Principles of Biology in Environmental Engineering: Molecular Biology-Based Identification of Microorganisms

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Abstract.

Technology from molecular biology increasingly is used by environmental engineers to screen for microorganisms in environmental samples and to monitor microbial biocatalysts in reactors. Training graduate and undergraduate environmental engineering students in fundamental molecular biology and applications of molecular biology in environmental engineering is a national challenge. At the University of Cincinnati, the author has begun to address this challenge by developing a new course entitled, "Molecular Methods in Environmental Engineering." The objective of the course is to teach students to adapt techniques from molecular biology to address important issues in environmental engineering. A hands-on laboratory format encourages students to develop scientific questions, learn appropriate methodology, conduct careful experimentation, analyze data, and draw conclusions worthy of presentation to peers. Although the initial offering of this course to well prepared graduate students was considered a success, future offerings must continue to address the daunting challenge of providing a supportive, yet independent learning environment for undergraduate students or poorly prepared graduate students. As molecular biology becomes as common to engineering curricula as chemistry and physics, engineering faculty need to take the lead in developing courses that introduce biology from an engineering perspective with a focus upon quantitative approaches and the application of science to find cost-effective solutions to society's demands. In particular, engineering faculty need to consider the implications of directly adapting existing course material from programs in the biological sciences to teach engineering students. Although memorization and recitation are effective tools for preparing undergraduate biology majors for careers in medically related disciplines, engineering curricula employ introductory materials that emphasize the derivation of advanced concepts from first principles using design examples. Engineering faculty must distill biology into first principles and relative design examples if we plan to follow traditional pedagogical approaches for teaching engineering students about biology. The author suggests that the approach followed in "Molecular Methods in Environmental Engineering" represents an effort to do just that.

Introduction.

The role for biology courses in engineering curricula is unclear. Historically, introductory courses in physics and chemistry have provided first and second year undergraduate engineering students with strong fundamentals in the basic sciences upon which engineering applications are developed. For example, courses in rigid body mechanics are built solidly upon first principles introduced in Newtonian physics while courses in heat transfer draw directly from topics

presented in physical chemistry. In contrast, a traditional ABET accredited undergraduate engineering curriculum does not reflect the same degree of dependence upon introductory courses in the biological sciences. Instead of tailoring biology courses to meet the needs of undergraduate engineers, one of the primary services of biology courses is the instruction of undergraduate pre-medicine students. Thus, the relationship between first principles of biology presented in introductory courses and subsequent applications of biology in engineering fields are unclear in engineering curricula.

Increasingly, engineers are applying basic and fundamental biology to meet society's demands. For example, biomedical engineering relates technology to biology through the development of improved medical devices, electrical and computer engineering harness the power of biology to create improved information storage and retrieval systems, and agricultural engineering continues to genetically modify plants to provide a sustainable food supply. In a similar manner, environmental engineering witnessed a recent explosion in applications of biology.

Environmental engineers use biology in at least two distinct ways. First, biology is understood from the perspective of public health including toxicology and the epidemiology of infectious disease. Second, biology is understood from the perspective of biocatalysis. Thus, biology for environmental engineering focuses primarily upon microorganisms, their metabolisms, and their capacity to induce infection.

The principal biological concern for environmental engineering is the identity of microorganisms. For example, coliform bacteria are used as indicators of the presence of fecal pollution while the presence of methanogens indicates a capacity to biogenerate methane. In the case of coliforms, design considerations in environmental engineering include the disinfection of potable water through drinking water treatment. In the case of methanogens, design considerations in environmental engineering include stabilization of sewage sludge through anaerobic digestion wastewater treatment. Historically, the identification of microorganisms in environmental engineering has employed technology developed in the field of determinative microbiology. Cultivation on semi-selective media as well as direct microscopic examination of environmental samples have been used by environmental engineers to identify, enumerate, and characterize microorganisms. Screening for microorganisms has been used to determine the efficacy of disinfection to remove bacteria in drinking water treatment processes and to monitor efforts to optimize the transformation of environmental pollutants in bioremediation and biological waste treatment.

