JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION
DOI: https://doi.org/10.1128/jmbe.v19i2.1373

Curriculum

Student-Centered Microbioassay Laboratory Activity Utilizing Bioluminescent Bacteria †

Kerri Younkin[1,*] and Christina Romano[2]
[1]*Southern York County School District, Glen Rock, PA 17327;* [2]*Scholastic Inc., New York, NY 10012*

Student-centered teaching allows students to be actively engaged in hands-on, minds-on activities that emphasize creativity and collaboration, enabling them to ask questions and design their own investigations to real-world problems. One such problem is water contamination, which causes human health and environmental issues. However, chemical water quality testing for pollutants can be timely and expensive. In addition to chemical testing, researchers have developed assays using unicellular organisms to determine which pollutants are present and in what concentrations. In this three-hour laboratory activity, high school students and undergraduate biology or microbiology students work in pairs to help a fictional company develop a water quality microbioassay. Students design their own laboratory protocols to test the reaction of a bioluminescent bacterial species (i.e., *Photobacterium phosphoreum* or *Aliivibrio fischeri*) to exposure of common aquatic pollutants such as fertilizer, household cleaners, and motor oil. During this laboratory activity, students apply previously learned components of experimental design, including positive and negative controls, constants, and experimental groups. In addition, students gain experience writing a scientific explanation for a recommendation regarding the bioluminescent bacteria's suitability in a bioassay. Pre- and post-evaluation data revealed that students were successful in achieving the activity's objectives as well as in designing their investigations and writing their protocols using scaffolds within the lesson.

INTRODUCTION

Trends in science education encourage a student-centered approach, in which students actively engage in hands-on, minds-on science activities that promote thinking and acting like scientists and engineers. Although student-centered instruction is not without its challenges (1), student-centered activities frequently integrate disciplinary content with laboratory practices and interdisciplinary conceptual themes and may include project-based or inquiry-based instruction. However, many traditional laboratory activities limit opportunities for students to design investigations and write their own protocols, instead providing a list of procedural steps, a data table, and sometimes the expected outcome. This laboratory activity provides scaffolds to support students as they design an investigation and write protocols to evaluate bioluminescent bacteria for a bioassay in response to a fictional company's real-world request.

Monitoring water quality serves many purposes, from protecting ecological systems to human water sources. Traditional water quality monitoring programs employ chemical testing, which can be costly in time and expense (2, 3). Bioluminescent bacteria, such as *Photobacterium phosphoreum*, *Aliivibrio fischeri*, and *P. harveyi*, possess the *lux* gene, which permits them to emit light in response to an environmental stimulus in an active process involving the enzyme luciferase and the electron transport chain (3, 4). Due to bioluminescence energy requirements via the *lux* gene, a reduction in light emissions can indicate that cells are biochemically compromised, stressed, or dead. Careful selection of unicellular organisms combined with genetic engineering and recombinant DNA techniques have allowed researchers to develop assays to determine which pollutants are present and in what concentrations (5). These tests are commercially available today, and there is a movement to continue validating these tests and use them for regulatory monitoring in addition to chemical testing (2, 3).

In this laboratory activity, students act as lab researchers for a fictional company interested in developing a bioassay to test water quality. Students learn about microbioassays and design a lab protocol to test the reaction of bioluminescent bacteria (*P. phosphoreum* or *A. fischeri*) to exposure to common aquatic pollutants. Students use the data they collect to make a recommendation to the

*Corresponding author. Mailing address: Susquehannock High School, 3280 Fissels Church Road, Glen Rock, PA 17327.
Phone: 717-235-4811. E-mail: kerri.younkin@sycsd.org.
Received: 20 June 2017, Accepted: 20 June 2018, Published: 31 July 2018.
†Supplemental materials available at http://asmscience.org/jmbe

fictional company regarding the continuing development of the microbioassay as well as to identify next steps in the development process. This activity is modeled after the commercially available, widely recognized and used water quality microbioassay, Microtox (6).

Intended audience

This activity targets high school and lower-division undergraduate students in biology and microbiology. It may be suitable for students in lower-division ecology courses with the addition of focused material regarding ecological relationships of microbes in an aquatic system.

