BASIC Exam Preparation – Subclass L

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Wisconsin Department of Natural Resources
Wastewater Operator Certification

Basic Laboratory Study Guide
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- DNR Lab Certification

A whole new world!

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13.1.1 Abbreviations:

- 53 pages…down from 70 (25% reduction!)
- Tried to keep “knowledges” short and sweet
- Added a few knowledges (or combined several)
- Cut a number of knowledges…
- …but still 116 of them
- But that’s down nearly 50% from 216

Chapter 1

Safety

1.1.1 General safety practices in a wastewater lab related to food & beverages in the lab.

- Eating, drinking, and even applying makeup in the laboratory is not recommended.
- Pathogens such as hepatitis, typhus, giardia, cryptosporidium, and toxic chemicals could contaminate food and beverages, and makeup and subsequently be ingested. This can lead to illness and even death.
- Conversely, food, cigarettes and drinks can also contaminate samples and lead to erroneous results. Tests particularly susceptible to contamination are BOD, TSS and phosphorus.

1.1.2 Food storage concerns in a wastewater laboratory

- Food and beverages must not be stored in refrigerators or areas of the laboratory where samples are stored or testing activities take place.
- Pathogens and toxic chemicals could contaminate food and beverages.

1.1.3 Proper preparation of dilute acid solutions from concentrated acids.

- Always pour acid into water (NOT vice versa) and swirl or mix to dissipate the heat of the reaction. This activity should be performed in a fume hood or in a well-ventilated area.
- When ~~acid~~ water is poured directly into ~~water~~ acid, an exothermic (heat generating) reaction results and can cause splash-back of acid onto the analyst.
- "Do what you otter...add acid to water"

1.1.4 Material safety data sheets (MSDS).

- Material Safety Data Sheets (MSDS) are designed to provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance.
- MSDS's include information such as physical data (melting point, boiling point, flash point etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill/leak procedures. These are of particular use if a spill or other accident occurs.
- Wastewater treatment plants must have MSDSs for chemicals used at the facility to comply with the Federal Hazard Communication Standard 29 CFR 1910.1200.

1.1.5 Proper venting of muffle furnaces and drying ovens.

This has safety and sample contamination implications. Volatiles driven off during the drying process could consist of hazardous substances which may pose a health risk for those working in the lab. Forced air ovens could aerosolize bacteria and viruses which could expose workers to disease causing organisms. Moisture driven off during sample drying can create a corrosive environment which can cause premature instrument failure.

These same substances could also contaminate samples. For example, drying raw wastewater could result in venting ammonia into the air causing samples and blanks to become contaminated.

1.1.6 Proper storage of laboratory chemicals.

- Chemicals must be stored in a cool, dry location out of direct sunlight.
- Storage in a cabinet is recommended.
- Liquid chemicals should be stored below human face level to avoid splattering in case of bottle spillage or breakage.
- Chemicals must be dated upon receipt and the expiration date recorded as well.
- Acids should be carefully stored, separate from other chemicals, and below eye level.
- The operator should always check the label on a chemical container for information regarding hazards with the use or storage of that chemical.
- Keep all Material Safety Data Sheets (MSDS) that come with the chemicals available for review for health, handling and disposal information.
- Chemicals should never be stored in unmarked containers.
- All reagents that are prepared and stored in separate containers should be labeled.

Chapter 2

Sampling, Preservation, and Sample Handling

2.1.1 Grab Samples

- A grab sample represents a single instant in time. It is subject to much more fluctuation than a composite sample. Generally, a grab sample is only used if the sample holding time would significantly change the parameter being tested, or if measurement of a slug loading is desired.

2.1.2 Flow Composite Samples

- A composite sample represents a longer period in time. It is far more likely to represent the average quality of the water being received or discharged, especially if waste characteristics change with time.
- Flow proportional composite samples are required for most wastewater plants. The exception is pond effluent, which is thought to have such a long detention time that the variations of loading do not affect it.

2.1.3. Guidelines to ensure proper collection of a representative sample.

The WPDES permit states the location where samples must be taken, what type of samples are to be taken, at what frequency to be taken, what samples are taken, and which parameters must be tested. For example whether the sample should be flow composite, time composite or grab.

Collect grab samples and locate the inlet tube for a composite sampler…

- In a well-mixed area
- In the center of the waste stream (away from channel sides)
- Off of the floor of the flume/channel

It is very important that the flow channel near any metering or measuring device be kept very clean. Debris or grit buildup in a channel can give a false reading, as the channel depth may be affected or the detention time and flow rate changed. Also, grease scum and debris will alter readings when using pressure or ultrasonic (transponder) type devices.

2.1.4 What type of sample should be collected?

Grab samples are used for determining instantaneous values and to identify extreme conditions. Municipal wastewater tests requiring grab samples include: pH, chlorine residual, dissolved oxygen, and, fecal coliform bacteria. Other tests (for industrial wastewater or under toxics regulations) requiring grab samples are: *oil and grease, volatile organics, and cyanide*.

Flow proportional compositing is the most representative way to sample and is required by the Wisconsin DNR. Composite samples are generally appropriate for the calculation of loading rates. Normally, in municipal wastewater, the tests in this category include BOD, suspended solids, phosphorus, and ammonia.

When using flow proportional composite sampling, the automatic sampler pulls an aliquot of sample from the flow stream directly proportional to the flow rate. Plant flow meters send an electronic "pulse" to the sampler to indicate a set amount of flow, such as a pulse for every 1,000 gallons. Then the sampler is programmed to pull a set amount of sample at a certain flow pulse interval such as 100 mL every 10 pulses or every 10,000 gallons.

2.1.5. Proper sub-sampling or splitting a wastewater sample. (1/2)

Most composite autosamplers collect wastewater into a single large jug, generally 2-3 gallons in size. When the facility is required to test their wastewater for more than one constituent that requires a different preservative (e.g., metals, phosphorus, suspended solids), it is necessary to sub-sample or "split" the wastewater into one or more containers so the appropriate preservatives may be added. This process must be done in such a way to assure that each sub-sample is identical to the other. Mixing is the most critical step in the sub-sampling process.

The same process may be used to prepare “split” samples.

2.1.5. Proper sub-sampling or splitting a wastewater sample. 2/2

Split samples are samples that are divided into two or more equal sub-samples, each of which is submitted to one or more laboratories for identical analysis. Split samples are used to:

1. Assess the variability from sample processing and preservation.
2. Assess the precision of laboratory analysis by allowing a comparison of analytical results from two parts of the same sample from the same location.
3. Verify compliance with discharge permits when "split" with Wisconsin Department of Natural Resource staff during *compliance inspections*.

Split samples must be prepared and analyzed for the same parameter(s) by the same method(s) to demonstrate the reproducibility of the split sampling and analytical techniques.

2.1.6. Requisite sample collection information.

For each sample or sample container the following information must be recorded:

- The sample type (flow proportional composite, time proportional composite, grab, etc)
- The sample point (where taken)
- Unique sample identification
- The time and date of the sampling (grab) -or- time and date of the first and last subsamples for a composite sample
- Any preservation during sampling - chemical added, refrigerated, *etc.*
- The initials of person sampling

It is also recommended that the operator record sample description (color, odor, turbidity, or other unusual observations). The information recorded on sample containers must be sufficient to trace it back to the sampling information.

2.1.7. Defining the sample (collection) date for Discharge Monitoring Report (DMR) recording.

- The day on which the majority of the sample was collected is the date of the sample.
- For example, if George started the composite sampler at 7:00 am on July 10th and removed the sample from the autosampler at 7:00 am on July 11th, the results of that sample on the DMR would be recorded for July 10th, since the majority of the sample (7:00 am to midnight = 17 hours) was collected on July 10th.

Section 2.2 - Sample Preservation

2.2.1. Maximum holding times and preservation requirements for key wastewater parameters.

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Test	Holding Time	Preservation
BOD	48 hrs after compositing	Cool to 6°C or less, without freezing
TSS	7 days	Cool to 6°C or less, without freezing
Ammonia	28 days	Add sulfuric acid (H_2SO_4) to pH < 2 and cool to 6°C or less, without freezing
Total P	28 days	Add sulfuric acid (H_2SO_4) to pH < 2 and cool to 6°C or less, without freezing
Fecal Coliform	6 hours	Add sodium thiosulfate if sample was chlorinated and cool to 6°C or less, without freezing
Samples must be refrigerated at a temperature not to exceed 6°C and must not be frozen.		

Chapter 3

Lab Equipment and Instrumentation

Section 3.1 - General Labware

3.1.1 Appropriate use of lab glassware and pipets. 1/2

VOLUMETRIC FLASKS
Volumetric flasks should always be used when making standards, particularly for calibration.

BEAKERS & ERLENMEYER FLASKS
Used for digestions. NOT for standard preparation or anything requiring volumetric measurements.

GRADUATED CYLINDERS
BOD & TSS when using 50 mL or more of sample.

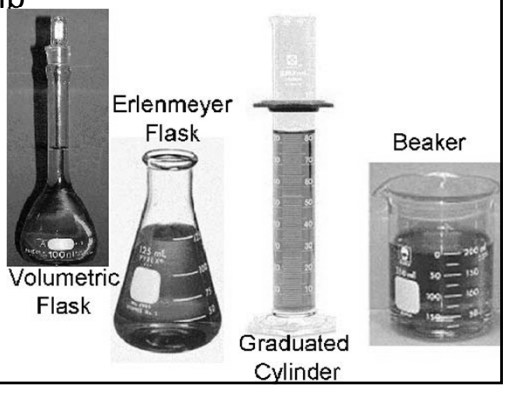
PIPETS, VOLUMETRIC (micro-bore), including mechanical pipets. Standard preparation.

PIPETS: WIDE-BORE
BOD & TSS samples.

PIPETS, MOHR
Color reagent (phosphorus).

3.1.1 Appropriate use of lab glassware and pipets. (2/2)

PIPETS, SEROLOGICAL (WIDE BORE)
BOD influents (low volume samples) also preservation of samples for phosphorus or ammonia.
Mohr pipets are measuring pipets in which the graduations stop well BEFORE the tip.
Serological pipets are very similar to Mohr pipets, except the graduations continue to the tip



3.1.2 Proper pipet technique. 1/2

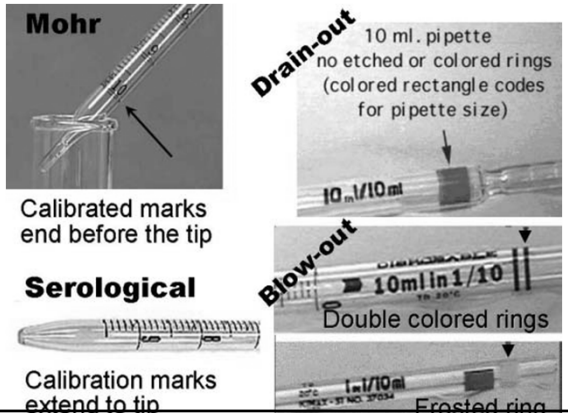
Volumetric pipets are classified as "TD" (To Deliver; the new international designation is "EX") and should never be blown out. These pipets are calibrated to deliver one specific volume and the calibration already takes into consideration the small amount of volume that may remain in the tip. Volumetric pipets may have a colored square at the top of the pipet which is a manufacturer's mark indicating the volume of the pipet.

Serological pipets can be used to measure various volumes from a single pipet due to their volume gradations. There are two kinds of serological pipets, "blow out" and "drain out".

Serological pipets from which the volume in the tip should be "blown out" typically are graduated right up to include the entire tip. These pipets are usually identified by the presence of two thin colored or etched (frosted) rings at the top of the pipet.

3.1.2 Proper pipet technique. 2/2

Serological or Mohr pipets which should be "drained out" typically have their graduated markings stop prior to the tip. Volume should not be drained below the last marking. These pipets are usually identified by the presence of a color-coded rectangle or square (but no frosted or colored rings) at the top of the pipet.



