SAMPLING AND LABORATORY

Sampling and laboratory analysis of wastewater and wastewater treatment processes are part of every wastewater treatment plant operator's daily routine. Inventories of biomass, the food coming to them, the water chemistry, the air available for them, and their removal efficiency must be calculated and recorded. There are requirements, both regulatory and operational, that dictate how and when these samples are collected, analyzed, and recorded or reported. Sampling for wastewater treatment is broken down into three categories: grab samples, composite samples, and proportional composite samples.

GRAB SAMPLES

Grab samples are single point real-time samples. A temperature reading or sludge depth sounding (Sludge Judge) of a clarifier are examples of a type of sample that would be a grab sample. A dissolved oxygen or pH reading would also be a grab sample. One of the reasons is because the DO and pH can change over the time it takes to collect a composite sample. Another is that this information must be known at specific points in the process. BAC-T samples for fecal coliform are also grab samples. Grab samples give a snap shot of a specific condition at a specific point in time and at a specific point in the system.

COMPOSITE SAMPLES

Sometimes a measurement of the average across a process is important. If you want to determine the total amount of solids that are under aeration in an activated sludge system, groups of grab samples from different spots in all of the different tanks or stages would need to be collected in a single container. This would be a composite sample representing the entire system. A single mixed liquor suspended solids (MLSS) test, run from a sample drawn from the collected composite, will indicate the average concentration for the system. Even when sampling a single stage or tank, a sample should be composited from different points in the tank. Since settleometer readings and MLSS are both used to calculate Sludge Volume Index (SVI), the settleometer sample should also be taken from the composite. Other operational concerns might require grab settleometer readings from specific tanks in other situations.

PROPORTIONAL COMPOSITE SAMPLES

A composite sample can also be taken over time to represent the average reading over the course of a full day. This is done when daily totals for BOD or suspended solids loading are needed. Since the influent and effluent flows change over the course of a day, representative sampling requirements dictate that smaller samples be taken at low flows and larger samples be drawn as the flow increases. This is known as a proportional composite sample. Some NPDES reports require flow-proportional samples for all daily effluent BOD and suspended solids testing. Proportional composite samples for BOD must be refrigerated at 4 degrees C (39^{o} F). The sample has a maximum holding time requirement of 48 hours if it is for BOD testing.

WASTEWATER LABORATORY

Smaller systems have fewer sampling and testing requirements. But all wastewater treatment plants should be equipped to run pH, DO, chlorine residual, and, if they are activated sludge, settleometer tests. Larger systems should have equipment and personnel to run, at a minimum, BOD, TSS, chemical oxygen demand (COD), and both mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). These tests are needed to manage and operate the plant efficiently on a daily basis. Anaerobic digester systems require testing for volatile acids and alkalinity. Tertiary nitrogen removal processes require analysis for ammonia and organic nitrogen (Total Kjeldahl Nitrogen) and nitrate nitrogen. These plants may also run their own fecal coliform tests. Many states require wastewater labs to be certified by the state regulatory agency. All testing must be done in accordance with "Standard Methods for Examination of Water and Wastewater" and 40 CFR 136.

GENERAL LABORATORY SAFETY

The following items are some of the general issues that pertain to laboratory operations:

- Make sure all lab chemicals are properly labeled and have not exceeded their expiration dates.
- Make sure the MSDS information on the different chemicals is available.
- Make sure that all of the personal safety gear required in the MSDS data is available and employees are following those safety procedures as indicated in the data sheet.
- Combustible chemicals must be store in approved fire cabinets.
- Acids and Bases must also be stored in approved cabinets
- Some chemicals have brown glass bottles because they are light sensitive. Store them in closed cabinets.
- Be careful when handling glassware, especially when connecting glassware to rubber hoses. Use water as a lubricant to avoid injury from breakage.
- Always dilute acids and bases by pouring them into the dilution water. Adding water to an acid or base can cause enough heat to boil the water and spew hot chemicals on you.