Learning time

This laboratory exercise requires a minimum of three hours to complete. As written, the three-hour block includes both the prelab, where students write the protocols, and the postlab, which focuses on analysis, with limited report time. Instructors could adapt this timeline so students complete their protocols prior to lab and use some class time for peer review prior to running the lab. Students will require additional time beyond the three-hour block to complete a full lab report and/or the Glow-Tel summary letter. With another laboratory session, students could extend the study by testing pollutant sensitivity using serial dilutions, evaluating the sensitivity of different microbes to the pollutants, or testing different protocols (e.g., disk diffusion method).

Prerequisite student knowledge

Students will have previously learned and had practice formulating hypotheses, and they should have learned the components of experimental design, including positive and negative controls, constants, and experimental groups. Although not required, students may benefit from an overview of water quality concerns focusing on pollution impacts. While it may be beneficial to students, prior experience with aseptic technique is not required for this lab and the students who completed the lab with us were unfamiliar with aseptic technique before completing this lab.

Learning objectives

During and upon completion of this investigation, students will be able to:

1. Design and test a protocol to determine whether bioluminescent bacteria are sensitive to common aquatic pollutants
2. Organize, collect, and analyze data
3. Write a scientific explanation for a recommendation regarding bioluminescent bacteria's suitability in a bioassay
4. Differentiate between bioluminescence and fluorescence
5. Describe the impact of pollutants on bacteria

PROCEDURE

Materials

Materials listed below are the required quantity per group of two students or per student. Please see Appendices 1 and 2 for student worksheets, as well as the media recipes, culturing notes, and supporting materials. Allow for incubation time of 12 to 16 hours for ideal bioluminescence of the bacteria prior to exposure to pollutants in the lab. Laboratory conditions should follow ASM Biosafety Guidelines (7) supporting appropriate considerations for a BSL1 organism, including personal protective equipment (eye protection; lab coats and gloves are recommended but optional), handwashing stations, biohazardous waste disposal (autoclave or 10% bleach solution), and a lockable lab door.

Pre-laboratory activity

- Copies of GlowTel letter (1/student)
- Fluorescence vs. luminescence table (1/pair)
- Small flashlights (1/group)
- Reflective strips (1/group)
- White boards, dry erase markers, erasers (1/group, optional)

Laboratory activity

Have a materials station for students to self-select the following:

- Test tubes (minimum of 6/group)
- Test tube racks (1/group)
- Permanent marker and labeling tape
- A. fischeri or P. phosphoreum in liquid broth, minimum of 15 mL/group
- p1000 micropipettes (1/group) and tips OR disposable 1-mL transfer pipettes
- 10–12 mL of the following samples:
  - Distilled water (1/group)
  - Diesel additive (1/every two groups)
  - Liquid fertilizer (e.g., Miracle Grow or Scotts, made according to manufacturer's directions) (1/every two groups)
  - Dirty and/or clean motor oil (1/every two groups)
  - Tea tree oil (1/every two groups)
  - Household cleaners (Lysol, Windex, Pine-Sol) (1/group)
  - Isopropyl alcohol (1/every two groups)

Student instructions

Student worksheets and supporting materials are available in Appendix 1. A materials list is prepared for students to use as they design their investigations and can be modified as necessary.

Faculty instructions

In this activity, students act as scientists in research and development for a fictional company called GlowTel. The lesson begins with students identifying GlowTel's research objectives and exploring differences between bioluminescence and fluorescence before learning more about bioluminescence. After students gain a base knowledge of bioluminescence and aquatic pollutants, they write their own protocols, which they review and revise. After instruction or review of sterile techniques, lab safety, and equipment use according to the ASM Guidelines (7), students follow their protocols and collect data. Students complete the activity by analyzing data and drafting a report with recommendations for future steps for GlowTel. Detailed instructor notes are available in Appendix 2.

Suggestions for determining student learning

Formative assessments during this activity include reviewing lab protocols during writing or prior to entering the lab, in-class discussions, and during-lab consultations with students. Options for formal assessments include writing a report for GlowTel or answering analysis questions (included in student materials in Appendix 1). A lab rubric is included in Appendix 4.

Sample data

Typically, *P. phosphoreum* and *A. fischeri* stop glowing when exposed to diesel additive, tea tree oil, isopropyl alcohol, and household cleaners. They continue to emit bioluminescence when exposed to distilled water and fertilizers. Samples of student work are included in Appendix 5.