Recently, the field of microbiology has seen an explosion in the development of new technology to identify microorganisms in environmental matrices. These new techniques have been generalized as cultivation independent, molecular biology-based, and ribosomal ribonucleic acid targeted (rRNA-targeted) (reviewed in 1). Environmental microbiologists and molecular microbial ecologists have applied these new techniques to assess the diversity of microorganisms in many environments including systems of direct interest to environmental engineers. The central theme emerging from these studies is the realization that traditional cultivation-dependent technology and direct microscopic examination of environmental samples have lead to a gross misrepresentation of the high degree of diversity among microorganisms (3). Some microbiologists have proposed that the five to ten thousand microbial species currently described

in pure culture represent less than one percent of the total microbial species on the planet. In some ways, this discovery can be likened to the discovery of quantum physics in a world dominated by the dogmas of Newtonian physics. A scientific revolution, of the type described by Thomas Kuhn (1962), has occurred in environmental microbiology, and the implications for applied environmental engineering are just beginning to take shape.

Although the identification of microorganisms remains the principal biological concern for environmental engineering, the technology available to screen for microorganisms is changing dramatically, and applications of these technologies are challenging some of the long-held views of environmental engineering education and practice. For example, the microorganisms long believed to be responsible for biological nutrient removal in activated sludge wastewater treatment included: *Nitrosomonas* spp., for ammonia oxidation; *Nitrobacter* spp., for nitrite oxidation; and *Acinetobacter* spp. for enhanced biological phosphorus removal. Within the past ten years, research to identify the predominant microorganisms in biological nutrient removal plants has discovered that *Nitrosomonas* spp. are the primary ammonia oxidizers only under certain conditions, *Nitrobacter* spp. are rarely found in activated sludge systems, and *Acinetobacter* spp. are not the primary microorganisms responsible for enhanced biological phosphorus removal (reviewed in 2). Thus, many on-going research projects in environmental engineering are applying these new technologies to reexamine the roles of microorganisms as biocatalysts and agents of infectious disease.

Integrating these new technologies and related discoveries into environmental engineering education is a national challenge. In what degree of detail should cultivation independent, molecular biology-based, rRNA-targeted techniques be introduced to students enrolled in environmental engineering? Should environmental engineering education wait for the forthcoming reorganization of environmental microbiology, or does providing the results of incomplete studies and on-going research improve environmental engineering education? How should undergraduate engineering curricula be modified to provide students in environmental engineering and other disciplines the appropriate knowledge of first principles of biological sciences? Finally, what role will introductory courses in biology play in future engineering curricula?

A new course for a new era.

At the University of Cincinnati, the Department of Civil and Environmental Engineering and the author are developing a course currently entitled, “Molecular Methods 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. The course introduced students to molecular biology technology through an extensive hands-on laboratory exercise conducted over a ten-week academic quarter. The laboratory exercise followed the “full-cycle 16S rRNA approach” employed in environmental microbiology research laboratories (described in 1). 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 included 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 (5). 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.

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.

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. Additionally, some of the students responded to three open ended questions on the summary survey.

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

- *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.*
- *Yes. I am equipped with knowledge about this approach, and I can interpret research results and publications from this developing field.*

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

- *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.*
- *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.*
- *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.*

- *Your perspective. We will never see "cutting edge" developments in a book.*
- *The whole structure of the course is similar to a research project.*
- *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.*

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

- *More theoretical basis especially for the background of molecular biology methods.*

These representative responses to the open-ended questions support the conclusion that the students felt that the first offering of the course was a success. Interestingly, the students indicated that they appreciated the effort to bring "state-of-the-art" research techniques into the environmental engineering classroom, and students commented that the course could be improved if relevant background information was provided. Two of the greatest challenges for developing a role for molecular biology in environmental engineering curricula are: (1) discovering successful approaches for moving research topics into formal student education; and (2) developing appropriate background information to introduce students to appropriate first principles of biology.

Course improvements and expansion.

Currently, the author is pursuing a number of options for improving and expanding the course. The author recently received funding through a Course Curriculum and Laboratory Improvement (CCLI) Educational Materials Development (EMD) proposal from the National Science Foundation (NSF) to purchase additional laboratory equipment and supplies as well as to support the development of a web-based laboratory manual and the conversion of the existing VHS-format laboratory instruction video to DVD-format (DUE-0127279, "Integrating Genomics Research into the Undergraduate Engineering Curriculum in Environmental Engineering.") The long-term goals identified in this NSF project include national dissemination through appropriate commercial distribution as well as an expansion of the existing course to include undergraduate students from related disciplines such as biomedical engineering and chemical engineering.

