

Operation Water Biology

Lesson One

Chlorination and Dechlorination



The purpose of a water treatment plant is to take raw water from a well or fresh water source, remove all of the **contaminants** and make the water safe to drink. The contaminants that should be removed include bacteria, ammonia, phosphorus, nitrogen, dissolved organic material, iron, manganese, arsenic and many more. Health Canada is a government organization which sets guidelines on the maximum level of different contaminants that can be present in the treated drinking water. As long as the treatment plant takes out enough contaminants to meet these guidelines, the water is assumed to be safe to drink. There are guidelines for levels of iron, arsenic and nitrate because these things may have direct aesthetic or health consequences, as we will discuss later. In Canada, there are no guidelines for ammonia or phosphorus because it is not usually dangerous to drink water with naturally occurring levels of these chemicals. The guidelines were set by looking only at the direct effect each contaminant could have on someone drinking the water. We now have more information about the way that some contaminants interact with each other and other consequences of their presence. If you look at the whole picture it seems that the contaminants that do not have guidelines should have them and the guidelines that are already in place are not strict enough. The experiments and lessons in this kit will use iron, ammonia and chlorine as examples to explain why this is a problem and show how it can be solved.

In this lesson we will begin talking about chlorine. When most people think about chlorine the first thing that comes to mind is the smell of swimming pools. Although you probably cannot smell the chlorine in your tap water it is added to most municipal drinking water supplies as part of the treatment process.

Much like in a swimming pool, chlorine is added to tap water to **disinfect** it. This is usually done as the last stage of water treatment after the water has been filtered in other ways. Chlorine can kill microorganisms like bacteria and viruses which could cause a wide range of diseases and health problems. Chlorine also reacts with, and removes, dissolved chemicals such as ammonia. Some of the chlorine gets used up every time it removes a contaminant from the water and these reactions produce small amounts of other chemicals called **chlorine byproducts**. These byproducts can sometimes be dangerous if they build up in large amounts. This can happen if a lot of chlorine is added to water that has not been filtered well and still has a lot of contaminants in it.

More information on Drinking Water Quality Guidelines can be found at www.safewater.org/fact-sheets-1/2017/1/23/purposeguidelinesregulations

More information on chlorination can be found at www.safewater.org/fact-sheets-1/2017/1/23/what-is-chlorination

Chlorine is useful to treat water because it reacts with so many different things and killing microorganisms is very important but if you want to study chemical reactions in water the chlorine there can sometimes get in the way. In a future lesson we will want to use some water that does not have any chlorine in it so today we will begin the process of taking the chlorine out of some tap water. This process is called dechlorination.

This experiment will show how granular activated carbon (GAC) can remove gases such as chlorine from water. The GAC has a very large surface area and is full of tiny holes. This allows it to grab and hold gases in the same way that a sponge holds liquids.

Materials needed for this experiment are:

- One total chlorine test strip
- Plastic cup
- 2mL of Granular Activated Carbon (GAC)
- Empty 5mL vial
- Empty, well rinsed, 2L plastic bottle
- Watch or timer
- Tape and a marker

1. To know how much chlorine gets taken out of your water by the GAC you will need to know how much is in it at the beginning. To find this, fill a cup about halfway with tap water then follow the instructions in the total chlorine concentration test procedure from the test procedure sheet. Be sure to keep the cup. You can rinse it out and use it again in the next few experiments.
2. Using the 5mL vial, measure out 2mL of GAC. When pouring GAC from one container to another you should do it over a clean piece of white paper so that any you spill can be poured back into its original container.
3. Put your 2mL of GAC into the empty 2L plastic bottle and then fill it up with tap water and put on the cap. The 5mL vial needs to be rinsed out right away to get the dust from the GAC out of it. This vial will be used in future lessons so it must be clean.
4. Swirl the water around so that the GAC spreads throughout the water. The GAC will settle to the bottom but you want it to be exposed to as much of the water as possible so hold the bottle upside down for a few seconds so that the GAC falls through the water then turn it right side up so it falls through again.
5. Label your bottle so it does not get mixed up with those of other groups.
6. You will open the bottle and test the chlorine concentration again in a few days after the GAC has had enough time to absorb the chlorine.

