Date: Tuesday, October 29, 2019

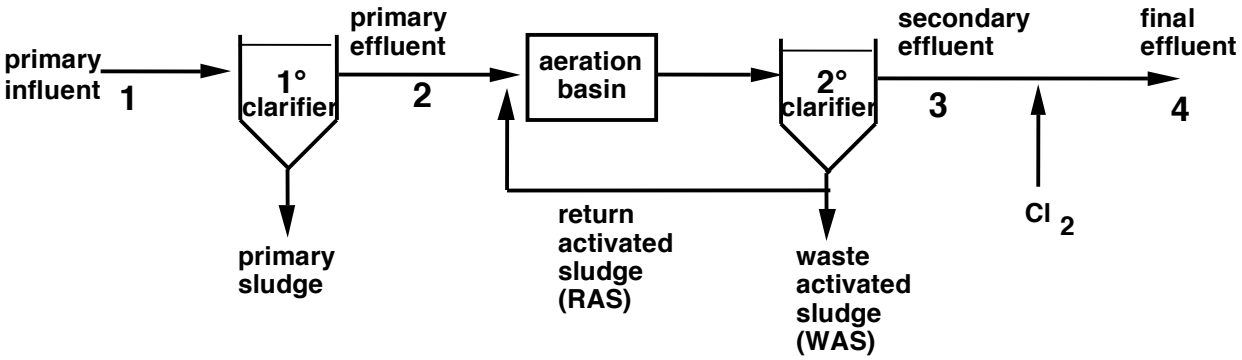
Topic(s): Analysis of wastewater samples throughout a treatment process

Report: Report will be due Tuesday, November 12

Preparation: Read lab handout, and be prepared for quiz that asks you to (1) define BOD in words, and (2) calculate the volume of sample added to a BOD bottle, given a dilution factor.

As we will discuss in class and read about in the textbook, parameters such as 5-day Biochemical Oxygen Demand (BOD_5) and Total Suspended Solids (TSS) are often used to assess the characteristics of wastewater. In this two-week lab, each group will measure BOD, TSS, and coliform bacteria in samples collected from a local wastewater treatment facility at different points within the treatment process. From these measurements, students will determine the removal effectiveness and efficiency of selected unit processes.

A schematic of a conventional water resource recovery facility is shown below:



The **primary clarifier**'s purpose is to remove suspended solids (particles that are more dense than water and will settle to the bottom in ~4 hours). The aeration basin and secondary clarifier comprise the **activated sludge process**. The **aeration basin** is a biological process that promotes the growth of aerobic bacteria, which biodegrade soluble organic material and some particulate organics that are not settled out in the primary clarifier. The purpose of the **secondary clarifier** is to remove suspended solids — mostly, the bacteria from the aeration basin— and produce effluent that is low in suspended solids and BOD. The **disinfection** process, often chlorine or ultraviolet light, is employed to destroy pathogenic microorganisms prior to discharge or reuse.

Safety Procedures. *Wear safety glasses, lab coats, and gloves for this experiment. You are working with real samples of wastewater that have the potential to contain pathogenic microorganisms such as E. coli. Use PPE and prudent lab technique. Separate your lab bench into a 'clean' side for notebooks, pens, etc., and a 'dirty' side, whereon you will perform your experiments. At the end of lab, place all used gloves, paper towels, and pipette tips into the biohazard containers for disposal.*

Biochemical Oxygen Demand (BOD)

The amount of oxygen consumed in a polluted water sample over time is directly proportional to the amount of biodegradable organic material in that sample. It is also indicative of the amount of oxygen depletion we could expect if we released this polluted water sample into the environment. To measure this effect, we first measure the amount of oxygen in a sample at the beginning of the test. Next, we seal the sample so no oxygen can enter from the atmosphere, and we incubate the sample at a constant temperature for a known amount of time. During this time, bacteria degrade the organic material and use some of the oxygen in the sample. At the end of the test, we measure the amount of oxygen left in the sample. The difference between the starting and ending oxygen concentrations is the amount of oxygen that was 'demanded' by the bacteria to degrade the organic matter. We call this the **Biochemical Oxygen Demand, or BOD,** and it is an indirect measurement of the amount of biodegradable organic pollution in the sample.

5 days is the common standard for measuring BOD. However, to fit with our weekly lab schedule, we will perform a 7-day BOD test. The tests will be set up the first week and completed the second week.

Each group will determine the BOD concentration in one wastewater sample. For a valid test, we need:

-
-

To meet these conditions, you will need more than one dilution of your water sample. Calculate the approximate amount of sample to start with as follows:

Choose a Dilution factor =

Calculate the volume of sample to add to bottle =

So, if you had landfill leachate with an expected BOD of 500 mg/L, and you wanted to set up your BOD bottle such that 5 mg/L of oxygen will be consumed during the test, the dilution factor would be 500 ÷ 5 = 100. The volume of the bottle is 300 ml, so you would add 300 ÷ 100 = 3 ml of sample to the bottle and make up the rest with dilution water. To make sure you get a valid test, you will also test two other dilutions, such as a dilution factor of 50 (6 ml sample) and 150 (2 ml sample) for this example.

