BIOREMEDIATION – MICROBE DETOX

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LESSON DETAILS

Subject Area(s): Bioremediation, Microbial chain elongation, reductive dechlorination/dehalogenation, carbon transformation, anerobic microorganisms, *Dehalococcoides mccartyi (needs hydrogen*)

Focus Grade Level: Community-college

Grade Level Range: Undergraduate

RESEARCH BACKGROUND

Contaminants in the environment pose health risks to humans and other organisms.

Using organisms to convert harmful contaminants to less harmful compounds can help redeem the environment (e.g., soil and groundwater) such as, cleaning of superfund sites - locations the EPA has deemed hazardous and in need of cleanup.

An example of one such hazardous chemical is chlorinated solvents; e.g., perchloroethylene (PCE) and trichloroethylene (TCE), common in groundwater. Industrial chlorinated solvents are found in the majority of many Superfund sites.

Bioremediation can be used to clean up contamination at a superfund site. A common form of bioremediation to cleanup chlorinated solvents is reductive dechlorination (or reductive dehalogenation), an anaerobic metabolism done by Dehalococcoides mccartyi. These bacterial microorganisms convert PCE, TCE, and other chlorinated solvents to non-toxic ethene or ethane.

Reductive dechlorination needs H_2 typically provided by fermentation. Fermentation is the breakdown of long-chain, complex organic molecules to produce hydrogen.

In a process called microbial chain elongation (MCE), anaerobic microorganisms produce H_2 through elongation of short-chain simple organic molecules to long-chain, complex organic molecules (the opposite of fermentation). MCE can support reductive dechlorination, like fermentation, through hydrogen production. Whether fermentation or MCE occurs in the subsurface will overwhelmingly depend on the substrate introduced: either long-chain, complex organics or short-chain, simple organics.

Current methods of bioremediation can lead to subsurface bioclogging, incomplete transformation of chlorinated solvents, methane production (a greenhouse gas), along with other issues.

Using MCE to support reductive dechlorination could be the only solution at certain sites where chemical/physical remediation has been shown to be ineffective. This is an import process to aid a return of sites to a more environmentally safe setting and can do so without high costs.

LESSON SUMMARY

Students will use principles of the scientific method to perform an experiment and gain knowledge of bioremediation (specifically cleanup of groundwater). Provided with an established protocol, students will use sugar (pollutant) and yeast (bacteria) exposed to varying environmental conditions to find the optimal setting (temperature, pH, salinity) for organisms to degrade pollutants. The measurement of degradation will be measured through CO_2 collection (conversion of pollutant to a harmless gas).

Materials and Equipment

Each group:

- small white board w/eraser
- balance
- digital thermometer
- tape measure
- 2 weigh boats or weigh paper
- 2 scoopulas
- 4 – 50 mL erlenmeyer flasks
- black sharpie marker
- 4 small balloons
- 2 T yeast
- 2 T sugar

To be shared among groups:

- salt w/scoopula
- baking soda w/scoopula
- calcium carbonate w/scoopula
- parafilm w/scissors
- pH strips
- thermometer
- white board markers
- ~250 mL refrigerated DI H_2O
- ~250 mL heated DI H_2O
- ~250 mL refrigerated tap H_2O
- ~250 mL heated tap H_2O
- ~250 mL refrigerated vinegar
- ~250 mL heated vinegar
- 6 - 5 mL graduated cylinders (for ea DI, tap, and vinegar)
- ice chest w/crushed ice
- 5 volumetric flasks (50, 100, 200, 250, 500 mL) – visual measure of CO_2 collection

Instructor: Question-- can another microorganism, or another substrate, do a better job?