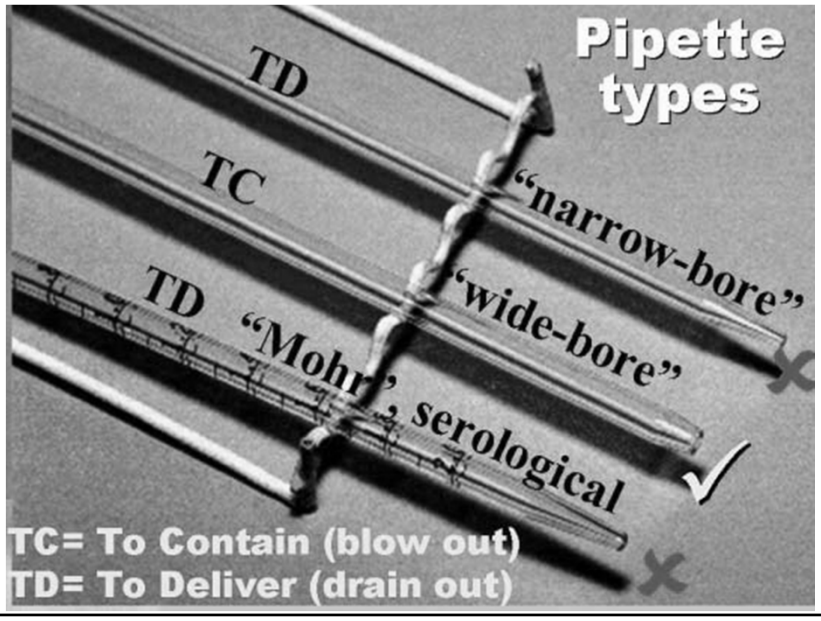
3.1.3 Proper pipets to be used for BOD and TSS tests.

Only wide-bore volumetric pipets are to be used for the BOD and TSS tests when less than 100 mL of sample is needed.

These pipets have a wide-bore opening which are useful when transferring samples that contain particulates such as raw wastewater. When pipetting samples with visible suspended solids, wide-bore pipets are more accurate because narrow-bore can serve to block or strain solids particles from the sample. This is why the use of wide-bore pipets is specified by Standard Methods when determining total suspended solids and BOD.

Other names for these pipets include macro bore and wide mouth tip. Volumetric factory made glassware must not be altered to "create" wide-bore pipets.

3.1.3 Proper pipets to be used for BOD and TSS tests.



3.1.4. Pipet accuracy

All pipets are associated with a degree of accuracy. Generally, the error (inaccuracy) increases as the volume of the pipet decreases. For example, a 1 mL Class A volumetric pipet may have an associated error of 0.6%, while the error for a 10 mL Class A volumetric pipet is 0.2%.

A single larger volume pipet, therefore, is more appropriate for measuring volumes than two smaller volume pipets. That is because error is additive. One could use a single 10 mL volumetric pipet to dispense a standard with 0.2% error, or use a 1 mL pipe ten times for a combined error of 6.0%. In addition, using a small pipet multiple times instead of a larger pipet a single time only increases your risk of introducing contamination.

Section 3.2 - Support Equipment

3.2.1 Explain the function of an incubator in a wastewater treatment plant laboratory.

An incubator is an enclosed chamber used in a wastewater treatment plant laboratory to provide a constant temperature in a controlled environment. It is used to maintain required conditions for the development of microbial or chemical analyses.

3.2.2 Desiccator function and considerations.

A desiccator is used to maintain a humidity-free environment. Oven-dried samples, glassware, and some chemicals should be stored in a desiccator before weighing to prevent moisture absorption. In the wastewater laboratory, a desiccator is used to cool TSS samples filters after drying. Absorbed moisture adds weight to the samples, causing incorrect weight measurements. In order for a desiccator to work properly, it must contain color indicating desiccant and the lid must have a silicone based grease seal or a rubber gasket seal.

The color of indicating desiccant changes from blue to pink as it absorbs moisture. Blue silica gel changes from deep blue to pale pink as it absorbs moisture. Once the material turns pink, the desiccant must be re-generated by heating in a drying oven at the manufacturer-specified temperature to drive off absorbed moisture or be replaced.

3.2.3 Types of balances used in a wastewater laboratory.

Analytical balance: Analytical balances are most often found in a laboratory where extreme sensitivity (0.1 mg) is needed for the weighing of items. Analytical balances usually measure mass and range in capacity from 0.1 mg to 150 grams. They are typically used for the suspended solids (TSS) testing in the wastewater laboratory where weight changes of 0.1 mg must be measured.

General purpose or top-loading balances: Another balance used primarily in a laboratory setting. They usually can measure objects weighing around 150-300 g. They offer less readability and accuracy than an analytical balance but allow measurements to be made quickly thus making it a more convenient choice when exacting measurements are not needed. Top-loaders are also more economical than analytical balances and suitable for weighing chemicals when preparing reagents.

3.2.4 Proper operation and verification requirements for balances. 1/3

"TOP-LOADER" BALANCES

This type of balance can generally weigh materials to one or two (rarely, three) decimal places depending on the model and price (e.g., 0.01 to 6200 g capacity). Allow the balance to warm-up before use to prevent electronic drift.

Balance verification is required at least once per month using one weight in the expected range of use.

ANALYTICAL BALANCE

This type of balance is a high quality, precision instrument that can typically measure mass in the range of 0.0001 to 100g. If the components of an electronic balance are cold when you start, they will drift while warming up, causing changes in your measurements. Avoid this problem by leaving balance on at all times or by allowing the balance to warm-up before use.

3.2.4 Proper operation and verification requirements for balances. 2/3

Analytical balance verification must be performed at least monthly using one weight in the gram range (e.g., 1-2 g) and one in the milligram range (e.g, 100 mg).

• Electronic balances require adequate warm-up time. Follow manufacturer instructions for warm up.

• Balance must be situated on a sturdy foundation.

• Protect the balance from vibration. Vibration can significantly affect measurement accuracy, even leaning on a balance table without a damping pad can affect results by several milligrams.

• Balance must be level to function properly. Check the balance bulls-eye (spirit) level to make sure bubble is inside the target.

• Balances need controlled temperature & humidity. Wide swings in humidity & temperature can damage the sensitive parts and electronics.

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3.2.4 Proper operation and verification requirements for balances. 3/3

Balances should be placed away from direct sunlight.

• The pan and balance floor must be clean. Use a camel-hair brush. Use a mild detergent, lab reagent water *and lint-free wipe if necessary*.

• Samples being weighed must be at room temperature. Warm or hot objects placed in a pan within a closed balance chamber can create air currents that buoy the pan, resulting in erroneous measurements.

• Do not weigh chemicals directly on the pan. Always use weigh boats or glassine paper.

• Sliding doors must be closed when making measurements. Avoid the effects of any draft or air currents on the balance.

• Have the balance checked by a professional. Have balance serviced annually.

3.2.5 Proper care, handling, and storage of certified ASTM Type 1 weights.

Weights can become scratched, worn, or corroded from atmospheric conditions and subsequently experience changes to their certified weight over time.

- When not in use weights should be stored in the original case to keep them as free from contamination as possible.
- Handle Type 1 weights with plastic tipped forceps to prevent damage to the surface of weights through metal-to-metal contact. Never use a metal forceps or your fingers to handle certified weights.
- Wear soft cotton gloves when handling larger weights (for example, weights heavier than 100 grams). Never handle weights with your bare hands because skin oils, skin acids and finger prints will damage the weights and change their mass.
- Weights should not be stacked on top of each other or come in contact with other weights.

Putting two or more of them in a plastic vial and letting them roll around against each other is NOT appropriate.

- If necessary, weights may be cleaned by lightly dusting them with a brush specifically designed for weights such as a soft camel hair brush.

3.2.6. Analytical weight set types and re-certification requirements.

Industry and certification authorities require weights to be cleaned and re-certified on a regular basis. Have weights re-certified by an external metrology firm at least every 5 years. Remember that these are precision calibration tools.

When ASTM Type 1 weights are purchased, individually or as sets, they are certified traceable to standards provided the National Institute of Standards and Technology (NIST). This process must only be done by a certified metrology company.

Certified weights are used to verify the calibration status of laboratory balances. These weights must be certified to be ASTM Type 1 (formerly Class "S") specifications. Type 1 weights are made from high grade stainless steel. Type 2 (formerly Class "P") are typically made of brass.

Type 2 (non-stainless steel) weights should not be used for analytical balance calibration verification, as brass is a soft metal alloy that is subject to oxidative pitting, tarnishing, and scratching. These conditions will result in significant changes to the standard's certified weight.

3.2.7 Critical considerations for drying ovens.

The following will affect the ability of a drying oven to maintain temperature:

- Repeated opening and closing of the oven door
- An overloaded oven (too many samples for size of oven)
- Oven defects (a poor door seal, etc.)
- An inadequate or defective thermostat
- Placement under an HVAC air vent

In order to ensure proper temperature measurement, place the thermometer bulb in a jar of clean sand or vermiculite (traceable thermometers like this are available commercially).

Doing so will insulate the bulb from rapid temperature shock associated with opening the oven door and provide an accurate temperature of the inside of the oven.

Chapter 4

General Lab Practices

Section 4.1 - Reagent Water

4.1.1 What is reagent water.

Reagent water is water which has been treated to remove any impurities that may affect the quality of sample analysis. You may see this referred to as laboratory reagent water, laboratory pure water, distilled water, or deionized water. Reagent water used for analyses is typically classified as either ASTM Type I or Type II.

4.1.2 Proper storage of reagent water.

Store only in materials that protect the water from contamination, such as Teflon and glass for BOD analysis or polyethylene, polycarbonate and polypropylene for other analyses.
Avoid storing water for extended lengths of time once it has been opened. If stored water appears cloudy or the general quality has appeared to change, discard.

4.1.3 Evaluating and troubleshooting laboratory reagent water quality. 1/5

Conductivity, which is the ability of a solution to conduct an electrical current, provides a measure of water quality. Pure water is actually a poor conductor. If water has even a tiny amount of ionic impurities, then it can conduct electricity much better, because ions such as salts separate ("ionize") into free ions in aqueous solution by which an electric current can flow. Therefore measurable conductivity means ions are present and the presence of ions clearly means that the water is not "pure". Conductivity is useful as an indication that ion exchange resin is overloaded, that a reverse osmosis membrane has been breached, or simply that your reagent water may not be of sufficient quality for use in testing.

4.1.3 Evaluating and troubleshooting laboratory reagent water quality. 2/5

In theory, lab reagent water should be "pure" and thus contain no dissolved solids or ions. Therefore one would expect the conductivity of lab reagent water to be zero. The American Society of Testing and Materials (ASTM) has defined Type I reagent water as water having a maximum conductivity of 0.056 µS/cm at 25°C. ASTM "Type II" water has a maximum conductivity of 1.0 µS/cm at 25°C.
The drawbacks to using conductivity alone as a means of verifying water quality are:
1. Conductivity ONLY measures substances that ionize…i.e. form ions. You can dissolve 1000 ppb (or more) of sugar in pure water and still not exceed ASTM Type I water criteria for conductivity.
2. It is virtually impossible to measure conductivity accurately to Type I or Type II levels without a closed system and VERY sensitive conductivity equipment.

4.1.3 Evaluating and troubleshooting laboratory reagent water quality. 3/5

The nominal levels of CO2 in the atmosphere will cause gaseous carbon dioxide (CO2) to enter pure water causing a chemical ionization reaction which increases conductivity well above even Type II water standards.
Some of the most critical sources of contamination that affect reagent water and the analytical tests which are performed using reagent water are as follows:

HIGH CONDUCTIVITY
Elevated conductivity is an indication of an increase in dissolved ions in the water. Some of these can be interfering substances, such as, copper, chromium, or ammonia. The more substances which are dissolved in the theoretically "pure" lab reagent water, the more likely it will be to have interferences in the tests. Elevated levels of trace metals could affect BOD results, while elevated ammonia levels will impact ammonia test results.

4.1.3 Evaluating and troubleshooting laboratory reagent water quality. 4/5

HIGH CONDUCTIVITY
Some municipal water systems add phosphate to the water source to remove iron and manganese. Without proper treatment (distillation or deionization) phosphate can interfere with total phosphorus determinations, leading to high bias.
HEAVY METALS
Copper and chromium are quite toxic to organisms. High levels of either could significantly affect any biological tests (BOD, fecal coliform) by inhibiting biological growth.

4.1.3 Evaluating and troubleshooting laboratory reagent water quality. 5/5

DISSOLVED ORGANICS

Many systems employ a carbon filtration step to remove dissolved organics. In the absence of such a system, or if the carbon filter is not functioning contamination from compounds such as volatile organic compounds (VOC, product of industrial pollution and runoff) can occur.

