Date: Tuesday, October 8, 2019

Topic(s): Drinking Water Treatment

Due Date: Group Lab Memo due Tuesday, October 22, 2019

Preparation: 1. Read the lab handout.
2. Calculate the volume of alum stock (1 g/L) to add to 1 L of raw water to achieve doses of 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, and 50 mg/L.
3. Calculate the volume of liquid bleach to add to 200 mL to achieve a dose of 6 mg/L. (The bleach is approximately 1% by weight, or ~10,000 mg/L free chlorine)
4. Bring a hard copy of your calculations to lab, they will be collected during lab.

Lab Overview.
Most of the processes at a drinking water treatment plant (DWTP) are focused on removing particles, and turbidity is used as the measure for the amount of particles in the water. Turbidity is a measure of the cloudiness of the water due to particles. The units for turbidity are called 'nephelometric turbidty units' or NTUs. A nephelometer is a device that measures the turbidity in a water sample by passing a beam of white light (400-680 nm) through the sample and measuring how much of that light is deflected as a result of the particles in suspension. You may remember that according to the drinking water regulations, the DWTP had to produce water with a turbidity of less than 0.3 NTUs, and they had a goal of less than 0.1 NTUs at the plant.

By removing the particles in the water, we are also removing potentially pathogenic organisms that could cause illness. Large particles like sand and gravel settle rapidly and can be easily removed. However, ***colloids*** are very small particles (1 nm – 1 um) that do not settle out by gravity in a reasonable time frame. These particles cause ***turbidity*** which results in poor water aesthetics.

These small particles have electrical charges on their surface, usually negative charges. Like with any electrical charge, "like repels like", meaning that these particles will not stick together into larger particles that would settle. So, during the treatment steps of ***coagulation*** and ***flocculation***, we use chemicals to neutralize these negative charges and bring the particles together into larger ***flocs***. Typical coagulants include different forms of Al and Fe salts which produce positively charged species in water, see Table 1. Subsequently, we separate the particles by ***sedimentation*** and ***filtration***. Finally, we use ***disinfectants*** such as chlorine to inactivate any pathogenic microorganisms remaining after filtration.

In this lab, we will perform a 'jar test' to mimic the coagulation, flocculation and sedimentation processes in a DWTP to determine an effective dose for the removal of turbidity. In addition, we will also evaluate the removal of indicator bacteria, namely *E. coli* and coliforms, by these processes and by disinfection with chlorine. *E. coli* and coliforms are often used as 'indicator' organisms. *E. coli* and coliforms are found in the intestines of warm blooded mammals (and other animals). The idea is that if we find them present in a sample, this 'indicates' that fecal contamination has likely occurred and it is possible that other pathogens are present in the sample.

Table 1. Examples of coagulants often used in water treatment.

Commonly used			
Aluminum sulfate (Alum, $Al_2(SO_4)_3$)			
Ferric sulfate ($Fe_2(SO_4)_3$), Ferric chloride ($FeCl_3$)			

Laboratory Objectives. The objectives of this lab are for students to perform:

1. a jar test to evaluate the effect of coagulant dosage on the removal of turbidity and bacteria (fecal coliforms and *E. coli*) from a raw water source;
2. a disinfection test to determine the effect of a disinfectant on the fecal coliform and *E. coli* concentrations.

Each team will write one lab memo. Make sure your group collects all relevant data during the lab. Variability is important here, so collect replicate samples (at least 3) during the experiment.

Part 1 – Raw Water Quality – Perform this part while your coagulant test (Part 2) is running

1. Using the turbidimeter provided, measure the turbidity (in units of NTU) of the source water. Repeat two more times for a total of at least three independent turbidity measurements. Be sure to wipe the outside of the vial each time with a Kim wipe.

