Microscale Gas Chemistry, Part 29.
The Mini-Ozone Generator and Bacteria

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Description.

In 2005 we published an article in the ***Journal of Chemical Education*** describing a microscale ozone generator built from a disposable thin-stemmed transfer pipet.[2] The generator electrochemically splits water into H_2 and O_2 with small amounts of O_3. Typically, the generator produces about 10 mL/minute, of which about 0.25 – 0.40 % of the total gas produced is O_3. This level of ozone represents 800 nanomole of ozone per minute — it would take 18 days of continuous operation to generate one gram of ozone. Despite the small amount of ozone generated, there is enough to cause a variety of interesting oxidations. In our original article,[2] we described the oxidation of food coloring over the course of minutes. In the January, 2007 issue of this ***Journal***, we described ozone's effect on over two dozen household substances ranging from fruit and vegetable juices to rubber bands, moldy cheese, wood fibers and silver.[3] In this article we describe the use of this mini-ozone generator to treat and kill bacteria in natural waters. Specifically, we treated water samples collected from Carter Lake in Omaha, Nebraska and from the Missouri River as it passes by Omaha.

Apparatus.

At the heart of ozone apparatus is a thin-stemmed pipet (Beral pipet) with the stem stretched to form a capillary delivery tube. A graphite and platinum electrode completed the device as shown in the Figure. The pipet is held upright with an optional framework constructed from soda straws and a 96-well plate as the base. Complete details for stretching the pipet and constructing the device are provided in our previous articles in this series[2, 3] as well as at our website.[4] The pipet bulb is filled about 3/4-full with 3 M H_2SO_4.

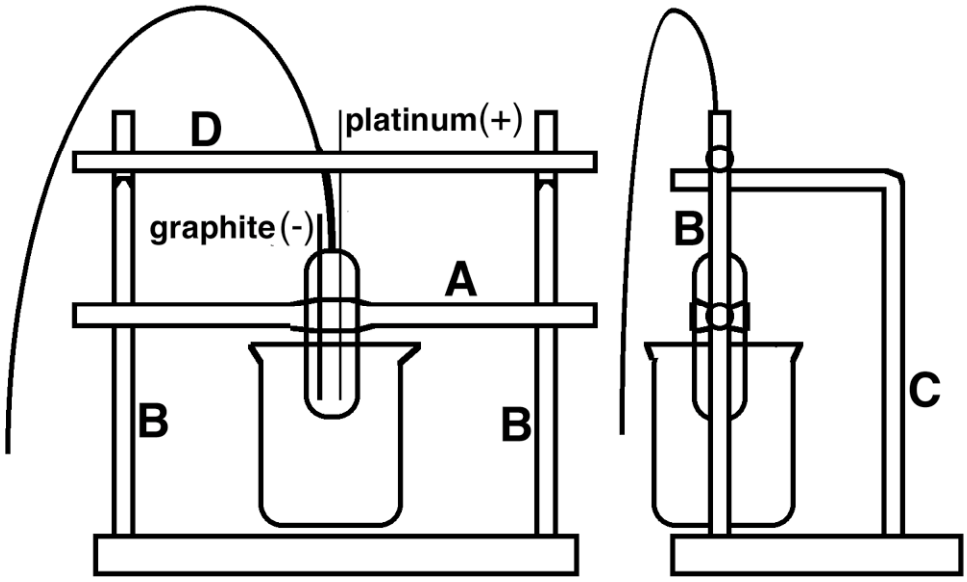

Figure. Left: front view; right: side view.

Ice bath

The beaker shown in the Figure is for an ice bath necessary to keep the system cool. Without the ice bath, the electrodes would heat up and could enlarge the holes through the pipet. If this occurs, the gases will no longer be delivered through the capillary tube but rather will leak out the enlarged hole(s) around the electrode(s). The ice bath should contain more water than ice so that every surface of the pipet bulb is in contact with ice water. The ice bath also improves the yield of ozone.

Power Supply

The power supply is set to deliver 6-volt direct current. The positive wire is connected to the platinum electrode (anode) and the negative wire is connected to the graphite electrode (cathode.) Two wires with alligator clips on both ends are used to connect the power supply to the electrodes. More information about the power supply is provided in our previous article[3] as well as at our website.[4]

Electrolysis of Water.