BOD is calculated as follows:

Where: DO_i (or DO(0)) =
DO_f = (or DO(final)) =
B_i =
B_f =
DF =

BOD concentrations in some common types of water sample:

Water Sample	BOD, mg/l	Seed Needed?
Distilled water	0	
River water	0-25	
Raw wastewater	200-500	
Primary effluent	50-300	
Secondary effluent	5-20	
Final effluent	2-15	
Landfill leachate	500-5,000	

Two factors that we need to consider are:

1. First: Some of the decrease in dissolved oxygen concentration is due to the dilution water we use. So, we test a **'blank'**, or a bottle that has no sample but only dilution water. Then, we correct our result accordingly.

2. Second: Some samples are too clean to have any bacteria in them, so we add bacteria to kick start the process. This is called **'seed'**. When we add this, sometimes we get a drop in DO due to these organisms even in clean water, so we must also correct for that. We test a **'seeded blank'** with only dilution water and microbes (no sample), and we correct our result accordingly.

For dirtier samples that don't need seed, we use the unseeded blank for correction, and for clean samples to which we add seed, we use the seeded blank to correct.

Coliforms as Indicator Organisms

As you will recall from class and previous labs, **coliforms** are a group of microorganisms that are useful as **indicator organisms**. That is, they are not necessarily pathogenic themselves, but their presence in a water signals the likelihood of fecal contamination, which in turn implies the possibility of pathogens being present. They are an environmentally relevant parameter for obvious reasons.

Coliforms have five characteristics that make them good indicator organisms:

1.
2.
3.
4.
5.

The procedure for measuring coliforms in your samples involves filtering a water sample, placing the filter on a growth medium, incubating it at **enteric** (intestinal) temperatures, and seeing what grows. We the calculate the number of organisms as # of colonies or colony forming units (CFUs) per unit volume (per 100 ml) of sample passed through the filter.

BOD Procedure:

1. You will perform BOD analysis on your sample only. However, you will conduct this analysis using three dilutions with duplicate (i.e., two) bottles for each dilution as shown in the table below.
2. Determine the volume of sample to use in your bottles, using the approach above.

   Sample:_____ Expected BOD (from table above):_____ mg/l

	More Dilute	Target Dilution	More Concentrated
Dilution Factor			
Volume of sample added to bottle			

   Check your dilution factors and sample volumes with Prof. Sills before proceeding.

3. Record the bottle numbers (already stenciled in black) of your six bottles, and assign two bottles to each of the dilutions above.
4. ***If you are seeding (i.e., adding microorganisms to) your BODs,*** add 1mL of primary effluent to each bottle.
5. Add the specified amount of sample to each bottle, and fill up the remainder of the bottle with the dilution water provided. This water contains a pH buffer and nutrients essential for growth.
6. After calibrating the DO meter, insert the DO electrode into the top of a bottle. Turn on the probe's mixer and allow the reading to stabilize. Record this DO concentration as the initial DO for that bottle. Repeat for each bottle. Our lab's standard operating procedure for measuring DO is appended to this lab handout.
7. After measuring the DO, insert a glass stopper and cap the bottle securely with a plastic cap.
8. Incubate the bottles for one week. Next week you will measure the DO in the bottles and calculate the BOD concentrations, determine which dilutions were valid, and calculate removal efficiencies for each unit process investigated. Because we have so many groups, it is essential that you record the number of your bottles: use the numbers printed in black on the bottles themselves, do not use tape.

Measuring Coliforms

1. You will perform analysis for one plate on only the single sample given to your group. For the analysis, you will use triplicates for each sample type, one of the three triplicate samples will come from each lab section.
2. Connect a filter assembly to the house vacuum line.
3. Remove the top lid of the filter apparatus, without touching the inside.
4. Apply an appropriate amount of sample into the filter cup:
   a. Raw Influent: 5 ml of a 1:10,000 dilution
   b. Primary Effluent: 5 ml of a 1:1,000 dilution
   c. Secondary Effluent: 10 ml of a 1:100 dilution
   d. Final Effluent: 10 ml of undiluted sample

You will need to perform the dilution calculation, and use deionized water for your dilutions.

5. Gently open the vacuum valve until all the sample has passed through the filter.
6. Remove the entire top part of the filter apparatus. Using sterile forceps, transfer the filter to one of the empty plates provided, keeping the filter face up.
7. Label the dish with "CEEG340, [time]am, Group[____], [sample name], [date]." Use tape to avoid obscuring the view of the plate. Incubate the filter upside down at 35°C overnight.
8. After ~24 (18–36) hours, examine the plate. Count all the colonies (total coliforms) and the blue colonies (*E. coli*), if any. Ideally, your plate will have 30-300 colonies. If the colonies have completely taken over the plate, write "TNTC", or too numerous to count. ***Report in the Google Sheet for Lab 9 and 10, the number of total colonies, the number of E. coli colonies, and the volume and dilution for your plate.***

Priming filters for TSS measurements next week:

1. Using forceps, place one glass fiber filter on the plastic rim of the vacuum filter apparatus, and replace the cup on top.
2. Pour 20-50 mL of deionized water through the filter three times while under vacuum. This helps to rinse off any loose glass fibers.
3. Turn off the vacuum, remove the filter, and place it in an aluminum pan.
4. Repeat until you have a total of 8 filters prepared. Leave the filters in the pans on your lab bench.

More information will be provided in next week's lab.