

# Chapter 16

## Use of Bioluminescence in Detecting Biohazardous Substances in Water

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## Introduction

The objective of this laboratory exercise is to provide students with a direct method of detecting potentially biohazardous materials in water by observing the reduction of bacterial bioluminescence. This versatile, quick, and easy exercise requires minimal preparation without expensive equipment.

The exercise employs non-pathogenic marine organisms, such as *Vibrio harveyi* or *Vibrio fischeri*, as the indicator of toxicity. It can be presented completely as a demonstration or be conducted entirely by the students. This exercise can be used in several different settings: basic biology, environmental biology, and microbiology laboratories. It can be used either to demonstrate the effects of environmental stresses on bacteria or the use of bacteria to detect and monitor environmental stress.

The intensity and level of the experiment, the amount of quantitation, safety precautions, and waste disposal can be regulated depending on the sophistication and ability of the students. In addition, if inexperienced students are involved, the instructor can utilize dilute solutions or use common household materials that are not extremely toxic to humans such as commercially-available hydrogen peroxide, laundry bleach, ammonia cleaner, vinegar, or bicarbonate of soda.

As students examine the potentially hazardous nature of various chemicals and household products, they develop critical thinking skills and enhance their collaborative working skills. Their analytical, synthesis, and evaluative cognitive skills can be strengthened, thus, allowing the student to move from a concrete operational level to the abstract.

## Materials

*For each group of students (size of group determined by instructor):*

Test tube rack (1)  
 Test tubes, 18 × 150 mm (10)  
 Test tubes, 13 × 100 mm (3)  
 Pipets, 1 ml (8)  
 Pipettor for 1.0 ml pipets (1)

*Preferred, but not required:*

Eppendorf pipet tips, 100 µl (4)  
 Eppendorf pipet tips, 1000 µl (4)  
 Eppendorf pipet, 10–100 µl (1)  
 Eppendorf pipet, 100–1000 µl (1)

*For the entire class:*

Erlenmeyer flask, sterile, 1000 ml, with aluminum foil cap or cotton stopper (1)  
 Saline (1 liter)

Graduated cylinder, 250 ml (1)  
 Pipet, 10 ml (1)  
 Pump/pipettor, 10 ml (1)  
 Incubator (optional), 20–23°C (1)  
 Ethanol, stored in a freezer at –18°C (0°F) (250 ml beaker)  
 Water bath or a beaker of hot water and thermometer, set at 50–55°C (1)  
 Dish pan, large, for discard (1)  
 Vortex mixer (optional) (1)  
 HCl (5 N) or vinegar (100 ml)  
 NaOH (5 N) or bicarbonate of soda (100 ml)  
 Overnight culture (ONC) of bacteria (200 ml in 1000 ml erlenmeyer flask; see Appendix A)  
 Demonstration streak plates (24-hour cultures) (several)  
 Nutrient agar plates (organisms applied with a sterile synthetic fiber swab in the shape of a single letter; incubated for 24 hours; suggested message: “The solution to pollution is dilution.”) (32)  
 Red-light flash lights (several)  
 Very dark room (equivalent to a closet with no windows; plug cracks around the door) (1)  
 Suggested chemicals (to be supplied by students): Windex, ST-37, antifreeze, witch hazel, Scope, Mr. Clean, tincture of iodine, Tilex, Fantastic, Clorox, Lysol, hydrogen peroxide, Liquid Plumber, rubbing alcohol, Listerine, Wisk, Raid, Pine Sol, liquid dish detergent

### Notes for the Instructor

This versatile procedure can be adapted to a variety of laboratories and situations. The type of equipment and procedure used can vary from institution to institution depending on available resources. If more sophisticated equipment are available (such as photometers, scintillation counters, and spectrophotometers) then accurate quantitation in parts per million or in some instances parts per billion is possible. In addition, the procedures can be modified to show that intermediate environmental stresses such as subtle changes in pH, temperature, oxygen concentration, and salt concentration can have intermediate effects on bioluminescence and thus the environment.

This exercise can be done in a 3-hour laboratory period. It requires a room or closet that can be darkened and routine laboratory equipment as indicated in the materials section. A flash light with red cellophane taped over the lens is helpful to facilitate some operations and movements in the dark. The red filter reduces the effects of night blindness.

It is essential that all safety measures are employed. Students should have aprons or laboratory coats, safety glasses, and disposable gloves.