Total chlorine concentration of tap water

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Lesson Two

Chlorine



Since chlorine gets used up when removing contaminants from water, the more contaminants there are the more chlorine is needed to get rid of them. The total amount of chlorine that must be added to water to fully disinfect it is known as that water's **chlorine demand**. For good sources of water the chlorine demand is low and the water treatment facility only has to add a small amount of chlorine to disinfect the water and make it safe to drink. Some extra chlorine is usually added to make sure that no bacteria can grow in the pipes and distribution system and that the water is still clean when it comes out of your tap. In fact, unlike most of the other water quality guidelines which state the maximum recommended amount of a contaminant in your drinking water, the guideline for chlorine is the minimum allowed amount. Any time that all of the chlorine gets used up before the water comes out of the tap there is a risk that bacteria could begin to grow in the water pipes. The current guideline for total chlorine from the Guidelines for Canadian Drink Water Quality is a minimum concentration of 0.5mg/L. Did your tap water meet this requirement when you tested it in the previous lesson?

More information on chlorination can be found at www.safewater.org/fact-sheets-1/2017/1/23/what-is-chlorination

It is easy to show the effect that a contaminant can have on the total chlorine concentration of a sample of water. In this experiment you will see that the chlorine demand created by a little bit of tea is enough to use up all of the chlorine in a water sample.

Materials needed for this experiment are:

- Two total chlorine test strips
- Two plastic cups
- Tea bag
- Empty 5mL vial
- Watch or timer

1. Use the 5mL vial to collect 5mL of the chlorine solution that your teacher has prepared.
2. Pour this sample into a cup then fill that cup about 3/4 of the way up with tap water to **dilute** the solution. Swirl the water around a little bit to make sure it is well mixed.

3. Fill the second cup about 1/4 of the way up with this diluted chlorine solution. The first cup should still be about half full; set it aside for now and perform a total chlorine concentration test on the sample in the second cup.
4. Once you have the result from that test you should empty and rinse the second cup.
5. Dip the tea bag into the first cup two or three times until the water just begins to turn colour. Do not let the tea bag sit in the water. If the water changes colour too much it could affect the colour of the test strip when you do the test. The tea bag can be thrown away or composted.
6. Do a total chlorine concentration test on the sample that you dipped the tea bag into. When you have the results from the test all the water can be disposed of. The cups should be rinsed and kept.

Initial total chlorine
concentration of chlorine
solution

Total chlorine
concentration of solution
after tea bag exposure

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Lesson Three

Ammonia and Chloramine Part One



One of the most important chemicals to the water treatment process is ammonia. The chemical formula for ammonia is NH_3 . Ammonia is something that many water treatment facilities deal with in one way or another. It is common for ground and surface water sources to contain ammonia because ammonia can come from so many sources. Ammonia can be added to soil by nitrogen-fixing bacteria as part of the nitrogen cycle, decay of plants and animals or agricultural and industrial processes. Ammonia is highly soluble so it gets dissolved and transported by surrounding ground water.

More information on the nitrogen cycle can be found at <http://chemistry.about.com/od/geochemistry/ss/nitrogencycle.htm>

In the areas that do have ammonia in their raw water it is a very problematic source of chlorine demand. For each milligram of ammonia in the water it takes 10-15 mg of chlorine to react with it and get rid of it. The reaction between ammonia and chlorine is much faster than the rate that chlorine kills bacteria so you cannot use chlorine to disinfect water that contains ammonia. Unfortunately the most widely used method of removing ammonia is to add chlorine. In a process called "break-point chlorination" chlorine is continuously added to water until all of the ammonia and bacteria have been removed, or in other words, until the chlorine demand has been met.

