Chapter 4 Laboratory Equipment

Laboratory Space Requirements

Sufficient laboratory space is essential for precise and accurate data measurement. While there is no specific design size, the laboratory must have adequate lighting at bench sites, multiple electrical outlets, a source of filtered air, oil-less vacuum, and natural gas. Electrical outlet design is critical and many laboratories are drastically undersized. The facility should be temperature and humidity controlled.
The laboratory should have sufficient bench top area for sample preparation and analysis, storage space for media, glassware, and portable equipment such as pH meter, DO meter, desiccator, oven, water bath, muffle furnace, centrifuge, microscope, and incubator.
Ample floor space is also needed for free standing equipment such as autoclave, refrigerator, BOD incubator, and analytical balance. Space must also be available for safety equipment such as fume hoods, safety shower (with adequate drain) and eye wash.
Bench tops and floors should be of a material that is easily cleaned and disinfected.
When planning for a new laboratory, it is beneficial to seek advice from other laboratories rather than just the architectural firm.

Temperature Controlled Equipment

Thermometers

Glass, dial, or electronic thermometers can be used to measure temperature. Mercury filled thermometers have gone out of favor due to the high toxicity of liquid mercury and the spill handling problems associated with a broken thermometer. Accuracy should be checked at least annually against a certified NIST thermometer. Thermometers should be checked over the entire range of intended use to verify the thermometer is accurate at all potential temperatures. Never exceed the maximum temperature rating of the thermometer. Discard thermometers that differ by more than 1^{o}C from the reference thermometer. Reference thermometers should be recalibrated every 5 years. Currently, the State of NM Department of Health, Scientific Laboratory Division will calibrate thermometers for free. Maintain a record of the thermometer calibrations. Thermometers not in service should be stored in their protective sleeve. Mercury thermometers should be read at the top of the mercury meniscus.

Figure: Notice the thermometer correction factor on the label.

Drying Oven

Drying ovens generally serve two common purposes, removing moisture and sterilizing. Drying ovens are set at 104 + 1^{o}C to commonly remove moisture from TSS samples. At 104^{o}C, the heat will evaporate water but is not hot enough to drive off organic molecules. The laboratory should purchase a forced air oven to avoid differences in temperature within the oven. The temperature in the oven is commonly measured by a digital thermometer on the door panel. This thermometer is convenient but is not acceptable unless it can be calibrated against a NIST thermometer. If the digital thermometer cannot be calibrated, the bulb of a calibrated thermometer graduated in at least 1^{o}C increments should be immersed in sand and placed on each shelf in the oven. The sand will hold the heat and reduce rapid fluctuations in the temperature reading when the door is opened. Calibration-corrected temperatures should be recorded for each thermometer being used. Record at least twice per day during each day the incubator is in use. The readings should be separated by at least 4 hours.

***SAFETY:* Be sure to use asbestos gloves or tongs when removing objects from the oven.**

Sterilizing Oven
The drying oven temperature can be increased to 170-180^{o}C. At this temperature, objects in the oven for at least 2 hours will become sterilized. Objects commonly sterilized in the sterilizing oven are inoculating sticks and glassware such as pipets. Spore strips can be used to confirm sterilization.

***SAFETY:* Be sure to use asbestos gloves or tongs when removing objects from the oven.**

Refrigerator
The laboratory refrigerator is commonly used to preserve samples, and store perishable chemicals (most commonly prepared biological broth). Laboratory refrigerators should maintain a temperature of 1-5 ^{o}C. Calibrated thermometers should be graduated in at least 1^{o}C increments and the bulb immersed in liquid. A thermometer can be inserted into a rubber stoppered Erlenmeyer flask to prevent evaporation. The reading should be recorded at least once per day.

