Chapter 14 : Wastewater Laboratory

Purpose of This Chapter

Performing accurate laboratory analysis of wastewater samples is a skill that takes time and practice to master. While advanced degrees and higher education are not required, critical thinking and trouble shooting abilities are a must. This chapter is intended to *introduce* the reader to laboratory tests, equipment and procedures. It is *not* intended to provide a comprehensive explanation of how to perform the tests.

Importance of the Laboratory

Laboratory tests are critical for the efficient control of the wastewater treatment plant as well as for the effluent monitoring required by discharge permits. Having a laboratory at your disposal is one of the most valuable assets a wastewater operator can have. Good operators turn to the laboratory for much of the information they need to troubleshoot problems in the treatment process. In fact, laboratory data can often be used to prevent problems from developing in the first place. Most medium and large plants in New Mexico have a full time lab analyst and the necessary laboratory equipment to run most NPDES and NMED-GWQB monitoring test as well as most process control tests. Small systems typically have only a limited ability to run process control test and take all of their effluent monitoring samples to a contract laboratory (with the exception of pH and total residual chlorine, which must be analyzed immediately).

Laboratory Certification

Many states require that lab analysts be certified, but New Mexico is not one of them. Laboratories and lab personnel that conduct monitoring test for NPDES and NMED-GWQB permits in New Mexico do not have to meet any certification requirements, however, laboratories that conduct drinking water analysis do. Lab experience does count toward operator certification, but it is awarded at one-quarter time. (Four years as a wastewater lab analyst counts as one year toward wastewater operator certification testing requirements). Many lab analysts are also certified operators.

Laboratory Safety

Many of the activities performed in wastewater treatment systems are dangerous, and working in the laboratory is no exception. Lab workers handle dangerous chemicals such as acid, bases and volatile compounds as well as infectious wastewater samples. Because of the hazards, it is important that lab workers consistently wear personal protective equipment (PPE) whenever appropriate. The PPE that is required for common daily tasks in the laboratory include; safety glasses, a face shield, goggles, rubber gloves and a lab coat. Laboratories themselves must be equipped with a variety of safety equipment. This equipment may include; a fume hood, an emergency shower and eyewash, fire extinguishers, a first aid kit and hazardous materials storage cabinets. Access to the laboratory should be limited (doors always closed during working hours and locked after hours).

Basic Laboratory Equipment and Procedures

Wastewater laboratories are filled with specialized instruments that must be used in a precise manner in order to obtain the desired accuracies. Understanding what level of accuracy is required is the first step in choosing what piece of equipment to use. Knowing which equipment delivers the desired level of accuracy is the second. Mastering the proper procedures and techniques associated with each instrument is also very important. The following is an overview of the basic lab procedures that all lab analysts should understand.

Weights and Measures

In the wastewater laboratory, the ability to precisely weigh items is very important. When a very high degree of accuracy is called for (+/- 0.1 mg), an *analytical balance* is used for weighing items. Other types of scales, such as a pan balance or a triple beam balance are used when less accuracy is needed. Whatever type of scale is used, it should be in good working order and checked for accuracy regularly. For analytical balances, this means verifying the accuracy against National Institute of Standards and Technology (NIST) certified Class 1 weights on a regular basis (generally monthly). It is also good laboratory practice to have balances checked and calibrated by an instrument service technician once a year.

Glassware

The glassware used in wastewater laboratories is constructed from specialized borosilicate glass. There are several types of glassware. Each type has a variety of uses based on the level of accuracy required. To properly read the volume of liquid in glassware, the level is measured to the bottom of the meniscus. (See Figure 14.1.)

Glassware should always be kept scrupulously clean and is easiest to clean immediately after use. Thorough cleaning with non-phosphate soap and scrubbing followed by several rinses with de-ionized water is the preferred method. The main types of glassware are as follows:

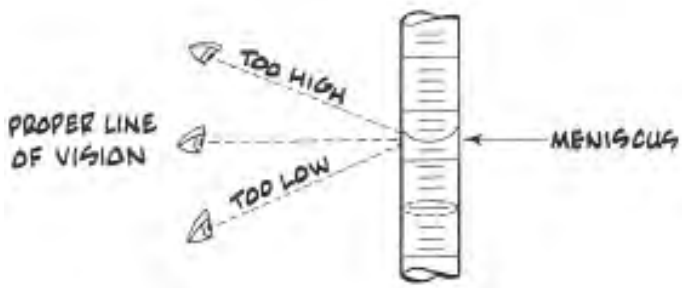

Figure 14.1 - *How to Read the Meniscus*

Beakers & Flasks

- Made of Borosilicate glass or plastic and used for mixing, heating, settling and other general procedures.
- Beakers and flasks are not used when accurate measurements of volume are required.

Graduated Cylinders

- Made of glass or plastic and used where good accuracy is required.
- Glass graduated cylinders are calibrated "To Deliver" (TD) or "To Contain" (TC). TD cylinders deliver the stated volume, TC cylinders are used for creating specific dilutions.
- Plastic are calibrated TC *and* TD, because the drops left behind when the contents are poured from the cylinder are accounted for.
- Use a size close to the volume being measured.
- Use Class "B" tolerance or better.

Volumetric Glassware

- Made of high-grade borosilicate glass and used where the *highest* level of accuracy is required, always labeled TC.
- Used to make up primary standards.
- Should be designated Class "A" tolerance.

Pipettes

- Pipettes are used to accurately measure and transfer small amounts of liquids.
- There are two main types of pipets; transfer (volumetric; Class "A"), and measuring (Mohr or serological; Class "B").
- The accuracy of a pipet is related to its type and the analyst's technique.
- Pipets are classified by their operation:
  - Volumetric pipets will deliver the specified or desired volume when drained and "tipped" to the edge of the receiving vessel. This type of pipet should not be "blown out".
  - Mohr pipets are graduated, but not calibrated to the tip. If allowed to drain completely, too much liquid will be delivered. Because of this, Mohr pipets are never "blown out".
  - Serological pipets are graduated, so they can deliver different measured volumes. Serological pipets must be "blown out" to deliver the measured volume and are designated with a frosted band or double lines near the top.

Burets

- Burets are used for volumetric titrations where high accuracy is required.
- They are made of borosilicate glass with a glass or Teflon stopper.
- When used properly, burets can deliver Class "A" tolerance.
- Digital titrators are now commonly used in-place of burets.