This works if there is only a little bit of ammonia but if there is more than 0.3 mg/L ammonia in the raw water then so much chlorine would have to be added to get rid of it that it would result in dangerous levels of **chlorination byproducts**. You can see that there are cases where the only options seem to be, a) not using break-point chlorination and thus leaving bacteria in the water, or b) disinfecting the water at the risk of adding harmful amounts of chemicals to it. This means that some treatment facilities have to use very complicated and expensive methods, which often still involve the use of other chemicals, to take ammonia out of the water before they add chlorine.

More information on disinfection byproducts can be found at <http://www.lenntech.com/processes/disinfection/byproducts/disinfection-byproducts.htm>



One new option that communities with ammonia problems have is **biological filtration**. This is a safe, chemical free, method of removing ammonia. In a biological filtration facility one of the stages of filtration is to pass the water through a special filter that is full of nitrifying bacteria. These bacteria take in the ammonia and some oxygen and perform a **bio-oxidation** reaction. They **oxidize** the ammonia into nitrite, $\text{NH}_3 + \text{O}_2 \rightarrow \text{NO}_2^- + 3\text{H}^+$. Then further oxidize that into nitrate, $\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 2\text{H}^+$. The bacteria gain energy from these reactions and are specialized to do them very efficiently. This process is part of the natural nitrogen cycle and does not produce any harmful byproducts. The nitrate that is produced by this process can easily be removed from the water by the reverse osmosis membrane in the final stage of the filtration process.

More information on biological filtration facilities can be found at www.safedrinkingwaterteam.org/ibrom.html

The reaction between chlorine and ammonia can be written as $\text{NH}_3 + \text{HOCl} \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O}$. In this chemical equation NH_3 is ammonia and HOCl is hypochlorous acid which is formed when the chlorine first dissolves in the water. The primary result of this chemical reaction is NH_2Cl , a chemical known as **chloramine**. Chloramine is a disinfectant like chlorine, it is a weaker disinfectant than chlorine but it lasts much longer in water. The chlorine concentration in water can gradually decrease as the chlorine evaporates out but chloramine does not do this. This makes it useful for making sure water stays disinfected throughout drinking water distribution systems. In areas where there is no, or very little, ammonia in the raw water treatment facilities might still want to use chloramine for this purpose. After chlorinating (disinfecting) the water, as the last step in the treatment process they add ammonia and more chlorine to the water so that they react and create chloramine.

With this information, you may be wondering if there is chloramine in your own tap water and how you might be able to measure it. The fact of the matter is, part of the process of finding chloramine concentration is the **total chlorine** concentration test that you have already done. The other part is a second kind of test which is called a **free chlorine** test. Understanding the difference between free and total chlorine is very important. The free chlorine test finds the concentration of regular unreacted chlorine like the kind added to water during the chlorination process or the kind found in chlorine bleach. The total chlorine concentration test finds the combined concentrations of the regular unreacted chlorine and the chlorine that has been in a reaction and is now chloramine. Since total chlorine is free chlorine plus chloramine, the total chlorine concentration must always be greater than or equal to the free chlorine concentration. This also means that the total and free chlorine tests can be used together to find the chloramine concentration of a water sample. Subtracting the free chlorine concentration from the total chlorine concentration will give you the chloramine concentration.

Example
If you test a water sample for both free and total chlorine and get values of 2.0 mg/L free chlorine and 2.5 mg/L total chlorine then you know that the concentration of chloramine in that sample must be 0.5 mg/L.

Consider an experiment that could put this information to use. Imagine two beakers, each with exactly 100mL of water. The first has a free chlorine concentration of 20mg/L, a total chlorine concentration of 20mg/L and an ammonia concentration of 0mg/L. The second beaker has both free and total chlorine concentrations of 0mg/L and an ammonia concentration of 4mg/L. What do you think would happen if the contents of these two beakers were poured together into a larger beaker?

This problem can be broken into smaller pieces that should be considered one at a time.