- 50 mL pre-filled erlenmeyer: sugar, DI H_2O, baking soda (different microorganism or substrate)
- 5 mL vinegar
- 1 balloon

ATTACHMENTS

Image 1 - equal grams of sugar and yeast

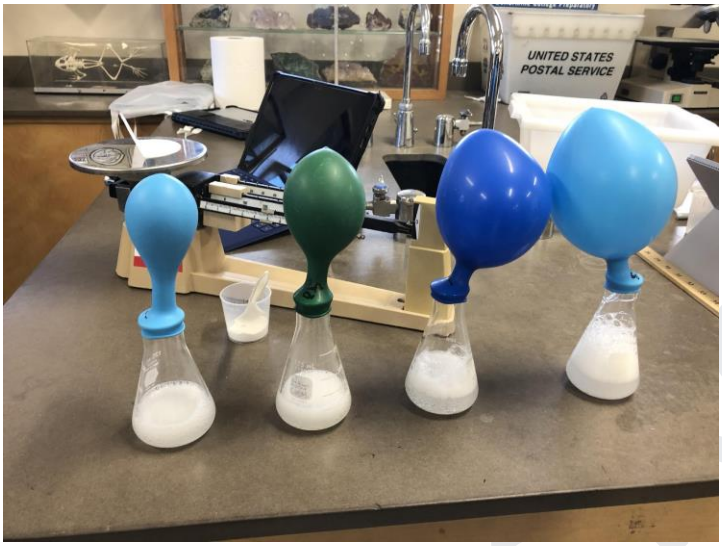

Image 2 - experiment set-up (one is a control

Crushed ICE	DI H_2O HOT	Vinegar COLD	Vinegar HOT	salt	baking soda	Tap H_2O COLD	Tap H_2O HOT	sugar	yeast	DI H_2O rm T
X								X	X	X
	X							X	X	X
		X						X	X	X
			X					X	X	X
				X				X	X	X
					X			X	X	X
						X		X	X	X
							X	X	X	X
X		X						X	X	X
X				X				X	X	X
X					X			X	X	X
X				X	X			X	X	X
X		X		X				X	X	X
	X		X					X	X	X
	X			X				X	X	X
	X				X			X	X	X
	X			X	X			X	X	X
	X		X	X				X	X	X

Image 3 - Bioremediation-Microb Detox_exp conditions (three provided to students)

Name _____

BIO156 – Bioremediation Microbe Detox
Individual Pre-assessment (10 pts)

1. Describe bioremediation (2 pts)

2. Tell what a Superfund Site is, and what the Environmental Protection Agency (EPA) wants to do at these locations. (4 pts)

3. **T** or **F** Trichloroethylene is a chlorinated solvent. (2 pts)

4. TCE is a completely _____ (safe/toxic) compound. (2 pts)

Bioremediation Microbe Detox Individual Pre-assessment

Name _____

Bioremediation Microbe Detox
Summative Assessment (15 pts)

Reflect on your thinking, learning, and the *process* of your group experiment.

Do you think you benefited from this group experiment? How so? If not, why?

Do you think your learning was enhanced when you shared your results with other groups? Why?

In what ways will this lesson, and performing the experiment, prepare you for quizzes and exams?

What did you learn from working on this task – ***about the process and yourself***?

How comfortable was it to work with your (new) class partner(s)?

- o Very comfortable
- o Somewhat comfortable
- o Not comfortable

What aspect of your work (or this lesson) do you think was least effective? Why?

Give three new vocabulary words and their definitions you learned while performing this investigation.

Would you be interested in becoming more involved in undergraduate work involving bioremediation and environmental engineering?

Educational Standards

AZ Maricopa County Community College District Course Competencies (MCCCD) – Introductory Biology for Allied Health.

1. Describe principles of scientific method and apply these in conducting laboratory investigations. (I, XIV)
8. Describe the structure, reproduction, and human impact of bacteria and viruses. (VIII)
19. Perform laboratory activities/experiments that demonstrate the principles of the scientific method. (I, XIV)
20. Perform appropriate mathematical calculations, conversions and representations (e.g., tables, graphs) of data generated via laboratory activities/experiments. (XIV)
21. Analyze and interpret data to draw logical conclusions. (XIV)

Learning Objectives

Students will use the scientific method, be introduced to the importance and impact of living organisms (bacteria), their contributions to bioremediation, and make connections between biology and engineering. Discuss implications of real-world ASU research that is ongoing, that this lesson is modeled after.