Safety issues

Prior to entering the lab, students must complete a lab safety discussion to participate in the lab and ensure that they understand lab safety. The lab safety discussion explicitly addresses wearing closed-toed shoes; tying back long hair and avoiding dangling jewelry and loose clothes; not eating or drinking in the lab or bringing food, drink, or gum into the lab; leaving personal belongings, including cell phones and pencils/pens, outside of the lab; and wearing personal protective equipment. Because the lab requires students to use broth, goggles are required and lab coats and gloves are recommended. Students have time to ask questions and get clarification following the safety discussion, and instructors check that students are following safety protocols before and during the lab. Once in the lab, instructors directly teach sterile techniques and pipetting skills, allowing time for student practice using distilled water before students are given access to the microbes.

Bacteria and materials contaminated with bacteria should be autoclaved according to the minimal standards set by the ASM Biosafety Guidelines (7) or state regulations. Alternatively, bacteria and materials contaminated by bacteria can be disinfected by soaking in a 10% bleach solution for a minimum of two hours before disposal. While the chemical pollutants named in this lab are readily available for consumer use and do not require consumers to use personal protective equipment, we recommend taking appropriate precautions with the pollutants in the lab and disposing of them according to labeled instructions.

If using the organisms and chemicals described in this article, BSL1 lab safety guidelines are appropriate. If BSL2 organisms or chemicals with higher risk concerns are substituted or added to this exercise, BSL2 safety guidelines should be followed (7).

DISCUSSION

Field testing

This activity was developed and field tested through a university-based informal science education program. This education program hosts three-hour, self-contained, bioscience field trips for students in grades 3 to 12 as well as undergraduate groups. Program instructors develop and teach classes, guiding students through all aspects of the preliminary materials and activities, lab safety, and the lab investigation, concluding with analysis and assessment. Class sizes vary, but range from 15 to 32 students.

This laboratory activity was piloted with three high school classes during the 2016–2017 school year and offered as an option for teachers to select for their class's field trip during the 2017–2018 academic year. Two pilot classes were higher level, research-based classes in which many students were also advanced-placement science students. The third pilot class was a ninth grade general environmental science class. Three high school classes in the 2017–2018 academic year completed the lab and the pre- and postlab survey.

Students in all six classes were partnered into groups of two or three. Students were actively engaged in the lab activity throughout the lab. We found that students were successful in designing their investigations and writing their protocols using hints provided and scaffolds within the lesson. The instructor provided additional support by asking directed questions to guide students, as necessary.

Evidence of student learning

To assess this activity, we compared pre- and postlab responses on a Likert-scale survey, photocopied and used

the rubric to score student work from each of the three pilot classes (see Appendix 4), and collected free-form responses from students and teachers following the lab. Appendix 3 contains a table pairing the specific learning objective with the teaching activity(ies) and assessment.

Using a Likert scale, students responded to the same six questions before and after the activity. The first three questions were attitudinal, and the last three were content-specific for the lab. Data were unpaired and all student pre-responses were compared to all student post- responses for each question. Students showed improvement in each of the six questions when comparing pre- and post- responses. See Appendix 3 for survey questions, percent of students selecting the preferred responses before and after the lab, and the improvement of preferred responses.

Summative assessment of student work was completed by using the rubric to score student work from each of the pilot classes. Students performed well on the summative report as scored by the rubric, indicating that they showed competence in writing hypotheses, designing and testing a protocol, organizing and collecting data, and analyzing their results. Class averages on the assignment were 88.8%, 91.1%, and 89.8%. Table 1 shows the averages per rubric section and summarizes common student errors by section.

In post- attitudinal free-form responses, 100% of responding students and teachers reported positively on this lab experience. Students shared that they learned how to create and conduct their own experiments, that they enjoyed having the freedom to design their own experiments, and that this lab made them more interested in pursuing science as a career. Students and teachers indicated a high level of engagement and interest throughout the activity. The most frequently named new content learning items were the difference between bioluminescence and fluorescence, that bacteria can be used to measure water quality in a bioassay, and that fertilizer (a known pollutant) does not kill bacteria. Some ninth grade students initially expressed frustration that they had to write their own protocols, and they indicated concern that they would not get the "right" results, and several students suggested extending the time available to work within the lab.