Additionally trihalomethanes (THMs) may be present in municipal systems that rely on chlorination for disinfection. While disinfection kills or inactivates most bacteria and many viruses, microbial byproducts and cell fragmentation can release compounds such as endotoxins to the water system. Many of these contaminants can serve as toxins and interfere with BOD test.

Section 4.2 - Standard Preparation

4.2.1 What is a standard?

A standard is a solution which consists of a known and documented concentration of a target analyte (e.g., 100 ppm phosphorus solution). Standards are used as reference material to determine the concentration of a target analyte in an unknown sample. The instrument response relative to the concentration of standards is compared to that of the unknown samples to determine the concentration. Standards should be prepared in a solution similar to the samples being tested. For example, if you are measuring phosphorus in wastewater, you should prepare the standards in water.

4.2.2 Proper preparation of calibration standards

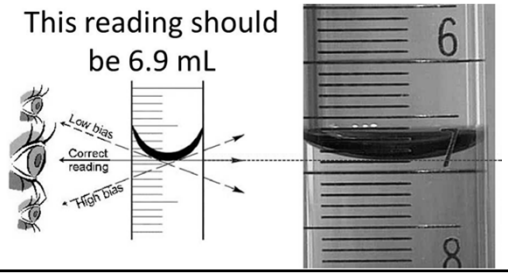
A good calibration curve is dependent on many factors. The first thing you need is a very accurate measuring device for making your calibration standards. When preparing a standard solution, be sure to use a volumetric pipet or mechanical pipet for accurate volumetric measurement.

If volumetric pipets are inaccurate then your curve is most likely going to show it. The main thing you need to be careful with is how you handle your standards before you measure them; any errors you make will show in your curve. Calibration standards are made by diluting a stock analyte solution in volumetric flasks

4.2.3 Reading a water level meniscus correctly.

When water or liquid is held in a small diameter tube such as a pipet or buret, the water level will form a concave curved surface (meniscus). The liquid in contact with the container walls will rise slightly, leaving a low point in the center. When taking a measurement, the low point of the curve is considered to be the correct measuring point.

Always read the meniscus at eye level. If the meniscus is not read at eye level, incorrect readings will result.



Chapter 5

Biochemical Oxygen Demand (BOD) Analysis

Section 5.1 - Definitions (BOD)

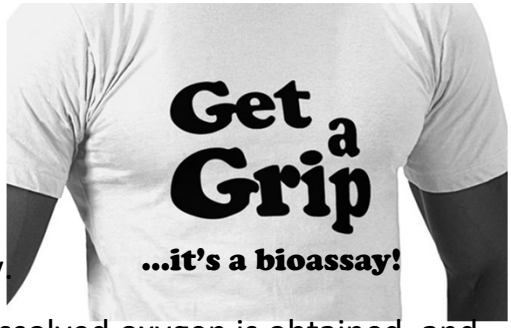
5.1.1 Define BOD 1/2

BOD = Biochemical Oxygen Demand
BOD is a test used to measure the amount of oxygen consumed by bacteria as they decompose organic matter in a sample of water. It can be used to infer the general quality of the water and its degree of pollution by biodegradable organic matter. It is used in water quality management and assessment, ecology and environmental science.
In a nutshell, BOD gives a measure on the impact of a waste(water) on the oxygen content of a receiving system(stream/river/lake). Wastes are broken down by microbial organisms (frequently referred to as "bugs"), and the bugs, in turn, require oxygen. Thus, in order for this test to "work", you need **(1)** a food source, **(2)** a population of bugs, **(3)** available oxygen fuel, and **(4)** a system which provides a hospitable environment for the bugs.

5.1.1 Define BOD 2/2

A series of dilutions with nutrient-rich, buffered dilution water is performed on each sample.

Samples may also be seeded with a population of microorganisms as necessary.



An initial measurement of dissolved oxygen is obtained, and then again following a 5-day incubation period at 20 ± 1 °C. The amount of oxygen depleted (during the 5 day incubation period) is used to calculate BOD.

5.1.2 Define carbonaceous BOD (cBOD)

Carbonaceous BOD (cBOD) is a specific part of "total" BOD which represents the amount of oxygen demand required to break down carbon sources only. Total BOD measures the oxygen consumption associated with microbial breakdown of a waste, including both carbonaceous and nitrogenous sources.

cBOD is measured by adding a pyridine-based inhibiting agent to the sample prior to incubation during the BOD test. This chemical inhibits the ability of the bacteria, Nitrosomonas sp., to convert ammonia to nitrite, which is the first phase of the nitrification process. By inhibiting this activity, the test measures only the oxygen utilized by microorganisms to breakdown carbonaceous waste.

5.1.3 Define dissolved oxygen

Dissolved oxygen (DO) is defined as oxygen (O2) molecules dissolved in water. Dissolved oxygen cannot be seen, so small bubbles are not actually considered to be "dissolved" oxygen. Oxygen becomes dissolved in water in one of two ways: it either dissolves into water from contact (diffusion) with the atmosphere or is produced by plants as a byproduct of photosynthesis. Dissolved oxygen concentration is related to the following:

1) **temperature** (as water temperature increases, it is able to hold LESS oxygen)

2) **atmospheric pressure** (as air pressure increases, water is able to hold MORE oxygen)

5.1.4. Define super-saturation.

Supersaturation means that the water contains more DO than it SHOULD contain according to physical tables. According to tables, the saturation point of oxygen in water at 20° and 760 mm pressure - which is standard temperature and pressure at sea level - is 9.06 mg/L.

So, yes, at sea level and 20°C, anything over 9.06 mg/L represents supersaturation. The method suggests that super-saturation is anything above 9.0 mg/L. However, in reality saturation will vary with temperature and pressure. Consult a DO saturation table.

CONDITIONS THAT FAVOR SO SUPER-SATURATION IN SAMPLES

- Super-saturation can occur during winter months (the colder the water, the more oxygen it can hold).
- Super-saturation can also be a problem in localities where algae are actively growing (e.g., lagoons).

Section 5.2 - BOD - General considerations

5.2.1 The significance of BOD in wastewater testing.

BOD is used to assess the relative strength of a waste. It measures the amount of oxygen required to stabilize a waste if it is discharged to a surface water.

BOD is the most commonly required test on WPDES and NPDES discharge permits. It is widely used in facility design planning and is used to gauge the effectiveness of wastewater treatment plants once in operation. The critical function of the BOD test is to provide a means to assess waste loading on surface waters. By looking at the potential *for a waste to utilize oxygen*, we can evaluate the potential impacts downstream, including those on fish.

5.2.2 BOD incubators specifications.

Thermostatically controlled at 20 ± 1°C. All light *must be excluded* to prevent photosynthesis during the 5-day incubation period. The temperature of the incubator must be documented daily when samples are being incubated.

5.2.3 Preparation and storage of BOD dilution water.

BOD dilution water nutrient buffer solutions can also be a source of contamination. If you prepare your own solution make sure you store the phosphate buffer in a refrigerator.

Phosphorus is the limiting factor in microbial populations, so once the phosphate buffer is added, dilution water becomes a prime growth medium for microorganisms. Discard any solution if it becomes cloudy or has "chunks" floating in the solution. Using single-use nutrient buffer pillows will avoid many of the pitfalls.

Before use bring dilution water temperature to 20 ± 3°C. It is best to bring the temperature to 21-22°C initially. Saturate dilution water with oxygen by shaking vigorously for several minutes, aerate with organic-free filtered air, or store "long enough to become saturated" in bottles covered with a clean coffee filter secured by a rubber band. Shaking is the best method.

It is preferable to store dilution water in a glass container. Plastic containers can leach BOD.

5.2.4 "Instantaneous" BOD requires minimizing the time before initial DO measurement.

Don't let samples sit too long between adding dilution water and the initial DO measurements. Standard Methods requires measuring the initial DO no longer than 30 minutes after adding the dilution water. The rationale behind this requirement is that some samples exhibit what is termed "instantaneous BOD". These samples contain materials that become oxidized quite rapidly, thereby rapidly reducing the available DO in the diluted sample. In these types of samples, any lag between dilution of the sample and measuring the initial DO will fail to capture this rapid initial utilization of oxygen, which results in a low bias for sample results.

You must determine an initial DO on each sample bottle.

5.2.5 How to determine the proper sample dilution volumes for the BOD test.

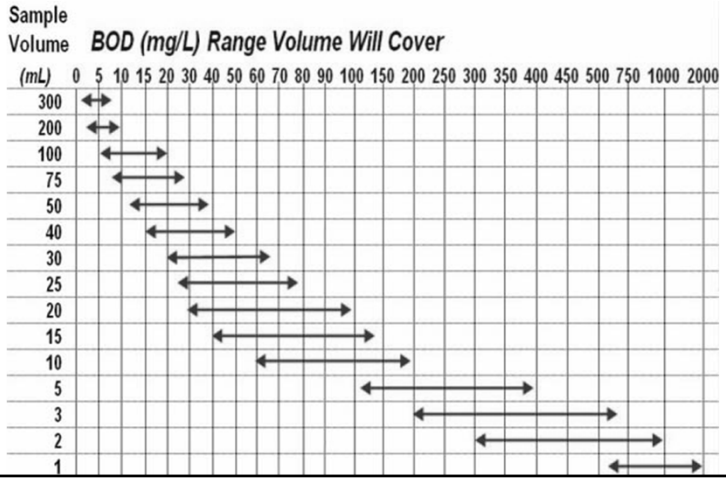
There are tables and charts that have been developed to assist analysts in making the best dilutions for any given sample. To use these charts, however, the analyst needs to have some idea of the sample BOD. All of these tables work off of a simple concept:

- Under typical conditions, at saturation in Wisconsin, initial DO should be about 8.2 - 8.5 mg/L
- The FINAL DO cannot be less than 1.0 mg/L
- Therefore the working range of DOs for any dilution is about 7.5 mg/L

If uncertain, or if you have variable BODs, use more dilutions or be flexible in what dilutions you use to ensure that you will have enough dilutions that meet depletion criteria. The correct approach is to always use enough dilutions to ensure that at least two dilutions will meet method-defined DO depletion criteria.

5.2.5 How to determine the proper sample dilution volumes for the BOD test.

In the absence of prior knowledge, use the following guidelines for dilutions:
-Strong industrial wastes: < 3 mL of sample (<1% dilution)
-Raw and settled wastewater: 3 - 15 mL of sample (1 - 5% dilution)



5.2.6 The affect of sample dilution volume on LOD for BOD. 1/2

As a bioassay procedure, the BOD test does not lend itself well to the typical EPA procedure (40 CFR Part 136 Appendix B) for determining the limit of detection (LOD).
Therefore, the LOD for any given sample is determined based on the method specified minimum required DO depletion (2 mg/L) and the sample volume in the least diluted (i.e., the one with the greatest volume of actual sample) sample.
BOD detection limits are theoretically based. A key assumption on which the concept of detection for BOD is based is that the LEAST amount of depletion allowable is 2 mg/L. The LOD is also based on the highest volume of sample used in a dilution series. What results is the detection capability for a specific dilution series, or sample. This technique should include seed correction, because we want to identify depletion due to the sample itself, and not due to the seed material.

5.2.6 The affect of sample dilution volume on LOD for BOD. 2/2

The calculation involved is simply:
LOD mg/L = 2 mg/L X [300 mL ÷ mLs sample in LEAST dilution]
EXAMPLE: sample with dilutions of 100, 50, and 25 mL
LOD = 2 mg/L X [300 mL ÷ 100 mL]
= 2 mg/L X 3
= 6 mg/L LOD for that sample

Highest Vol.used	Dilution	Sample LOD
300 mL	1.0	2 mg/L
200 mL	1.5	3 mg/L
100 mL	3.0	6 mg/L
75 mL	4.0	8 mg/L
50 mL	6.0	12 mg/L

In order to report an LOD of 2 mg/L, one sample dilution containing 300 mL of sample must be included.

Section 5.3 - DO Meter Calibration

5.3.1 Equipment required to calibrate a DO meter.

Assuming one is not using the Winkler technique for calibration, which requires no additional equipment, the following are required to accurately calibrate a DO meter.
A barometer, or pressure obtained from a local source and adjusted for elevation, is required to obtain an accurate pressure reading that is corrected for altitude. All modern DO meters are equipped with on-board barometers. On-board barometers are more than satisfactory so an external *should not be used.*
A thermometer is required to determine the temperatures of the dilution water used to calibrate the DO meter.
A DO saturation table, chart (or properly vetted algorithm) is required to determine the saturation point based on calibration solution temperature and barometric pressure.