Dry Air Incubator
Incubators are similar to ovens except they are primarily used for bacteriological work and require more precise control temperatures. If the temperature varies from the proper temperature or if the wrong temperature is used, good test results may be reported from bad water. As with ovens, forced air incubators are preferred to convection incubators and are less likely to form "hot" spots. Calibrated thermometers should again be inserted in liquid and placed on each shelf of the incubator. The temperature should be 35 $\pm$ 0.5^{o}C. Calibration-corrected temperatures should be recorded for each thermometer. Record at least twice per day during each day the incubator is in use. The readings should be separated by at least 4 hours. A thermometer graduated in at least 0.1^{o}C increments should be used.

Figure: Dry Air Incubator

Waterbath
The water bath incubator is similar to the dry air incubator except the temperature must be at 44.5 $\pm$ 0.2^{o}C. To maintain this temperature a recirculating water bath is required with a gable cover. A thermometer graduated in at least 0.1^{o}C increments should be used. Record at least twice per day during each day the waterbath is in use. The readings should be separated by at least 4 hours.

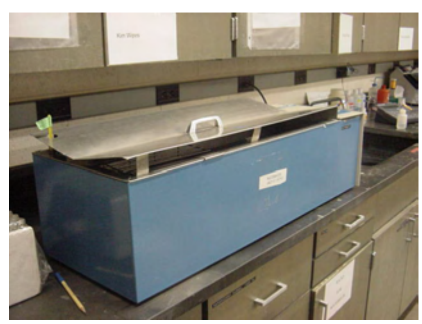


Figure: Waterbath

BOD Incubator
The BOD incubator is a specially converted refrigerator used in the BOD test. The temperature must be maintained at 20 $\pm$ 1.0^{o}C Calibration-corrected temperatures should be recorded for each thermometer being used at least once per day during each day the incubator is in use. A thermometer graduated in at least 0.1^{o}C increments should be used.

Muffle Furnace
Muffle furnaces heat materials from about 200-1100 ^{o}C. The most common use of a muffle furnace is to "burn" or "volatilize"off the organic portion of a sample leaving only ash behind. Muffle furnaces usually do not run continuously, so allow a 30 minute warm up time.

SAFETY: Be sure to use asbestos gloves and long tongs when removing objects from the muffle furnace.

Hot Plates
Electric hot plates are commonly used by the laboratory to heat solutions in either beakers or Erlenmeyer flasks. They usually have a temperature range of about 50- 350^{o}C. Hot plates heat up slowly but also cool down slowly. The amount of heat produced is controlled by an adjustable controller or rheostat (knob). The numbers on the knob are not assigned a calibrated value so the laboratory must calibrate the knob if the temperature is important. To calibrate the numbers on the rheostat, set the beaker containing a high boiling oil such as mineral oil on the hot plate. Turn on the hot plate and measure the temperature at each setting on the rheostat. Record the temperature values for each knob setting.

Burners
Burners are primarily used for bacteriological work in water and wastewater analysis laboratories. They are used occasionally to heat solutions, dry samples, and soften glass for glass repair. Most burners have an air vent and fuel adjustment valve.

To light a burner, open the air vents. Bring a lit match or striker up the side of the burner to the top and carefully turn on the gas valve. If the gas flow is too fast, the gas may flare up or go out. Reduce the gas flow and repeat the ignition procedure. After the flame is lit, adjust the flame height using the gas valve. If a hot flame is needed the air valve should be completely open and the flame will be blue. If a cooler flame is needed, reduce the air flow and the flame will become more yellow.

SAFETY: Natural gas burns very clean and the tip of the flame may be almost clear. Be careful not to reach across on open flame especially wearing flammable synthetic materials.

Burners are also used for glass bending and fire polishing. To bend glass tubing, attach a flame spreader, and gently roll the tubing back and forth until the glass becomes soft. Remove the glass tubing from the flame and gently bend it to the desired shape. To cut a piece of glass tubing, etch the tubing with a triangular file. Using gloves to protect your hands, place your thumbs close together on the opposite side of the tubing from the scratch. Gently push out on the scratch until it snaps. Fire polish the ends by gently and continuously rolling the ends in a flame until the glass begins to soften. Remove from the heat and cool. The ends of the glass should be free from rough, jagged or sharp edges.