Vocabulary

bioremediation	Use of microorganisms (introduced or naturally occurring) to break down/consume environmental pollutants.
substrate	Base material involved in a reaction that undergo chemical transformation.
groundwater	Water that is held underground in saturated zones beneath the land surface.
superfund sites	Locations polluted with hazardous materials
chlorinated solvents	Industrial chemical used widely for metal cleaning, production of lacquers, perfumes.
reductive dechlorination	Chemical reaction that cleaves the bond between carbon and chlorine, to release the chloride ions.
anerobic bacteria	Microbes that thrive in an environment with little to no oxygen.
D. mccartyi	Anaerobic bacteria, known dechlorinators
microbial chain elongation	Anerobic metabolism is which microorganisms grow and gain energy by combining carboxylates with reduced compounds to produce longer carboxylate chains.
TCE (trichloroethylene)	Chlorinated solvent used degreasing, also found in adhesives, paint and stain removers.
pH	Measure of how acidic or alkaline a substance is based on a scale from 0-14.

Lesson Procedure

Assigned the Flipped-Lesson videos a couple days before performing the Erlenmeyer flask/CO_2 collection experiment.

Pre-assessment questionnaire – individual quiz.

Introduction/Motivation

Overview and Brainstorming questions.

Introduce the experiment:

Preparation of 50 mL Erlenmeyer flasks, students mix the pollutant (sugar) with yeast (bacteria) and add other chemicals. They stretch a balloon over the top of each Erlenmeyer to "collect" CO_2 gas produced.

1. Divide the class into groups of two or three students each and provide each group with three of the conditions (Image 3).
2. Explain to groups they get four Erlenmeyer flasks total in which to test their assigned conditions. The sugar represents a toxic contaminant, and the yeast represents the microorganism, bacteria.
3. Students are to use the balance to measure out equal amounts of sugar and yeast, 5 grams (Image 1). Remind students to 'tare' the balance either before or after placing the chemical on the weigh paper/boat. They want to have four piles of 5 grams of sugar and three piles of 5 grams of yeast (equal amounts). Taking three of the Erlenmeyer flasks, they will place one of each pile of sugar into each of the three flasks. They will also place one of each pile of yeast into each of the three flasks. To reduce contamination of chemicals, tell students to make sure they use the same scoopula for measuring the sugar, and a different scoopula for measuring out the yeast.
4. Some of the groups will vary the three flasks environment by adding cold/hot vinegar, cold/hot tap water, baking soda, salt, ice, according to their protocols (provided in step 1).
5. The fourth flask is designated as the control; the last pile of sugar is to be mixed with only with DI water in this flask.
6. They will add DI water (or any liquid) at the same time to each of the flasks, and stretch a balloon over the top to see, if any, gas is released.
7. Instruct groups to discuss the (environmental) conditions they are exposing their yeast to through their experiments. Ideas: vinegar lowers pH, baking soda raises pH, salt raises salinity, hot/cold, etc.
8. Pass out group supplies and show students where the shared products are. Have students check their balloons for holes and are not stuck together at the neck.
9. In three of the flasks, students use equal parts of sugar and yeast to keep their samples consistent with each other. The procedure for preparing the flasks is as follows:
    - Use a marker and tape to label the flask with mixture content (that is, sugar, yeast, water, etc).
    - One student puts one pile each of sugar in the flask (NO Yeast yet), along with salt or baking soda, other dry chemical (if they wish to test using that chemical); For example, if no salt is desired, skip to the next step.
    - Add DI water (and other liquid) to the flasks as close to the same time as possible. Each flask will be filled with an equal amount of DI water (bring up to 50 mL), and then add additional liquid (25 mL) of choice (hot water, vinegar plus hot water, cold water, ice, etc.).
    - Place thumb over the top of the flask and shake it to dissolve the sugar in the liquid.
    - Add one pile of yeast to each of the flasks and quickly stretch the balloon over the top.