Broader implications of the course.

In what degree of detail should cultivation independent, molecular biology-based, rRNA-targeted techniques be introduced to students enrolled in environmental engineering? One of the primary concerns during the development of "Molecular Methods in Environmental Engineering" was the appropriateness of selecting the "full-cycle 16S rRNA approach" to serve as the sole example of molecular biology in environmental engineering. Although techniques targeting 16S rRNA have proven invaluable for the identification of microorganisms in environmental samples, alternative techniques are available. For example, antibody staining and phospholipid analysis have been used to screen for microorganisms in environmental samples. Furthermore, the "full-cycle 16S rRNA approach," as presented in the class, does not include all of the techniques targeting 16S rRNA. Thus, although the author selected the "full-cycle 16S rRNA" approach as a representative molecular biology-based technique, alternative course

development could include a survey of a variety of unrelated techniques instead of a thorough development of a single collection of related techniques. On a positive note, at least one student indicted, "*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,*" suggesting that the selection of the "full-cycle 16S rRNA approach" was appropriate.

Should environmental engineering education wait for the forthcoming reorganization of environmental microbiology, or does providing the results of incomplete studies and on-going research improve environmental engineering education? For graduate students in the environmental engineering discipline, courses that infuse state-of-the-art research topics, such as molecular biology, into the classroom and in formal laboratory training are invaluable. For graduate students in related disciplines or for undergraduate students in civil and environmental engineering, the advantages of introducing evolving technology are less clear. Thus, the current course format for "Molecular Methods in Environmental Engineering" strikes a balance between teaching advanced topics to graduate students within the environmental engineering discipline and introducing less experienced and less prepared students to confusing topics. As the field of environmental microbiology emerges from the current state of confusion into a new era of understanding, environmental engineering curricula need to be ready to integrate new concepts and new techniques into courses designed to teach biological principles.

How should undergraduate engineering curricula be modified to provide students in environmental engineering and other disciplines the appropriate knowledge of first principles of biological sciences? and what role will introductory courses in biology play in future engineering curricula? The development of a set of first principles of biology appropriate for undergraduate engineering curricula should be a national priority for engineering faculty. Unfortunately, many engineering faculty are ill-prepared to meet this challenge. At one time, introductory courses in calculus, physics, and chemistry were taught by engineering faculty. To reduce teaching loads and to permit engineering faculty to explore advanced courses, the responsibility for teaching introductory mathematics and science courses were transferred from engineering faculty to colleagues in the arts and sciences. Nevertheless, engineering faculty maintained an appreciation for the content of introductory mathematics and science courses, and mid-level engineering courses continued to build directly upon first principles presented in introductory courses. Teaching biology to undergraduate engineering students represents a unique challenge. Engineering faculty are unfamiliar with the content of introductory biology courses, and colleagues in the arts and sciences responsible for introductory biology courses have been working more closely with medically related disciplines as compared to engineering related disciplines. Thus, the simple approach of requiring existing introductory biology courses to provide first principles of biology to engineering students may be doomed to failure. Instead, engineering faculty need to distill biology into first principles if engineering curricula will follow traditional pedagogical approaches for teaching engineering students about biology.

Conclusions.

To address the national need for integrating techniques from molecular biology into the environmental engineering curricula, in cooperation with the Department of Civil and Environmental Engineering at the University of Cincinnati the author is developing a new course

currently entitled, "Molecular Methods in Environmental Engineering." The objective of this course is to introduce engineering students to molecular biology through hands-on laboratory exercises complimented by weekly lecture-discussion sessions. The course is designed to teach molecular biology from an environmental engineering perspective with a focus upon quantitative approaches and the application of molecular biology to find cost-effective solutions to society's environmental problems. In particular, environmental engineering students are introduced to advanced techniques to identify microorganisms in environmental samples. "Molecular Methods in Environmental Engineering" represents the type of new course that needs to be developed by engineering faculty to distill the principles of biology and to present these principles in a format that can be drawn upon in advanced engineering design courses. In the future, "Molecular Methods in Environmental Engineering" can be used as a mid-level engineering course building upon first principles of biology presented in reformulated introductory biology courses. The author suggests that engineering faculty need to take an active role in the redevelopment of course content in introductory biology courses if engineering curricula will follow traditional pedagogical approaches for teaching engineering students about biology.

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