Safety: Fire polished glass may be very hot even though it looks cool.

Digestion Apparatus
In addition to hot plates, wastewater laboratories have specially dedicated heating equipment for specific tests such as COD, TKN and phosphates. Heating blocks are not easily adjusted to a specific temperature and are therefore usually dedicated to a specific test. Sealed test tubes containing digesting chemicals are placed in the heating cells. These devices

should be used in a fume hood in the event one of the tubes breaks.

Figure: Hach COD digester

<u>SAFETY:</u> Be sure to use gloves, goggles and the tube sleeve when removing hot tubes from the digester.

Balances

Balances are an essential tool in most wastewater laboratories. Balances are used to determine a variety of solids analysis such as TSS, MLSS, VSS, TDS and to measure chemicals needed to prepare standard solutions and reagents. There are usually two types of balances, the top loading which is used for low accuracy measurement (sensitivity of 0.01 gm) and the analytical balance which is used for high accuracy measurement (sensitivity of 0.0001 gm).

Top Loading Balance

The top loading balance is used when the weight of a substance or object is not needed to a high degree of accuracy. When an object is placed on the balance pan, the balance will display the weight to the nearest 0.01 gm. This balance is often used to weigh chemicals to prepare reagents or bacteriological broth.

Figure: Mettler Electronic Top Loading Balance

To weigh an object on the top loading balance, first clean the pan using a camel hair brush. Next zero the balance by gently pressing the bar. The meter display will show zero. Now carefully place the object or tare on the balance. Read and record the weight to the nearest 0.01 gm. If the object is going to be used as a tare weight, the bar can be pressed again to make the tare weight go to zero. Chemicals can now be added to the tare using a spatula. The weight on the electronic display represents the weight of the chemicals. The maximum capacity of the top loading balance is around 600 gm and should not be exceeded.

Analytical Balances

Analytical balances are the workhorses of the wastewater laboratory. Analytical balances come in a variety of sensitivities ranging from 0.0001 gm to the highly analytical 0.000001 gm balance.

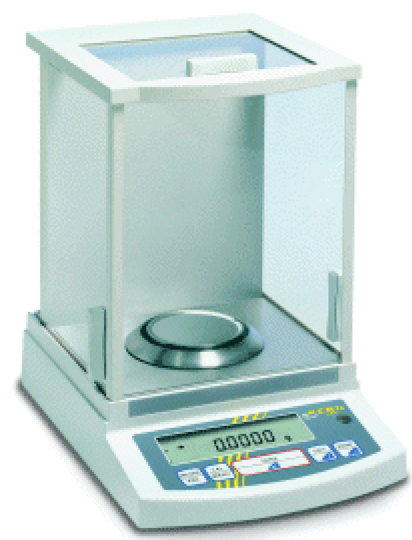


Figure: Electronic Analytical Balance

Most wastewater laboratories use the single pan electronic analytical balances having a sensitivity of 0.0001 gm. Because of this very high sensitivity, special handling is required for all objects placed on the pan. The maximum capacity of analytical balances will be around 150 gm.

Balance Rules

Because of the high sensitivity of the analytical balance, there are a number of "rules" that should be followed to avoid erratic and inaccurate balance readings.

1. Always use a tare. A tare is any object placed on the balance and used to contain the material to be weighed. The tare prevents contamination of the weighed material. If the pan has not been cleaned properly, dust or previous chemicals may contaminate the weighed material. In addition, chemicals placed directly on the pan may damage (corrode) the balance pan itself. Using a tare also allows chemicals to be easily and accurately transferred from the pan.

2. Balances should be placed in a constant temperature, constant humidity atmosphere. They should be located away from direct sunlight, heating and cooling vents, heavy laboratory traffic, and corrosive fumes. Both the top loading and analytical balances are sensitive enough to be affected by ambient room air currents. Close the balance doors before taking the final weight.

3. Balances should be leveled before using. This is generally a one-time operation performed during installation but should be checked periodically. The balance is leveled when the bubble is in the center of the centering circle. If the bubble overlaps the circle, the balance is out of adjustment and should be corrected by turning the screws on the balance feet.