5.3.2
Options for calibrating a DO meter 1/2

WATER-SATURATED AIR (WSA)

- Place the probe in a BOD bottle containing about 1 inch of water.
- Shake BOD bottle prior to inserting probe to assure saturation. We recommend turning the stirrer OFF to avoid heating the thermistor during calibration.
- The probe may need to sit in the bottle for 30-35 minutes in order to match the temperature of the air.
- Determine barometric pressure from the on-board barometer if so equipped.
- Check the temperature of the air (in the bottle) to be sure the probe thermistor is working correctly. This is also determined using the thermometer on-board the DO meter.
- Use the meter's auto-calibration function to calibrate the probe and meter.
- The WSA and auto-calibration function on modern DO meters is the most common DO calibration technique used by wastewater laboratories.

5.3.2
Options for calibrating a DO meter 2/2

AIR-SATURATED WATER (ASW)

- Place the probe in a BOD bottle filled with air-saturated (well-shaken) water.
- Leave probe in the water w/ stirrer operating long enough for the probe temperature to equalize with the water temperature; generally about 5 minutes.
- Determine barometric pressure from the on-board barometer if so equipped.
- Check the temperature of source water to be sure the probe thermistor is working correctly. This is also determined using the thermometer on-board the DO meter.
- Use a detailed DO saturation table to determine the theoretical DO concentration.
- Manually adjust the meter to read the DO concentration determined from the saturation table. Winkler Titration should be avoided because its accuracy depends on the accuracy of the chemicals used in the test, and frankly, the DO probe technology has advanced to the point that Winkler titrations are obsolete.

NOTE: With LDO probes, WSA calibrations work best, as long as a consistent procedure is used.

5.3.3 How atmospheric pressure and temperature affect DO calibration. 1/2

The concentration of dissolved oxygen (DO) in water is dependent on both temperature and atmospheric pressure. As the temperature of water increases, its ability to hold oxygen (DO saturation point) decreases. Conversely, as temperature decreases, the water can hold more DO. This explains why fish in an aquarium become stressed and may die if the water temperature is too high. The water can simply no longer hold enough oxygen for the fish to survive. In natural systems, this can result in a "fish kill". On the other hand, as atmospheric pressure increases, the ability of water to hold oxygen (DO saturation) actually increases.

5.3.3 How atmospheric pressure and temperature affect DO calibration. 2/2

Oxygen is a dissolved gas, and gases tend to escape, but increased atmospheric pressure reduces the ability of oxygen to escape the system. This also explains why the oxygen is "thinner" at higher altitudes, where pressure is much lower than at sea level. In order to make accurate DO measurements for the BOD test, we must calibrate the DO meter. We must know the temperature of the calibration solution or air and the atmospheric pressure in order to determine the specific DO saturation point under these conditions.

Once we know the saturation point we can calibrate the meter properly. Because temperature and pressure are constantly changing, we need to calibrate the DO meter daily before analysis to ensure accurate results.

5.3.4 Verification of barometer accuracy.

All barometers (electronic and aneroid) must be checked to verify they are functioning properly. The internet or local airport can provide information that should be used to check laboratory barometers.

Note that measurements obtained from airports or the internet have generally been adjusted for sea level. Therefore, these values must be uncorrected" to the laboratory elevation before they can be used to verify the accuracy of a wall or "on-board" barometer.

Generally speaking, barometer accuracy should be verified monthly. In no case should verification be performed less frequently than annually.

Section 5.4 - Seed Material and Seeding

5.4.1 What is the purpose of "seeding" BOD samples.

The purpose of seeding is to ensure that an adequate population of viable microorganisms is available to breakdown any waste material, utilizing oxygen during the process.

Microorganisms are a critical component of the BOD test, as it is the oxygen they utilize during the breakdown of organic matter which is measured by the BOD test.

Consequently, whenever there is suspicion that a sample may not contain an adequate population of microorganisms, sample dilutions must be "seeded" with microorganisms obtained as either commercially purchased synthetic seed, or preferably as a portion of process water, such as settled primary or mixed liquor, from a treatment plant.

5.4.2 How are BOD samples "seeded"?

A small volume of concentrated seed material can be added to individual sample dilutions.

Alternatively, you can seed dilution water and then all samples that are diluted to any extent are provided with a population of microorganisms. Either practice is acceptable. Seeding dilution water ensures all samples are seeded, but seeding individual samples makes calculating seed correction factors a much easier task. Seeding individual samples works best.

5.4.3 What options are available for use as BOD seed material. 1/2

There are really two options for obtaining a seed source. The first is to utilize a sample from some aspect of the treatment process. Settled primary wastewater or mixed liquor are generally the best sources. Alternatively, there are several commercially available synthetic seed sources available.

If domestic primary wastewater or mixed liquor supernatant is used as a seed source, it should first be allowed to settle for about an hour at room temperature. Labs should not allow primary or mixed liquor to settle for more than an hour. If they do, they will lose too much solids. Under no circumstances should it be settled overnight because the population of bugs will start to crash.

Commercial seed (e.g., BOD seed, Polyseed) is supplied as microorganisms which are freeze dried onto an inert material.

5.4.3 What options are available for use as BOD seed material. 2/2

The general procedure is to open a capsule of synthetic seed into a quantity of BOD dilution water and mix for a period of time. The sequence is important. The seed can only be introduced after nutrients have been added to reagent water and mixed completely. Some synthetic seeds may require longer or different mixing procedures than the manufacturer recommends. DO NOT mix seed in distilled or deionized water! Adding a seed capsule to deionized water causes an osmotic imbalance that will kill seed organisms.

The purified water -- containing no salts -- rushes across the bacterial cell walls. The bacteria subsequently swell up and burst (or lyse) which, if it does not kill them, will severely impair their ability to utilize oxygen.

Effluent from a biological treatment system is NOT recommended, as it creates the potential for nitrification to occur. If you do use effluent from a biological treatment system (or recycle effluent) as your seed source, it's very likely you have nitrification and BOD results will be biased high.

5.4.4 When is seeding required for wastewater samples?

Seeding is required whenever any sample is collected downstream of any disinfection, whenever sample pH requires adjustment, and also when inhibitor is added for cBOD determination.

Sample pH extremes will severely shock or kill microorganisms. Therefore, when sample pH adjustment is required, subsequent sample dilutions must be seeded.

If collected downstream of disinfection processes, samples must be tested for chlorine residual, and if present, the residual must be quenched prior to sample dilution and analysis. Disinfection agents, *such as chlorine or UV, kill or prevent microorganism populations* from reproducing. Therefore, any sample which has an initial chlorine residual, or any sample collected downstream of any disinfection process must be seeded.

Finally, the inhibiting agent used to disrupt the nitrification process for cBOD determinations may have a toxic effect on other microorganisms. Therefore, samples for cBOD determinations (to which nitrification inhibitor is added) must be seeded as well.

5.4.5 Important considerations for seed controls. 1/2

Seed material itself causes a DO depletion. Therefore depletion due to seed material must be subtracted from the total DO depletion of each seeded sample dilution prior to calculating BOD for the original sample.

When samples are seeded, an accurate BOD determination depends on the ability to account for the DO depletion due to the seed material in each sample dilution. This is accomplished by determining a seed correction factor. The seed correction factor is generated based on the analysis of "seed control" samples, which are aliquots of dilution water containing known volumes of seed material. The amount of DO depletion in each control is then calculated as the amount of DO depletion per mL of seed material added.

The results of seed controls are then averaged to determine the final "seed correction factor" to be used to adjust the BOD calculation of seeded samples.

5.4.5 Important considerations for seed controls. 2/2

Seed controls must be treated just like any other sample, since we are really trying to accurately determine the BOD of the seed material. Therefore at least two dilutions of seed material are required, and the method specified DO depletion criteria must be met. The intent of the 0.6 to 1.0 mg/L range is guidance, rather than a requirement. The true test of a seed material is to utilize a sufficient quantity of seed material such that method acceptance criteria for GGA determination are routinely achieved.

Seed controls are required on each day that any samples/QC are seeded.

Seed control results must be reviewed for consistency in the data. Individual seed control factors (DO depletion per 1 mL of seed) should agree well with each other and the average. If there is a considerable range in these values, it will likely result in high variability in GGA results.

5.4.6 How is a seed correction factor (SCF) calculated?

EXAMPLE:

Seed Control 1
Volume = 10 mL
Initial DO = 8.50 mg/L
Final DO = 5.50 mg/L

Seed Control 2
Volume = 20 mL
Initial DO = 8.50 mg/L
Final DO = 2.70 mg/L

SCF for Seed Control 1:
Depletion = (8.50 - 5.50) - 0 = 3.0 mg/L
SCF 1= 3.0 mg/L ÷ 10 mL
SCF 1= 0.30 mg/L / seed mL

SCF for Seed Control 2:
Depletion = (8.50 - 2.70) - 0 = 5.80 mg/L
SCF 2= 5.8 mg/L ÷ 20 mL
SCF 2= 0.29 mg/L /seed mL

Average SCF = (0.30 + 0.29) ÷ 2
Average SCF = 0.295 mg/L /seed mL

Thus, for each mL of seed added to samples, 0.295 mg/L DO must be subtracted from the individual sample DO depletion prior to calculating BOD.

Section 5.5 - Method Specifications

5.5.1 Sample pretreatment required for BOD testing. 1/4

Because the BOD test is a bioassay, it is critical to maintain optimal conditions for the "bugs" (bacteria) to stay live and viable. Temperature, pH, oxygen levels and the presence of disinfectants can all influence the outcome of the BOD test. Before proceeding with the BOD test, the following items must be checked. In many cases, pretreatment may be required.

pH

pH extremes kill (or at least severely injure) the "bugs". Consequently, you must test each sample to confirm that the pH is in the appropriate range before proceeding with the BOD test. pH extremes are defined as less than pH 6 or greater than pH 8.5.

If the undiluted sample requires pH adjustment, adjust the pH with 1N H_2SO_4 or 1N NaOH.

Do not dilute sample by >0.5% (1.5 mL in a 300 mL bottle). If more acid or base is needed, use more concentrated solutions (i.e., 5N).

Phosphate buffer in the dilution water will often adjust the pH into the acceptable range, particularly in influent samples, and effluent dilutions which contain significant volumes of dilution water. However, this must be confirmed and recorded.

5.5.1 Sample pretreatment required for BOD testing. 2/4

CHLORINE RESIDUAL/DISINFECTION

Check wastewater samples for residual chlorine unless it can be demonstrated that the sample was collected prior to where chlorine is added. A plant diagram with the sampling point shown in relation to the chlorine contact tank is usually satisfactory. If chlorine is detected, quench the chlorine residual as per Standard Methods and seed the sample(s). If ANY disinfection process is employed (UV, chlorine) and the sample(s) is collected downstream of disinfection, seed the sample(s).

5.5.1 Sample pretreatment required for BOD testing. 3/4

SUPER-SATURATION

Water only has a limited capacity to hold oxygen. This capacity, or saturation point, is driven by temperature and barometric pressure. If the sample DO is greater than the saturation point when the bottles are placed in the incubator, oxygen will physically come out of the solution (will appear as micro bubbles) and appear to be an oxygen demand. The resulting BOD will be falsely high.

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5.5.1 Sample pretreatment required for BOD testing. 4/4

SUPER-SATURATION

EXAMPLE: What should be done if the initial DO of a BOD sample dilution is 9.5 mg/L and the saturation point has been determined to be 8.8 mg/L?

1. Warm samples to room temperature (20 ± 3°C) and THEN shake them VIGOROUSLY to prevent super-saturation problem. It is best to warm to 21-22°C.
2. Reduce excess DO by shaking sample(s)vigorously or aerate with filtered compressed air.
3. Know the saturation point at your facility/your conditions.
4. Supersaturation is a concern if DO > 9.0 mg/l at 20°C, but the saturation point could be below 9.0 mg/L. The exact saturation point can only be determined using a saturation table.