Temperature Measurements

Accurate temperature measurements are critical to many of the tests that are performed in the wastewater laboratory. Temperature measurements should be made with good mercury thermometers or digital thermometers. Never rely upon the temperature display built into an incubator or drying oven for an accurate indication of the instrument's temperature. Thermometers located in BOD incubators and drying ovens should be placed in stoppered beakers that contain clean sand, water or mineral oil to protect the thermometer from breakage and mitigate rapid fluctuations in temperature that occur when the unit is opened. For water bath incubators, thermometers should be held upright with their mercury bulb submerged in the water bath itself. The temperature of all operating instruments should be recorded twice a day in an instrument temperature log. Use thermometers that have the sensitivity required for each test. For most tests, use a thermometer with graduations of 0.1° C. To verify the accuracy of laboratory thermometers, each thermometer should be calibrated against a NIST *certified thermometer* in its working range at least once per year. Once calibrated, laboratory thermometers should be flagged with the date of their last calibration and any correction factor. When a laboratory thermometer is read, the correction factor is included when the temperature is recorded to ensure that the most accurate temperature possible is maintained in the instrument.

Solutions and Standards

Solutions consist of a liquid which has a solid dissolved or dispersed throughout it. The liquid medium is known as the *solvent* and the solid is known as the *solute*. The

concentration of a solution is expressed in terms of how many milligrams of solids are contained per liter of liquid (mg/L). A standard solution is a solution for which the concentration is known. Standards are often made up so that 1mL = 1 mg/L. Lab analysts can easily dilute standard solutions to a lower strength, such as when preparing a calibration curve.

There are two main types of standards: primary standards and secondary standards. A primary standard is a standard that is prepared by dissolving a weighed amount of a substance of a known composition in a measured final volume. A secondary standard is a standard for which the concentration is derived by comparison, such as by titration. For the highest level of accuracy, the solids used to make up a primary standard should be weighed using an analytical balance and the liquid volumes should be measured using Class "A" volumetric glassware.

Sometimes it is convenient to purchase and use solutions that have a specific concentration, which is based on the molecular weigh of the solute. These are known as Molar Solutions (M). A molar solution consists of one gram (molecular weight) of a compound made up to one liter with distilled water. For example, the molecular weight of CaSO4 is 136. If you create a solution that contains 136 grams of CaSO4 made up to 1 liter, you will have a 1M CaSO4 solution.

Another solution that is sometimes convenient is the Normal Solution (N). A normal solution contains one gram-equivalent weight of reagent per liter. An equivalent weight of a substance is defined as that weight which releases or accepts 1 mole of electrons. It takes equivalent volumes of acidic 1N solutions to neutralize equivalent volumes of caustic 1N solutions. (Caution, never directly mix strong acids and bases). Most laboratories maintain 0.05N, 0.1N and 1.0N working solutions of the most commonly used acids and bases.

The solutions used in the lab on an everyday basis are known as Stock Solutions. Stock solutions are made stronger than those used in various lab tests because stronger solutions are generally more stable than weak solutions. Stock solutions are easily diluted to give a desired working concentration. Stock solutions are considered standards when the concentration is very accurately known.

Overview of Individual Tests

Biochemical Oxygen Demand (BOD5)

Being able to measure the "*strength*" of wastewater is important for controlling treatment systems and for measuring the effectiveness or treatment. The Biochemical Oxygen Demand (BOD) is a test that measures the biological and chemical oxygen demand of wastewater. In the BOD test, wastewater samples are incubated at 20° C for five days. During the incubation, microorganisms metabolize nutrients in the sample. In doing so, they use oxygen. If a lot of nutrients are present, the organisms with reproduce actively, creating a larger population and thus, using a lot of oxygen. Furthermore, chemical substances in the wastewater sample (such as hydrogen sulfide or sulfur dioxide) will react with oxygen, which also causes an oxygen demand. Because both types of oxygen demand are measured, the test is called the Biochemical Oxygen Demand.

BOD Sample Collection and Preservation

Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values. For this reason, analyze samples promptly or cool samples to 4º C for storage. Warm stored samples to 20ºC before analysis. For grab samples, analysis should be performed within two hours or the sample should be cooled to 4º C at time of sample collection. Standard Methods states that every effort should be made to begin analysis within 6 hours, but under no circumstances start analysis of grab samples more than 24 hours after sample collection. For composite samples, keep sample aliquots at 4º C during compositing and limit the compositing period to 24 hours. Use the same holding time criteria as for grab samples, starting the measurement of holding time from the end of the compositing period. Under the BOD methodology detailed in the Federal Register under the Code of Federal Regulations, (40 CFR 136), a maximum sample holding time of 48 hours following the last composite sample aliquot is allowable. However, be aware that this longer holding time should only be used out of clear necessity and that 40 CFR 136 should be cited as the sampling protocol. For all BOD samples, state the storage time, temperature and conditions as part of the result.

Setting up the BOD

The BOD test is conducted in special 300 mL glass bottles, known as "BOD bottles". To begin setting up the test, a measured volume of sample is added to a BOD bottle. The amount of sample that is used depends upon how strong the lab analyst suspects the wastewater to be. For example, if the sample is raw influent, the analyst may only use a small portion, say 20 mL, whereas if the sample is very clear effluent, the analyst may use 250 mL. If the sample was disinfected, (effluent), there may not be enough live microorganisms in it to conduct the test, and so extra microorganisms must be added. These extra microorganisms are known as "seed". Usually, 1 – 3 mL of settled influent is used as a seed, which is added to the

BOD bottles containing sample. After the addition of seed, the BOD bottles are filled up the rest of the way with buffered dilution water that contains all of the things (other than food) that the microorganisms need to reproduce. Using a dissolved oxygen meter equipped with a special stirring probe, the dissolved oxygen in the BOD bottles containing sample, seed and buffered dilution water is then measured. This beginning dissolved oxygen level is known as the *initial D.O.*

Incubating the BOD samples

After the initial D.O. has been measured in each of the BOD bottles, the bottles are sealed so that no oxygen can get in or out. Then, the BOD bottles are placed in an incubator that is specially designed for the BOD test. The samples are incubated at 20º +/- 1º C for 5 days. It is critical that the incubation temperature stay as close to 20º C as possible if the test is to be accurate. When samples are placed into the incubator in the morning, they should be read in the morning 5 days later. When placed into the incubator in the afternoon, they should be read 5 days later, in the afternoon. If the incubator allows the temperature to exceed 21 ºC or go below 19 ºC, all samples in the incubator become invalidated and cannot be used for reporting purposes.

Reading the BOD

After the 5 days of incubation, the BOD bottles are removed from the incubator and the level of dissolved oxygen is again measured in the bottles. This is known as the *Final D.O.* The amount of dissolved oxygen that was consumed during the incubation is related to the strength of the sample and the volume of sample added to the bottle. Because the lab analyst knows the sample volume, a simple calculation can be performed to determine the concentration of BOD in milligrams per liter. (See Figure 14.2)

Seed Corrections

As discussed earlier, when we are testing samples that have been disinfected (like effluent), there are not enough microorganisms alive in the sample to allow the test to run. Therefore, we add "seed" microorganisms to these types of samples in order to ensure there are live bugs to use the dissolved oxygen during the incubation time. Good seeding material is usually obtained by settling raw influent for at least 1 hour but less than 36 hours and then pipeting from 1 cm below the surface of the settled liquid. Primary effluent, non-disinfected secondary effluent and commercially prepared seed can also be used.