SETTLEOMETER TEST

A settleometer test is used to determine how well activated sludge MLSS settles in the clarifier. It is used to calculate the SVI for the process. A settleometer test can be performed using a 1-liter tapered cone called an Imhoff cone, a 1-liter breaker or a 1000-ml graduated cylinder. A 2-liter beaker can also be used. The settleometer is filled with mixed liquor and allowed to sit for either 30 or 60 minutes. As the sludge settles, its volume is recorded every 5-10 minutes. The final reading at the end of test is recorded as ml or as a percentage. 300 ml in a 1000-ml settleometer would then be a 30% sludge volume. 300 ml in a 2-liter beaker would be a 15% volume.

The settleability of the sludge will depend on sludge age, condition and bioactivity. Settleometer readings early in the morning may be higher due to lack of food during low flow conditions. Never make a process change based on a single set of settleometer tests. But if a trend persists over the course of the day, process adjustments should be considered.

TESTING METERS

Dissolved oxygen and pH tests can be run using meters instead of laboratory chemical analysis. Both meters have electrodes that contain a fluid called an electrolyte in them. The electrolyte is held in the electrode by a semi-permeable membrane. The membrane allows the chemical or gas being analyzed to react with the electrolyte. This generates an electrical current proportional to the analyze strength. This current is converted to a display on the meter.

DO meters must be calibrated before each use. They are adjusted to compensate for atmospheric pressure and temperature. They can be used to continuously monitor aeration tanks. Once calibrated, it is a simple matter to take DO readings. The most important issue is to allow the meter time to react once it is immersed in the tank. This can take up to a minute to stabilize. Don't place a DO probe directly over a diffuser or it may read oxygen in the air bubbles instead of the water. Always make sure the meter probe is stored in distilled or de-ionized water when not in use. It should not be allowed to dry out. DO readings from samples must be taken as soon as possible or biological activity may cause a drop in the reading.

A pH meter must be standardized before each use. They are standardized using buffer solutions of a known pH, usually 4, 7, and 10. A buffer solution has chemicals that neutralize acids and bases while maintaining a constant pH. If a drop of acid is added to a pH 7.0 buffer solution, the pH will remain at 7.0. These solutions allow the operator to correct any deviation in the pH meter by adjusting it to match the pH of the buffer solution. Fresh buffer solution should be used each time the unit is standardized. Electrodes must be rinsed with distilled water between readings. Some should be stored in distilled water when not in use. Others may require storage in a buffered pH 4.0 solution with potassium chloride added. The electrode membrane can be cleaned with isopropyl alcohol when slime or scale builds up on it.

A field-test for pH may include color strips or indicator chemicals. Both contain chemical indicators that change color as the pH changes. Chemical indictors are also used in laboratory titrations for nitrogen, dissolved oxygen, alkalinity and chlorine residual.

BACTERIOLOGICAL TESTING

Bacteriological tests are conducted using either the MPN fermentation test or the membrane filter test. The membrane filter test is easier to run and results are obtained by counting individual colonies on the membrane. These are visible without the need for magnification. A sample is drawn through the filter pad where the bacteria are trapped on the filter. The filter is then incubated in a nutrient broth at a temperature that allows the coliform bacteria to grow on the filter pad. After 24 hours, the individual bacteria that were caught on the filter will grow into a colony that can be visually counted using an optical scope.

Although the procedure is similar total coliform and fecal coliform use different nutrient media and incubation temperatures. Fecal coliform samples must be run within six hours of collection. The petri dishes must be placed in watertight bags and incubated in a water bath within 30 minutes of filtration. The box below identifies the differences in the tests.

Parameter	Total Coliform	Fecal Coliform
Media	M-Endo Broth	M-FC Broth
Incubation Time	24 $\pm$ 2 hours	24 $\pm$ 2 hours
Temperature	$35^{o} \pm 0.5$ C	$44.5^{o} \pm 0.2$ C
Colony Color	Red w/Green Metallic	Blue

MIXED LIQUOR SUSPENDED SOLIDS TESTING

MLSS testing also involves a filtration step. A sample of wastewater is filtered through a vacuum crucible. The solids in the crucible are dried for a minimum of 30 minutes to remove the moisture and weighed. The crucible must be dried to a constant weight before filtration and the sample is dried to a constant weight at 103-105^{o} C. The weight in grams is multiplied by the sample factor of 1,000,000 divided by the sample size to determine the MLSS concentration. If 50 milliliters of sample is dried to 0.00125 grams, then 0.00125 mg X 1,000,000 /50ml = 25 mg/L.