Oxygen and ozone are generated at the anode (platinum electrode) and hydrogen at the cathode according to the following reactions:

Predominant reaction: $2\ H_2O \longrightarrow O_2 + 2\ H_2$ $\quad E_{tot} = -1.23$ V

Minor reaction: $3\ H_2O \longrightarrow O_3 + 3\ H_2$ $\quad E_{tot} = -1.51$ V

Thus, the gas mixture collected is about 2/3 hydrogen and 1/3 oxygen with trace levels of ozone.

Experiments and Results.

We used three different methods to culture bacteria. All three work well and are described below. The first method is recommended for its simplicity and low cost.

1. Nutrient agar plates.

Nutrient agar plates are prepared and shipped in a sterile sleeve containing ten plates. They are ready to use, but must be refrigerated until used. They have a short shelf-life so they should be ordered shortly before they are needed. We ordered ours from Fisher Scientific S716931A (about $20 for a sleeve of 10 plates.)

We collected two 500 mL samples, one from Carter Lake in Omaha, Nebraska and another from the Missouri River as it passes Omaha. Both samples were collected on 23 August, 2007. The samples were refrigerated overnight, which allowed them to settle. Using a disposable pipet, we carefully transferred about 15 mL of water from each sample, one at a time to a clean 30-mL test tube (15 x 150 mm.) Before we began our treatment with ozone, we transferred 3 mL water sample to a nutrient agar plate. The agar plate was covered and then gently swirled in order to coat the entire surface with the water sample. This sample was labeled "t = 0 min."

The ozone delivery tube was then placed all the way to the bottom of the test tube containing the water sample. Ozone generation commences immediately when the power supply is plugged in. We removed 3 mL samples of water with a disposable pipet after 5, 15 and 30 minutes. As before, the samples were added to fresh agar dishes, covered, and swirled. The entire process was repeated for the second water sample using a new test tube and four more agar plates. All eight samples were incubated at room temperature.

Three days later, the results were clear. Murky, yellow and cream-colored bacteria completely covered the surfaces of the t = 0 min samples from both Carter Lake and the Missouri River. In addition, the t = 5 minutes sample from Carter Lake also was covered with bacteria. All of the other Petri dishes were bacteria-free.

We repeated this experiment using time-lapsed photography. The camera took one picture per hour for 4.5 days. This time, the bacteria grew in clumped colonies in the untreated water and not at all in the sample treated with the ozone stream for 15 minutes. The sequence of photos were assembled into an eighteen second movie in which the bacteria can be seen to grow at about 20,000 times faster than in the actual experiment (1 s = 6 hr.) The movie is available at our website.[4]

2. Nutrient-rich agar media.

We prepared our own nutrient-rich agar media (2.5 g tryptone, 1.25 g yeast, 2.5 g NaCl, 3.75 g agar, and 7.5 mL 0.1 M NaOH in water to make 250 mL.) The solution is poured into Petri dishes and then autoclaved for 1.25 hour. Rich agar media are stored in a refrigerator until needed. Samples from Carter Lake and the Missouri River were treated and prepared as described above. Incubation took place in the refrigerator and colonies of bacteria appeared within a week. Overall, the results were very similar to the Nutrient Agar results, above: We found that colonies of bacteria and coliform formed in great numbers in the natural water samples, but in the samples treated even for a few minutes had far fewer colonies and those treated for 30 minutes or more had no bacteria whatsoever.. Pictures of the bacteria colonies that grew in ozone-untreated samples are shown at our website.[4]