Unfortunately, no matter what the origin of the seed, some oxygen-demanding material (BOD) will be carried along with the seed microorganisms. In order to obtain the true BOD of the sample, we must subtract out the amount of organic material that came along with the seed microorganisms. To do this, we determine how many mg/L of D.O. are used per mL of seed by running seed controls along side the regular samples. (see Figure 14.3)

Knowing the BOD of the each seed control allows us to calculate the seed correction factor, which is the average of the seed controls multiplied by the mLs of seed added to each of the sample bottles. (See Figure 14.4.)

Ideally, the Seed Correction Factor should be between 0.6 and 1.0 mg/L. If it is too high, too much seed has been used in the sample bottles. If it is too low, more seed should be used in the sample bottles next time. Often, lab analysts have to adjust the amount of seed (and even the source of the seed) in order to stay within this range.

Calculating BOD

To calculate the BOD for a seeded sample, we use the same equation shown above, except that we eliminate the oxygen demand caused by the seed by subtracting out the seed correction factor.

It is important to note that not all samples require seeding. Influent samples typically have a multitude of live microorganisms and therefore do not require seeding. If a lab analyst does not know if a sample has been disinfected or not, it is safest to seed the samples to ensure good test results. (See Figure 14.5)

$$\text{BOD, mg/L} = \frac{(\text{Initial D.O.} - \text{Final D.O.}) \times 300}{(\text{Volume of Sample used (mL)})}$$

Figure 14.2 - ***Concentration of BOD***

$$\text{Seed Control} = \frac{(\text{Initial D.O. Seed} - \text{Final D.O. Seed})}{\text{mL of seed added to seed control bottle}}$$

Figure 14.3 - ***Seed Control***

$$\text{Seed Correction Factor} = \text{average of seed controls} \times \text{mL of seed added to each sample bottle}$$

Figure 14.4 - ***Seed Correction Factor***

$$\text{BOD, mg/L} = \frac{(\text{Initial D.O.} - \text{Final D.O.} - \text{Seed Correction Factor}) \times 300}{\text{Volume of sample used (mL)}}$$

Figure 14.5 - *Calculating BOD for Seeded Samples*

BOD Quality Control

Because this test relies in part upon living microorganisms, many things can go wrong, with the outcome being inaccurate results. To avoid this, quality controls are used when running the BOD. These are:

- Dilution water blanks
- Oxygen Depletion Rules
- Sample pH adjustments
- Dechlorination of Chlorinated Samples
- Dissolved Oxygen Meter Calibration
- Careful control of the incubator temperature
- Routine analysis of a standard (a stabilized sugar called Glucose/Glutamic acid is used)
- Annual analysis of an externally supplied standard

Dilution Water Blanks

If the water that is used to make up the buffered dilution water contains organic matter or chemicals that will cause an oxygen demand, the test results will be incorrect. To ensure against this problem, two BOD bottles that contain only buffered dilution water are incubated along with the sample bottles and the seed correction bottles. These two bottles are known as "dilution water blanks". If the difference between the initial D.O. and the final D.O. of the dilution water blanks is >0.2 mg/L, it indicates that something is wrong with the water used to make the dilution water, that the D.O. meter calibration is off, or that the glassware used in the test is contaminated. Whatever the cause, the analyst should work to correct the problem. When over-depletion of the dilution water blanks occurs, it should be highlighted on the benchsheet. If this data is used for permit reporting purposes, a notation should be made on the discharge monitoring report detailing the over-depletion problem. However, excessive oxygen demand in the dilution water blanks is not a reason to invalidate (throw out) the data from a BOD test.

Oxygen Depletion Rules

Because the BOD test is essentially based on oxygen depletion, we must insure that enough oxygen is in the bottles at the beginning of the test and that enough oxygen remains in the bottle at the end of the test for us to accurately measure. The oxygen depletion rules outline the various aspects of oxygen levels that are acceptable during the test. The depletion rules are as follows:

1. At least 2.0 mg/L of dissolved oxygen must be consumed in sample bottles during incubation or the results from that bottle are not included in calculating the BOD.
2. At least 1.0 mg/L of dissolved oxygen must remain in sample bottles following incubation or the results are not included in calculating the BOD.

If no bottles containing sample meet these depletion rules, the results can still be used for reporting purposes, but the data is suspect and the results should be recorded in such a way that the problem is indicated. If the data is used for permit reporting purposes (NPDES or NM Ground Water DPs), a notation should be made on the discharge monitoring reports that explains the problem.

Sample pH adjustments

If a sample contains caustic alkalinity or acidity (defined as a pH or > 8.5 or < 6.0 respectively), the sample pH must be adjusted to near neutral. If samples have a pH of > 8.5 or < 6.0, they should be adjusted to a pH of between 6.5 and 7.5 before the analysis.

Dechlorination of Chlorinated Samples

We have already discussed the need to seed samples that have been disinfected. However, if samples were disinfected with chlorine, any residual chlorine remaining in the sample could kill our seed microorganisms. THIS WILL RESULT IN A BOD MEASUREMENT THAT IS MUCH LOWER THAN THE ACTUAL BOD. In order to avoid this problem, all chlorinated samples must be checked for residual chlorine and dechlorinated with a freshly prepared 0.025 N sodium sulfite solution if any residual chlorine is found. After dechlorination, samples must be checked again to verify that no residual chlorine remains.

Dissolved Oxygen Meter Calibration

The D.O. measurements conducted as part of the BOD test can be made in two ways; (1) chemically, through the Winkler titration, or (2) electrometrically, with a polarographic D.O. meter. Because of the difficulties involved with the Winkler titration method, almost all laboratories in New Mexico use D.O. meters for performing the BOD test.

D.O. meters can be temperamental devices. To be accurate, D.O. meters must be calibrated before each use, preferably close to the time that they will be used to make measurements. Calibration is typically done using the theoretical dissolved oxygen value of saturated air, after

correcting for variations caused by temperature and atmospheric pressure.

To obtain the accuracy required for the BOD test, an atmospheric pressure reading from an accurate (calibrated) barometer should be used to obtain the theoretical dissolved oxygen calibration value. This is different from the "altitude correction factor" which is acceptable when calibrating field D.O. meters, but the high degree of accuracy required demands it. Be aware that laboratory barometers should be calibrated against a local airport barometric reading, but the airport reading must be converted to a true barometric pressure that has not been adjusted for altitude.