Make sure there is enough oxygen! Always start with an initial DO close to the saturation point for your facility (typically about 8.2 to 8.5 mg/L in Wisconsin). If the DO is low, shake or aerate with filtered compressed air to increase the DO concentration. Remember, starting with a higher initial DO will allow you to cover a wider BOD range with each dilution.

5.5.2 Incubation time and temperature requirements for BOD samples.

Samples must be incubated for 5 days in the dark at a temperature of 20 ± 1°C. The 5-day incubation period was originally set by early BOD pioneers based on the length of time it took water to flow from London, England, down the Thames River to the North Sea. Although this may seem like an arbitrary time, it has been internationally adopted as the "standard" incubation period for the BOD test.

For optimal defensibility of results, you should strive to stay *as close to the actual 5 day incubation period as you can*, but certainly stay within 5 days ± 4-6 hours. The current edition of Standard Methods indicates ± 6 hours.

5.5.3 Minimum number of dilutions required for BOD.

With the exception of blanks and GGA samples, at least three (3) dilutions are recommended; however, a minimum of two (2) sample dilutions must meet DO depletion and residual criteria for any BOD determination.

Note that, while three dilutions are recommended for BOD analysis, three dilutions are not sufficient to identify sample toxicity. When analyzing samples for which they have no previous historical data to guide them, analysts are cautioned to use an appropriate number of dilutions. Standard Methods suggests using at least five dilutions for unfamiliar samples.

5.5.4 What is the smallest sample volume for BOD that can be used without performing apreliminary dilution?

The smallest volume which can be used for BOD without performing a dilution of the original sample is 3 mL. If less than 3 mL of original sample volume is used for a single dilution(<1% dilution), a preliminary dilution (of the original undiluted sample) is required.
This is best done by preparing a simple dilution and then pipetting a portion of the diluted sample into the BOD bottle. For example, if sample volumes of 0.5, 1.0 and 2.0 mL of original; (undiluted) sample are desired, make a 10 fold preliminary dilution of the original sample. Prepare the 10 fold dilution by pipetting 10 mL of well mixed sample into a 100 mL volumetric flask (or 100 mL graduated cylinder) containing about 50 mL of BOD dilution water. Bring the flask to 100 mL by adding additional BOD dilution water and then mix thoroughly. Then pipet 5, 10 and 20 mL of the diluted sample into the BOD bottles which represents 0.5, 1 and 2 mL of the original sample.

5.5.5 DO depletion requirements for BOD testing.

In order to be used to calculate sample results without qualification, dilutions must deplete at least 2 mg/L of DO, and the final (or residual) DO must be at least 1.0 mg/L.

5.5.6 BOD samples that require additional nutrients.

When larger sample volumes are used to prepare BOD dilutions (> 200 mL), it will be necessary to add extra nutrients. Additional nutrients are required to ensure sufficient nutrients are available for bacterial growth and to stabilize pH. Use the following guidance to determine the amount of supplemental nutrients to add:

CURRENT GUIDANCE:

After dilution, BOD bottles containing more than 67% sample (> 200 mL) may be nutrient-limited and subsequently reduce biological activity. The resulting BOD will be biased low. In such samples, add the nutrient/buffer solutions directly to each BOD bottle at a rate of 1 mL/L (0.3 mL/300-mL bottle) or use commercially prepared solutions/pillows designed to dose the appropriate bottle size. When individual nutrient pillows are used, it's OK to use dilution water that may contain some nutrients already. Generally, it's far easier to use the nutrient pillows designed for individual 300 mL BOD bottle.

Section 5.6 - Calculations and Reporting Results

5.6.1 Given data, calculate BOD for a sample 1/3

1. The formula for the calculation is:
BOD (mg/L) =[(iDO - fDO) - SCF] × DF
iDO = Initial DO (mg/L)
fDO = Final DO (mg/L)
SCF = Seed Correction Factor (if applicable)
DF (Dilution Factor) = 300 mL ÷ [sample volume (mL)]

2. Given the following data:

Dilution	IDO	FDO	Sample Volume
A	8.30 mg/L	0.80 mg/L	300 mL
B	8.30 mg/L	1.30 mg/L	250 mL
C	8.30 mg/L	4.25 mg/L	200 mL
D	8.30 mg/L	5.90 mg/L	100 mL
E	8.30 mg/L	7.40 mg/L	50 mL

5.6.1 Given data, calculate BOD for a sample 2/3

3. Calculate BOD of individual dilutions:

BOD #A: Depletion = (8.30 - 0.80) - 0 = 7.5 mg/L
Excess depletion. DO NOT USE

BOD #B: Depletion = (8.30 - 1.30) - 0 = 7.0 mg/L
BOD = 7.0 mg/L × (300 ÷ 250)
BOD = 7.0 mg/L × (1.2)
BOD = 8.4 mg/L

BOD #C: Depletion = (8.30 - 4.25) - 0 = 4.05 mg/L
BOD = 4.05 mg/L × (300 ÷ 200)
BOD = 4.05 mg/L × (1.5)
BOD = 6.075 mg/L

BOD #D: Depletion = (8.30 - 5.90) - 0 = 2.4 mg/L
BOD = 2.4 mg/L × (300 ÷ 100)
BOD = 2.4 mg/L × (3)
BOD = 7.2 mg/L

BOD #E: Depletion = (8.30 - 7.40) - 0 = 0.9 mg/L
Insufficient depletion. DO NOT USE

5.6.1 Given data, calculate BOD for a sample 3/3

4. Average all useable results to obtain a BOD for the whole sample.

Average BOD = (8.4 + 6.075 + 7.2) ÷ 3
Average BOD = (21.675) ÷ 3
Average BOD = 7.225
Report BOD = 7

5.6.2 Given data, calculate BOD for a GGA standard, which requires the application of the seed correction factor. 1/2

1. The formula for the calculation is:

BOD (mg/L) =[(iDO - fDO) - SCF] × DF
where: iDO = Initial DO (mg/L)
fDO = Final DO (mg/L)
SCF = Seed Correction Factor (if applicable)
= depletion (mg/L) from seed ÷ seed volume (mL)
DF (Dilution Factor) = 300 mL ÷ [sample volume (mL)]

---

2. Given the following data calculate the result for the GGA standard:

DILUTION	IDO	FDO	mL SEED	SAMPLE VOLUME
Seed Control 1	8.45 mg/L	5.45 mg/L	10 mL	N/A
Seed Control 2	8.45 mg/L	3.65 mg/L	15 mL	N/A
GGA	8.30 mg/L	3.75 mg/L	2 mL	6 mL

5.6.2 Given data, calculate BOD for a GGA standard, which requires the application of the seed correction factor. 2/3

3. Calculate individual seed correction factors (SCF) for Seed Controls:

SCF for Seed Control 1:
Depletion = (8.45 - 5.45) - 0 = 3.0 mg/L
SCF 1 = 3.0 mg/L ÷ 10 mL
SCF 1 = 0.30 mg/L per mL of seed
SCF for Seed Control 2:
Depletion = (8.45 - 3.65) - 0 = 4.8 mg/L
SCF 2 = 4.8 mg/L ÷ 15 mL
SCF 2 = 0.32 mg/L per mL of seed
Average SCF = (0.30 + 0.32) ÷ 2
Average SCF = 0.31 mg/L per mL of seed

5.6.2 Given data, calculate BOD for a GGA standard, which requires the application of the seed correction factor. 3/3

4. Calculate BOD for the GGA solution:
Depletion = (8.30 - 3.75) = 4.55 mg/L
Seed correction = 0.31 mg/L per mL x 2 mL = 0.62 mg/L
Adjusted depletion = (4.55 - 0.62) = 3.93 mg/L
BOD = 3.93 mg/L × (300 mL ÷ 6 mL)
BOD = 3.93 mg/L × (50)
BOD = 196.5 mg/L

5. Evaluate GGA result:
Result (196.5 mg/L) falls within required 167.5 - 228.5 mg/L

5.6.3 Procedures for reporting results when all dilutions under-deplete (fail to deplete at least 2 mg/L). 1/2

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged. If all of the dilutions over-deplete, the result must be reported as "greater than" the BOD calculated using the smallest sample volume used (e.g., most dilute).

For example: Given the following data:

Sample Bottle #	A	B	C
Sample Size (mL)	300	250	200
Initial D.O. (mg/L)	8.48	8.50	8.47
Final D.O. (mg/L)	7.40	7.70	7.95
Depletion (mg/L)	1.08	0.80	0.52
Dilution Factor	1	1.2	1.5
BOD (mg/L)	----	----	----

5.6.3 Procedures for reporting results when all dilutions under-deplete (fail to deplete at least 2 mg/L). 2/2

In the example, all three dilutions fail to meet the minimum requirement of 2.0 mg/L for depletion. Because at least one of the dilutions is a full bottle (300 mL), the requirements to report a result of the target detection limit have been met. Report result as "< 2 mg/L".

NOTE: If no 300 mL dilution had been made, and the only two dilutions were 250 mL and 200 mL, then the result would have to be reported as "< 2.4 mg/L", based on the dilution with the largest amount of original sample. This situation indicates that a dilution of 300 mL should routinely be incorporated.

5.6.4 Procedures for reporting results when all dilutions over-deplete (final DO less than 1.0 mg/L). 1/2

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged.

Example: Given the following data:

Sample Bottle #	A	B	C
Sample Size (mL)	300	200	100
Initial D.O. (mg/L)	8.48	8.50	8.47
Final D.O. (mg/L)	< 1.0	< 1.0	< 1.0
Depletion (mg/L)	> 7.48	> 7.50	> 7.47
Dilution Factor	1	1.5	3
BOD (mg/L)	**>7.5**	**>11.3**	**>22.4**

In the example, all three dilutions fail to meet the residual DO requirement of 1.0 mg/L.

Report result as "> 22 mg/L".

5.6.4 Procedures for reporting results when all dilutions over-deplete (final DO less than 1.0 mg/L). 2/2

NOTE: If this represents an unusual situation, then a comment should be included on the DMR indicating a non-routine event. Additional dilutions may be required over the next several days until the plant settles down. If, however, this situation occurs regularly, then the lab must be routinely using higher dilutions (a smaller sample volume) when preparing samples

Section 5.7 - Nitrification

5.7.1 What is nitrogenous oxygen demand?

Nitrogenous oxygen demand is the amount of oxygen consumed during the biochemical conversion of organic nitrogen and ammonia to nitrate nitrogen. This process is called nitrification. We are primarily concerned with the inorganic forms, and specifically, ammonia nitrogen. This means that if the wastewater contains ammonia (and most do, particularly lagoons) AND the nitrifying bacteria are present, then oxygen can be consumed in the conversion of nitrogen forms. The oxygen consumed is measured as BOD, leading to BOD results biased high.

The key equations are as follows:

Ammonia + Oxygen -> Nitrite -> Nitrate.

5.7.2 Using BOD:TSS ratios as a tool to identify nitrification.

Generally speaking there should be a 1:1 relationship between BOD and TSS results for domestic municipal wastewater effluent. This relationship only applies to domestic wastewater effluents. This is because the majority of the BOD comes directly from the material that makes up the TSS. Industrial wastes, such as dairy and food processing wastes, contain a high dissolved BOD component. Milks and sugars from food wastes will pass through the TSS filters causing the BOD to be significantly higher than the TSS values.

If BOD is always significantly higher than TSS (e.g., TSS 10, BOD 25), nitrification is likely occurring. Confirm by performing side-by-side BOD tests with and without nitrification inhibitors (e.g, determine both BOD and carbonaceous BOD (cBOD). If the inhibited (carbonaceous) BOD results are significantly lower and closer to the TSS results, nitrification is occurring. Repeat side-by-side tests to confirm your findings.

NOTE: Always seed samples when nitrification inhibitor is used.

5.7.3 Testing differences between BOD and cBOD.

The only difference between samples analyzed for BOD and those analyzed for cBOD is NOT the letter "c". The key difference between samples analyzed for BOD and those analyzed for cBOD is more precisely the addition of a chemical inhibitor to all samples for which cBOD is determined.

If nitrification was occurring in the original sample, the cBOD result is expected to be lower than the BOD result. The difference between the results represents the amount of oxygen which is consumed during the nitrification process. Since the BOD test only measures oxygen consumed, performing both BOD and cBOD is the only way to distinguish the amount of oxygen consumed during decomposition of organic waste from that which is consumed during nitrification.

