Integrating Biological Principles in Environmental Engineering Education: Summary Results of a Three-Year Pilot Study

Daniel B. Oerther

Department of Civil and Environmental Engineering, University of Cincinnati

Abstract.

Teaching biology to engineers is a challenge. For environmental engineers, biological principles are necessary to understand microorganisms that are removed from drinking water as well as microorganisms that treat waste in sewage treatment plants and bioremediation field sites. At the University of Cincinnati, we integrated state-of-the-art research in environmental microbiology within our graduate and undergraduate environmental engineering curricula. For the past three years, we taught a novel course entitled, "Molecular Biology in Environmental Engineering." Course evaluations over the past three years suggested that the course was successful for primarily two reasons, namely: (1) the course employed a problem-based learning approach to underlie all learning activities; and (2) experiments were conducted by student teams facilitating interpersonal communication as a primary means of learning from peers. This paper outlines the specific experimental procedures employed in the laboratory, as well as evaluates the results of student input from assessment tools including: one-on-one interviews with the instructor; anonymous student surveys; group interviews with an independent third party; and follow-up surveys conducted with graduates of the course. The results from our three-year pilot study suggest that the approaches followed in this course could be adapted to introduce engineering students to advanced research topics from many fields of fundamental science.

Teaching Biology to Engineers at the University of Cincinnati.

Biology has become a pervasive science; underlying many of the societal questions of the early 21st century including the threat of bioterrorism and the ethical dilemmas associated with cloning of human beings. As technologically proficient scholars, all engineers need to be trained to understand the basic tenants of biology. In a more advanced manner, environmental engineering students need to understand the microbiology of public health as well as the capabilities of microorganisms to degrade environmental pollutants. At the University of Cincinnati, we have developed a new course entitled, "Molecular Biology in Environmental Engineering." This course serves as a capstone in a series of four courses that introduce engineers to biology.

During the third year of a five-year undergraduate B.S. program, all civil engineering students at the University of Cincinnati are required to enroll in "Introduction to Environmental Engineering." In this course, all of our students are first introduced to biological waste treatment and public health microbiology. The first exposure of students to biology occurs in a lecture-discussion format with assigned readings from the required textbook. The primary emphasis of

this course is providing an overview of biology as it relates to engineering and helping to link high school biology with college-level biology.

We have found that many engineering students in our program have not participated in a biology course since their sophomore or junior year in high school. Thus, although our ABET accredited program provides adequate emphasis on chemistry, physics, and mathematics, we find that our students lack an appreciation for and an understanding of the basic tenants of biology. For example, most students cannot define biology beyond the "study of life", and we spend a significant portion of class time developing a definition of "life."

The condition of biology education for engineers at the University of Cincinnati may not be unique. Engineering students often do not have sufficient exposure to college-level biology, and engineering programs across the country need to reevaluate the emphasis placed upon chemistry and physics as part of ABET accreditation. Perhaps the time has come to encourage the study of biology to a degree equivalent to our current emphasis on chemistry and physics.

The second course in our four-course series is offered to seniors in civil engineering who have elected to concentrate in environmental engineering. This course is entitled, "Chemistry and Microbiology of Environmental Systems," and the primary objectives include introducing seniors to the biology of activated sludge sewage treatment including advanced topics related to biological nutrient removal. This course is intended to provide students interested in environmental engineering with sufficient background to excel in graduate work in environmental engineering. Accompanying the lectures for this course, students also participate in a term-long lab course. For two weeks during the lab course, pairs of students meet daily with a graduate student teaching assistant to perform maintenance, sampling, and analysis of pilot-scale activated sludge systems. Also, the students tour a full-scale municipal activated sludge sewage treatment plant, and complete a two-week, extended homework assignment that requires the development of a preliminary design for an industrial activated sludge sewage treatment system. Thus, "Chemistry and Microbiology of Environmental Systems" provides our seniors opting for a concentration in environmental engineering with an opportunity to experience extensive "hands-on" activities in biology and engineering design related to biology.