Incubator Temperature Control

BOD incubators look like refrigerators, but they actually can heat or cool as needed to maintain an inside temperature of 20º C +/- 1º C. Good laboratory practices dictate that the incubator temperature should be carefully determined and recorded at least two times a day for the entire incubation period. The thermometer used to measure the BOD incubator temperature must be calibrated against a thermometer certified by NIST at least once a year. If the temperature inside the incubator goes above 21º C or below 19º C at any time during the incubation period, the test is invalidated and the results cannot be used for reporting purposes.

Glucose Glutamic Acid Standard (GGA)

Microorganisms metabolized Glucose (a simple sugar) very rapidly and at an unpredictable rate. Glucose that has been stabilized by the addition of Glutamic acid is metabolized by microorganisms at a steady rate. Because of this, GGA can be used as a BOD standard. [Remember, a standard is a substance whose chemical constituents are very accurately known]. Carefully weighing 150 mg of dry Glucose and 150 mg of dry Glutamic acid with an analytical balance and making it up to exactly one liter with pure water will yield a BOD standard.

A 2% dilution of this standard (6 mL in a BOD bottle) will result in a BOD of 198 mg/L. If the BOD of the standard is close to 198 mg/L, we assume that the measurements of the samples were accurate as well. The tolerance for the GGA standard is 198 mg/L +/- 30.5 mg/L.

In order to ensure that BOD tests are providing reliable results, a GGA standard must be run at least 10% of the time along with samples. If the GGA standard does not fall within the tolerances, the entire set of BOD sample data associated with the failed GGA standard becomes invalid (the sample data is thrown out and cannot be used for reporting). If one GGA bottle is within the tolerance and one is out, the data can still be used.

Because of the consequences of failing the GGA standard, most laboratories run GGAs with every sample set that is analyzed. This way, if the GGA fails, only that sample set is invalidated, as opposed to invalidating several weeks of data (if the GGA is run infrequently).

Externally Supplied Standard

Even with all of the previously discussed quality control measures, the BOD test typically has an accuracy of +/- 15%. Because of this, it is important that externally supplied standards be analyzed at least annually to ensure that the level of accuracy being delivered is acceptable. Externally supplied BOD standards can be purchased commercially.

BOD benchsheet

The following benchsheet shows how a BOD test looks on paper. Using the information on the benchsheet, try to calculate the BOD of this effluent sample. Remember to exclude data that does not meet the depletion rules and correct for the seed that was added to the sample bottles. Calculate the GGA standard to make sure that the test met quality control requirements. (See Figure 14.6)

Fecal Coliform Membrane Filter Procedure

The Membrane Filter Procedure for determining the density of Fecal Coliform bacteria is commonly used to monitor the effectiveness of the disinfection process of wastewater treatment plants in New Mexico. The procedure involves filtering known volumes of effluent samples through a membrane with a nominal pour size of 0.45 microns. The membrane containing the trapped Fecal Coliform microorganisms is then placed onto a growth medium (M-FC medium), sealed in a Petri dish and incubated for 24 hours. Any Fecal Coliform bacteria trapped on the membrane will grow into characteristic blue colonies that can be counted, yielding how many colony-forming units (CFUs) were contained in a 100 mL sample portion.

Fecal Coliform Sample Collection and Transport

Fecal Coliform samples must be collected and transported in specially prepared bottles that have been sterilized and contain a small amount of dechlorinating chemical (Sodium Thiosulfate). If the sample has been disinfected with chlorine and does not get completely dechlorinated, the chlorine will continue to disinfect the sample in the bottle on the way to the lab, yielding a false low result. During preparation, Sodium Thiosulfate is added to Fecal Coliform sample bottles as a dechlorinating agent, and then they are sterilized in an autoclave.

Care must be taken to draw the sample aliquot into the bottle without washing out the dechlorinating agent. Only grab samples are taken, and they should be drawn directly from

Figure 14.6 - *Biochemical Oxygen Demand Worksheet*

Sampler: Joe T. Operator
Name of Facility: New Mexico WWTF
Date of Sampling: 3/6/04
Time(s) of Sampling: 10:00, 11:00, 12:00, 1:00, 2:00, 3:00
Exact Sampling Location: Effluent V-notch weir
Type of Sample: Grab ___ Composite ✔ Hour 6 hour
Flow at sample time:(GPM) 749, 695, 854, 347, 550, 465, 391

Sample Preservation: Refrigerated at 4°C
Date of Analysis: 3/6/04
Method Used: Method 5210, Standard Methods for the Examination of Water and Wastewater, 18th ed.

Note: If sample must be stored over 2 hours, refrigerate to 4°C. Sample temperature should be 20°C before analysis. Warm stored samples to 20°C in water bath.

SAMPLE PRETREATMENT

Sample pH: 7.2
Sample pH adjusted to: N/A
Seed Source: Settled Influent
Seed Collection: Date 3/6/04 Time 8:31 am

Sample Chlorinated? Yes ; Cl_2 present 0.1 mg/L
Date of sodium sulfite prep.: 3/6/04
Volume of sodium sulfite used/liter sample: 0.3 mg/lL
Sample rechecked for Cl_2: <0.099 mg/l

UNSEEDED DILUTION WATER BLANKS

Bottle #	1	2
D.O. Initial (mg/L)	7.2	7.2
D.O. Final (mg/L)	7.1	7.2
Difference		

Note: Should be <0.2 mg/L after 5 days of incubation.

D.O. Meter calibration value 7.4 initial

D.O. Meter calibration value 7.3 final

SEED CORRECTION

Bottle #	3	4	5
mL Seed Used	3	6	9
D.O. Initial (mg/L)	7.1	7.1	7.1
D.O. Final (mg/L)	5.9	4.6	3.6
Difference			
D.O. Used/mL Seed			
Seed Correction Factor =			

Note: The Seed Correction Factor is the average of the D.O. used/mL of seed in the seed control bottles multiplied by the mL of seed used to seed the GGA and sample bottles.

GLUCOSE-GLUTAMIC ACID STANDARD

Bottle #	6	7
ml Standard	6	6
ml Seed Used	2	2
D.O. Initial (mg/L)	6.9	6.8
D.O. Final (mg/L)	2.2	2.2
Difference		
Seed Correction Factor		
Corrected Difference		
BOD mg/L		

Note: GGA should be 198 ± 30.5 mg/L

SAMPLE DATA

Bottle #	8	9	10	11
ml Sample	30	50	75	100
ml Seed Used	2	2	2	2
D.O. Initial (mg/L)	6.9	6.9	6.8	6.7
D.O. Final (mg/L)	4.3	3.0	1.7	0.4
Difference				
Seed Correction				
Corrected Difference				
BOD mg/L				
Average BOD (mg/L) =	(Should be) 17.9 mg/L			

Analyst (preparer): JTO
Analyst (reading): JTO

Date & Time of initial reading: 3:35 pm, 3/6/04
Date & Time of final reading: 2:45 pm, 3/11/04

- Dilutions that result in a residual D.O. of at least 1 mg/L and a D.O. uptake of a least 2 mg/L after 5 days of incubation produce the most reliable results.
- Before use, seed should settle for at least 1 hr. and no longer than 36 hours at 20°C.