Mixed liquor volatile suspended solids or MLVSS is determined by taking the dried MLSS sample and firing it in an oven at 500 +/- 50^{o} C. The ash is cooled in a desiccator and then weighed. The mg/L of non-volatile ash can be calculated the same way as MLSS. The MLVSS is calculated by subtracting the non-volatile ash number from the MLSS. It is MLVSS that should be used for F:M calculations.

BOD/COD TESTING

The biochemical oxygen demand measures the amount of oxygen that the biomass (bugs) uses to stabilize (eat) the organic material in the wastewater. The BOD_5 test is used for calculating organic loading for the plant processes. A sample is allowed to sit in an incubator for 5 days at 20^o +/- 1 C. The amount of dissolved oxygen that is used or depleted from the BOD bottle is used to calculate the BOD strength.

BOD bottles hold 300 ml for the test. The amount of sample used in the test depends on the strength of the wastewater. If the DO at the end of the test is less than 1.0 mg/L, the sample must be diluted. This means that the only sample that might not need dilution would be a plant effluent sample. Tests on digester supernatant may require the use of less that 1-milliliter of sample in the dilution water. The diluted sample must have an oxygen depletion of at least 2.0 mg/L and a residual of at least 1.0 mg/L for the results to be valid. The dilution water must be de-ionized to remove chemicals that might inhibit the biological processes. Micronutrients like magnesium, iron, and phosphate are then added to it to improve the biological activity during the test. Samples are run in pairs with one bottle containing only dilution water, unless samples are seeded. Tests on disinfected effluent will have to be seeded with raw sewage to replace the bugs that were killed during disinfection. The sample must also be dechlorinated using sodium sulfite, since chlorine will inhibit bioactivity. A seed blank will also need to be run in addition to the BOD test. The uptake of oxygen that results from decomposition in the seed portion will have to be subtracted from the total oxygen uptake in the BOD test.

Chemical oxygen demand (COD) is sometimes used in larger systems as a parallel test to BOD. The COD test involves a chemical reduction of organics in the sample. It will result in a higher strength than the BOD results, but the test can be run in about 3 hours. This means that the results of a process change can be determined the same day instead of waiting 5 days to find out. When enough COD's have been run and compared to BOD results, a conversion factor can be established that can be used to approximate the BOD strength for daily process adjustments. COD results are more likely to reflect the results from tertiary treatment.

BASIC STUDY QUESTIONS

1. What are some of the issues regarding storage of laboratory chemicals?

2. Which tests use grab samples?

3. How do you determine the results of a membrane filter test?

BASIC SAMPLE TEST QUESTIONS

1. The chemical that changes color during a pH or alkalinity test is called:

   A. A buffer
   B. An indicator
   C. An acid
   D. A micronutrient

2. How do you lubricate a rubber-to-glass connection?

   A. Water
   B. Light machine oil
   C. Vegetable oil
   D. Hydrochloric acid

3. A pH meter should be standardized every:

   A. Day
   B. Week
   C. Shift
   D. Use

ADVANCED STUDY QUESTIONS

1. What must be added to BOD dilution water?

2. What is a buffer?

3. What must be done to a chlorinated BOD sample?

4. Which media are used for total and fecal coliform membrane filter test?

5. What is the advantage of using a COD test for process control?

ADVANCED SAMPLE TEST QUESTIONS

1. Fecal coliform tests are incubated at:

   A. 20^{o} +/- 2 C
   B. 35^{o} +/- 0.5 C
   C. 44.5^{o} +/- 0.2 C
   D. 103-105^{o} C

2. The maximum holding time for a 24-hour BOD composite sample is:

   A. 24 hours
   B. 48 hours
   C. 72 hours
   D. 96 hours

3. MLVSS samples must ignited at:

   A. 35^{o} C
   B. 135^{o} C
   C. 255^{o} C
   D. 500 ± 50^{o} C

4. What color is a fecal coliform colony in the membrane filter test?

   A. Red
   B. Blue
   C. Green
   D. Black