Two additional courses are offered each year as split-level and include both seniors as well as graduate students in environmental engineering. The first of these courses is "Biological and Microbiological Principles of Environmental Systems." Following a lecture-discussion format, this course is intended to provide a broad-based overview of the biology and microbiology of environmental engineering including agents of infectious disease and specific microorganisms that are known to degrade pollutants. Significant emphasis is placed upon providing a basic understanding of the biochemistry and physiology of microorganisms. We have found that seniors from our program are well equipped to participate in this course because of the background they have received from our previous two courses covering biology. Typically, first year M.S. students represent the largest portion of students in this course for biological treatment systems (e.g., the design of sewage treatment works). Most of our graduate students fit the model of typical civil engineering education as discussed briefly above. Therefore, this course often represents the first exposure of our graduate students to biology since high school.

The final course in our four-course series is entitled, "Molecular Biology in Environmental Engineering," and this course is the focus of this paper. "Molecular Biology in Environmental Engineering" is primarily a "hands-on" laboratory course that provides students with an opportunity to conduct a term-long experiment as part of a problem-based learning approach. Although the original intent of this split-level course was to provide a capstone experience in biology for seniors and graduate students in the department of civil and environmental engineering, we have found that many additional students opt to take advantage of this course. During the first three years of this course, participants have included students from civil and environmental engineering as well as the depart of chemical engineering and the department of electrical engineering from the College of Engineering, the department of environmental health and the department of molecular genetics from the College of Medicine, and the department of biological sciences from the College of Arts and Sciences. Enrollment in the first offering of our course was exclusively the intended target audience, namely civil and environmental engineering students, but enrollment in each subsequent offering has grown to include a cross-disciplinary pool of students. One reason for this increase in the breadth of enrollment is that students are drawn to the opportunity for a "hands-on" laboratory experience to learn about molecular biology as it can be applied to solve problems in a particular field, namely environmental engineering. Thus, we have found that providing an opportunity for "hands-on" laboratory work coupled with a problem-based learning approach is an effective strategy to attract crossdisciplinary participation in courses.

An Introduction to Molecular Biology in Environmental Engineering and Science.

Molecular biology refers to the science of studying molecules within and extracted from living systems. The primary classes of molecules that are of interest to environmental engineers include carbohydrates, lipids, proteins, and nucleic acid. Environmental engineers study carbohydrates and lipids because these molecules provide information about the physiology of microbial populations in bioreactors and bioremediation field sites. The study of proteins provides information about the biocatalytic properties of microbial populations, and nucleic acids provide a means of determining both the potential for biocatalytic capacity as well as the identity of specific microbial populations.

Recently, studies of nucleic acids have been used extensively to document the identity of microbial populations in various environmental samples including soil, sewage sludge, and drinking water. This increased interest in molecular biology procedures targeting nucleic acid has been driven primarily by the interest of researchers to positively identify microbial populations. Before the development of nucleic acid-based microbial identification, the primary means of identifying microorganisms included direct microscopic examination coupled with simple staining procedures or labor-intensive culture-based assays (e.g., most probable number [MPN] assays, filter counts, or plate counts). Both microscopic observation and culture-based assays are prone to error and often fail to uniquely identify microbial populations. In fact, head-to-head comparisons of the results of nucleic acid-based molecular biology procedures and culture-based procedures showed that culture-based procedures could only properly identify less than one percent of the microorganisms identified using nucleic acid-based molecular biology procedures using nucleic acid-based molecular biology procedures. Based upon these results, many researchers have adopted strategies using nucleic

acid-based molecular biology procedures to document different groups of microorganisms in environmental samples.

One of the most widely applied nucleic acid-based molecular biology procedures is known as the "full-cycle 16S rRNA approach." The 16S rRNA (16S ribosomal ribonucleic acid) molecule is an important structural component of a major macromolecular complex that can be found in all cells, namely the ribosome. The ribosome is responsible for the cellular information processing reaction known as translation. During translation, information contained in messenger RNA (mRNA) is "translated" into peptides and proteins. Thus, the "language" of nucleic acid (DNA and RNA) is "translated" into the "language" of amino acid (peptides and proteins). Because all life forms require ribosomes to conduct the translation reaction to produce proteins, the 16S rRNA molecule is a very old molecule in an evolutionary sense. In other words, the 16S rRNA molecule exists today in much the same form as it originally existed approximately 2-3 billion years ago when life on earth first began. Because the 16S rRNA molecule is found in all life, researchers can compare the sequence of nucleic acid in this molecule isolated from two different organisms and use the information to determine unique molecular signatures for each organism. These unique molecular signatures are then used to positively identify microorganisms in the environment.

