Learn How to Disinfect Contaminated Water

https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p025/microbiology/disinfect-contaminated-water (http://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p025/microbiology/disinfect-contaminated-water)

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Experimental Procedure

Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: Project Involving Potentially Hazardous Biological Agents (http://www.sciencebuddies.org/science-fair-projects/competitions/biological-agents-regulations) and Scientific Review Committee (http://www.sciencebuddies.org/science-fair-projects/competitions/scientific-review-committee-src). You can also visit the webpage ISEF Rules & Guidelines (https://www.societyforscience.org/isef/international-rules/) directly.

Caution: Do not drink any of the water during or after you have completed the experiment, or you might risk getting sick. The SODIS process is a disinfection process and not a sterilization process. It also doesn't remove chemicals from the water. The resulting water may not fulfill EPA standards (https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations) for drinking water.

Note: There will be many steps going on at the same time with this experiment, so be sure to read through the procedure carefully beforehand, and keep careful track of samples, upcoming steps, dates, and times as you perform the experiment.

Preparing the Setup

1. Go to your local creek or stream with the clean, plastic 1-gal jug and a pair of disposable gloves. Place the jug into the water and fill it up. Avoid trapping any large particles or foreign objects in the jug. Once the jug is full, replace the cap. Take the jug back to where you are conducting your testing.
2. Now put the clamp-lamp and the Daylight Blue Reptile Bulb together, following all instructions that came with the lamp. Find a quiet location near an electrical outlet. Clip the lamp onto something, such as the bottom of a cabinet door over a counter, allowing the lamp to face downward. You could also clip the lamp assembly to the top of the rod of a buret stand. Figure 1 shows this configuration with a homemade buret stand.
3. Sterilize the medicine droppers. Sterilization kills all of the bacteria on the tools. Using sterilized tools prevents you from adding bacteria to the samples.
    a. Wash the glass part of the medicine droppers with soap and water.
    b. Dip the outside of the medicine droppers in isopropyl alcohol. Suck up a dropper full of the alcohol with each dropper then squirt it out. Repeat twice.
    c. Let the droppers dry completely before using.

Figure 1. This image shows the experimental setup, with the UV light shining on the test samples (*Note:* the samples in the picture have not been placed on the dark, metal sheet yet, but yours should be.)

Preparing the SODIS Samples

1. Carefully transfer some of the water from the jug into two of the clean 16-oz. clear plastic water bottles. Follow the SODIS procedure:
    a. Fill the bottles ¾ full with the creek or stream water and screw on the lid.
    b. Shake the bottles for 20 seconds each in order to aerate them.
    c. Now fill the bottles fully and screw on the lids tightly.
    d. Keep the rest of the water in the jug in a cool, dark place.
2. When the sample bottles are prepared, plug the lamp into the outlet and turn on the lamp. The lamp should be about 6 inches away from the counter, or from the bottom of the buret stand. Place both samples onto the dark metal sheet beneath the lamp, directly in the path of the light, to mimic the SODIS process as much as possible. Based on your background research, what does the metal sheet represent, and why it is important? Leave the light shining on the samples. Do not disturb the light or the samples until you are ready to test their bacterial content, 12 hours later.
    a. *Note:* When the SODIS process is applied in a real situation, the minimum time that the bottle of water sits in direct sunlight is 6 hours (longer if the water is very turbid). In this science fair project, you are using the lamp as a substitute for the sun. Since the lamp doesn't produce the same amount of UVA or heat as the Sun does, you will need to keep your bottles of creek or stream water under the lamp for longer than 6 hours.

    In your lab notebook, note the time on the clock and the date on which you placed the samples under the lamp. Warn others in your household to be careful around the lamp and bottles on the counter.

Preparing and Testing the Boiling and Untreated Water Samples

Note: It is important that you observe the following agar plates at the same time. You will apply the boiled water and untreated water samples to the agar plates while the SODIS samples are still under the UV lamp. You must keep track of the time when you start and stop tests so that the observations can be compared.