Section 5.8 - Toxicity

5.8.1 Identifying sample toxicity affecting BOD results.

The first symptom of sample toxicity is evidenced by a decrease in BOD concentration as sample volume increases. What this really means is that we're looking for a trend, and a trend realistically requires more than two data points. If only two dilutions are used, and the dilution with greater sample volume yields a lower BOD result, it COULD merely be a function of sample homogeneity. Having an additional dilution which confirms the initial two dilutions serves as a referee. Therefore, at least three (3) dilutions are necessary to effectively detect sample toxicity.

There must be a distinct trend in the data for the sample to be designated "toxic" and reported as such on the DMR.

5.8.2 Procedures for reporting results when data suggest toxicity. 1/2

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged.

Example: Given the following data, what result should be reported:

Sample Bottle #	A	B	C	D
Sample Size (mL)	2	5	10	20
Initial D.O. (mg/L)	8.6	8.5	8.3	8.0
Final D.O. (mg/L)	7.4	6.1	4.3	1.5
Depletion (mg/L)	1.2	2.4	4.0	6.5
Dilution Factor	150	60	30	15
BOD (mg/L)	(> 180)	144	120	98

5.8.2 Procedures for reporting results when data suggest toxicity. 2/2

In the example, dilution/bottle A is not acceptable, because it does not meet the minimum requirement of 2.0 mg/L for depletion. Samples B, C, and D are acceptable.
Because these dilutions show a distinct "sliding" effect, with BOD dropping as dilutions contain more of the original sample. In addition, if the dilution in bottle "A" were calculated out, it would indicate an even higher BOD (> 180). This may be an indication of toxicity.
Because there are insufficient dilutions to demonstrate that the threshold inhibition point has been reached (as evidenced by consistent BOD values among a dilution series), we cannot report an accurate result.
The best result, in this case is to report the results from the most dilute sample that meets depletion criteria, and use a ">" sign to indicate that the result is at least this high. Therefore a result of "> 144 mg/L" should be reported. In addition, a comment on the DMR should be made to indicate that this result is biased low due to indicated toxicity.

Section 5.9 - BOD Quality Control Requirements

5.9.1 Assessing the accuracy of the DO meter calibration

"Laboratory control sample" or "LCS" means a sample of an inert matrix or a matrix with a consistent concentration of the analytes of interest, fortified with a verified known amount of the analytes of interest. The purpose of an LCS is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

The BOD method blank, assuming it has been brought to saturation, can serve as an LCS for DO measurements. If we know the temperature of the blank and the atmospheric pressure (properly adjusted for elevation) then we can calculate the theoretical dissolved oxygen saturation point.

If the DO meter is properly calibrated, the initial DO measurement of the BOD method blank should be very close to the theoretical saturation point. How close? Reasonably, we should expect the IDO to fall within 0.1-0.2 mg/L of theoretical saturation. If this proves difficult, then corrective action should be taken to ensure that the blank has been shaken to ensure saturation, that temperature and pressure measurements are accurate, and that the DO probe is working properly.

5.9.2 BOD blank frequency and acceptance criteria.

At least one dilution water blank must be prepared on each day that samples are prepared.

The maximum allowable depletion in a method blank is 0.2 mg/L.

Due to rounding, depletion amounts as much as 0.24 mg/L are acceptable.

5.9.3 What causes excessive DO depletion in blanks?

- Calibration errors
- Supersaturation
- Contamination (organic matter + micro-organisms)

5.9.4 Assessing the accuracy of the BOD test.

A laboratory control sample (LCS) is prepared from a solution (GGA) containing 150 mg/L of glucose and 150 mg/L of glutamic acid.

These laboratory control samples shall be processed at a frequency of at least one sample per analytical batch for laboratories that analyze more than 20 samples per week. Laboratories that analyze fewer than 20 samples per week shall analyze, at a minimum, one LCS per week.

IN SHORT: An LCS (G/GA) is required to be analyzed at least once per week, or after every 20 samples--whichever is more frequent.

The acceptance criteria for each LCS is 198 +/- 30.5 mg/L (167.5 - 228.5 mg/L).

LCS samples may not be averaged to achieve a result that falls within the acceptance criteria; each individual LCS shall be assessed against these criteria.

5.9.5 Potential reasons why GGA results could be unacceptably high or low.

HIGH BIAS of GGA results is caused by:

- Nitrification
- Cold GGA solution
- Contamination: Organic matter
- Contamination: Microorganisms (“bugs”)

NOTE: Contamination from either “bugs” or BOD material alone will cause high bias in GGA but is not likely to cause an exceedance in blanks. There must be contamination from BOTH “bugs” AND waste material for contamination to result in blank exceedances. This explains a common statement from lab analysts that “my GGA is failing high, but my blanks are fine”.

LOW BIAS of GGA results is caused by:

- Not enough seed
- Seed materials too weak or variable
- GGA too old or contaminated

Chapter 6

Total Suspended Solids (TSS) Analysis

Section 6.1 - Total Suspended Solids (TSS)

6.1.1 What is Total Suspended Solids (TSS)?

Total suspended solids (TSS) is a water quality measurement, generally required for wastewater treatment plant outfalls. This test can also be called non-filterable residue (NFR), a term that refers to the identical measurement: the dry-weight of particles trapped by a filter, typically of a specified pore size.

TSS of a water sample is determined by pouring a measured volume of water through a pre-weighed filter of a specified pore size, then weighing the filter again after drying to remove all water. The gain in weight is a dry weight measure of the particulates present in the water sample expressed in mg/L calculated from the volume of water filtered.

6.1.2 What is the significance of the TSS test in wastewater testing?

Total suspended solids (TSS) are those which are visible and in suspension in the water.

They are the solids which can be removed from wastewater by physical or mechanical means such as sedimentation, flocculation, or filtration. TSS will include the larger floating particles and consist of silt, grit, clay, fecal solids, paper, fibers, particles of food, garbage, and similar materials.

Suspended solids are approximately 70% organic and 30% inorganic. TSS determinations may be used to assess wastewater strength, process efficiency, and loadings.

By reducing the TSS in your effluent discharge, you are going to get better disinfection, which will reduce your fecal coliform and/or E. coli counts, allowing you to maintain compliance.

6.1.3 Critical requirements for a TSS drying oven.

Drying ovens used for TSS determinations must be able to consistently maintain a temperature of 103-105°C (104 ± 1°C). The purpose of the method temperature is to drive off water but not lose volatile solids.

Drying ovens should be vented properly as a health and safety precaution. Do not position a drying oven directly beneath an HVAC blower vent because drafts can be forced through the top of the oven. Direct contact with blowing cold air prevents the ability of the oven to maintain constant temperature. Ovens can be vented, but should not be placed directly inside any hood. Placing the oven in a hood will disrupt air flow and the hood will not work properly. Preferably, ovens should be vented using a snorkel or canopy type vent. ~~**???** can be vented too, but should not be placed inside any hood~~. Check and document the oven temperature daily when samples are being dried.

6.1.4 Filter type and preparation requirements for TSS testing.

Standard Methods 2540 D requires glass fiber filters without organic binders such as Whatman 934AH or equivalent. All filter papers are NOT alike! Standard Methods cites the following brands of filters as being equivalent: Millipore AP40, Gelman type A/E, ED Scientific Specialties Grade 161.

Filters must be rinsed to remove loose materials and assure the filter weight remains constant.

- **PRE-RINSE**: Filters must be pre-rinsed with several volumes of reagent water before use. (Omit if pre-rinsed/pre-tared filters are used).
- **INITIAL WEIGHT:** After pre-rinsing, filters must be dried to constant weight at the method specified temperature, desiccated, and then an initial weight obtained. Filters are now ready for use. (Omit this if pre-rinsed/pre-tared filters are used).
- **POST RINSE:** During and after sample filtration, direct a fine stream of reagent water along the sides of the filter funnel and over the filter surface. Also rinse any graduated cylinder used for transfer of the sample aliquot with a small volume of reagent water. The purpose of directing a fine stream of reagent water over the filter surface during filtration is to dislodge any finer particles from clogging the filter pores, and allowing the sample to filter properly.

6.1.5 TSS filter screen maintenance

Ensure filter support screens are not excessively clogged with particulates, which can result in uneven drying. Uneven drying can result in high bias due to “flash” surface drying similar to what can occur if more than 200 mg of residue is captured. In addition, clogged filter screens can lead to trapping of “fines”, or micro-suspended particles that normally would pass through the filter pores but can be held back by clogged pores and larger diameter TSS material.

6.1.6 Constant weight and drying time requirements for TSS testing.

The attainment of "constant weight", as discussed in Standard Methods procedures, is a process of repeated drying, cooling, and weighing of an object until the weight does not change from the previous weighing, or, until weight loss is less than 0.5 mg or 4 percent, whichever is greater. This process assures that the sample is completely dry and that there is no added weight due to moisture.

The DNR Lab Certification Program has developed an alternative to performing measurements to constant weight routinely for samples and filters. Samples dried overnight (at least 8 hours) are exempt from determination of constant weight. If samples are dried for less than 8 hours, labs must dry samples to a constant weight.

6.1.7 TSS requirements for solids capture on filters.

A residue of at least 1 mg, and not more than 200 mg, must be captured on the filter. If at least 500 mL of sample volume is filtered and 1 mg of residue is not obtained, the analyst is not required to repeat the analysis using more sample volume.

A minimum capture weight of 1 mg and 500 mL of sample filtered is required to report an LOD of 2 mg/L, the required LOD for Discharge Monitoring Report (DMR) reporting for effluent samples.

Residue amounts greater than 200 mg on a filter can lead to "flash" surface drying and the formation of a salt crust layer that traps moisture beneath it. This can cause sample results to be biased high. This is generally expected to be a problem related to process control samples with heavier solids loading.

6.1.8 Required LOD for TSS and what to do if you can't achieve it.

In order to achieve an LOD of 2 mg/L (as required for DMR reporting), one must filter 500 mL and fail to achieve a residue of 1 milligram of solids. More volume can be filtered, but as long as you filter at least 500 mL and you do not obtain at least 1 milligram of residue, then a result of "< 2 mg/L" can be reported.

If you are not capturing at least 1 milligram of residue and you are filtering LESS than 500 mL of sample, you will be required to filter a larger sample volume, up to a maximum of 500 mL.

6.1.9 How sample volume affects the LOD for the TSS test. 1/2

Like with BOD, the limit of detection (LOD) for TSS is theoretically based. A key assumption on which the concept of detection for TSS is based is that the MINIMUM capture weight of residue is 1 milligram (mg). The LOD is also based on the volume of sample used.

The calculation involved is simply:
LOD mg/L = [1 mg residue] ÷ [sample volume (L) filtered]

6.1.9 How sample volume affects the LOD for the TSS test. 2/2

EXAMPLE: sample volume filtered = 200 mL

1. Convert mL to L:
   1 L = 1000 mL, so 200 mL ÷ 1000 mL = 0.2 L
2. Formula for calculation of TSS=
   LOD = [1 mg] ÷ [0.2 L]
   1 ÷ 0.2 = 5
   LOD = 5 mg/L …..for that sample

highest sample volume used	Sample LOD
500 mL	2 mg/L [1 ÷ 0.5 = 2]
250 mL	4 mg/L [1 ÷ 0.25 = 4]
100 mL	10 mg/L [1 ÷ 0.1 = 10]
50 mL	20 mg/L [1 ÷ 0.05 = 20]

6.1.10 How is TSS calculated?

TSS (mg/L) = [Residue weight (mg)] ÷ [sample volume (L)]
TSS (mg/L) = [F+R - FW] ÷ [SV]
where: F+R = Weight (mg) of filter plus residue (after drying)
FW = Filter weight (mg)
SV = Sample volume (L)

Given the following data:
FW = 0.4955 g F+R = 0.4987 g SV filtered = 300 mL
Volume (mL) ÷ 1000 = Volume (L) Weight (g) × 1000 = Weight (mg)
F+R: 0.4987 g × 1000 = 498.7 mg
FW: 0.4955 g × 1000 = 495.5 mg
Residue weight = 498.7 mg - 495.5 mg = 3.2 mg
SV: 300 mL ÷ 1000 = 0.30 L
TSS = [3.2 mg] ÷ [0.3 L]= 10.7 mg/L TSS = 10.7 mg/L
NOTE: Report: 11; round to nearest whole number.
LOD = [1 mg] ÷ [0.3 L] = 3.3 mg/L for this sample
[Report: 3]

Chapter 7

Ammonia (NH3-N) Analysis

Section 7.1 - Ammonia Nitrogen (NH3-N)

7.1.1 The significance of the ammonia test in wastewater testing.

- Ammonia is a major excretory product of animals and is toxic to organisms at high pH levels.