4. Position the balance on a separate table free from vibration. Special balance tables are available but very expensive. If a table can not be purchased, the balance should be placed in a quiet space (away from the pump room) and given time to stabilize before the reading is taken.

5. Keep the balance clean. Always clean the pan and the chamber with deionized water or alcohol. Clean spills up immediately to prevent corrosion

6. Never put chemicals directly on the pan

7. Never overload the balance. Check the maximum rated capacity prior to use. Replacing the internal controls of an electronic balance is very expensive and may leave the lab without a balance for at least a month or more.

8. Never weigh a warm or hot object. Always cool to room temperature in a desiccator prior to weighing. Hot objects will heat the air within the balance chamber causing the pan to be elevated and giving a lighter weight.

9. Allow the balance reading to stabilize before taking the reading. Electronic balances indicate when the reading is stable.

10. Balances should be calibrated in-house at least monthly using NIST traceable Class 1 weights. Keep Class 1 weights clean and dry at all times.

Figure: Class 1 weights

Never touch the weights with fingers as the oils and acids will change the weight. Handle the weights with forceps or tongs. Class 1 weights should be calibrated every 5 years. Be sure to record and maintain all calibration results.

11. Calibrate and service the balance once a year using a certified balance technician. A good balance service will clean the interior and exterior of the balance and verify that the

balance is working within specification. A good service should show that each quadrant of the pan will produce an identical weight.

12. Always use forceps or tongs to place an object on or off the balance. Modern electronic balances are sensitive enough to weigh an oily fingerprint.

Sources of Error
Moisture, temperature, and static electricity are problems that should be considered. Many of the weighings in a wastewater laboratory involve driving off moisture. It is important to pre-dry dishes, filters, and in some cases chemicals before weighing in order to prevent moisture from adding weight that may be lost later in the test procedure. Some chemicals are defined as hygroscopic. Highly hygroscopic chemicals will absorb water under humid conditions. Analytical balances are sensitive enough to measure this hygroscopic water. It is very apparent when a hygroscopic chemical is placed on the balance because the weight never stabilizes and continues to increase. This error can be minimized by placing a small beaker of desiccant inside the balance chamber.
Temperature changes will also affect the weight of material on the balance. If the material is hotter than room temperature, convection air currents in the balance will push the pan upward causing the material weight to be less than the true value. If the material is colder than room temperature, convection currents will cause the material the weigh more than the true value. Materials removed from the drying oven or muffle furnace should never be immediately placed on the balance pan. Allow to cool in the desiccator for sufficient time to reach room temperature.
Static charges from objects coming in contact with the balance can be transferred to the balance weighing mechanism and cause erratic readings. These charges are difficult to remove. Sometimes touching the outside of the balance chamber with a drop of water will reduce the static charge error.

In-house Balance Calibration
Each laboratory should calibrate their balances at least monthly using Class 1 weights. Record the Make, Model, and Serial Number of the balance being calibrated. Place a Class 1 weight on the balance and record the displayed weight. Record the value as acceptable if it is within $\pm$ 0.0002 gm. Repeat this process with a minimum of 5 Class 1 weights covering the balance range. For instance, 1.0000, 5.0000, 10.0000, 50.0000 and 150.0000 gm weights could be used. The balance should be recalibrated if the recorded values are outside this allowance. Follow the manufacturers recommended procedure. Class 1 weights should be re-certified every five years. The NM Dept. of Agriculture Metrology office at NMSU will calibrate these weights for a fee. If the balance is frequently out of calibration, it may need to be calibrated more often. Most electronic analytical balances have an internal calibration procedure which takes about 2 minutes and can be performed daily.

Autoclave
The autoclave is a glorified pressure cooker that uses steam heat to sterilize. The autoclave should maintain a sterilization temperature of 121^{o}C during the sterilizing cycle and be able to complete the entire cycle within 45 minutes when a 12-15 minute sterilization period is used.

