RESEARCH ON LEARNING

An Open-Ended, Inquiry-Based Approach to Environmental Microbiology

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Abstract

An undergraduate environmental microbiology course was used to examine the hypothesis that students could best grow as biologists, inform career decisions, and experience the scientific process by engaging in a collaborative, research-based laboratory format. Students learned how to use scientific literature to formulate relevant questions and hypotheses and develop detailed experimental research proposals. They collected, analyzed, interpreted, and presented original scientific data in the form of a research-poster conference. Course objectives were measured using two Likert-style surveys, and the resulting data supported the original hypothesis of this work.

Key Words: *Environmental; microbiology; research; inquiry; collaboration.*

The best way to learn science is by doing science. This instructional concept received traction in the 1960s and early 1970s through the Biological Sciences Curriculum Study (1977). It was advanced in the 1980s by the National Science Foundation biology task force, which suggested that a main objective for undergraduate biology instruction be the acquisition of skills that allow students to "explore nature, raise questions, generate multiple tentative answers, and test these through the deduction of their logical consequences and comparison with evidence" (National Research Council [NRC], 1990). It was further developed through the 1990s by the American Association for the Advancement of Science (1993) and into the new millennium by the NRC (2000), which recommended that science educators promote independent learning, engage students in experimental design and execution, emphasize science as it is practiced, and promote scientific communication skills. There is an ever-growing body of educational research on the efficacy of inquiry-based biology instruction at the college level (Caccavo, 2009). According to a recent request (Leonard, 2010), detailed examples of how teachers can incorporate authentic inquiry into their courses are now needed. The purpose of this article is to provide a practical approach to open-ended inquiry learning in a university environmental microbiology course.

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○ Course Objectives

Environmental Microbiology (BI348) was an upper-division elective course within the biology curriculum. The students in this course were sophomore through senior level and had taken a prerequisite, laboratory-based, introductory microbiology course in which they were introduced to the basic laboratory techniques for manipulating and working with microorganisms (microscopy, staining, aseptic technique, isolation, use of selective and differential media, serial dilution, and plate counting). BI348 consisted of three 1-hour lectures and one 3-hour lab each week over the 15-week semester. The approach to the laboratory portion of the course over the eight semesters it has been taught has transformed in a way that parallels the national priorities in science education briefly described above. Original iterations of the course utilized a laboratory manual that could be ordered with the text (Maier et al., 2000). The students worked in pairs on weekly cookbook exercises that included the coliform MPN assay, the contact slide assay, detection of bacteriophages, microbiological analysis of food, and isolation of antibiotic-producing microbes. They were assessed through reports that were torn from the end manual at the end of each exercise. The students generally found these labs interesting and fun, but they were far from challenging. A review of the literature on science education led to the question of how environmental microbiology could be used as a platform for students to personally experience the scientific process through a collaborative, research-based laboratory format. I hypothesized that such an experience would help the students grow as biologists and inform their future career decisions and that it could best be addressed through a fundamental set of objectives designed to emphasize this format. The students should learn how to read and interpret the primary scientific literature and then formulate relevant questions and hypotheses within the context of that literature. They should develop a detailed experimental plan to test their hypotheses within the scope of the course and the resources of the department. The students should learn how to collect, analyze, interpret, and present original scientific

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data. Finally, the course should emphasize collaboration as a vital component of the scientific process.

○ Context Is Critical

An open-ended, inquiry-based approach (Eastwell, 2009) to the BI348 laboratory was developed and tested. Originally, the students were encouraged to choose any topic in environmental microbiology that interested them. The projects they developed were interesting but lacked depth. It was important that they began their project at the beginning of the semester so that they would have enough time to complete it, but at that point in the course they had not been exposed to sufficient content to ask relevant questions. Subsequently, the laboratory projects focused on wastewater treatment. The lectures on this topic were moved to the beginning of the course, and a field trip to the local wastewater treatment plant was conducted early in the semester. Equipping the students with appropriate content and context allowed them to develop research topics with texture and depth and was foundational to the inquiry-based nature of the course.