1. What is the chloramine concentration in each of the two original beakers?
2. Pouring the beakers together will result in a total volume of 200mL; this volume change could **dilute** the chemicals. Might this have an effect on the concentrations of chlorine and ammonia before any chemical reaction even occurs? Consider the result of two 100mL beakers being poured together if one had 20mg/L of salt and the other had 4mg/L of sugar.
3. Assuming that a free chlorine concentration of 10mg/L is exactly enough to react with 1mg/L ammonia, what should the final free chlorine and ammonia concentrations be?
4. If all of the free chlorine used in the reaction becomes chloramine what should the final chloramine and total chlorine concentrations be?

Ammonia and Chloramine Part Two



In this experiment you will combine an ammonia solution with a chlorine solution in the same way that was described in Lesson 3. You will test the two samples separately to find their individual ammonia, free chlorine and total chlorine concentrations. 100mL of each will then be poured together to see if a reaction resulting in the production of chloramine really takes place.

To make sure that there are no extra, unwanted reactions taking place and to control as many variables as possible, the solutions will be mixed using some of your dechlorinated water.

Materials needed for this experiment are:

- Four total chlorine test strips and three free chlorine test strips
- Three ammonia test strips
- Ammonia colour matching card/chart and square test vial or disposable beaker if you have the new version of the ammonia test.
- Two plastic cups
- Two 250mL beakers
- Empty 5mL vial
- 5mL ammonia solution vial
- Coffee filter
- Dechlorination bottle prepared in Lesson One
- Watch or timer
- Tape and marker
- Stir stick

First you can find out how much chlorine the GAC was able to remove from your tap water.

1. You will be using a coffee filter to separate the GAC out of the water. The easiest way to do this is by folding the filter into a cone. Begin by folding the filter in half and then half again so it is in the shape shown in Figure 1 to the right. Looking at the filter from above, you should see four edges of the filter like in Figure 2. You can pull three of these edges in one direction and the forth in the other direction to open the filter into a cone shape.
2. Hold the coffee filter in place over a plastic cup. Open the dechlorination bottle and slowly pour some of the water through the filter until the cup is about half full. This filter will be used again in step 5.

Figure 1



Figure 2



3. Perform a total chlorine concentration test on this sample.
4. Find the difference between the chlorine concentrations in the regular tap water that you tested in the first lesson and this water that has been exposed to the GAC.

Now begins the ammonia / chloramine experiment

5. Fold your coffee filter into a cone and hold it in place over one of the beakers.
6. Slowly pour water from your dechlorination bottle through the coffee filter until the beaker is full to the 250mL mark. Move the filter to the second beaker and fill it to 250mL as well.
7. Label one beaker as "Ammonia" and the other as "Chlorine".
8. Pour the contents of the 5mL ammonia vial into the ammonia beaker.
9. Swirl the beaker so that the ammonia solution will be well mixed. If you want to use an object to stir the solution use a clean stir stick, not a pen, pencil, finger or anything else.
10. Perform the Ammonia Test on this sample by following the provided procedure. You must wait 5 minutes before reading the result of the Ammonia Test so go on to step 11, the time should be up when you are finished the other tests.
11. Pour about 50mL of your diluted ammonia solution into a cup and perform a total chlorine concentration test. Empty and rinse the cup, pour another 50mL of your diluted ammonia solution sample into the same cup and do the Free Chlorine Test. Empty and rinse the cup again.
12. Use the 5mL vial (not the one that the ammonia came in) to get 5mL of the chlorine solution that has been prepared by the teacher.
13. Pour this sample into the chlorine beaker and swirl it to mix the solution. If you want to use an object to stir the solution use a clean stir stick, not a pen, pencil, finger or anything else.
14. Do all three tests on this chlorine sample in the same way you did for the ammonia sample. Use the second cup for the chlorine tests of this sample.
15. Pour some of the water out of the ammonia beaker until you have 100mL left in the beaker. You may want to pour the water into a cup so that if you pour too much you can put some back.

Total Chlorine
concentration of tap water
after exposure to GAC

Amount that the GAC
lowered the tap water's
total chlorine concentration

All results from these tests
should be recorded in the
space provided on page 4.