Figure: Steam sterilizer

The autoclave should have a pressure gauge, a safety valve and a thermometer sensor in the exhaust. It is also common to have a temperature recording device and a digital timer. The sterilization temperature of 121^{o}C must be confirmed by using a spore strip, integrator strip or a calibrated high temperature thermometer. Overloading the autoclave by placing objects very close together may prevent complete sterilization and should be avoided. The autoclave is commonly used to sterilize bacteriological media, phosphate dilution water and contaminated microbiological samples.

Magnetic Stirrers

A magnetic stirrer is a common apparatus used during titrations and to mix solutions. Teflon covered magnets are placed in the flask to be stirred.

Figure: Magnetic stirrer

The solution is added then the speed is slowly increased causing a vortex to form. Do not stir the solution so violently that the solution splashes up the side of the flask. If a large amount of solids needs to be mixed, add the solids slowly as the magnet turns to avoid jamming the magnet. If excessive solids prevent the magnet from spinning, swirl the flask to dislodge the magnet, and then continue. If the magnet begins to wobble excessively or starts vibrating without spinning as the speed is increased, stop the stirrer then restart slowly.

Desiccator

A desiccator is used to allow hot objects to be cooled without condensation. *A desiccator is NOT used to remove water from samples*. If humidity is allowed to condense on the surface of the object, the weight will be inaccurate. Hygroscopic chemicals such as bacteriologic media are often stored in a desiccator to reduce moisture absorption into the media. Dehydrated bacteriological media should be stored upside down in tightly closed bottles in the dark at less than 30^{o}C.   If they become discolored and caked they should be discarded.

The desiccator has a pan in the bottom containing a chemical called a desiccant.

Figure: Desiccator

There are a variety of different desiccants but the most common one is called Indicating Drierite. Indicating Drierite will change colors from blue to pink as the material absorbs moisture (humidity). It is important to keep the door closed to prevent excessive humidity absorbance. It is important that the seal on the desiccator be tight. If the seal or lock is poor, moisture will infiltrate the desiccator and prematurely exhaust it. Once the Drierite turns pink it must be replaced or regenerated. It can be regenerated at 180°C for 2 hours. Never place any object directly on the desiccant as it may pick up contaminants.

There is a second type of desiccator that looks like a glass jar. It works the same way but has a sliding top that is sealed by silicone grease. When placing hot objects into the desiccator, be sure to leave the top cracked slightly to allow the heat to escape for a couple of minutes. If the top is closed immediately the hot sample will heat up the air in the desiccator causing the pressure to increase and the expensive lid may pop off.

Laboratory Grade Water

A source of laboratory grade water is essential. The two most common methods of producing laboratory grade water are distillation and deionization. Distillation involves heating water to produce steam. The steam is then re-condensed to a receiving bottle, leaving the contaminating solids behind. The solids remaining in the boiler will eventually scale the heater elements and require maintenance. Deionization involves passing water thru a demineralization cartridge which removes contaminating ions. When the capacity of the demineralizer cartridge is exhausted, the cartridge must be replaced or regenerated. The life of the demineralizer cartridge can be extended by pretreatment with a cartridge filter and RO membrane. Laboratory grade water must be checked for quality monthly. The most important parameter is conductivity which should be less than 2 uSiemans. Other parameters will be discussed later.

Ultraviolet Sterilizer

The ultraviolet sterilizer uses high intensity UV light to sterilize. A minimum exposure time of 2-3 minutes is required for sterilization.