○ Collaboration Is the Key

Originally, the students worked independently on their research projects. However, a further review of relevant literature, and a concomitant increase in enrollments in our major, suggested that the research projects would be strengthened through collaboration. This took two forms. Experience suggested that the optimum size for a research team is four students. A smaller group often has difficulty handling the workload at the project's apex, and a group of more than four often results in at least one student being left out as they struggle to arrange their busy schedules. The bulk of assessment in the laboratory was done by the group, so that the research teams worked together for a common good (their grades). The students were individually graded on attendance and participation so that there was a mechanism for rewarding students who assumed leadership roles or went the extra mile. An equally important aspect of collaboration was faculty interaction with each group. It would be careless to merely say that faculty should play the role of facilitator in open-ended inquiry research projects. This role is subtle, and faculty must gracefully walk a very fine line to be encouraging and supportive rather than omnipresent and overbearing. Only experience can tell faculty when to be in the lab asking research teams probing questions and giving timely advice, and when to leave the lab to allow the students to work out problems on their own. Ultimately this relationship hinges on faculty trusting their students in the lab, and the students trusting that their success is a faculty priority. A laboratory humming with the activity of 20 students organized into five research teams is a crucible for learning these skills and optimizing such relationships.

○ The Beginning

The students were glassy-eyed when the laboratory research project was described on the first day of class. Since most of them had never done research, this seemed like a daunting task, and it was important to help them transition from the introductory cookbook type of lab experiences they were used to. Therefore, the first lab began with a brief lecture on the process of the scientific method and how it is related to the expectations of BI348. After the students chose their research teams, they were taught how to begin this process. Starting with a fairly broad topic (e.g., disinfection) that they found interesting, they narrowed that broad topic down to a specific topic (e.g., the use of ultraviolet light as a means of mixed liquor disinfection) by collecting a base of information on the topic through reading books and review articles and scanning journal article titles and abstracts. This literature review provided the students with a general idea of what has been done in the field and of the different subdisciplines of study within the field, so that they could choose which one interested them the most. They next formulated a question and hypothesis concerning that specific topic. In order to do this they had to acquire a deeper base of knowledge by actually reading a number of primary-literature articles (i.e., articles in peer-reviewed scientific journals). As they read these articles, they obtained ideas about what the investigators did not pursue or angles that were not addressed. The authors may even have suggested future experiments that could be done. These were ideas that the student groups could develop into a research project that was within the context of the literature, the scope of the course, and the means of the department. The only way they could develop a relevant question and hypothesis was by gaining an understanding of the work that had been done before. The natural tendency of the students was to start by thinking about the experimental methods they could use, instead of realizing that the experiment should flow from the question and the question should flow from a base of knowledge rooted in the literature. Table 1 shows some examples of student-derived questions that resulted from the process described above.

○ The Proposal

A key tipping point in the success of this course came when the students were required to submit a research proposal before they began working on their projects in the laboratory. In its most effective form (Table 2), this assignment was a tool used by the students to organize their thoughts, focus their efforts, and provide a structural framework for the execution of their experiment. There were two elements to the proposal that made it particularly useful in this capacity. First, the rubric for the proposal was as detailed as possible. The students used the introduction for background information that

Table 1. Student-generated research questions on wastewater treatment.

1	To what extent do sonic wave amplitude (3, 5, 7 and 9 watts mL^{-1}), exposure duration (3, 5, 7, and 10 minutes), and ambient water temperature (4, 20, 30, and 40°C) influence the death of fecal coliforms after secondary clarification of wastewater?
2	How do varying levels of oxygen exposure (anaerobic, naturally aerated, and actively aerated) affect the concentration of fecal coliforms in compost piles composed of biosolids?
3	How do varying concentrations of tetracycline (0.015, 0.15, 1.5, and 15 μg mL^{-1}) affect the elimination rate of heterotrophic bacteria in a wastewater-treatment aeration basin?
4	Which temperatures (10, 22, 35, or 45°C) result in the greatest level of fecal-coliform photoreactivation after ultraviolet-light disinfection of postsecondary-clarification wastewater?

Table 2. Research proposal rubric.

Proposal Component	Point Value
Introduction with background information providing context and references	20
Research question	10
Research hypothesis and rationale	10
Chronological description of the experimental methods, including: • Positive control • Negative control • Materials list	20 10 10 10
Description of the expected results, including: • Type of data collected • Relationship of data to hypothesis • Sample graph	2.5 2.5 5

provided context and a rationale for their proposed work that was rooted in the scientific literature and fully referenced. The explanation of the experimental methods entailed a chronological, step-by-step list of the procedure for the entire experiment, including positive and negative controls, and a detailed list of all of the materials the team would need to conduct the experiment. In order to obtain this materials list, the students had to know exactly what they would be doing when they stepped into the laboratory to begin their experiments. The students were also required to describe the results they expected to obtain. This specifically included the type of data they would collect, critical analysis of how those data might reflect on the hypotheses, and even the structure of the graphs they would use to illustrate their research.

