

## Observation and stimulation of bioluminescence and chemiluminescence.

### Purpose

Stimulate and observe the phenomena of bioluminescence, fluorescence and chemiluminescence.

### Overview

This experiment introduces the students to chemical reactions, reaction rates, chemiluminescence, fluorescence and bioluminescence.

### Time

1 hour

### Key Concepts

Chemiluminescence occurs when a chemical reaction produces light. Bioluminescence is chemiluminescence in living organisms. Several different types of organisms are bioluminescent. However, the chemistry of bioluminescence can vary considerably between organisms. This fascinating phenomenon has evolved independently in nature five different times (five different chemistries are known).

### Skills

Collecting data  
Making observations  
Forming hypotheses

### Materials

1. Light sticks (three per student). These can be purchased at party stores, however you can get them much cheaper if you buy from the Oriental Trading Company (a mail order toy company-they are on the web). You can get a bulk pack of 50 bracelet size sticks for about \$35.00.
2. Warm water bath (40°C).
3. Ice and an ice bucket.
4. A solution of luminol in DMSO (1 mg/mL 2 mL per student).
5. Sodium hydroxide pellets.
6. An aqueous solution of fluorescein in a dropper bottle.
7. *Pyrocystus lunula* culture (two 10mL portions per student, on alternate circadian cycles).
8. Vinegar (in a dropper bottle).
9. Transfer pipettes
10. 10 mL test tubes (4 per student).

## Facilitator Preparation

Glow sticks are used to demonstrate the effect of temperature on the rates of chemical reactions. The glowsticks contain two chemicals that are mixed when the glass tube on the inside is broken. This initiates a chemical reaction that gives off light. The higher the reaction temperature, the faster the reaction, the more intense the chemiluminescence. Reaction rates increase about 2 fold for every 10°C rise in temperature. If you want to save on glowsticks, you can separate the class into three groups and give each group one glow stick.

The luminol experiment demonstrates chemiluminescence and fluorescence. Luminol is oxidized (with molecular oxygen) in the presence of sodium hydroxide. When the students shake the tube, they are introducing oxygen into the solution. That's why the reaction doesn't work unless they shake the tube and why chemiluminescence stops when they set the tube aside. In the second part of this experiment, students add a fluorescent dye to the solution. This dye absorbs the light emitted by the luminol and re-emits light at a longer wavelength, changing the color that is observed. This is the phenomena of fluorescence.

In the bioluminescence experiment, students will notice that the intensity of dinoflagellate bioluminescence is much greater at night. Ask students to speculate on why this is so. It is also interesting to note that dinoflagellates emit blue light. This is the wavelength that is transmitted the farthest in seawater. Obviously dinoflagellates want to be seen. But why? The accepted hypothesis is that bioluminescence cuts down of feeding by other organisms. If a predator sees a flash of light (induced because somebody is eating a dino) then the predator will go and try to eat what is trying to eat the dino.

The intensity of dinoflagellate bioluminescence has been used as an indicator of water quality.

Anecdotal evidence suggests that bioluminescent intensity increased just before a bloom of the Florida Red Tide dinoflagellate, *Karenia brevis*. (IS THIS CORECT?). Does this apply to other organisms as well?

## Background

In 1887 a scientist named Raphaël Dubois isolated light producing chemicals from the common piddock, which is a clam that bores holes in rocks. He discovered that if he ground this clam up in cold water, he saw light in the water, which glowed for several minutes, indicating that he had extracted the light producing chemicals from the clam's tissues. He then found that if he made a hot-water extract from another clam and added this to the original cold-water extract, he could reactivate the light reaction. Dubois called his hot-water extract luciferin and the cold-water extract luciferase. It turns out that luciferase is an enzyme that catalyses a chemical reaction. The luciferin is the substrate for that reaction. The reaction produces a molecule that is in an electronically excited state. When it relaxes (gives off energy and goes back to the ground state) a photon of light is released. Although these terms are used to describe the substrate and the enzyme in any bioluminescent reaction, different creatures produce very different versions of these chemicals. Dinoflagellates are just one type of bioluminescent organism. The most famous site of bioluminescence is called (**Phosphorescent Bay-in PR- I don't know the name in Spanish-can somebody fill in for me?**). This is actually a misnomer since bioluminescence and phosphorescence are really two different phenomena. It is interesting to note that dinoflagellate bioluminescence differs in intensity depending on the

time of day. You will compare two cultures of dinoflagellates; one that is on a normal circadian cycle and one that is on a reverse circadian cycle (they think it is night time).

## Procedure

**These experiments are best done in a darkened room. It is especially important for the bioluminescence experiment (Part III) that the room be completely dark and that students allow a minute or two for their eyes to adjust.**

### Part I. Rates of Chemical Reactions.

1. Obtain three light sticks from your instructor and break the glass tube on the inside.
2. In a room that can be made completely dark, put one light stick in an ice bucket. Put one light stick in the warm water bath. Keep one light stick at room temperature.
3. Go and do Part II then come back when you are done.
4. Turn out the lights and let your eyes adjust to the lack of light.
5. Compare the intensity of light coming from your glowsticks.

### Part II. Rates of Chemical Reactions.

1. Explain the relative brightness of the glowsticks and the effect of temperature.
2. Why does chemiluminescence decrease when the test tube is not shaken (what else is in the tube?)
3. What happened when you added the dye to the luminol solution? Speculate on why.
4. Speculate on the differences in bioluminescence between day phase and night phase cultures.

### Part III. Chemiluminescence and Fluorescence.

1. Dispense about 2 mL of a solution of luminol (1 mg/mL in DMSO) into each of two test tubes.
2. To the first tube, add one (1) pellet of NaOH.
3. To the second tube, add a few drops of a solution of fluorescein and one (1) pellet of NaOH.
4. Take the tubes to a dark room. Stopper and shake each tube vigorously. What color are the solutions? Approximately what wavelengths of light do these colors correspond to? What happened and why?
5. Set both tubes down for 5 minutes. Has the chemiluminescence stopped? Shake the tube again. What happened and why?

### Part IV. Stimulation of dinoflagellate bioluminescence.

1. **DAY PHASE:** Label screw cap test tube Day PHASE.  
**NIGHT PHASE:** Label screw cap test tube Night PHASE.
2. **DAY PHASE:** Obtain about 5 mL of *Pyrocystus lunula* culture that is on the day PHASE from your instructors.  
**NIGHT PHASE:** Obtain about 5 mL of *Pyrocystus lunula* culture that is on the night PHASE from your instructors. **MAKE SURE YOU SHIELD IT FROM LIGHT!**

3. Go into one of the dark rooms and shake the tubes. What do you see? Record your observations in your notebook.
4. Place each tube on ice. What happened? Why? Record your thoughts in your notebook.
5. Add a few drops of 5% glacial acetic acid (vinegar). Write down what you saw happen and why you think it occurred.

**Student Assessment???**