Note: Calculate data in shaded blocks

the flow stream, (not collected with a sample dipper and then poured into the bottle).

Sterile sample bottles should only be opened once, at the time of sampling. The lid of the sample bottle should never be allowed to become contaminated. Samples that will take longer than 1 hour to analyze should be cooled to between 1º – 4º C on ice or in a refrigerator and then transported in a cooler on ice to the laboratory. The maximum holding time for Fecal Coliform samples that will be used for state or federal permit reporting purposes is 6 hours.

Setting up the Fecal Coliform Membrane Procedure

All materials used for the Fecal Coliform Membrane Procedure must be sterilized prior to running the test. Most labs purchase pre-sterilized Petri dishes, membranes, absorbent media pads and M-FC media. The equipment used to filter the samples is typically sterilized using an autoclave or ultra-violet light-sterilizing box. At the start of the test, the lab analyst disinfects the counter top where the test will be run using alcohol. Petri dishes are laid out for each sample volume to be filtered and absorbent pads are placed in the Petri dishes. Next, M-FC media is poured onto the absorbent pad. Any excessive M-FC media on the absorbent pad is then discarded by pouring it out into a sink or trash can.

Filtering Samples

Using a set of tweezers that has been flame sterilized, a sterile membrane filter is placed upon a special filter funnel apparatus. Using a vacuum pump, a measured portion of sample is drawn through the membrane. A variety of sample volumes, ranging from dilutions that contain < 0.0001 mL of sample all the way up to 100 mL of sample, may be filtered through successive membranes. The sample volume range that is used is dependent upon the expected Fecal Coliform concentration.

Ideally, sample volumes or dilutions that yield between 20 and 60 colonies per plate should be selected. After the samples have filtered through the membrane, three successive 20 – 30 mL volumes of buffered dilution water are used to rinse the sides of the filter vessel and then drawn through the membrane filter. Next, the membrane is removed (using flame sterilized tweezers) and is carefully placed upon the absorbent pad containing M-FC media in the Petri dish. The Petri dish is then sealed. This procedure is repeated for all sample volumes to be filtered.

Incubating Samples

When all of the sample volumes have been filtered and placed into Petri dishes, they are collectively sealed in sterile, waterproof plastic bags and placed into a water bath incubator. Fecal Coliform samples are incubated at a very specific temperature: 44.5º C, +/- 0.2º C. Water bath incubators are generally used for this test because they can hold the temperature much more accurately than air incubators. After 24 hours of incubation, the Petri dishes are removed and the blue Fecal Coliform colonies are counted.

Determining Fecal Coliform Densities

The number of Fecal Coliform in an effluent sample is reflected by how many colonies grew on the membrane during incubation and how much sample was filtered through the membrane. After incubation, remove the samples and count all of the blue colonies on each filter.

$$\text{Fecal Coliform CFUs/ 100 mL} = \frac{\text{(Number of Colonies Counted on Filter) X (100)}}{\text{Volume of Sample Filtered, (mL)}}$$

Figure 14.7 - ***Fecal Coliform Densities***

Samples should ideally have at least 1 plate with 20 – 60 colonies; however, samples are not rejected if there is a countable number. (See Figure 14.7.)

It is important to understand that the volume of sample filtered could be very small or up to 100 mL because the volume has to be adjusted for the expected Fecal Coliform concentration. For samples that are suspected to contain very high numbers of Fecal Coliform, a dilution is made that may contain only 0.1, 0.01, 0.001 mL/100mL of actual sample. Sometimes, it is necessary that dilutions are taken even further. For most wastewater treatment plant effluents, undiluted sample volumes of 10, 50 and 100 mL will commonly be filtered. If the sample is diluted, the actual volume of sample in the dilution (not the total volume, including dilution water) must be entered into the equation in order to obtain the correct CFU/100 mL density.

Fecal Coliform Reporting

Fecal Coliform densities are reported using specific rules:

- Filters having 20 – 60 colonies growing on them are preferred. (Report only the results from plates with 20 – 60 colonies when they occur).
- If no plate has between 20 – 60 colonies, all the counts are added from the sample plates and divided by the total volume of sample filtered.

- Samples with growth covering the entire plate with no distinct colonies are reported as confluent growth.
- Samples with more than 200 colonies are reported as too numerous to count (TNTC).
- Confluent growth and TNTC plates cannot be used for DMR reporting purposes (re-sampling is required).
- If no sample plates have blue colonies and all Quality Control checks out, report result as < 1 CFU /100 mL.
- For DMR purposes - < 1 CFU /100 mL = 1

Quality Control

Because the M-FC test is based on cultivating small numbers of microorganisms, many variables exist that can affect the accuracy of the test. The quality control procedures for the M-FC test attempt to eliminate sources of error.

The following quality controls are required at a minimum:

- Positive control
  Anything that is toxic in the materials used in the test will suppress their growth, giving false negative results. By testing the materials on a sample known to contain Fecal Coliform, the materials can be shown to perform as intended. Typically, either a diluted pure strain of E. coli or 1 – 2 mL of raw influent is used as a positive control. These sources should create the characteristic blue colonies filtered and incubated.
- Sterility checks
  Any contamination from sources containing Fecal Coliform bacteria will give false positive results. Sterility checks verify that the materials used in the test are not contaminated with bacteria. Tryptic Soy Broth (TSB) is used as a media to demonstrate sterile conditions. If Petri dishes, membrane filters, absorbent pads and other materials used in the test are incubated with TSB but show no growth, the material are sterile. If growth occurs, the materials are contaminated and should not be used in the test.
- Negative control
  In order to determine which bacteria are Fecal Coliform and which bacteria are not, the media that the bacteria are grown upon (broth) contains a chemical that is only taken up by Fecal Coliform, turning them blue. A negative control proves that the media will differentiate between Fecal Coliform and all other bacteria. Usually, an organism such as *Enterobacter Aerogenes* is used as a negative control. Colonies of *Enterobacter Aerogenes* will grow on the membrane, but will be some color other than blue, usually tan.
- Pre-blank
  A pre-blank is used to demonstrate that the filter funnel apparatus was properly sterilized before the test. Pre-blanks are just like samples, except that 100 mL of sterile buffered dilution water are filtered. Pre-blanks are filtered BEFORE any of the samples. No growth should ever be observed on the pre-blank. If growth occurs, the filter funnel sterilizing procedures should be improved.
- After-blank
  An after-blank is used to demonstrate that the rinsing following filtering of the samples was adequate. After-blanks are just like pre-blanks, except that they are done AFTER the samples have been filtered. No growth should occur on the after-blanks. If growth occurs, sample carry over is occurring and the rinsing procedure following each sample filtration should be improved.