The second essential element of the proposal was the rough draft. A rough draft of the proposal was not required but was encouraged by awarding extra-credit points for its timely submission. In this way, the students were free to make mistakes without consequences, and there was thus a lot less pressure as they made their first tentative steps as scientists. The draft was due approximately 1 month after the start of the course, typically 1 to 2 weeks before the final proposal was due. The students were permitted to submit as many draft revisions as they wanted before the final proposal was due, and each revision was an attempt to help the students hone their proposals to perfection. The fine line of facilitation was again at play here: the corrections provided only enough information for the students to find their own solutions to the problems raised in their drafts. The research proposal ultimately served as a laboratory manual for each specific project. The students exhaustively thought through the details of their projects so that they could then conduct their experiments with confidence and competence.

○ Putting It All Together

There was a palpable, collective sense of relief and excitement when the students were able to leave the confines of the library, step away from their computer keyboards, and enter the laboratory to begin their experiments. The students prepared all of their own cultures, media, and solutions, set up their own equipment, and obtained their own samples. Many required samples from the wastewater treatment plant, and the earlier field trip there served as a point of contact between my students and the plant staff. Laboratory sessions were completely unstructured, and the students soon realized that successful completion of their projects required many hours of work outside of the scheduled laboratory period. Faculty should again play a supportive role in the lab by answering questions, giving pointers (e.g., on the best way to pour agar plates), or making suggestions about the optimum number of dilutions to perform, but being always mindful to avoid hovering. It is important for the student research teams to understand that each step of the experiment would take them far more time than they could anticipate, so they should avoid procrastination. Interestingly, the students not only learned to collaborate within their research teams, but also learned to collaborate with other teams as everyone jockeyed for space in the laboratory and the use of equipment. An unanticipated and fun camaraderie developed in the class. Approximately 1 month before the end of the semester, the students analyzed the data that they had collected to that point and used that analysis to make some decisions on the direction of the project. For example, should they adjust sampling frequency, or increase the number of dilutions they were using, or start the experiment over after making procedural adjustments?

The projects culminated with the presentation of research posters at an in-house Environmental Microbiology Research Conference conducted during the final laboratory period. Once again, the students produced the best posters when provided with a detailed rubric (Table 3). Meetings between faculty and each research team a few weeks before the conference were established to discuss their

Table 3. Research poster rubric.

Poster Component	Point Value
Descriptive title, including researcher names	5
Introduction, including: • Referenced background information • Question • Hypothesis and rationale	5 5 5
Materials and methods summarized in complete sentences (not a list of steps)	10
Results, including: • A written description in complete sentences • At least one table of data • At least one graph of data	10 5 5
Discussion, including: • Significance of your findings • Whether results support or refute the hypothesis • What could be changed to make the experiment more effective	10 5 5
Visual appearance of poster • Consider appearance, design, organization, content • Organize in logical and uncongested procession • Incorporate visual aids (pictures, diagrams, drawings) • Construct using PowerPoint and print in campus copyshop	5 5 5 5
Attend Environmental Microbiology Research Conference	20