- Place thumb over the top of the flask (bending the balloon), and shake it to mix the solution.
- It takes about 10 minutes to notice results; note the time the yeast is added so that 10 minutes can be calculated.
- Swirl or gently shake each flask, as needed, during the 10 minutes.

10. After the full 10 minutes, students should note the size of the balloons - measure the circumference with the tape measure.
11. They should also take the pH and temperature of each flask.
12. As a class, discuss which conditions resulted in the most pollutant (sugar) consumed by the bacteria (yeast) broken down to make CO_2 (as indicated by the balloon size)?

Finish lesson with CLOSURE activities, and Summative Assessment.

Introduction/Motivation

How important is clean water to you?

Clean water is essential to our communities, our economy, and our health. Our overall environmental health.

The Environmental Protection Agency (EPA) lists the most serious uncontrolled or abandoned hazardous waste sites as Superfund sites. These areas affect not only the soil, but groundwater. Arizona has nine active superfund sites.

Engineers have utilized bacteria to break down dangerous toxins i.e., oil, toxic metals, and chlorinated solvents and clean up the pollution. This is a process called bioremediation.

Why do you think that bioremediation might be a good, or even better, treatment to use over other available processes or technologies? (Possible answers: Taking advantage of natural processes, lower cost, better for the environment, etc.)

Learning Activities/Strategies

Flipped Learning:

- Have students view provided videos below on their own (pre-assessment questions are based on these).
  - https://youtu.be/N6Nzc4pfJP4
    - Biological Degradation of Chlorinated Solvents and Groundwater Remediation
  - https://youtu.be/c_MaLs5lhrU
    - CDC Trichloroethylene (TCE) Overview
  - https://youtu.be/UMoXRFyUCYw
    - CERCLA Superfund Act - What it is and its Purpose.
  - https://youtu.be/uAyVcR17COs
    - What is Bioremediation?

Overview with students:

- https://www.epa.gov/superfund/search-superfund-sites-where-you-live
    - This site has information about Superfund sites (locations, history, contaminants, etc).
- WPD | Superfund Sites | ADEQ (azdeq.gov)
    - View a Superfund site in AZ with contaminants of concern (see chlorinated solvents).

Brainstorm:

- What are some ways you know of that have been used to clean up any kind of hazardous spill?
- What are some ways nature might help with this process?

Closure

Once students have gathered data on each of their flasks, they will create a bar graph (white board) to share results of remediation and the most successful conditions (low pH was best, cold temperature was favored, etc.).

I will pose one last question, "Do you think there could be other microorganisms/substrates that might do a better job?". The instructor will then pour vinegar into the pre-filled Erlenmeyer flask (baking soda) to simulate a larger pollutant conversion. This a conversation starter about how both locations and microorganism communities can vary - some might perform a better than another.

Students learn about Superfund sites, toxic chlorinated solvents, and the importance of in situ bioremediation and its advantages.

This experiment will be tied to ongoing research currently happening at ASU to make connections between biology, chemistry, and environmental engineering. Students will be introduced to research and other job opportunities in science. They also get to have a little fun!

Assessment

The formative assessment will be a simple (individual) quiz to assure they participated in the flipped-lesson.

The summative assessment is to be taken as a group, and will review the experiment they covered, and a few background questions.

Formative Assessment

Questionnaire based on the four YouTube videos students are to view before class, "Bioremediation Microbe Detox Pre-assessment".

Summative Assessment

Students will take the summative assessment, "Bioremediation Microbe Detox - Summative Assessment". Covers experiment and background questions about learning and comprehension.

Contributors

Individuals

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References

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