A positive control, negative control and sterility check are required for each new lot of materials to be used in the test. Pre-blanks and after-blanks should be run once for every 5 sample volumes filtered. Additionally, duplicate analysis must be conducted at least 10% of the time, (and more often if following good lab practices). If any problems with the positive control, negative control, sterility check, pre-blank or after-blank are observed, the data generated with the associated materials and procedures is suspect and should not be used for reporting purposes. If the incubator temperature ever falls below 44.3º C or exceeds 44.7º C, the sample data must be rejected. Rejected data must never be used for reporting purposes.

Fecal Coliform Benchsheet

Figure 14.8 shows what the Fecal Coliform test looks like on paper. Using the information provided on the benchsheet, try to calculate the correct number of Fecal Coliform CFUs/100 mL.

Total Suspended Solids Procedure

Solids in wastewater can be classified as Total Solids (TS), Total Suspended Solids (TSS) and Total Dissolved Solids (TDS). TS represent all of the solids in a wastewater sample, after the water has been evaporated off. TDS represent all of the solids in a wastewater sample that has passed through a 2-micron (or smaller) filter, after the water has been evaporated off. TSS represent all of the solids in a wastewater sample that remain trapped on a 2-micron (or smaller) filter, which has had all water evaporated off. From the perspective of process control of wastewater treatment plants and NPDES permit monitoring, TSS is the parameter of most importance. The TSS procedure

Figure 14.8 - *Fecal Coliform Membrane Filter Method Benchsheet*

Name of Facility: New Mexico WWTF
Time of Sampling: 10:03 AM
Date of Sampling: 3/6/04
Exact Sampling Location: Effluent V-notch weir
Sample Preservation: Refrigerated at 4°
Signature of Sampler: Joe T. Operator

Time of Analysis: 1:35 PM
Date of Analysis: 3/6/04
Analyst: Joe T. Operator
Method Used: Method 9222D, Standard Methods for the Examination of Water and Wastewater, 18th edition

Quality Control	Membrane Filter	m-FC Broth	Adsorbent Pads
Date of Purchase	2/06/04	2/23/04	2/06/04
Lot number	8113912	908315	3453
Date of Expiration	4/01/05	11/11/05	4/01/05
pH		7.4	

Waterbath 44.5° ± 0.2°	
Time In: 1:40 PM	Date In: 3/6/04
Temp In: 44.4°	
Time Out: 1:30 PM	Date Out: 3/7/04
Temp Out: 44.5°	

Filter funnel sterilized: UV light, 2 min.	Work area disinfected: Yes, alcohol

Dish	Sample Volume (mL)	Colonies on Membrane	CFU/100 mL	Plates Used in Count
Pre-blank	0	0	0	
1	10	3	30	
2	30	17	57	
3	50	37	74	
4	70	48	69	
5	100	74	74	
After-blank	0	0	0	

Sample Result: ***(Should be)*** 72 CFU/100 mL

Key:

	calculate data in shaded blocks

involves filtering known volumes of wastewater through pre-weighed glass fiber filters and then drying the filters at 103º – 105º C in a drying oven. The residue trapped on the filter is then weighed to determine the TSS concentration in mg/L.

Total Suspended Solids Sample Collection and Preservation

Samples to be analyzed for Total Suspended Solids content should be collected in clean polypropylene or glass bottles (typically 500 – 1000 mL). It is important that the material in suspension does not adhere to the container walls. Analyze samples as soon as possible because of the impracticality of preserving samples. Refrigerate samples at 4º C up to the time of analysis to minimize microbiological decomposition of solids. Preferably, do not hold samples more than 24 hours, but in no case hold samples more than 7 days. Bring samples to room temperature before analysis, because changes in the sample temperature/density will affect volumetric measurements.

Preparation of Glass Fiber Filters

The filters used in the TSS test are specialized glass fiber filters with a nominal pour size of < 2 microns. Various companies supply these filters. These are a few examples: Whatman grade 934AH, Gelman type A/E, Millipore type AP40 and E-D Scientific Specialties grade 161. Other products that are demonstrated to give comparable results are allowable.

To prepare filters for use, insert the filter disk with the wrinkled (rough) side up in a filtration apparatus. Never handle the filter or aluminum dish by hand. Oil from your skin could cause an inaccurate test result. Always handle filters and aluminum dishes with forceps. Apply a vacuum and wash the filter with three successive 20 mL portions of reagent-grade water. Continue suction to remove all traces of water. Remove the filter from the filter apparatus and place it into an aluminum dish (known as a planchet). Dry filter and dish in an oven at 103 – 105º C for at least one hour.

If the filter will be used in the Volatile Suspended Solids test, ignite at 500 +/- 50º C for 15 minutes in a muffle furnace. Cool filter and dish in a desiccator and then weigh filter and dish on an analytical balance. Repeat cycle of drying and desiccating until a constant weight is obtained or until the weight change is less than 4% of the previous weighing or 0.5 mg, which ever is less.

The cleaning and complete drying of the filters is critical to obtaining accurate results with the TSS test. Rinsing the filters removes any loose debris from the filter. Verifying that the filters are completely dry before being used in the test prevents the introduction of error attributable to wet filters. It is important that good records be maintained that demonstrate that the filter rinsing and drying was carried out correctly.

Most laboratories prepare a week or even a months worth of filters at a time. Store prepared filters in a desiccator until needed for analysis.

Selection of Volume to be Filtered

The volume of sample to be filtered depends upon the amount of suspended matter in the sample. In general, only about 50 – 100 mL of raw influent can be filtered; while filtering 1000 mL of clean effluent is common. High solids content samples like mixed liquor from an activated sludge plant may only allow filtration of 10 – 20 mL. Samples should be carefully measured in clean graduated cylinders or using clean wide-tipped pipets. Choose sample volumes that yield between 10 and 200 mg dried residue. If more than 10 minutes are required to complete filtration, use less sample volume or use a larger diameter filter. When very low suspended solids wastewater is encountered, less than 10 mg of dried residue is acceptable, but compensate by using a high-sensitivity analytical balance (capable of measuring 0.002 mg) if very high accuracy is required.

Sample Filtration

To begin sample filtration, assemble the filtration apparatus, place a filter with the wrinkled side up on the apparatus and begin suction. Wet the filter with a small amount of rinse water to seat it. While stirring the sample with a magnetic stirrer or after thoroughly mixing the sample in the sample container, transfer a measured volume to the filter funnel with a pipet or graduated cylinder. After the sample has been drawn through the filter, rinse the pipet or graduated cylinder and the walls of the filter funnel down with three successive volumes of about 10 mLs of rinse water, allowing complete drainage between washings. Some samples require more thorough rinsing. Continue suction until all liquid is removed from the filter. Carefully remove the filter and residue with forceps and transfer to the planchet. Dry filter, residue and dish in an oven at 103 – 105º C for at least one hour. Cool filter, residue and dish in a desiccator and then weigh filter, residue and dish together on an analytical balance. Repeat cycle of drying and desiccating until a constant weight is obtained or until the weight change is less than 4% of the previous weighing or 0.5 mg, which ever is less. Carefully record each drying/ desiccating/ weighing on the TSS benchsheet.

