

UREASE EFFICACY IN ENZYME INDUCED CARBONATE PRECIPITATION (EICP) IN SELECTED SOIL SAMPLES AND EXPERIMENTAL CONDITIONS

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LESSON DETAILS

Subject Area(s): Biology, Geology, Geotechnical Engineering

Focus Grade Level: College Freshmen

Grade Level Range: High School - College Sophomore

RESEARCH BACKGROUND

Enzymes play a major role in mediating a variety of biochemical processes that are essential to all living organisms and can also be used by engineers and scientists for beneficial purposes; for example, control of fugitive dust emissions by enzyme induced carbonate precipitation (EICP). Erosion and generation of dust are natural or man-caused phenomena that have significant economic, societal, environmental, and health-related consequences. Strategies for dust control based on naturally occurring or induced chemical or biological processes present a more sustainable alternative to traditional dust control methods, especially those that rely on large volumes of water (1). This project attempts to analyze the efficacy of Enzyme Induced Carbonate Precipitation (EICP), a bio-mediated dust control method, on selected soil samples under experimental conditions applying techniques routinely used in geotechnical engineering research.

LESSON SUMMARY

Students in BIO 181 (Introductory Biology for Majors I) are introduced to the role the enzyme urease can play in making carbonate precipitation more effective by increasing the rate of precipitation by a factor of 10^9 . Students are then introduced to the problems associated with erosion and dust generation and to how EICP can be harnessed to provide a more sustainable method for controlling dust than conventional methods, highlighting the importance of urease in this process. Students are then asked to list environmental factors that can impact the efficacy of urease in catalyzing EICP, and what experimental design needs to be in place to test that. The project is designed and implemented as a CURE (Course-based Undergraduate Research Experiences) in BIO 181 labs. BIO 181 labs host groups of 24 students; each lab session is 3 hours long. All students enrolled in these lab courses execute the project from start to finish with the assistance at all times of the instructor and student mentors. If possible, experts in the topic are invited to the lab to give a brief introduction to the topic and to stress its relevance to the community and to society at large. The experiment expands for 6-8 weeks out of a 16-week semester. Through this project, students gain a better understanding of enzyme activity, bio mediation, applications of bio mediation to environmental problems and geotechnical engineering, interdisciplinary collaboration, engineering design, and the process of science.

MATERIALS AND EQUIPMENT

- Basic laboratory materials and instrumentation (test tubes, flasks, pipettes, micropipettes, graduated cylinders)



- Spectrophotometers, scales
- Scanning Electron Microscopy (SEM) photos of EICP soil improved with ad without dry milk
- 4 Vernier conductivity probes: <https://www.vernier.com/product/conductivity-probe/>
- 3 Pocket soil penetrometers: <https://www.humboldtmg.com/soil-penetrometer-pocket-type.html>
- 50 lb box of Ottawa 20/30 sand
- 20 round baking pans
- Jack beans, 500 g (Sigma-Aldrich):
https://www.sigmaaldrich.com/US/en/product/sigma/j0250?gclid=Cj0KCCQjwy9-kBhCHARIsAHpBjHgeUJZMEsm3G48sH66kBHtmxW_mXU9AVFpooDX9vmtWOa8RZnSrTQUaAl8fEALw_wcB&gclidsrc=aw.ds
- Dry milk
- urea
- urease
- calcium carbonate
- 12 hand sprayers
- materials for processing SEM soil samples (TBD)

ATTACHMENTS

[Dust Control Water Trucks](#)

[Lab Tour Biocementation](#)

[Fundamentals of Biocementation](#)

[Student Handout](#)

EDUCATIONAL STANDARDS

COMMUNITY COLLEGE FACULTY

MCCCD Course Competencies for [BIO 181 \(Introductory Biology for Majors I\)](#)

1. Describe and apply the scientific method to investigate biological phenomena. (I, XI)
5. Describe the relationship between atoms and molecules and the importance of chemical bonds to atomic stability, molecular structure, and chemical characteristics. (II)
6. Describe the relationships between the structure and functions of the four major kinds of organic macromolecules found in living things. (II)
8. Compare and contrast prokaryotic and eukaryotic cells. (III)
12. Explain the importance of enzymes to metabolic processes and their mode of action. (V)
22. Demonstrate laboratory procedures and safe practices. (XI)
23. Apply principles of scientific methods while conducting laboratory activities and experiments. (XI)



24. Perform laboratory activities using relevant equipment, chemical reagents, and supplies to observe biological specimens, measure variables, and design and accurately conduct experiments. (XI)

26. Demonstrate the ability to accurately use pipettes, micropipettes, and other volumetric devices, chemical glassware, balances, pH meters or test papers, spectrophotometers, and separation techniques such as chromatography, differential centrifugation and/or gel electrophoresis to perform activities relevant to other course competencies. (XI)

27. Demonstrate the ability to construct a graph that accurately portrays quantitative data. (XI)

28. Calculate appropriate proportions of solvent and solute(s) to make molar and/or percent solutions of varying concentrations. (XI)

LEARNING OBJECTIVES

Students will be able to:

- Describe connections between geotechnical engineering, soil science, and biochemical processes
- Describe fugitive dust control and soil erosion and current methodologies used for their control
- Describe the economical, environmental, and health related problems associated with fugitive dust and erosion
- Describe the role enzymes play in facilitating biochemical processes
- Describe the most common types of bio mediated methodologies to control fugitive dust and erosion
- Describe the process of Enzyme Induced Carbonate Precipitation (EICP)
- Identify and describe the role of enzymes and supplemental proteins in biogeochemical reactions through EICP
- Demonstrate how experimental conditions affect the efficacy of urease and of EICP
- Design experimental protocols, analyze results, and draw conclusions
- Acquire proficiency with geotechnical laboratory techniques