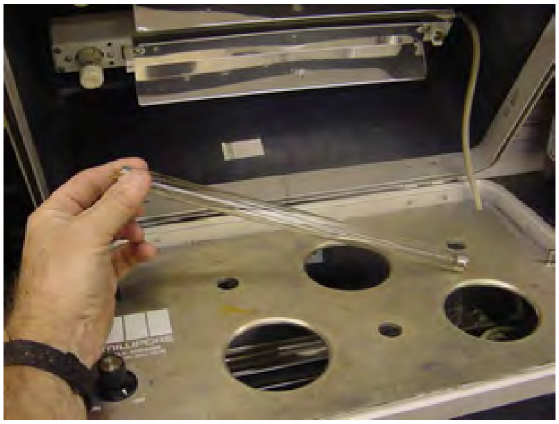
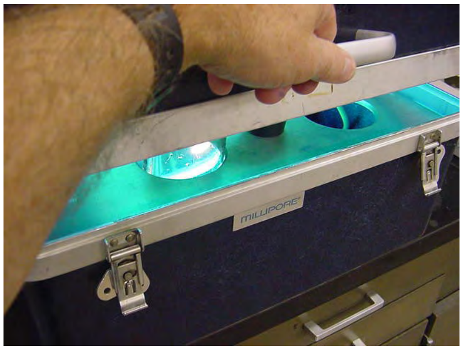


Figure: Ultraviolet lamp

The UV lamps should not be touched with the fingers and should be cleaned monthly using Isopropyl alcohol and a lint free cloth. The lamps should be replaced when they no longer produce a 99% kill. Lamps typically have a shelf life of 2 years.

<u>SAFETY</u>*: *Do not look at UV light. The light can damage the retina of the eye.



Electronic Instrumentation

The wastewater laboratory may also use a variety of instruments to measure contaminants such as pH, DO, and metals. These instruments all operate by comparing millivolts generated by a standard with millivolts generated by the sample. All instruments must be calibrated frequently against either prepared or purchased standard solutions. Specific meter operation will be discussed in later chapters.

Light Microscope
The microscope can be a valuable tool to the wastewater laboratory technician. Monitoring the microbiological population of an activated sludge process can help diagnose the operating condition of the activated sludge process and guide the operator to process control changes. The laboratory technician can use the microscope to identify, count and check on the activity of various types of protozoans and metazoans to:
1. Determine which organisms are present in a WWTP that is running well.
2. Determine if a toxic shock to the activated sludge system has taken place.
3. Provide data for trend charts relating protozoan/metazoan numbers to specific plant treatment parameter.
4. Identify filamentous bacteria types to help in determining the cause of bulking or foaming.

The light microscope is a sturdy instrument which when handled properly can work for many years. The microscope should always be carried using two hands- one on the base and one on the neck. This will help reduce crashing the microscope into the lab bench and misaligning the lenses. The use to the microscope will be discussed in a later chapter.

Visible Light Spectrophotometer
Spectrophotometry uses the Beer-Lambert Law to determine the concentration of a compound by creating colors that are proportional to the concentration. This instrument will be discussed in a later chapter.

Figure: Spectrophotometer

Atomic Absorption Spectrophotometer
An atomic absorption spectrophotometer is a very expensive high end spectrophotometer used to measure very low levels of metals. It operates in a similar manner as the basic spectrophotometer but measures metals in their atomic state rather than as a molecular compound.

Inductive Coupled Plasma (ICP)
This is also a very expensive high end spectrophotometer that measures a nebulized sample into a "plasma" which dissociates molecules into their atomic form. The emission of colors by these ionized materials is measured using a spectrophotometer.

Maintenance/Service Contracts
In order to produce valid answers, the equipment must be in proper operating conditions. Whenever the equipment calibration cannot be obtained, the data becomes invalid. To avoid this, laboratory personnel can perform both routine maintenance and preventative maintenance. An example of routine maintenance would be to simply press the CAL button on the analytical balance. Preventative maintenance would be to replace the pH electrode or DO membrane at some time interval, long before the data becomes questionable. If the maintenance is beyond the capabilities of the lab technician, a specialist may be needed. These are often expensive but a necessary part of doing business as a laboratory. Some manufacturers offer service contracts. These are also expensive and should be evaluated closely to be sure they cover your maintenance expectations. It is important for all levels of management to understand the role and cost of maintaining laboratory equipment. The laboratory must have sufficient budget to cover maintenance and repair cost. Laboratory data is often required to meet Federal law and intentional filing of invalid data carries civil and criminal penalties. You, the lab technician, are responsible for the data you generate!!