All groups will follow the procedure you used in Lab 2 (Water Quality Testing for the Lewisburg Neighborhoods Corporation) to filter 5 mL of the raw water onto a membrane. Incubate this membrane for 24 hours at 35°C. After filtering your samples for fecal coliform and *E. coli*, immediately label the dish with a descriptive label, such as "8-A-Raw, 10/18/18, 5 mL." Arrange for at least one person from your group to come to the lab 24 ± 2 hrs to count and record the number of total colonies (total coliforms) and the number of blue colonies (*E. coli* colonies). Record this data in a Spreadsheet, which you should post in the Lab 7 Google Folder. Make sure you also record the volume of water filtered. ***Not posting this data in way that others can use by the end of the day on Wednesday 10/9 will result in a 10 pt reduction on the lab assignment for all members of your group.***

Part 2 – Chemical Coagulant Optimization Test (Do this part first!)

1. Measure 1 liter of the raw water sample into each of the 6 containers. Label these with tape as Control, 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, and 50 mg/L. These labels represent the coagulant dose for each container.
2. Place each container under one of the stirring bays in the test apparatus.
3. Measure the appropriate volume of the coagulant stock solution and pour each into one of the small plastic beakers.
4. Start the stirrers. Set the speed to 250 rpm.
5. Simultaneously add the alum solutions to each container. Start a stopwatch. This is Time Zero.
6. After 1 minute, reduce the speed to 30 rpm. Leave at this speed for 15 minutes.
7. After 15 minutes, turn off the stirrers and allow the suspension to settle for 20 minutes.
8. Using a pipette, extract sample from each container and measure the turbidity. Measure 2 to 3 replicates for each container.
9. Choose the sample with the lowest turbidity.
10. Filter 25 mL of the settled water (with the lowest turbidity) through a membrane. Incubate this membrane for 24 ± 2 hours at 35°C.

After filtering your samples for fecal coliform and *E. coli*, immediately label the dish with a descriptive label, such as "8-A-Settled, 10/16/18, 25 mL." Arrange for at least one person from your group to come to the lab 24 ± 2 hrs to count and record the number of total colonies (total coliforms) and the number of blue colonies (*E. coli* colonies). Record this data in a Spreadsheet, which you should post in the Lab 7 Google

Folder. Make sure you also record the volume of water filtered. ***Not posting this data by the end of the day on Wednesday 10/9 will result in a 10 pt reduction on the lab assignment for all members of your group.***

Part 3 – Disinfection

1. Using the sample with the lowest settled turbidity, measure out 200 mL of the settled water using a graduated cylinder.
2. Pour this into a glass beaker with a stir bar, and position the beaker over a stir plate.
3. Using the bleach provided and the volume you calculated in preparation, dose 6 mg/L of chlorine bleach to the 200 mL of settled water.
4. Turn on the stirrer for 5 minutes.
5. After 5 minutes of contact time with the chlorine, measure the final turbidity of the disinfected water, with several replicates, leaving at least 50 mL.
6. Filter 50 mL of the disinfected water through a membrane, and incubate at 35°C for 24 hours.

After filtering your samples for fecal coliform and *E. coli*, immediately label the dish with a descriptive label, such as "8-A-Disinfect, 10/16/18, 50 mL." Arrange for at least one person from your group to come to the lab 24 ± 2 hrs to count and record the number of total colonies (total coliforms) and the number of blue colonies (*E. coli* colonies). Record this data in a Spreadsheet, which you should post in the Lab 7 Google Folder. Make sure you also record the volume of water filtered. ***Not posting this data by the end of the day on Wednesday 10/9 will result in a 10 pt reduction on the lab assignment for all members of your group.***

Part IV. Enumeration of *E. coli* and Total Coliforms

After 24 hours of incubation, you will need to return to read your plates and count the colonies that have formed. Follow these steps:

1. Put on all safety gear including a lab coat, gloves, and safety classes.
2. After incubation for 24 ± 2 hours, remove the plates from the incubator.
3. Since you incubated them upside down, turn them over to view the membrane filter more easily. You shouldn't need to open the plates, so keep the lids closed.
4. If it is helpful, you can view the plates under the magnifying glass that is in the lab.
5. You should see some blue or red dots on your plate if you had any *E. coli* or total coliforms in your sample. Figure 1 shows a plate with both blue and red colonies, as you can see they are small dots on the membranes.
6. The blue dots are *E. coli* while the red dots are other coliforms. The total coliforms are the sum of *E. coli* and other coliforms. For this plate, there are four blue dots and two red dots. So, there are four *E. coli* and two coliforms. The total coliforms would be 6 (*E. coli* plus the other coliforms). If you do not see any colonies, leave in the incubator for another 24 hours and try again. In some cases, only part of the colony has color, often the center. If any part of the colony is blue, count it as *E. coli* and if any part is red, count it as a coliform. Count any blue to purple colonies as *E. coli*.
7. After you have completed your counting, discard the plates and your gloves in the biohazard cans. Be sure to wash your hands with soap and water and the hand sanitizer before leaving lab.

8. Add your data to the spreadsheet file that you posted on the Google Drive during lab. ***Not posting this data by the end of the day on Wednesday 10/9 will result in a 10 pt reduction on the lab assignment for all members of your group.***

9. Typically, we express the concentration of microbes as # of colony forming units (CFUs) per 100 mL of sample. In this sample, we filtered 5 mL through the membrane; therefore, the *E. coli* concentration in CFU per 100 mL would be

   E. coli concentration = $\frac{4\ CFUs}{5\ mL} * 100 = 80$ CFU/100 mL

   Total coliform concentration = $\frac{6\ CFUs}{5\ mL} * 100 = 120$ CFU/100 mL

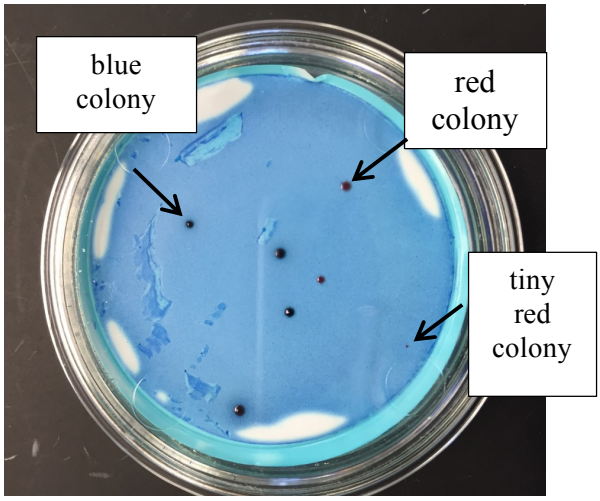

Figure 1. Picture of membrane filter plate after incubation showing colonies.

Before you leave lab:

Upload an Excel spreadsheet (to the Google Drive Folder for Lab 8) with the turbidity data you collected. After you count coliforms and E. coli, you will add that data (including the volume of water filtered) to the spreadsheet. Make sure that your spreadsheets have descriptive files names that include all of your names.

Deliverables:

This will be a group lab memo. Items/analysis to include:

1. Describe the lab testing that you did and how it replicates the processes in a full-scale DWTP.

2. Describe (concisely) the results from your testing. Include a graph showing settled turbidity (dependent variable) versus coagulant dose (independent variable). Use error bars to represent the variability of the results, and make sure to note what the error bars represent (i.e., ± standard deviation). Recommend an optimum alum dose to achieve the lowest turbidity. Your choice must be justified by your data and your interpretation of it.

3. Calculate the log reduction in total coliforms and in *E. coli* for each step in the treatment process, relative to the raw water. Present your results in a bar graph that shows both the concentration for each step (raw, settled, disinfected) and the log reductions. Log reduction is defined as:

$$log\ reduction = log\left(\frac{'initial'\ colony\ count}{'final'\ colony\ count}\right)$$

   The 'initial' colony count is just the value you measured before a treatment step and the 'final' is after the particular treatment step. That way, the log reduction for a given treatment can be calculated. For samples with zero colony counts, calculate log reduction as "N/A". Note: CFU = colony forming units. To compare across samples, you must normalize your colony counts to the volume of sample passed through the filter, and convert to per 100 mL. To present the variability of this data, you will need to use data from other teams.