16. Pour some of the water out of the chlorine beaker until you have 100mL left. You may want to pour it into the other cup so that if you pour too much you can put some back.
17. Pour your 100mL chlorine solution into the ammonia beaker so that there is 200mL in this beaker. Swirl this solution a bit to mix it, if you want to use an object to stir the solution use a clean stir stick, not a pen, pencil, finger or anything else.
18. Do all three of the tests on this mixed sample. Either of the cups can be used for the chlorine tests.

All results from these tests should be recorded in the space provided on page 4.

1. When you have all nine of your test results calculate the chloramine concentrations of each of the three solutions tested. Remember that you can do this by subtracting the free chlorine concentration from the total chlorine concentration. Record these values in the spaces provided.
2. Calculate the concentrations of all four chemicals that would have been in the mixed solution after pouring together the chlorine and ammonia solutions but before the chemical reaction started (just like the salt and sugar example). The differences between these calculated values and the actual concentrations you found experimentally are due to the chemical reaction.
3.
 - a. Did the chemical reaction appear to change the concentration of all four of the chemicals you tested for?
 - b. Did any of your calculated concentrations from question 2 match, or almost match, the actual experimental results? If so, does this mean that these things were not involved in the chemical reaction? How might you explain this?
 - c. Were any of your calculated concentrations from question 2 very different from the actual experimental results? What does this mean?
4. In the Lesson 3 questions we made two assumptions. First we assumed that a free chlorine concentration of 10mg/L is exactly enough to react with 1mg/L ammonia and secondly we assumed that all of the free chlorine used in the reaction becomes chloramine. Do you think your experimental results confirm these assumptions? Why or why not?

Lesson Four Results Sheet

Ammonia Solution	Chlorine Solution	Mixed Solution
Ammonia Concentration <input data-bbox="276 575 529 697" type="text"/>	Ammonia Concentration <input data-bbox="685 575 938 697" type="text"/>	Ammonia Concentration <input data-bbox="1094 575 1347 697" type="text"/>
Total Chlorine Concentration <input data-bbox="276 863 529 984" type="text"/>	Total Chlorine Concentration <input data-bbox="685 863 938 984" type="text"/>	Total Chlorine Concentration <input data-bbox="1094 863 1347 984" type="text"/>
Free Chlorine Concentration <input data-bbox="276 1152 529 1274" type="text"/>	Free Chlorine Concentration <input data-bbox="685 1152 938 1274" type="text"/>	Free Chlorine Concentration <input data-bbox="1094 1152 1347 1274" type="text"/>
Chloramine Concentration <input data-bbox="276 1440 529 1562" type="text"/>	Chloramine Concentration <input data-bbox="685 1440 938 1562" type="text"/>	Chloramine Concentration <input data-bbox="1094 1440 1347 1562" type="text"/>

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Lesson Five

Consequences of Inadequate Drinking Water Treatment



Discovering if a community has a problem with ammonia is as simple as doing a single test on a sample of tap water but since there is no guideline for ammonia in the Guidelines for Canadian Drinking Water Quality this test is rarely done. People in these communities often do not know that their water is not being properly disinfected. In this lesson your class will perform ammonia tests on the water samples that you have collected.

Your class might find ammonia in some of the water samples that you have collected and tested during this lesson. Recall from Lesson Three when you learned that some water treatment facilities add ammonia to the water. This is done after the water has been disinfected so that they can use it to create chloramine which will keep the water disinfected throughout the distribution system. In these cases the water is properly disinfected even though an ammonia test might show that there is still ammonia in the water. This practice is usually only used by water treatment facilities serving very large distribution systems and is not common in rural communities that have small facilities. If you do find ammonia in these samples you should investigate whether the facility that treated that water added the ammonia after disinfection or if the ammonia was in the raw water. Only if the ammonia was in the raw water is there cause to be concerned that the water was not disinfected. This means that there could be disease-causing micro-organisms in that water. When a situation like this is discovered a boil water advisory is usually put in effect.