- Approximately 60% of the nitrogen entering a wastewater plant is ammonia.

- Ammonia toxicity is based on pH and temperature.

- Ammonia affects wastewater plants differently based on type or design.

- High levels of ammonia and the presence of nitrifying organisms will result in increased BOD values (i.e, biased high).

7.1.2 The operating principle of ammonia electrodes (ISE). 1/2

A "gas-sensing" type electrode is used for ammonia (NH3) analysis. Dissolved ammonia (NH3 gas and NH4 ions) in the sample is converted to NH3 gas by raising pH to above 11 with a strong base. NH3 gas diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale. The millivolt response can then be correlated to the ammonia concentration.

In aqueous solutions, ammonia exists in an equilibrium between two forms, the ionized form (NH4+, ammonium ion) and the un-ionized form (NH3, ammonia gas). The relative amount of each form present in any sample is controlled solely by pH and temperature. As temperature and pH increase, the predominant form becomes the NH3 (gaseous) form.

7.1.2 The operating principle of ammonia electrodes (ISE). 1/2

This [un-ionized form (NH3, ammonia gas)] is, of course, the form which can cross the gas permeable membrane of the ammonia probe. Since ammonia is a gas, however, it can dissipate quickly from solution. To prevent these rapid losses of ammonia from being measured, the probe is placed into the solution and readings initiated BEFORE the buffer solution is added to a sample or standard.

NOTE: At least one manufacturer sells a buffer solution which remains blue in color as long as the solution pH is above 11.

7.1.3 The relationship between ammonia concentration and electrode response time.

As ammonia concentration decreases electrode response time increases. The lower the concentration of ammonia in the sample, the fewer the number of ammonia molecules as gas that pass across the membrane to react with water and trigger a pH change observed as a millivolt increase on the meter.

The response time for very low concentration samples, such as blanks and even low calibration standards, can be so slow that the meters "jump the gun" and lock in on a millivolt response even though the millivolts are continuing to increase...very slowly.

This phenomenon explains why blank measurements are often incorrectly believed to be above the limit of detection for ammonia. It also explains why some people have trouble meeting calibration acceptance criteria.

7.1.4 Why temperature is so critical when using the ion-selective electrode.

Ammonia electrodes function according to the physical constraints of the Nernst equation, and in that equation temperature is the only variable. Each one degree (°C) change in temperature is associated with a 1-2% error due to changes in the electrode slope.

Because ion selective electrode measurement is affected by temperature, calibration standards and samples must be at the same temperature.

7.1.5 When is distillation of samples for ammonia required?

Unless exempted, all wastewater samples must be distilled. An exemption is provided for domestic municipal wastewater effluents that do not have any significant industrial or waste component that are analyzed using ion selective electrodes.

7.1.6 Ammonia calibrations - minimum number of calibration standards required.

Calibrations must consist of at least 3 standards. In addition, a blank is required as part of the calibration for colorimetric analyses. Due to the physics governing ion-selective electrodes, a blank must be analyzed, but it is not included as part of the calibration.

7.1.7 Ammonia calibrations - standard concentration levels.

ION SELECTIVE ELECTRODES (ISE)

Each calibration standard should be exactly 10 times the concentration of the next lowest calibration standard. This is frequently referred to as a "ten-fold" or a "decade" difference.

The reason for this requirement is that electrode response is dictated by the Nernst equation. The Nernst equation states that at 25°C, each ten-fold rise in concentration will result in an increase in electrode response, or slope, of 59.1 millivolts.

Temperature is the only variable involved; as temperature increases, the slope increases.

COLORIMETRIC PROCEDURE

The concentration of the standards chosen to establish a calibration function shall be within the same orders of magnitude as the expected concentration of samples to be analyzed.

Therefore, if you don't expect to analyze samples containing ammonia levels greater than 10 mg/L, then limit your calibration to that range.

In addition, laboratories must include in initial calibrations a standard at a concentration near the limit of quantitation of the analysis.

7.1.8 Calibration acceptance criteria for ammonia testing.

ION SELECTIVE ELECTRODE

The calibration must yield a slope within the range of 57 ± 3 (54-60) millivolts (mV). A slope within these parameters represents a valid calibration.

Slope is based on the Nernst equation. The only variable is temperature; slope varies directly with temperature.

Theoretical slope is 59.1 mV @ 25°C or 58.1 mV @ 20°C. Some ion meters display the slope as a percentage of the theoretical (based on 25°C). For example, a 98.5% slope = 58.27 mV (which represents 98.5% of 59.1 mV).

COLORIMETRY

The correlation coefficient of the calibration linear regression must be greater than or equal to 0.995. In addition, on days when a calibration curve is generated, a second source standard must be analyzed as an initial calibration verification (ICV) standard. Results of the ICV standard must fall within 10% of the true. On days when a calibration curve is not generated, a mid-calibration standard must be analyzed as a continuing calibration verification (CCV) standard. Results of the CCV standard must fall within 10% of the true value.

7.1.9 QC sample types and frequencies required for ammonia testing.

METHOD BLANK

- Required on each day of analysis
- Method blanks should be below the LOD, but must be less than the greater of: the LOD, 5% of any regulatory limit, or 10% of sample concentration or affected sample results must be qualified.

LAB CONTROL STANDARDS (LCS)

- Required every 20 samples per batch of samples processed.
- Labs are required to establish acceptance criteria for LCS samples. Generally these criteria are 90-110% recovery.

NOTE: If using methods other than manual chemistry procedures in Standard Methods, additional QC samples, including matrix spikes and replicates, may be required..

Chapter 8

Total Phosphorus Analysis

Section 8.1 - Total Phosphorus (TP)

8.1.1 The significance of total phosphorus in wastewater testing.

Phosphorus is a vital nutrient for plant growth and for metabolic reactions of all other life forms. Phosphorus is often a limiting factor for algal growth. Consequently, the more phosphorus that is discharged to receiving stream systems, the greater the likelihood of algae and aquatic weed growth downstream.

Phosphorus in wastewater is usually present in the form of organic compounds and phosphates that can easily be removed by *chemical precipitation*. This process, however, increases the volume and weight of sludge. It has been estimated that the human body releases about a pound of phosphorus per year.

8.1.2 Sources and control of contamination in phosphorus analysis. 1/2

The phosphorus test is extremely sensitive at even trace levels. Phosphorus is all around us, so it is a very easy test to contaminate. Common sources of contamination include: treated (for sequestration of iron and manganese) tap water, oils on fingers, tobacco residue, soap and some detergents, and laboratory buffers (including solutions used to calibrate pH meters) or other reagents.

Be sure to clean glassware thoroughly, and maintain laboratory cleanliness.

Blanks and standards are critical in monitoring for phosphorus contamination.

Keeping records of the absorbance of blanks and standards over time will help detect changes in reagent water quality or reagents.

8.1.2 Sources and control of contamination in phosphorus analysis. 2/2

General considerations to control phosphorus contamination include, but are not limited to:

- Wash glassware well, using a non-phosphate detergent.
- DO NOT use Alconox (contains ~8.7% phosphorus).
- Rinse glassware with dilute (1-10%) hydrochloric acid. (Note: Always use a fume hood when rinsing with hydrochloric acid.)
- Never re-use HCl solution to wash glassware (Used acid soon becomes contaminated and can contaminate all of your glassware).
- Even new glassware needs to be washed.
- DO NOT touch inside of glassware with bare hands.
- DO NOT smoke or use air fresheners in the laboratory.
- Cover samples if you use autoclave for digestion.
- Segregate glassware for TP (but still rotate glassware).

8.1.3 Calibration requirements for total phosphorus

- Lab certification code requires at least three calibration standards plus a calibration blank.
- The more calibration standards used to develop the relationship between phosphorus concentration and absorbance, the more accurate the calibration.
- Calibration standards must bracket sample concentrations.
- Linear regressions developed from phosphorus calibrations must be evaluated using the correlation coefficient which must be greater than or equal to 0.995.
- On days when a calibration curve is generated, a second source standard must be analyzed as an initial calibration verification (ICV) standard. Results of the ICV standard must fall within 10% of the true value.
- On days when a calibration curve is not generated, a mid-calibration standard must be analyzed as a continuing calibration verification (CCV) standard. Results of the CCV standard must fall within 10% of the true value.

8.1.4 QC sample types, frequencies, and acceptance criteria required for total phosphorus testing.

METHOD BLANK

- Required on each day of analysis
- Method blanks should be below the LOD, but must be less than the greater of: the LOD, 5% of any regulatory limit, or 10% of sample concentration or affected sample results must be qualified.

LAB CONTROL STANDARDS (LCS)

- Required every 20 samples per batch of samples processed.
- Labs are required to establish acceptance criteria for LCS samples. Generally these criteria are 90-110% recovery.

NOTE: If using methods other than manual chemistry procedures in Standard Methods, additional QC samples, including matrix spikes and replicates, may be required.

Chapter 9

Total Residual Chlorine (TRC)

Section 9.1 - Total Residual Chlorine (TRC)

9.1.1 Sample handling procedures for total residual chlorine.

- Collect samples in amber, glass bottles treated with bleach to remove chlorine demand.
- Treat bottles by filling with reagent water, adding a few mL of household bleach, allow to soak about 30 minutes, and then rinse thoroughly with tap water followed by reagent water.
- Minimize the time between sampling and analysis (preferably less than 15 minutes) to avoid loss of chlorine.
- Warm samples to room temperature before testing with the ISE method.
- Fill sample completely to minimize contact with the air until samples are tested.

9.1.2 Preferred reagent used to prepare standards in the DPD (colorimetric) determination of total residual chlorine.

- A potassium permanganate ($KMnO_4$) solution is used as a chlorine equivalent. A solution
- containing 0.891 g/L of potassium permanganate has a chlorine equivalent of 1000 mg/L.
- Permanganate standards have a bright pink-red color.

9.1.3 Preferred reagent used to prepare standards in the ISE determination of total residual chlorine.

A potassium iodate (KIO_3) solution is used as a chlorine equivalent.
A solution containing 0.1005 g/L of potassium iodate has a chlorine equivalent of 100 mg/L.
Chlorine standards can be prepared using household bleach formulations, but standards prepared from bleach are unstable. The iodate standards are much more stable.

9.1.4 Department permit reporting limit and LOD requirements for total residual chlorine.

Permits are written to include a detection limit requirement of 38 ug/L (0.038 mg/L) residual chlorine. However, laboratories are considered to be in compliance as long as their reporting limit for residual chlorine does not exceed 100 ug/L (0.100 mg/L).

Chapter 10

Process control

Section 10.1 - Process control

10.1.1 The importance of pH in process control.

pH of the biological mass in treatment facilities must be monitored to determine if the levels are too acidic or caustic for the microbes in the plant or the receiving water. Most wastewater plants in Wisconsin have an effluent discharge limit of 6.0 to 9.0 standard units (s.u.) in their permit, but pH levels of 6.8 to 7.2 are optimum for biological activity of most aerobic organisms. Laboratories may use bench-top pH meters for the analysis, using two or more calibration pH buffers that bracket the pH levels measured. The pH meter should be calibrated daily using fresh buffers. So, if samples are expected to pH values between 7.0 and 7.5, calibration of the pH meter should be performed using buffers of pH 7 and pH 10. There is no holding time for pH analysis, so all analyses for permit use must be done onsite.

Generally, the lower the pH of wastewater, the greater the disinfection capacity with chlorine compounds, to a point. Below the pH of 4.0, very little disinfection takes place. The higher the pH, the lower the disinfection capability of chlorine. A pH of 5.5 to 7.5 is optimal. The optimum pH for nitrification is 7.5 to 8.5 su. As ammonia is converted to nitrite and nitrate, alkalinity decreases and pH of the wastewater may drop.

10.1.2 The importance of dissolved oxygen (DO) in process control.

- Dissolved Oxygen (DO) field analysis is used to monitor the amount of dissolved oxygen in various areas of the facility. A dissolved oxygen meter with a submersible field probe is used. Some facilities also have in-line DO monitors. Aerobic organisms require a minimum DO level of about 2.0 mg/L for proper health. When a facility is accomplishing biological nitrogen and phosphorus removal, the oxygen levels in the anaerobic, anoxic, and oxic sectors must be carefully monitored.
- DO limits are required in some permits to ensure that there is adequate DO in the receiving stream.