3. Hach Millipore method.

Hach Millipore sells complete coliform bacteria and *E. Coli* analysis kits.[5] The kits are easy to use and give separate results for coliform bacteria which appear as red colonies after incubation and *E. Coli* which appear as blue colonies after incubation. The kit utilizes a Microfil S filtration device (100 mL funnel, sterile, one time use) with white grid filter paper and the use of a prepackaged broth that leads to the two different colors for the coliform and *E. Coli* colonies. Incubation takes place over a 24 hour period at 37 ^{o}C. Detailed instructions are available from Hach/Millipore. The main disadvantage of these kits is that they are considerably more expensive than either of the other two methods described above. Each experiment using this method requires 100 mL of sample. We passed the ozone-containing gas through 100 mL water samples in a 250 mL volumetric flask containing a spinning stir bar for 15, 30 and 45 minutes. After each timed experiment, we must start over with a new 100 mL sample. Experimental results are shown at our website[4]: untreated samples of the Missouri River showed 26 counts of coliform and 4 counts of *E. Coli*. After treating a fresh 100 mL sample for 15 minutes with ozone, followed by analysis as above, the sample showed only 3 counts of coliform and no *E. Coli*. Samples receiving 30 and 45 minute exposures showed neither coliform nor *E Coli*.

Destroying bacteria samples.

All samples cultured on agar plates were treated with household chlorine bleach for 24 hours. The plates were then collected in a sealed plastic bag and placed in the trash. As always, local regulations should be followed.

Hazards and Safety Precautions

Sulfuric acid (3 M) is corrosive and should be handled with care. Eye protection must be used.

Suggested activities.

1. Collect and test water for bacteria from local streams and rivers.
2. Collect and test water from rain run-off. Samples can be collected near downspouts, near residential streets, and so on.
3. Collect and test water from your pet's water bowl.
4. Collect and test water throughout various seasons.
5. Collect and test water from local streams before and after weather events such as heavy rainstorms.
6. Test tap water that is (a) fresh and (b) has been allowed to sit on the counter for a few days.
7. Test bottled water that has been opened for several days.

Teaching tips.

1. If no gas is flowing, the electrodes are probably in contact with each other.
2. Do not switch the wire leads to the electrodes. If the solution becomes suddenly dark, the wires are backwards.
3. Check for the odor of ozone near the mouth of the test tube as evidence that the device is working.
4. The microscale ozone generator can be stored indefinitely. It is not necessary to remove the acid from the pipet bulb.
5. The acid level will drop, but the moles of acid present is unchanged: simply add water when the level is low. Refer to our previous articles (Ref. 2 and 3) for complete details on preparing and maintaining the ozone generator.
6. Using the commercially available nutrient agar plates is inexpensive and easy. Each experiment takes only a few minutes more than the ozone exposure time.
7. Water samples should be fresh. Bacteria die in sealed containers after prolonged periods of time, even with refrigeration.
8. Allow students to propose "studies" similar to those above.
9. Incorporate the use of "controls" in all projects.

Questions

1. Where do coliform bacteria come from in natural waters? *E. Coli*?
2. How does ozone kill bacteria?
3. What other chemicals are commonly used to kill bacteria? What are the pros and cons to ozone and these other chemicals?
4. Using the microscale ozone generator, it takes more than five minutes to completely kill all of the bacteria in the water samples that we tested. Why does it take so long? Are some bacteria simply harder to kill? Or is the ozone the limiting reagent?
5. Use the Internet or your chemistry book to read more about the use of ozone to disinfect water samples.

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Endnotes and References:

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2. "Laboratory Experiments on the Electrochemical Remediation of the Environment, Part 7. Microscale Production of Ozone"; Ibanez, J. G.; Alatorre-Ordaz, A.; Mayen-Mondragon, R.; Moran-Moran, M. T.; Bruce Mattson, Scot Eskestrand; ***Journal of Chemical Education*** 2005, **82**, 1546-1548.
3. "Microscale Gas Chemistry, Part 28. Mini-Ozone generator: 800 nanomole/minute," Bruce Mattson, Janel Michels, Stephanie Gallegos, Jorge G. Ibanez, Alejandro Alatorre-Ordaz, Rodrigo Mayen-Mondragon, M. T. Moran-Moran, ***Chem13 News***, 344; pp 6 – 11; January 2007.
4. Our Microscale Gas website is http://mattson.creighton.edu/Microscale_Gas_Chemistry.html. From there, click "All Gases" and then "Ozone." The bacteria work is all located in "Part 8."
5. Millipore Corporation, website: www.millipore.com. Microfil S filtration device: catalog item MVHAWGS24 and m-ColiBlue24 broth: catalog item M00PMCB24.