Location that this water sample was collected

Ammonia concentration of the water sample

More information on drinking water advisories in Canada can be found at <https://www.ec.gc.ca/indicateurs-indicators/default.asp?lang=en&n=2C75C17A-1>

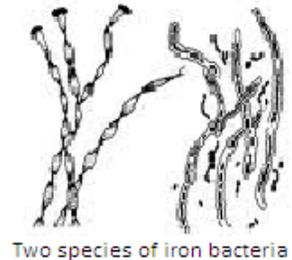
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Lesson Six

Removing Iron from Drinking Water



Iron is an essential element for humans. People usually get all the iron they need from the food they eat so extra iron in water is not necessary for good health. The guideline for iron in the Guidelines for Canadian Drinking Water Quality is 0.3mg/L. This guideline is in place primarily for aesthetic reasons since iron discolours water and promotes bacterial growth. It is not a health-based guideline because iron, and the kinds of bacteria that grow around iron, are not usually harmful to peoples' health. These **iron bacteria** do cause many other problems though. When iron is present in water, bacteria that use iron as their energy source will grow and build up anywhere that water flows. These bacteria form thick layers called biofilm in reservoirs and pipes, causing a lot of damage to distribution systems that is difficult and expensive to repair. Excessive bacterial activity also results in water with an unpleasant taste and smell which makes it bad for both cooking and washing. These bacteria can still grow in water that has less iron than the 0.3mg/L guideline. If there is any iron at all that these bacteria can use for energy then they will grow and spread. To keep iron bacteria out of drinking water all of the iron must be removed, not just enough to meet the guideline.



Two species of iron bacteria

Take a look at the tubes of raw well water that are included in the kit. You will see that the iron in this water has settled to the bottom of the tubes, you should see many very small orange particles there. If you shake up one of the tubes and look very closely you can see the tiny orange particles floating all around in the water. These particles are visible because this iron is insoluble in water. This is not always the case though, when this water first comes out of the well there would not be any tiny orange particles visible in it. This was because of a difference between the two chemical states of iron. Iron can either be in a **reduced** state or an **oxidized** state (also known as rust). One of the biggest deciding factors of which state iron is in is exposure to oxygen. When the iron is deep in a well it is not exposed to oxygen so it will be in its reduced state. This reduced iron is soluble in water so it dissolves and you can not see it even though it is still there. When the water is brought up from the well and is exposed to oxygen it becomes oxidized. This is when the iron separates from the water because it is no longer soluble.

Making sure that all of the iron in the water is oxidized is an important step in filtering it out. This is because when the iron is still reduced it is dissolved in the water and can pass right through filters along with the water. To filter out the iron it must first be oxidized so that the particles separate from the water and then can be picked up by the filter. There are a few different methods that treatment facilities use to oxidize iron and other contaminants. The simplest is to let the water sit in a large open pool called a reservoir so that it is exposed to the oxygen in the air, most of the iron will eventually oxidize and settle to the bottom but this process is slow and often leaves some reduced iron in the water. Another option is to use oxidizing chemicals; these can be added to the water to oxidize all of the iron more quickly and efficiently than the reservoirs. These processes create water like the raw well water that you saw in the tubes with many very small iron particles floating around. Even though the iron is no longer dissolved in the water it is still very difficult to filter out because the particles are so small. This iron can pass right through most regular filters and when very fine filters that can catch particles of that size are used they clog up very quickly and need to be constantly cleaned and replaced. The usual solution to this is to add another chemical called a flocculent. The flocculent makes the little particles stick together in clumps which are called **floc**. This floc is easier to filter out because its larger size makes it easier to catch.

A different option for filtering iron is to use a biological process like the one for ammonia that was discussed earlier. Rather than putting chemicals in it, the water can be passed through a filter containing bacteria that do an even better job of oxidizing all of the iron. These are actually the very same iron bacteria as we mentioned at the beginning of this handout, we may want to keep them out of the water pipes but we can still put them to work for us in our filters. Iron bacteria take in reduced iron and oxygen and perform a bio-oxidization reaction on them which produces oxidized iron. These iron bacteria gain energy from performing this reaction and they are specialized to do it very effectively. There is a second benefit to using these bacteria as well, as they are oxidizing the iron they are also making a little bit of sticky gel which automatically forms a floc with the iron. This allows the bacteria to do the job of both the oxidizing and flocculating chemicals at once. During the experiment for this lesson you will see an iron floc formed by iron bacteria in the other tubes included in the kit. These bio-oxidized water samples were taken from real **biological filters** in a water treatment facility where iron bacteria oxidized the iron and formed the floc. Once this floc is formed it gets caught in the filter and the water passes through iron free. There is no longer an energy source in this water for any other iron bacteria to use so they can not grow and form biofilm and thus all of the problems associated with iron in water are avoided.