Chapter 11

QA/QC (Quality Assurance/Quality Control)

Section 11.1 - Precision / Accuracy

11.1.1 What is a "second source" standard and how are they used? 1/2

Second source standards are actually a form of QCS sample, analyzed more frequently.

The greater frequency helps to identify preparation errors made during dilution of stock standards to prepare working standards. It is recommended that stock standards be purchased from each of two different suppliers or different lots of the same solution may be purchased from a single supplier.

- Stock A= primary source used to prepare calibration standards.
- Stock B= secondary source used to verify the prepared calibration standards.

Note that second source standards will also help to identify *discrepancies in the concentration of* original stock standards. Second source standards are frequently used to prepare QC samples, including laboratory control standards (LCS).

11.1.1 What is a "second source" standard and how are they used? 2/2

If there is no independent verification (such as second source standards) and the same solution that is used to prepare calibration standards is also used to prepare spiked QC samples, errors made in the preparation of the stock standard cannot be easily identified.

Second source standards need only be analyzed any time that a new dilution of a stock standard is prepared which will then be used to prepare calibration standards. Many labs, however, routinely include a second source standard, by using second source standards to prepare LCS. This practice provides a much more frequent, independent verification that standard solutions used to generate calibrations are accurate.

11.1.2 What is a Laboratory Control Standard (LCS)?

"Laboratory Control Standard" or "LCS" means a sample prepared by the laboratory, using reagent water, to which a known amount of the analyte of interest is added. The purpose of an LCS is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements. In many EPA methods, the term "lab-fortified blank" is substantially equivalent to a laboratory control sample.

11.3 Define Quality Control Standard (QCS) 1/2

Quality Control Standard or "QCS" means an externally obtained solution or sample containing method analyte of known concentration, accompanied by specified analytical acceptance limits, and different from the source of calibration standards. These samples are distinguished from proficiency test samples in that the acceptance limits are provided with the sample, rather than after analysis. Quality control standards are used to check either laboratory or instrument performance.

These types of samples are important indicators of quality because instantaneous feedback on performance is provided. This offers the analyst an opportunity to correct any analytical problems in a timely manner. Quality Control Standards are analyzed every four months as a check on analytical performance. Appropriate measures are taken to investigate problems when the result of a QCS analysis is inconsistent with past results.

11.3 Define Quality Control Standard (QCS) 2/2

Unlike PT samples, whose results are not received for some time after the testing is performed, QCS samples can be used at any time there is concern about the control of a specific analysis. Having immediate access to the validated concentrations allows the analyst to take immediate action to identify and correct the problem.

QCS standards are often referred to as "blind standards". QCS sample analysis is not required for tests in which the laboratory incorporates second source standards.

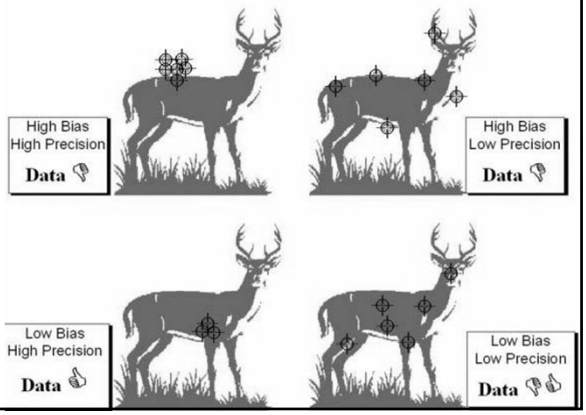
11.1.4 Define Bias and Precision 1/2

Bias (Accuracy) is the systematic or persistent error in an analysis which results in the expected sample measurement being consistently different than the sample's true value.
A systematic bias is a bias resulting from a flaw integral to the system within which the bias arises (for example, an incorrectly calibrated thermostat may consistently read - that is 'biased' - several degrees hotter or colder than actual temperature). As a consequence, systematic bias commonly leads to systematic errors, as opposed to random errors, which tend to cancel one another out.
Precision is a measure of how closely multiple determinations performed on the same sample will agree with each other. Accuracy is the degree of closeness to the actual value while precision is the degree of reproducibility.

11.1.4 Define Bias and Precision 1/2

Bias (Accuracy) is the systematic or persistent error in an Using the typical "target" analogy, if a large number of arrows are fired at the target, the size of the arrow cluster on the target represents the shooter's precision. When all arrows are grouped tightly together, the cluster is considered precise since they all struck close to the same spot.

Note that the cluster may be very precise, by virtue of a tight grouping, but if the cluster is nowhere near the bullseye, then the shoot er is precise but not accurate.



Section 11.2 - Limit of Detection (LOD)

11.2.1 Procedure for determining an LOD.

The most common, and simplest, procedure for determining the LOD can be summarized as:

1. *Determine a spike concentration*, which approximates the LOD.
2. Prepare 7 or 8 replicates of reagent water spiked at an appropriate level.
3. Analyze the replicate spikes. NOTE: To ensure that day-to-day precision is considered, these replicates should be analyzed on different, preferably non-consecutive, days.
4. Calculate the LOD: Multiply the standard deviation of replicates by the "t-value" (99% confidence) associated with the number of replicates.
5. Perform the "Wisconsin DNR 5-point" check of the LOD. Repeat *the LOD determination* as appropriate.

IMPORTANT: This procedure is NOT used to determine the LOD for BOD and TSS!!!!!

NOTE: The EPA procedure for determining detection limits can be found in 40 CFR Part 136 Appendix B, Revision 1.11. The procedure also contains t-table values required for step 4 above.

11.2.2 LOD determination frequency

The limit of detection will change over time for a variety of reasons, and it is necessary to periodically update the calculated LOD value. Many analytical methods require that the LOD be determined prior to using a new analytical system, and some even require annual updates. The frequency of the determination specified in the analytical method should be followed. If the method does not specify a frequency, the Department recommends that LODs be recalculated whenever a new analyst begins generating data or the performance of the analytical system changes (in addition to the initial LOD). LODs must also be recalculated whenever the analytical procedure is modified (e.g. new analyst and equipment). Laboratories must give their LODs an "expiration date" of one year and check their LODs at least annually.

11.2.3 Define LOD and LOQ.

Limit of detection (LOD) means the lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is statistically different from a blank (or, not a false positive value). For department purposes, the LOD approximates the "method detection limit" (MDL, the term used by the EPA and in many methods). The LOD is determined using the procedure in 40 CFR Part 136 Appendix B, Revision 1.11.

Limit of quantitation or (LOQ) means the lowest concentration or amount of an analyte for which quantitative results can be obtained with a specified degree of confidence. The limit of quantitation is typically considered to be a value 10/3 or 3.333 times the limit of detection (LOD).

Section 11.3 - Corrective Action

11.3.1 What does "corrective action" mean?

Corrective action means any measure taken to correct and prevent the recurrence of the causes of any exceedance of expected analytical operating conditions or quality control acceptance criteria. Corrective action must be documented.

Corrective action must be designed to identify the reason for the failure, and then correct it.

There should also be a plan to quickly verify that the action taken has the desired effect.

11.3.2 Documentation required for corrective action.

- WHEN did you become aware of the problem?

- HOW did you become aware of the problem?

- WHO initiated corrective action to resolve the problem?

- WHAT action did you take to fix the problem? WHAT data was affected?

- WHY do feel the action was appropriate (how do you know the problem has been resolved)?

Chapter 12

Documentation and Traceability

Section 12.1 - SOPs and Quality Manual

12.1.1 Basic contents of a laboratory Quality Manual.

The quality manual must include, or make reference to, at least the following elements:

- Organization and management structure of the laboratory.
- Procedures for handling samples.
- Procedures for retention, control and maintenance of documents used in or associated with analyses.
- Procedures for achieving traceability of standards, reagents and reference materials.
- Lists of major analytical instruments and support equipment.
- Analytical Methods: Lists of all test methods used by the laboratory.
- Procedures for calibration, verification and maintenance of major analytical instruments and support equipment.
- Summary of the types and frequency of analysis of QC control samples for each test.
- Procedures for evaluating quality control samples.
- Procedures for initiating, following up on and documenting corrective action addressing quality assurance and quality control failures, discrepancies or nonconformance.
- Procedures for reviewing analytical data and reporting results.

12.1.2 Four critical aspects of QC sample analysis that should be considered in a QA Manual.

What is being reviewed? (**PARAMETER**)
How often does this parameter need to be checked? (**FREQUENCY**)
What is it being reviewed against (how is it evaluated)? (**CRITERIA**)
What if it doesn't meet specifications? (**CORRECTIVE ACTION**)

PARAMETER	FREQUENCY	CRITERIA	CORRECTIVE ACTION
TSS oven	Daily	103-105 °C	Adjust temp. up or down
BOD incubator	Daily	19.0 to 21.0 °C	Adjust temp. up or down
NH3 slope	Daily	-54 to -60 mV	1) Enough time to stabilize? 2) Low standard too low? 3) Change membrane 4) Perform inner body ✓
Phosphorus known standard	Daily	90-110%	1) Replace standards/reagents 2) If high…contamination? 3) Re-do calibration 4) Reanalyze/ qualify smpls

12.1.3 What are SOPs (Standard Operating Procedures). 1/2

- Standard operating procedures (SOP) are used to describe a procedure or set of procedures to perform a given task, analysis, or in response to a specific event or situation.
- SOPs often offer guidance where official methods are lacking, or are extremely broad.
- SOPs typically provide practical detail to the sometimes generic language provided in methods or to identify specific situations where choices are available. Every good quality system is based on its standard operating procedures (SOPs).
- SOPs may be documents written by laboratory personnel or may consist entirely of copies of published documents, manuals or procedures if the laboratory follows the chosen source exactly. SOPs must indicate their dates of issue or revision.

12.1.3 What are SOPs (Standard Operating Procedures). 2/2

- SOPs may partly consist of copies of published documents, manuals or procedures if:
- - Modifications to the published source are described in writing in additional documents.
- - Clarifications, changes or choices are completely described in additional documents, when published sources offer multiple options, ambiguous directives or insufficient detail to perform or reproduce an analysis.

Section 12.2 - Traceability

12.2.1 Traceability of reagents and standards.

The laboratory must maintain records that detail the preparation of intermediate and working standards and reagents. These records must link the intermediate and working standards and reagents to their respective originating stocks or neat compounds and must indicate their date of preparation, expiration and the identity of the preparer. The laboratory must also be able to link the reagents and standards used to the analysis records.

12.2.2 Mechanisms to ensure records are both permanent and unalterable 1/2

HAND-WRITTEN RECORDS.....

Records must be produced in a manner that both ensures their permanence and ensures that they are un-alterable. Records generated in pencil or erasable ink can be easily altered.

When original information is altered in this manner, reviewers such as a lab auditor or even the court could suspect that the alterations made represent a fraudulent practice.

Consequently, even the use of correction fluid or tape in a laboratory is not recommended. If you make an error while recording laboratory-related information, draw a single line through the incorrect information and write the correct information above or adjacent to the incorrect information.

Always initial and date any such changes you make. It is also a good corrected in this manner it is very clear what error was made, how it was corrected and who made the correction. This is very defensible and it will improve the credibility of the data.

12.2.2 Mechanisms to ensure records are both permanent and unalterable 1/2

ELECTRONIC RECORDS.....

Many labs are routinely using computers to create and store their data. Just as pencil can be erased, so can a value in a spreadsheet be easily deleted with a single keystroke.

Security of electronic records begins with the use of password-based computer systems.

Access to computer applications used to record and store laboratory records must be controlled through the use of unique user IDs and passwords. These are standard options for virtually all current operating systems. In addition, systems must be designed to automatically log a user off after a certain period of inactivity at the keyboard (or mouse).

All electronic data must be regularly backed up onto media which will survive record retention requirements. This may mean changing media used to back-up data as technology advances.

Whatever system you use, there must be built in assurances that data is free from alteration.

Section 12.3 - Records Retention

12.3.1 Discuss record storage and retention time.

- Records such as bench sheets, sampler logs, and DMRs must be unalterable, readily retrievable and stored for a minimum of 3 years.