In our class, students determine the sequence of 16S rRNA molecules isolated from samples of their own choosing. They use this sequence information to design "probes" which are used subsequently to positively identify microorganisms directly in their samples. Thus, the students conduct a "full-cycle 16S rRNA approach" by starting with a sample, generating and analyzing sequence information, designing probes, and going back to their original sample to find the microorganisms they have elected to identify.

Our Course, "Molecular Biology in Environmental Engineering."

At the University of Cincinnati, the Department of Civil and Environmental Engineering and the author have spent three years pilot-testing a course entitled, "Molecular Biology in Environmental Engineering." The objective of the course is to teach limited fundamentals of molecular biology in the context of quantitative engineering design and practice. The course was offered for the first time in the spring of 2001 with an enrollment of fifteen graduate students from the Program of Environmental Engineering and Science of the Department of Civil and Environmental Engineering. In the spring of 2002, the course was offered to an enrollment of eleven students including students from the Department of Civil and Environmental Engineering, department of electrical engineering, and the department of environmental health. The third offering of the course will occur in the spring of 2003. Preregistration shows enrollment by students from the Department of Civil and Environmental Engineering as well as students from the department of biological sciences and the department of molecular genetics. Thus, enrollment in our course has grown beyond the original target audience to include cross-disciplinary participation.

The course introduces students to molecular biology procedures through an extensive hands-on laboratory exercise conducted over a ten-week academic quarter. As discussed briefly above, the laboratory exercises for the course follow the "full-cycle 16S rRNA approach" employed in

environmental microbiology research laboratories. A hands-on laboratory format and multidisciplinary team approach to an open-ended problem allowed students to develop scientific questions, learn appropriate methodology, conduct careful experimentation, analyze data, and draw conclusions worthy of presentation to peers. Thus, the final outcomes of the course include the preparation of "peer-review quality-like" manuscripts by the students as well as one-on-one personal discussions with the instructor in lieu of a final examination.

The "full-cycle 16S rRNA approach" as applied in the class has been described previously (1, 2). Briefly, the genetic material from a mixed community of microorganisms is isolated, documented, and the nucleotide sequence information is used to construct molecular probes to interrogate the identity of single-celled microorganisms in samples removed from environmental matrices. The steps in the cycle include:

- The extraction of genomic deoxyribonucleic acid (DNA) from an environmental sample;
- The selective amplification of 16S rRNA genes;
- Cloning of amplified genes and subsequently determining primary nucleotide sequence information;
- Using the nucleotide sequence information to develop molecular probes; and
- Determining the abundance of target microorganisms in samples removed from the environment through whole-cell 16S rRNA-targeted fluorescence *in situ* hybridizations (FISH) with fluorescently labeled oligonucleotide hybridization probes.

Results of Course Evaluations.

The first offering of the course in the spring of 2001 was a considerable success. Thirteen of the fifteen students enrolled in the course responded to a detailed summary survey provided on the last day of the course. A detailed discussion of the results is provided elsewhere (5), but the most relevant finding are summarized herein. Similarly, the second offering of the course in the spring of 2002 was also successful. Six of the eight students enrolled in the course responded to a detailed summary survey provided on the last day of the course. In addition, during the second offering of the course the assessment tools were expanded to include a nongraded test of vocabulary and basic concepts administered in a pre- and post-format before and after each lecture. This allowed us to quantify the amount of information learned and retained by each student. A third method of assessment was provided by an informal interview of the entire class conducted by Dr. Cathy Maltbie of the Evaluation Services Center of the University of Cincinnati. All three methods of assessment will be used to evaluate the upcoming offering of the course in the spring of 2003.

First course offering in the Spring of 2001. The class had an almost equal number of male and female students with a median age of 27-30 years. About one-third of the students had previous laboratory experience in biology, but some students categorized themselves as inexperienced in biology and poorly prepared for the course. Overall, the students enrolled in the course could be generalized as mature graduate students working toward graduate degrees in environmental engineering. All of the students were enrolled in the Department of Civil and Environmental Engineering as graduate students pursuing either a M.S. or a Ph.D. in environmental engineering or environmental science.