More information on biological water treatment can be found at www.safedrinkingwaterteam.org/ibrom.html

In this experiment you will be able to see many of the things discussed in this lesson for yourself. You will filter water samples that have been oxidized in different ways and see if they give different results.

Materials needed for this experiment are:

- Three small plastic beakers
- Two 250mL beakers
- Plastic cup
- Raw well water sample
- Bio-oxidized water sample
- Two coffee filters
- Four aluminum packets of iron test reagent
- Marker
- Scissors
- Stir stick

1. Label one of the small plastic beakers “raw unfiltered” then shake up the raw well water sample, open it and pour 10mL into that beaker. Set this beaker aside for now.
2. Fold your coffee filter into a cone just like in Lesson Four and hold it in place over the cup.
3. Slowly pour the rest of the raw well water sample through the filter into the cup.
4. Label one of the small plastic beakers “raw filtered” then pour 10mL of filtered raw well water from the cup into that beaker. This beaker should also be set aside for now. Any water still in the cup can be disposed of.
5. Put 100mL of tap water into one of the large beakers.
6. Very gently and slowly turn the tube of bio-oxidized sample upside down. If the floc is stuck to the bottom of the tube turn it over a few times until all of the floc is knocked loose, you want all of it to come out when you pour the water out. You must do this carefully; you do not want to break the floc apart too much.
7. Open the tube of bio-oxidized sample and very carefully pour it into the beaker with 100mL of water (not through a filter). Try to hold the tube close to the surface of the water when pouring because the floc might break apart when it hits the water if it falls too far.
8. Fold your second coffee filter into a cone and hold it in place over the second large beaker.
9. Very slowly pour the sample you just mixed in step 7 through the coffee filter. Try to hold the beaker close to the filter when pouring so that the water does not fall as far.
10. Label the third small plastic beaker “filtered bio-oxidized” and pour 10mL of the filtered bio-oxidized sample into that beaker.

Once everyone has prepared their three samples the teacher will prepare the unfiltered bio-oxidized samples and give one to your group.

11. You can now begin observing and comparing the different samples.

The pairs of samples that you should compare are:

- a. Unfiltered raw water to unfiltered bio-oxidized
- b. Unfiltered raw water to filtered raw water
- c. Unfiltered bio-oxidized to filtered bio-oxidized
- d. Filtered raw water to filtered bio-oxidized

On a piece of paper write down the names of the two samples you are comparing and then write down your observations in a sentence or two. Leave lots of space to add things later then put the names of the next pair of samples and continue.

Examine them closely, does there seem to be differences between them? Are there different sizes or number of particles visible? Does one look like it has more or less iron than the other?

The aluminum packets contain a reagent that is used to indicate the presence of iron. When it is added to a water sample it will turn pinkish red if there is iron in it. The darker the colour gets the more iron there is. This is very useful for comparing samples, it can be put into a number of samples and you will know that the one that turns the darkest shade of red has the most iron or if some turn the same colour you will know that they have the same iron concentration.

12. With scissors very carefully cut open the tops of your four packets and pour one of the packets into each of your four samples. Use a stir stick to mix the powder into the water samples. You must wait at least three minutes for the reaction to finish but once the colour changes there is no hurry, the samples will stay that colour for a long time.

13. When the three minutes are up compare the same four pairs of samples again. Which sample really had more iron? Judging by how different the colours of the samples are does the difference in iron concentration seem to be large or small? Write down your observations in a sentence or two in the spaces that you left under your first observations.