The use of *Vibrio harveyi* or *Vibrio fischeri* has many advantages. They grow best at 23°C in the presence of 3% salt. These are ideal organisms to grow in a student laboratory as they do not require an incubator or extensive media preparation that more fastidious or delicate organisms require. The 3% salt helps cut down on contaminants. As far as we can tell, these organisms are completely non-pathogenic for humans and thus the risk of a student infection is essentially non-existent.

During the class immediately preceding the laboratory, give the students directions for a BYOP (Bring Your Own Pollutant) party. The students are asked to find a household product (water soluble) that if disposed of improperly could cause an environmental hazard. Give the students screw cap tubes and plastic bags. Caution must be stressed in the transport of these materials. Instruct them not to carry materials in their pockets because minor accidents could result in a wound containing both broken glass or chemical.

This exercise is a good forum for evaluating the data and discussing why there is data variability. To challenge the students and stimulate critical thinking the exercise could be amplified by asking the students to predict how this method could be improved? How could it be applied to a different type of situation?

### Student Outline

#### Background

Most individuals believe that water pollution exists but have no concept of how to screen for the presence of biohazardous materials in water or what types of chemicals pose a threat. By the use of this concrete exercise, students can demonstrate biotoxicity in a short period of time which is not possible with routine chemical tests.

One bioassay employed is the observation of bioluminescence reduction in the non-pathogenic marine bacteria, *Vibrio harveyi* or *Vibrio fischeri*. This method has wide application because there are many chemicals found in aquatic environments—industrial and domestic effluents that can react directly with luciferase. The only requirement is that the pollutant be soluble and stable for 1 hour in 3% saline. Students can be requested to obtain from the home or community potentially toxic materials that are routinely used and if disposed of improperly could pose an environmental threat.

#### Objectives

The student will be able to:

1. Visualize bioluminescence.
2. Visualize the instantaneous effects of environmental changes on microbes.
3. Visualize the effect of aeration on luminescence.
4. Visualize the influence of acid and alkali on luminescence.
5. Visualize the thermal denaturation of an enzyme.
6. Visualize the reversible inhibition of an enzyme by cooling.
7. Heighten his/her environmental awareness.
8. Improve problem solving skills.
9. Work efficiently and effectively in a cooperative learning situation.
10. Demonstrate leadership ability.
11. Assume leadership roles.

#### Effect of Oxygen on Bioluminescence

##### *Materials*

Overnight culture (ONC) of bacteria (200 ml) in a 1000 ml erlenmeyer flask

Demonstration plate (streak agar plate; incubated for 24 hours)

Darkened room (use a windowless closet/room; if not available, place aluminum foil on windows/doors)

##### *Procedure*

1. Swirl flask by hand.
2. Observe increase in luminescence.
3. Let sit for 1–2 minutes.

4. Observe diminution of luminescence.
5. Re-swirl.
6. Observe increase in luminescence.
7. Examine petri dish cultures for individual luminescent colonies.
8. Record your data.

### **Effect of pH on Bioluminescence**

#### *Materials*

Overnight culture (ONC) and cell suspension (see Appendix A)  
Test tube rack, test tubes, pipets, pipettors  
At least 1 N of HCl and NaOH solutions (may substitute a weak acid and a weak base)  
Darkened room

#### *Procedure*

1. Add 1 ml of acid or base to a (18 × 150 mm) test tube.
2. Add 1 ml of 1:10 cell suspension to a separate (13 × 100 mm) tube.
3. Pour cell suspension into acid or base to cut down on spillage and dripping.
4. Observe immediate reduction in luminescence.
5. Record your data.

### **Effect of Temperature on Bioluminescence**

#### *Materials*

Overnight culture (ONC) and cell suspension  
Test tube rack, test tubes, pipets, pipettors  
250 ml beaker of alcohol that has been cooled in freezer  
250 ml beaker of water at 50°C  
250 ml beaker of water at room temperature  
Darkened room

#### *Procedure*

1. Add 1 ml of 1:10 cell suspension into two separate tubes.
2. Place tube #1 in 50–55°C water and tube #2 in a cold alcohol bath.
3. Allow each tube to reach the desired temperature.
4. Observe luminescence.
5. Allow each tube to come to room temperature in a beaker of water.
6. Observe luminescence.
7. Record your data.