Second course offering in the Spring of 2002. The class had an equal number of male and female students (four of each) with an average age of 26 years. Two students indicated that they had participated in fewer than two previous courses in biology. Four of the students indicated that they had participated in more than nine previous courses in biology. Therefore, overall the students enrolled in this course could be considered to be well trained in biology. One student was from the department of electrical engineering (studying BioMEMS – bio-micro-electrical mechanical systems for her research topic) and one student was from the department of environmental health in the College of Medicine (studying infectious disease as related to bioterrorism for her research topic). The remaining six students were from the Department of Civil and Environmental Engineering. Overall, the students enrolled in the course could be generalized as mature graduate students working toward M.S. or Ph.D. degrees in biology-related fields.

Third course offering in the Spring of 2003. Currently, enrollment is open for the third offering of the course. Already, early registration indicates that at least twelve students will be enrolled including seven students from the Department of Civil and Environmental Engineering, one student from the department of chemical engineering, two students from the department of electrical engineering, one student from the department of biological sciences from the College of Arts and Sciences, one student from the department of environmental health, and one student from the department of molecular genetics of the College of Medicine. Thus, the breadth of enrollment appears to be continuing to increase as students from diverse backgrounds and diverse departments across the campus participate in our course.

Comments from the class of 2001 and 2002. At the end of the course, the students were provided with an anonymous course evaluation form that asked a number of open ended questions. A summary of results from the class of 2001 and the class of 2002 is provided below.

In response to the question, "In your opinion, were the objectives of the course met?" students responded:

- 2001: The course met some of the objectives, but some students are not convinced why we use molecular biology to identify microorganisms in systems that have been proved or have been operating successfully.
- 2001: Yes. I am equipped with knowledge about this approach, and I can interpret research results and publications from this developing field.
- 2002: Yes. I learned how to conduct the lab procedures, but more importantly I learned the value of integration molecular biology tools into my research and I learned how interdisciplinary work could be performed by Environmental Engineers working with biologists.

In response to the question, "What was the best aspect of this course?" students responded:

- 2001: Most of the procedures are basic/universal operations in molecular biology, which means that we understand how to study biology and biotechnology at the molecular level.
- 2001: Experimental work because it is through applications that a student gets a tight grip on ideas and concepts. In addition, the challenging experiments and the value of the final result make the work more interesting.

- 2001: The lectures were interesting and informative. I learned a great deal, and my ideas about environmental engineering and science have been positively affected by the knowledge I have gained.
- 2001: Your perspective. We will never see "cutting edge" developments in a book.
- 2001: The whole structure of the course is similar to a research project.
- 2001: The best aspect was carrying the concepts from the classroom to the lab in a manner relevant to our field. Also, having a class that is new gives a fresh perspective into the future of environmental engineering.
- 2002: The availability of the professor to answer student questions during the lectures as well as during office hours and during the lab sessions.
- 2002: Working with a team of interdisciplinary students to complete an open-ended lab assignment.

In response to the question, "What part of the course would you suggest improving?" students responded:

- 2001: *More theoretical basis especially for the background of molecular biology methods.*
- 2002: Help us to make more of the experiments work its great to conduct these research projects, but what should we do if all of our work doesn't go as planned.
- 2002: Some group members overpowered less experienced group members and the less experienced people ended up learning less because they weren't allowed to do as much in the lab.

Conclusions.

These representative responses to the open-ended questions support the conclusion that the first and second offering of the course were successes. Both groups of students indicated that they really appreciated all of the effort put into providing an opportunity to use "state-of-the-art" research techniques in the classroom. As identified in the first course offering, one of the greatest challenges remains striking a delicate balance between providing too much basic information and boring students and providing too little background information leading to a lack of understanding of the theoretical basis for the work. In the second offering of the course, students participated in pre- and post-lecture quizzes. The students commented that the quizzes provides "sign posts" for the lecture by highlighting both key concepts and critical vocabulary. In a sense, the quizzes provides an outline for the students to follow when trying to understand the lectures and to identify key points.

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Biographical Information.

DANIEL B. OERTHER is a tenure-track Assistant Professor in the Department of Civil and Environmental Engineering at the University of Cincinnati. Dr. Oerther's research and teaching interface environmental engineering with fundamental principles of microbial ecology and advanced techniques in molecular biology and genomics.