Calculating TSS

Using the initial and final weigh of the filter, dish and residue, calculate total suspended solids as illustrated in Figure 14.9.

$$\text{TSS (mg/L)} = \frac{\text{(Weight of filter, dish and dried residue} - \text{Weight of filter and dish)} \times 1{,}000{,}000}{\text{Sample volume, mL}}$$

Figure 14.9 - *Calculating Total Suspended Solids*

TSS Quality Control

As is the case with all test methods, the TSS analysis' accuracy can only be relied upon when the methodology has been closely followed. In order to check the analyst's technique, run duplicate analysis on at least 10% of samples. Duplicate determinations should agree within 5% of their average. Externally supplied standards should be analyzed at least annually to verify the accuracy of the laboratory and analyst.

TSS Benchsheet

Using the information given on Figure 14.10, the TSS benchsheet, calculate the TSS of the samples.

pH Procedure

Measurement of pH is one of the most important and frequently used tests in water/ wastewater analysis. Practically every phase of water supply and wastewater treatment is pH dependent. At a given temperature, pH indicates the *intensity* of the acidic or basic character of a solution. A solution's pH is the outcome of the balance between hydrogen ions (H+) and hydroxide (OH^-) molecules, as well as its temperature. What is actually measured is the activity of hydrogen ions (H+). This measurement is then translated onto a scale that spans from 0 to 14 as the reciprocal of the logarithm of the hydrogen ion activity; - log [H+]. In general, pH values are reported to the tenth decimal in the standard units (S.U.) of the 0 to 14 scale.

Many laboratory procedures are pH dependent. Metabolic rates, organism reproduction rates, various chemical reactions and chlorine toxicity are all influenced by pH. Because of its effect upon so many aspects of water analysis, ACCURATELY MEASURING PH IS ONE OF THE CORNERSTONES THAT LABORATORY QUALITY ASSURANCE/ QUALITY CONTROL IS BUILT UPON.

Two methods for measuring pH values are approved by NMED and EPA for permit compliance reporting purposes. These are:

- EPA Methods For Chemical Analysis Of Water And Wastes, Method 150.1 (Electrometric Method)
- Standard Methods for the Examination of Water and Wastewater, 18th, 19th or 20th editions, Method 4500-H+ B. (Electrometric Method)

Both methods are electrometric, which involves the use of a pH probe from which the signal is read out upon a millivolt meter. Although both methods are acceptable (and very similar), this text specifically discusses Method 4500-H+ B from Standard Methods.

pH Sample Collection and Handling

Samples to be analyzed for pH should be collected in 100 – 500 mL polypropylene sample bottles. There are no methods of preservation, therefore, all pH samples are grab samples and must be analyzed within 15 minutes of sample collection. The quicker the samples can be analyzed, the more accurate the result will be. If fact, for many process control applications, pH is measured in-situ, such as in wastewater treatment lagoons. Effluent samples that are analyzed for permit reporting purposes should clearly indicate the sample time as well as the time of analysis to verify that the 15-minute holding time was not exceeded.

pH Meter and Calibration Buffers

Measurement of pH is made with a pH meter that consists of:

- A potentiometer (millivolt meter)
- A glass *sensing* electrode
- A *reference* electrode (half cell)
- A temperature compensating device

For routine work, the pH meter must be accurate and reproducible to the nearest 0.1 pH unit. Before use, the meter must be calibrated to at least two standards and then checked against a third standard. Most labs use calibration standards (known as buffers) that have pH values of 4.0, 7.0 and 10.0 at 25º C. Buffers with these values can be purchased that are color coded so that the buffer with pH value 4.0 is red, 7.0 is yellow and 10.0 is blue. The color-coding makes the buffers easier to identify while calibrating the pH meter.

Calibration of pH meter

In a laboratory, pH meters are generally calibrated once in the morning and once in the afternoon. In the field, calibrate for each set of samples or whenever field conditions change. Calibrate to two points that bracket the expected sample pH and are at least 3 pH units apart using the following sequence:

- Ensure that the meter is on, the electrode is connected and all needed materials are present.
- Engage the instrument calibration mode.

Figure 14.10 - *Total Suspended Solids Benchsheet*

Name of Facility: New Mexico WWTF
Date of Sampling: 4/6/04
Time of Sampling: 10:00 AM
Name of Sampler: Joe T. Operator
Type of Sample: Grab ✔ Comp ___
Flow at time of sampling: 0.88 MGD
Sample Preservation: none
Date of Analysis: 4/6/04

Time of Analysis: 10:15 AM
Analyzed by: Joe T. Operator
Method Used: Method 2540 D., Standard Methods for the Examination of Water and Wastewater, 18th edition

SAMPLE DATA

Sample Location(s):	Influent	Effluent	(Duplicate) Effluent	Blanks	
Dish Number	1A	1B	1C	2A	2B
Sample Volume (mL)	100	1000	1000	100	100
1st Weight fo filter, dry residue & dish (grams)	1.0261	1.0217	1.0193	1.0005	1.0089
Weight of dry filter & dish (tare weight, grams)	1.0008	1.0015	1.0012	1.0004	1.0090
Weight of dry residue (grams)				0.0001	-0.0001
Time of 1st weighing	11:30 AM	11:30 AM	11:31 AM	11:34 AM	11:35 AM
2nd Weight fo filter, dry residue & dish (grams)	1.0258	1.0215	1.0192	1.0004	1.0089
Weight of dry filter & dish (tare weight, grams)	1.0008	1.0015	1.0012	1.0004	1.0090
Weight of dry residue (grams)				0.0000	-0.0001
Time of 2nd weighing	1:20 PM	1:21 PM	1:21 PM	1:21 PM	1:23 PM
TSS (mg/L)				ND	ND
Average TSS (mg/L)		*(should be)* 19.0		Non-Detectable	

$$TSS,\ mg/l = \frac{Final\ Wt.\ grams - initial\ Wt.\ grams \times 1{,}000{,}000}{Sample\ Volume,\ ml}$$

Note: Refrigerate sample at 4°C up to the time of analysis.