1. While the SODIS samples continue resting under the UV light, measure 1 cup of the creek or stream water in the liquid measuring cup and pour it into the 1-qt. pot. Place the pot onto the stove top and bring the water to a rolling boil. Boil the sample for 5 minutes. Remove the pot from the burner, cover the pot, and let the water cool to room

temperature.
2. Put on a pair of disposable gloves. Get out three nutrient agar plates. Suck some of the boiled (but now room-temperature) water into a sterilized medicine dropper.
3. Remove the cover of one of the plates. Apply three drops of the boiled water to the soy agar. Use a sterile cotton swab to smear the water drops in a zigzag pattern on the surface of the nutrient agar, starting in the center—smear the water sample from the center of the plate to the edge of the plate. Replace the cover.
4. Repeat steps 2–3 with the two other nutrient agar plates. Reuse the same medicine dropper. Using the permanent marker, note down the time, the date, and the treatment process on the bottom of the plates (in this set of trials, it is *boiling;* for the next set of trials it will be *untreated*). Set aside the medicine dropper to sterilize again for future use.
5. Keep your nutrient agar plates in a warm location in your house that will not be disturbed.
6. Repeat steps 2–5 with untreated water from the 1-gal jug. Use a freshly sterilized medical dropper for the untreated water sample. You can reuse the same dropper for all three agar plates.
7. Let the boiled water and untreated water plates (6 in total) sit undisturbed for 24 hours. Do you observe any growth on the plates? Record the time, date, and your observations in your lab notebook. Check again in, 48 hours, 72 hours, and 96 hours. Record your observations each time you check. If you see any growth, count the number of bacterial colonies and record the number in your lab notebook.

Testing the SODIS Samples

1. In the meantime, you will need to also keep track of the time the water samples spend underneath the UV lamp. After 12 hours, remove one of the bottles from underneath the lamp. Repeat steps 2–5 of the previous section with this SODIS sample. Use a freshly sterilized medical dropper. Again, you can reuse the same one for all three agar plates. Then perform step 7 of the previous section, counting the number of bacterial colonies, and recording the time and date in your lab notebook.
2. After 48 hours have elapsed, remove the second SODIS sample from under the lamp. Repeat steps 2–5 and step 7 (all from the previous section) with this sample, using one freshly sterilized medicine dropper for all three agar plates.

Repeating the Experiment

1. Repeat the entire experiment one additional times, with fresh materials. Remember to record all your observations in your lab notebook. Repeating the experiment will help you determine how reliable your data is.
2. As you finish obtaining your sets of data, follow the procedure detailed in Microorganisms Safety Guide (http://www.sciencebuddies.org/science-fair-projects/references/microorganisms-safety) to safely dispose of your agar plates.

Analyzing Your Data

1. Now analyze your data. Plot the data on a scatter plot. For the first plot, label the x-axis *Treatment* and the y-axis *Bacterial Count at 96 Hours.* For the second plot, label the x-axis *Observation Time* and the y-axis *Bacterial Count.* For this second plot, you can plot all of your data on one plot or you can make a plot for each treatment.
2. How does the bacterial count change with each treatment? Is SODIS a viable treatment process? Do bacteria grow at different rates for different treatments?

Bacterial Safety

Bacteria are all around us in our daily lives and the vast majority of them are not harmful. However, for maximum safety, all bacterial cultures should always be treated as potential hazards. This means that proper handling, cleanup, and disposal are necessary. Below are a few important safety reminders.

- Keep your nose and mouth away from tubes, pipettes, or other tools that come in contact with bacterial cultures, in order to avoid ingesting or inhaling any bacteria.
- Make sure to wash your hands thoroughly after handling bacteria.
- **Proper Disposal of Bacterial Cultures**
    - Bacterial cultures, plates, and disposables that are used to manipulate the bacteria should be soaked in a 10% bleach solution (1 part bleach to 9 parts water) for 1–2 hours.
    - Use caution when handling the bleach, as it can ruin your clothes if spilled, and any disinfectant can be harmful if splashed in your eyes.
    - After bleach treatment is completed, these items can be placed in your normal household garbage.
- **Cleaning Your Work Area**
    - At the end of your experiment, use a disinfectant, such as 70% ethanol, a 10% bleach solution, or a commercial antibacterial kitchen/bath cleaning solution, to thoroughly clean any surfaces you have used.
    - Be aware of the possible hazards of disinfectants and use them carefully.