The evidence of student learning supports that this laboratory activity serves as an opportunity for students to practice and increase competence in writing hypotheses, developing protocols and recording data. It also maintains student engagement in a real-world concern involving aquatic pollution.

Possible modifications

Depending on time and resources available, faculty could modify this lab to include both a fluorescing bacterial strain, such as *Pseudomonas fluorescens*, and a bioluminescent strain, such as *P. phosphoreum* or *A. fischeri.* A series of photos depicting the steps required for this lab may be provided to students requiring additional scaffolding as they write their protocols.

Modifications for upper level biology and microbiology course could include students designing serial dilutions to test microbial sensitivity to the pollutants, identification of and incorporation of testing microbial response to locally relevant aquatic pollutants, or evaluation of microbial

TABLE 1.
Student response scores using rubric.

Rubric Section	Number of Students Scored	Average Scores for Section	Common Student Errors
Identification of pollutants	60	97.1%	The occasional student listed only one experimental pollutant instead of two.
Hypothesis	60	85.0%	Students who missed points here most commonly failed to explain (identify) the "why" or "because" of the hypothesis.
Controls	60	89.6%	Students who scored below 4 most commonly did not list the positive and negative control substances.They listed only the expected reaction (bacteria glowing or not glowing).
Protocol	39	87.2%	Students occasionally needed to include additional details in their protocol. One class piloted a scaffold for developing protocol-writing skills. These students sequenced picture steps and used an instructor-prepared protocol.
Data table	60	91.3%	Students occasionally only reported one trial or were not neat in organizing their data.
Analysis and conclusion questions	21	84.5%	We collected and scored one class's Analysis and Conclusions Questions. Due to time limitations in our three-hour field trip format and because we cannot collect student work completed after our class ends, we did not collect these from the other classes.

sensitivity to pollutants as compared with other testing methods, such as disk diffusion. Advanced students involved in interdisciplinary studies or with access to Arduino and similar resources may also incorporate designing and programming a luminometer to measure the lumens emitted prior to and post pollutant exposure.

SUPPLEMENTAL MATERIALS

Appendix 1: Student worksheets
Appendix 2: Faculty instructions
Appendix 3: Alignment and assessment
Appendix 4: Lab rubric
Appendix 5: Examples of student work

ACKNOWLEDGMENTS

Special thanks to Dr. Mary Stapleton for providing lab space and in-house resources to support lab and field testing of this activity. Additional thanks to Henry Jackson and Debbie Phillips for lab support, especially preparation of laboratory media and culturing bacteria. The authors also wish to thank the teachers and classes involved with the pilot: Elizabeth Schap, from Liberty High School, and Kim Bickerstaff, from Western School of Technology. The authors declare that there are no conflicts of interest.

REFERENCES

1. Felder RM, Brent R. 1996. Navigating the bumpy road to student-centered instruction. Coll Teach 44(2):43–47.
2. Mankiewicz-Boczek J, Nalecz-Jawecki G, Drobniewska A, Kaza M, Sumorok B, Izydorczyk K, Zelewski M, Sawicki J. 2008. Application of microbiotests battery for complete toxicity assessments of rivers. Ecotoxicol Environ Safety 71:830–836.
3. Nunes-Halldorson V, Leticia Duran N. 2003. Bioluminescent bacteria: LUX genes as environmental biosensors. Braz J Microbiol 34:91–96.
4. Thomulka KW, McGee DJ, Lange JH. 1993. Use of the bioluminescent bacterium *Photobacterium phosphoreum* to detect potentially biohazardous materials in the water. Bull Environ Contam Toxicol 51(4):538–544.
5. Sousa S, Duffy C, Weitz H, Glover A, Eckart B, Henkler R, Killham K. 1997. Use of a LUX-modified bacterial biosensor to identify constraints to bioremediation of BTEX-contaminated sites. Environ Toxicol Chem 17(6):1039–1045.
6. Wadia K, Thompson KC. (2007). Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive. Trends Anal Chem 26(4):300–307.
7. Emmert EAB. (2013). Biosafety guidelines for handling microorganisms in the teaching laboratory: development and rationale. J Microbiol Biol Educ 14(1):78–83.