Key:

	calculate data in shaded blocks

- Remove the electrode from its storage solution and rinse it with de-ionized water.
- Blot the electrode dry with a paper towel, (do not wipe).
- Immerse the electrode in pH buffer 7.0 (constantly mixed by a stir plate).
- Allow the meter to stabilize, record the reading.
- Calibrate the meter to buffer 7.0.
- Remove electrode from the 7.0 buffer, rinse with de-ionized water and blot dry with paper towel.
- Immerse the electrode in a second pH buffer (4.0 or 10.0).
- While mixing constantly with stir plate, allow the meter to stabilize and then record the reading.
- Calibrate meter to second buffer value.
- Remove the electrode from the second buffer, rinse it with de-ionized water and blot dry with paper towel.
- Immerse the electrode in the check pH buffer (buffer not yet used).
- While mixing, allow the meter to stabilize and then record the reading.
- The reading of the check buffer must be within 0.1 pH unit of actual value, if not, the calibration must be repeated.
- Remove the electrode from the buffer, rinse it with de-ionized water and blot dry with paper towel.
- The pH meter is now calibrated.

Measuring Sample pH

Use sufficient sample volume to cover the electrode and give adequate clearance for a stir bar. Mix the sample constantly, but be cautious not to introduce CO2 by overly rapid mixing (no vortex). Record the pH value when the reading is stable. Some pH meters indicate when the reading is stable with a light or through a "chirp". Record the sample temperature to the nearest degree Centigrade (^{o} C). Repeat measurement on another sample aliquot until values differ by < 0.1 pH unit.

Be aware that if the reading is not in the range bracketed by the calibration buffers used, the meter will need to be recalibrated using another buffer. For example, if you calibrated to the 7.0 and the 10.0 buffers, and then checked the calibration with the 4.0, the sample reading must be between 7.0 and 10.0. If the sample reading were below 7.0, you would need to go back and recalibrate to the 7.0 and the 4.0 buffers, and check the calibration with the 10.0 buffer.

Quality Control For pH Measurements

- Equipment
  - Do not store electrodes dry. Store in solution indicated by manufacturer.
  - Maintain electrolyte in reference electrodes. Re-fill when volume is low.
  - Uncover fill hole of the reference electrode (if so equipped) so that electrolyte can flow freely.
- Buffers
  - Record buffer date of purchase, date opened and expiration date.
  - Purchase buffers in quantities that will be consumed within six months.
  - Buffers should be used before their expiration date.
  - Store buffers in the dark at room temperature.
  - Discard buffers that are not clear or that show growth.
- Samples Measurements
  - Meter must be calibrated with two buffers that bracket the sample pH.
  - Performance evaluations on externally supplied standards should be run at least once a year.

Total Residual Chlorine Procedure

Chlorine is a common wastewater disinfectant. Residual chlorine in effluent is toxic to fish in receiving streams and so must be removed with a *dechlorinating* chemical, such as sulfur di-oxide. Many NPDES permits place limitations on the amount of total residual chlorine (TRC) that can be discharged after dechlorination. Often, a permit will impose a limit of <0.019 mg/L or <0.011 mg/L TRC, although many permits require that "no-detectable" TRC be discharged. For most treatment plants, "no-detectable" TRC is equivalent to <0.099 mg/L.

There are three EPA approved test methods for measuring low-level TRC; (1) The Amperometric Titration method, (2) the Iodometric Electrode method and (3) The DPD Colorimetric method. This text focuses on the most commonly used method; the DPD Colorimetric, as described in Method 4500-Cl G., Standard Methods for the Examination of Water and Wastewater, 18th edition. The DPD method is a colorimetric method that takes advantage of the reaction that occurs between residual chlorine and *N,N-Diethyl-p-phenylenediamine* (DPD) indicator solution. When chlorine is present, a reaction with the DPD indicator results in the development of a pink coloration. The more chlorine present in a sample, the more intense the color development. Using a photometric device, the color intensity can be measured through absorption.

TRC Sample Collection and Handling

Samples to be analyzed for TRC should be collected in clean 100 – 500 mL polypropylene bottles. Ensure that representative samples are taken, i.e.; peak flow, well mixed flow stream. NPDES permits specify the exact TRC sampling location (generally, following the last treatment process). Residual chlorine samples are not stable, and there are no preservation methods. TRC samples are subject to change from excessive holding times, exposure to sunlight, increase in temperature and agitation. Much like pH samples, TRC samples must be analyzed within 15 minutes (preferably less). Benchsheets should record both the time that the sample was collected and the time that it was analyzed to demonstrate that the holding time was not exceeded.

TRC Equipment

The DPD method of measuring TRC can be performed on two different (but similar) instruments; (1) a spectrophotometer, and (2) a filter photometer. A spectrophotometer is a device that measures the absorption of light at various wavelengths. For TRC measurements using a spectrophotometer, a wavelength of 515 nm with a light path of a least 1cm is required. A filter photometer is a device that measures the absorption of light in a fixed range of wavelengths. For TRC measurements using a filter photometer, a wavelength range of 490 - 530 nm with a light path of a least 1cm is required.

Instrument Calibration

Calibration to known standards is required for all photometric devices. The required frequency of calibration is dictated by individual circumstances. The recovery of known standards to check the instrument calibration is required as part of a quality control program. Because chlorine is unstable it cannot be used for making standards directly. Standards can be prepared either with Chlorine exposed Potassium Iodide (KI) or with Potassium permanganate (KMnO4). At least 5 calibration standards covering the Chlorine equivalent range of 0.05 – 4.0 mg/L should be used to prepare a calibration curve.

TRC Analysis

- Assure that the instrument is set to the correct wavelength and that the optical sample cells are clean and not damaged.
- Measure appropriate volumes of buffer and DPD reagents into a gradated cylinder and fill with appropriate sample volume (usually 50 mL).
- Stopper the graduated cylinder.
- Mix the buffer, DPD reagent and sample by inverting several times and allow 3 minutes for color development.
- Using a matched optical sample cell filled with sample, zero the instrument.
- When color of sample cell with buffer and DPD reagent is developed (3 min.), place cell in instrument and read absorption. (Most modern instruments display a reading directly in mg/L TRC).
- Using distilled water, prepare a reagent blank that contains:
  - A blanking agent
  - Buffer and DPD
- Read the absorption of the reagent blank with the instrument.
- Subtract the reading obtained for the reagent blank from the reading obtained for the sample.
- This yields the corrected sample TRC.

Quality Control - Sample Preparation

Samples containing significant turbidity should be filtered through a 3-micron membrane filter after reacting the sample with DPD reagent and buffer. When using this method, zero the instrument with a filtered sample blank.

Quality Control - Standard Recovery

Periodically prepare standards from Chlorine reacted with KI or from $KMnO_4$. Determine recovery of these standards and establish lower detection limit as the lowest standard that can be detected with 95% recovery.

Quality Control- Performance Evaluation Standards

Externally supplied performance evaluation standards should be analyzed at least annually.

References

Standard Methods for the Examination of Water and Wastewater, 18th, 19th and 20th editions

Operation of Wastewater Treatment Plants, Volume 2, 4th ed., Chapter 16