**BYOP** (*Bring Your Own Pollutant*)

*Materials*

Overnight culture (ONC) and cell suspension  
Test tube rack, test tubes (10 × 150 mm), pipets, pipettors  
Saline  
Darkened room  
Agents

*Procedure (performed by students on the second day)*

1. In the preceding class, your instructor will ask you to bring an agent to the next laboratory period—one that you think would create an environmental hazard if disposed of improperly. *Note:* We recommend that you place your agent in a small screw cap tube wrapped in a paper towel and placed in a zip-lock bag. *Caution:* Do not transport this package in garment pockets.
2. Make a 1:10 cell suspension.
3. Add 1.0 ml of saline to control tube #1.
4. Add no saline to tube #2.
5. Add 0.9 ml saline to four additional tubes (#3, 4, 5, 6).
6. Add 1.0 ml of agent to tube #2 (direct).
7. Add 0.1 ml of agent from stock to tube #3 (contains 0.9 ml saline = 1:10 dilution) and mix. By adding 0.1 ml from tube #3 to tube #4 and mixing, proceed to make a 1:100, 1:1000, 1:10,000 dilution (tubes #4, 5, 6).
8. Add 10 ml of cell suspension to a large test tube in each student’s rack.
9. Record time and set timer for 15 minutes.
10. From the large test tube add 1 ml of cell suspension to tubes #1–6 by using a 1 ml pipet.
11. Go into a darkened room.
12. When the timer goes off, visually inspect the tubes. By comparing with the control score on a “4+” system. A “4+” indicates the most amount of light with no toxicity, while “–” indicates a complete reduction in light with toxicity (as depicted below).

Least light				Most light	
–	+/-	+	++	+++	++++
Most effective			Least effective		

13. Record your data:

Control luminescence	
Direct	
1:10	
1:100	
1:1000	
1:10,000	

14. Create a class data table and record the class data.

15. Develop several conclusions based on the class data.

16. Discuss how this method could be improved and how it could be applied to a different type of situation.

APPENDIX A  
*Materials and Methods*

**Nutrient broth (NB)** for *Vibrio harveyi* or *Vibrio fischeri*: Follow manufacturers instructions but add 3% NaCl. Solid media (NA) is the same as NB except that it contains 15.0 g agar per liter. 20 ml of nutrient agar is dispensed into 100 × 15 mm plastic petri dishes.

**Saline** (per liter): 3% of NaCl, 1.25 ml of 40% sucrose, and 1.0 ml of NB—if only neutral compounds are to be tested. Otherwise, use phosphate buffered saline (see below).

**Stocks/organisms:** *Vibrio harveyi* / *Vibrio fischeri* are cloned weekly on NA plates. Bioluminescent organisms can be purchased from: American Type Culture Collection (ATCC), Sales Department, 12301 Parklawn Dr., Rockville, MD 20825, 1-800-638-6597.

**Overnight culture (ONC):** *Vibrio harveyi/Vibrio fischeri*. Inoculate 25 ml of NB in a 125 ml erlenmeyer flask from stock plate. Incubate for 24 hours at room temperature or 23°C. Store in refrigerator until use. For larger broth cultures use 5.0 ml of ONC as an inoculum and store in refrigerator until needed.

**Cell suspension:** Dilute ONC 1:10 with saline and store in an ice bath.

**Stock solutions:** Usually made as 1 g per liter. Commercially available materials are used directly or after an appropriate dilution in water.

**Reaction mixture:** Each tube contains 1.0 ml of cell suspension and 1.0 ml of compound/dilution.

**Control tubes:** (#1) 1.0 ml of cell suspension, (#2) 1.0 ml of saline.

**Incubation temperature:** All incubations are at 23°C although room temperature will suffice.

**Quantitation:** Use 1.0 ml of an agent/dilution. After incubation, the reduction in luminescence compared to the control is determined by visual inspection in a darkened room. Luminescence can be scored on a “4+” basis.

**Phosphate buffered saline (PBS;** for use with very acidic or alkaline samples): 5.67 g of Na<sub>2</sub>HPO<sub>4</sub>, 25.34 g of NaCl, 1 ml of nutrient broth, and 1.25 ml of 40% sucrose; about 1.2 g of KH<sub>2</sub>PO<sub>4</sub> is added to adjust pH to 7.0. Add distilled water to bring the final volume to 1000 ml. Refrigerate.

**Disposal:** Contaminated materials are autoclaved and disposed of in an appropriate hazardous waste container.

APPENDIX B  
*Further Reading*

- Thomulka, K. W., L. H. Peck, and D. J. McGee. 1993. Use of bioluminescence in detecting biohazardous substances in water. *Journal of College Science Teaching*, 23:109–112.
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