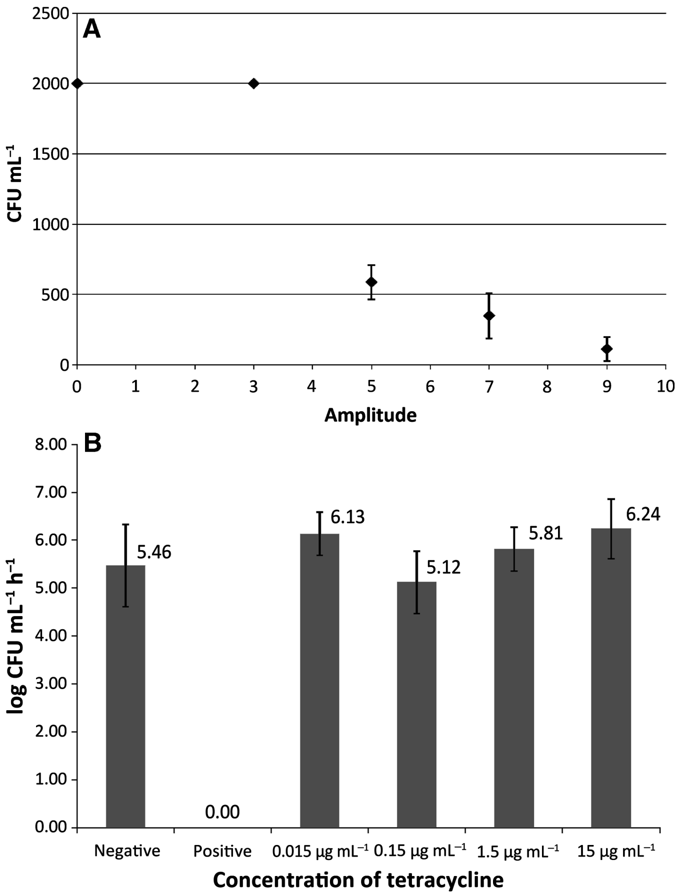

Figure 1. Examples of data collected by student research teams. (A) Effects of sonication amplitude on the concentration of fecal coliforms (measured in colony-forming units [CFUs] mL^{-1}) in secondary-clarification wastewater. Each point represents the mean of three replicates ± SD. These data show a positive correlation between sonication amplitude and fecal-coliform removal in wastewater. (B) Elimination rates of heterotrophic bacteria in aeration-basin wastewater treated with different concentrations of the antibiotic tetracycline. Each point represents the mean of three replicates ± SD. These results show that even high amounts of tetracycline do not affect the concentration of heterotrophic bacteria in wastewater.

results, explain relevant statistical analyses, and recommend different forms of data presentation. As the rubrics and interactions with research teams were gradually refined in this course, there was a concomitant improvement in the quality of data that the students generated (Figure 1). It is particularly interesting to note that the experiments described in these graphs were conducted only once (with three replicates per treatment). The reproducibility of the data (as seen by the small error bars) and the fact that the data coherently addressed the students' questions and hypotheses speak to both the utility of a detailed research proposal and the high quality of the science conducted by the students.

○ Evaluation

The hypothesis of this study was tested using two Likert-style surveys that the students were asked to complete during the class following the research conference. One survey allowed the students to evaluate

Table 4. Student evaluation of the open-ended inquiry laboratory.

Statement	Score[a]
The research proposal was an effective way to develop my project.	4.82 ± 0.39
This lab taught me how to do research science.	4.70 ± 0.46
This lab required me to synthesize and apply concepts learned in class.	4.29 ± 0.77
This lab provided some direction, positive or negative, in choosing a career.	3.05 ± 1.59
I enjoyed the collaborative nature of this lab.	4.41 ± 0.93
This lab required more critical thinking than non-research-based labs.	4.82 ± 0.39
The Research Conference was an effective way to learn about other research projects.	4.41 ± 0.61

[a]Mean score for the entire class (n = 17) ± SD. Students responded on a numerical scale from 0 (disagree) to 5 (agree). Data are from December 2010.

Table 5. Student evaluation of personal improvement from the open-inquiry laboratory.

Category	Score[a]
Reading, interpreting, and using scientific literature	4.05 ± 0.74
Creating a research question and hypothesis	4.52 ± 0.62
Considering all of the details involved in designing and executing an experiment	4.76 ± 0.43
Preparing and writing a research proposal	4.41 ± 0.61
Experience with microbiological techniques	4.11 ± 0.69
Collecting experimental data and documenting laboratory work	4.17 ± 0.63
Analyzing and interpreting experimental data	4.35 ± 0.60
Understanding the role of microbes in the wastewater-treatment process	4.41 ± 0.71
Presenting scientific information to an audience	4.05 ± 0.89
Working collaboratively in a scientific setting	4.41 ± 0.79

[a]Mean scores for the entire class (n = 17) ± SD. Students responded on a numerical scale from 0 (no improvement) to 5 (significant improvement). Data are from December 2010.

the goals of the laboratory section of the course (Table 4). These data suggested that almost all of the objectives of the course were achieved. The one exception was the influence that the course had on the career choice of the students. The low average and high standard deviation of this answer were most likely due to the highly personal nature of career development, such that the course reinforced the students who were previously considering research careers but had little effect on the future career goals of those who were not. The second survey allowed the students to evaluate their own learning (Table 5). The data suggested that most of the students experienced significant personal improvement in the assessed learning areas and supported the original hypothesis of this work.

In conclusion, development of an open-ended, inquiry-based laboratory in BI348 was transformative from both a teaching and student learning perspective. The success of this approach required a teacher willing to cultivate a unique skill set, not the least of which was a willingness to abdicate control and empower the students. Perhaps the experience was best summarized by one of the BI348 students who, when asked what the most important thing they learned in BI348 lab was, responded that "research is hard work and often does not go according to plan, but the excitement and fulfillment that comes with learning something new through your own experiment is well worth the cost."

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