VOCABULARY

<i>word/phrase</i>	<i>Definition</i>
geotechnical engineering	a branch of civil engineering concerned with earth materials. It uses the principles of soil and rock mechanics and rock mechanics for designing engineering solutions to address related problems.
engineering solution	a design conceived and carried out to produce the desired result.
soil	the upper layer of earth in which plants grow. A black or dark brown material typically consisting of a mixture of organic remains, clay, and rock particles.
bio-mediation	any process wherein a biological system, living or dead, is employed.



biogeochemical reactions	Reactions that involve mutual interactions (two-way) between the biology and chemistry of the Earth system.
bio cementation	a soil stabilizing and strengthening process which occurs when bacteria induce the calcite precipitation in soil.
EICP	Enzyme Induced Carbonate Precipitation
urea	a colorless, crystalline compound (CH ₄ N ₂ O). Urea is the main nitrogenous breakdown product of protein metabolism in mammals.
urease	a naturally occurring enzyme that hydrolyzes urea into ammonium carbonate

LESSON PROCEDURE

INTRODUCTION/MOTIVATION

I have found that the most effective way to engage students in a research project is by asking them a simple question, which can be integrated into the course through a Discussion Board. Here is how it reads:

For our last lab discussion, please picture yourself as a scientist, or as an engineer, or as a nurse working in clinical research, or as the professional you want to be or that you already are. Then think about the issues that affect you and your community and pick a topic or a project that you would like to work on if you had the opportunity. This will be a discussion with the entire class.

Discussion posts and student replies to the class allow me to be aware of what THEIR interests are. With that information, I tailor the research project in a way that targets their interest and learning outcomes (for instance, specifically how fugitive dust affects health and how current methods of fugitive dust control may not be sustainable in places like Arizona). I make every effort to bring experts (community or industry partners, researchers) the day I introduce the project and I ask them to talk about the discipline, major challenges, and their career path. If possible, I try to bring to the class an undergraduate student (in this case, it would be a Geotechnical or Material Science Engineer), and I ask them to mingle with the class. In my experience, these two strategies are crucial for sparking motivation and engagement.

LEARNING ACTIVITIES/STRATEGIES

Problem Based Learning: Students in BIO 181 (Introductory Biology for Majors I) are introduced to the problems associated with dust control and to alternative and more sustainable methods of dust control that rely on bio mediation (more specifically bio cementation). Students are then introduced to bio mediated methods of dust control and, more specifically, to the Enzyme Induced Carbonate Precipitation (EICP) method, and to urease, then enzyme that catalyzes EICP. Students are then asked to list environmental factors that can impact the efficacy of urease in catalyzing EICP, and what experimental design needs to be in place to test it.

Course-based Undergraduate Research Experiences (CUREs): The project is designed and implemented as a CURE (Course-based Undergraduate Research Experiences) in BIO 181 labs. BIO 181 labs host groups of 24 students; each lab session is 3 hours long. The lesson/experiment expands for 6-8 weeks out of a 16-week semester.



Collaborative Learning: The project is conducted collaboratively by groups of 4 students. Each student has a role in the project. The entire class participates in the final discussion of results and conclusions. Students are asked to take a role in the project depending on their preferred skills. I typically assign the following roles:

Team leader: keeps the group on task and ensures that steps and experimental design are followed; **Procedures leader:** ensures procedures are accurately followed. Describes procedures in detail to those team members that may have a hard time with some steps; **Results leader:** ensures results are recorded and correctly analyzed. Describes data collection and analysis in detail to those team members that may have a hard time with some methods; **Conclusion and Project Presentation Leader:** ensures all the components of the project are organized for presentation.

Even though each student has a role, they naturally collaborate and assume different roles as the project goes, but they know there is a part of the project they are responsible for.

Requirements for Teaching the Lesson:

Concepts: Freshmen introductory biology course concepts such as atomic structure, structure of organic molecules and macromolecules, enzymes and enzyme inhibition, structure of prokaryotic cells, and basic notions of geology and geotechnics, are indispensable. Additionally, concepts related to fugitive dust and erosion control and methodologies, biocementation, and benefits and challenges of biocementation are necessary for the success of the lesson.

Techniques: In addition to experience in general laboratory techniques and safety procedures, this lesson requires experience in spectrophotometry, soil sample preparation, enzyme activity measurements, and scanning electron microscopy (SEM).

CLOSURE

I require a one-page summary per group and a class PowerPoint presentation, to which partners, faculty, and students are invited to attend. Students work collaboratively with their group to complete the one-page summary and with the entire class to complete the presentation.

ASSESSMENT

FORMATIVE ASSESSMENT

SUMMATIVE ASSESSMENT

One-page summary per group and a class PowerPoint presentation, to which partners, faculty, and students are invited to attend.



CONTRIBUTORS

INDIVIDUALS

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REFERENCES

(1) Kavazanjian Jr. E & Iglesias E (2009). Biopolymer soil stabilization for wind erosion control. *Proceedings, 17th International Conf. Soil Mechanics and Geotechnical Engineering*, Alexandria, IOS Press - Mill Press, Vol. 2: 881-884

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