposes. The methods described here are designed for that purpose. If orthophosphate appears in a facility's permit as an NPDES required parameter, the sample will have to be filtered immediately upon collection through a 0.45-micron filter before analysis. For process control purposes filtering is not necessary.

The most common method of analysis of orthophosphate/reactive phosphorus in Vermont wastewater facilities is the Hach ascorbic acid TNT method. This method can be performed using the same colorimeter as used for Total Phosphorus without the need for the digestor / reactor.

Here is a link for the Hach orthophosphate/reactive phosphorus method.

https://www.hach.com/asset-get.download.jsa?id=7639983836

Note: Other companies may provide kits acceptable for this analysis. This link is provided because at this time the majority of Vermont wastewater facilities have the Hach equipment necessary to perform this analysis.

Quality Control

Because in most cases orthophosphate is measured for process control only, there are no required quality control procedures. However, it is generally a good idea to measure standard(s) in approximating the expected concentration of the sample in order to be sure the results generated are as accurate as possible. Typically, a 1.0ppm standard is purchased. Dilutions are prepared from that standard.

Recommended Quality control:

Supply Water Quality

- Conductivity < 10 micro siemens
- Phosphate Free

Sampling

- Sample Type – usually a grab but a check can be done on composites
- Sample Time -
- Sample Location

Glassware

- Acid washed - Distilled water rinses

- Dedicated to Phosphorus Analysis ONLY

Equipment
- Spectrophotometer
  - wavelength 880 nm
  - light path < or = 2.5 cm
- Photometer
  - filter Red
  - light path < or = 0.5 cm
- Service Records

Analytical Results
- Blank
  - The sample is used to "zero" the instrument
- Standards
  - Number and concentrations used
  - Frequency of Use - at least 1/set up
  - Results

Duplicate
- Replicate schedule

Reporting:

Orthophosphate is recorded as mg/L as P. Be sure that (either) the instrument used converts the result from orthophosphate as PO_4 to orthophosphate as P. If the instrument used does not convert the result automatically there is a simple conversion that can be used. Simply multiply the mg/L as PO4 result by 0.326. For example: If the instrument used gives a reading of 2.0 mg/L PO4, simply multiply the result by 0.326 to yield a result of 0.652 mg/L as P. **Be sure to record the result as xx mg/L as P.**

Reporting Orthophosphate Data

ORTHOPHOSPHATE BENCH SHEET
SAMPLE TYPE: (typically a grab)
SAMPLING TIME AND DATE:
SAMPLE VOLUME: (volume used in analysis, typically 10 mL.)
SAMPLE PRESERVATION: N/A for process control (filtered for NPDES required reporting)
ANALYST:
ANALYSIS TIME AND DATE:
METHOD: (ex: Hach method 8048)
STANDARDS
Concentrations Used:
Results (include calculations if applicable):
SAMPLE RESULTS
Raw Data and Calculations:

References:

Standard Methods for Analysis of Water and Wastewater 23rd edition Section 4500-P

Hach®methods can be found via links provided