Encouraging High School Students to Learn about Bioremediation

Richard O. Mines, Jr.[1], Janet Carlson Powell[2], Laura W. Lackey[1]

**Mercer University[1], Department of Environmental Engineering,
1400 Coleman Avenue, Macon, GA 31210 / BSCS[2], 5415 Mark Dabling Blvd.
Colorado Springs, CO 808919**

Abstract
This paper presents a laboratory activity for high school students used to stimulate their interest in environmental engineering and the role of bioremediation in cleaning up the environment. The proposed laboratory activity utilized six, 2-L plastic bottles that contain 100-grams of indigenous soil in each to serve as bioreactors. Varying amounts of glucose are added to the reactors, which are monitored with time for a period of five to ten days. Questions for assessing the exercise along with sample laboratory results are provided.

Introduction

The challenges of environmental engineers have historically focused on the design of drinking water treatment facilities, municipal and industrial wastewater treatment, solid waste collection and disposal systems, and air pollution control equipment. In recent years, these challenges have expanded to include the identification, removal, and treatment of hazardous chemicals and wastes that have resulted from inadvertent spills or illegal discharges to the land, water, and air. This paper presents a laboratory activity for high school students used to stimulate their interest in environmental engineering and the role of bioremediation in cleaning up the environment.

Bioremediation is a natural process in which indigenous microorganisms found in soil and water are utilized for treating primarily toxic organic compounds such as solvents, pesticides, herbicides, and precursors for industrial processes. These microorganisms transform the toxic organic compounds into less harmful products such as carbon dioxide and water. It can be used to treat contaminated media, excavated soil, soil in situ, groundwater, surface water, and gases emanating from soil. Bioremediation requires the control and manipulation of microbial processes, therefore, requiring the integration of scientific principles with engineering.

The proposed laboratory activity utilizes six soil bioreactors to measure the concentration of glucose over time to simulate how heterotophic bacteria in the soil would consume and transform gasoline or oil into innocuous products. Different glucose concentrations are added to the soil in five of the bioreactors and the sixth serves as a control. Parameters that may be measured over time include dissolved oxygen (DO), pH, glucose concentration, and chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Colony forming units (CFU) and turbidity analyses may also be conducted to quantify the microbial growth rate.

Background

Consider a scenario that leads to a hazardous material being released into the environment. For example, when a tanker truck overturns and gasoline percolates through the soil and into the groundwater, how does this impact the ecosystem? Some of the gasoline volatizes and is released to the atmosphere contributing to smog production. Gasoline that reaches the groundwater contaminates it making it unfit for human consumption. Aerobic, heterotrophic bacteria that proliferate in the soil are capable of using the organic components in the gasoline for energy and the synthesis (growth) of more bacteria. Engineers capitalize on this phenomenon and attempt to further stimulate the microbial degradation process. Microorganisms are used in environmental engineering for treating industrial and municipal wastewater; contaminated groundwater; digestion of sludges; and remediation of toxic and hazardous wastes.

Aerobic, heterotrophic bacteria require oxygen, carbon, nitrogen, and phosphorus in order to flourish in the environment. Heterotophic microorganisms use organic carbon for two purposes; synthesis of cellular components and the production of energy. The following equation denotes the synthesis of biomass assuming that the composition[1] of a typical microorganism can be expresses as $C_5H_7O_2N$:

$$C_6 H_{12} O_6 + O_2 + NH_3 \rightarrow C_5 H_7 O_2 N + CO_2 + 4 H_2 O \qquad \text{Eq [1]}$$

A portion of the organic carbon is also oxidized for the production of energy. The stoichiometric equation for the oxidation of carbon is as follows:

$$C + O_2 \rightarrow CO_2 \qquad \text{Eq [2]}$$

Bacteria typically grow at an exponential growth rate according to the following equation:

$$X = X_O e^{(-Kt)} \qquad \text{Eq [3]}$$

X_o, and X = Microorganism concentration initially and at the end of the monitoring period, mg/L,

K = Microorganism growth rate constant, $days^{-1}$, and

t = Monitoring time period, days.

Substrate such as glucose or sucrose is typically consumed by bacteria at an exponential rate according to the following equation:

$$S = S_O e^{(-kt)} \qquad \text{Eq [4]}$$

S_o, and S = Substrate concentration initially and at the end of the monitoring period, mg/L,

k = Substrate removal rate constant, $days^{-1}$, and

t = Monitoring time period, days.

Materials and Methods

This section presents the materials that are necessary for performing the laboratory exercise along with a step-by-step procedure. Table 1 presents the materials required to complete the laboratory exercises. A turbidimeter and materials for performing CFUs are not necessary unless microbial growth rates are going to be determined. The cost per student is approximately $2.05 with exception to the BOD or COD analysis. Table 2 lists the 5 steps for performing the study. Glucose stock solutions may be eliminated and the glucose added directly to the 2-L bottles or 1-L flasks. The bottles or flasks must be mixed for 1 to 2 minutes after adding the glucose.

Table 1. Materials necessary to complete laboratory exercise.

Six, 2-L plastic bottles with caps or six, 1-L Erlenmeyer flasks
600-grams of soil
Glucose stock solution of 100 g/L.
3-liters of tap water and funnel
Analytical balance
Dissolved oxygen (DO) meter and probe
pH meter and probe
Glucose determination reagent strips or equipment necessary to measure biochemical oxygen demand (BOD) or chemical oxygen demand (COD)
Fish pump compressor, tubing, and diffuser stone for aeration (Optional)
Heterotrophic plate count media and petri dishes and turbidimeter (Optional)

Table 2. Procedures for performing laboratory exercise.

1. Prepare six, 2-L plastic bottles by adding 100 grams of indigenous soil and 500 ml of distilled or tap water to each.
2. Prepare glucose stock solution of 100 g/L or weigh appropriate amounts of glucose to add directly into each 2-L bottle.
3. Add 2.5-ml of the 100 g/L stock glucose solution to bottle #1; add 5-ml of the 100 g/L stock glucose solution to bottle #2; add 10-ml of the 100 g/L stock glucose solution to bottle #3; add 20-ml of the 100 g/L stock glucose solution to bottle #4; 20-ml of the 100 g/L stock glucose solution to bottle #5, and no substrate is added to bottle #6 which serves as the control. Mix gently for 1 to 2 minutes. Aerate bottle #5 constantly (Optional).
4. Measure the initial pH, DO, glucose, and/or COD of the liquid portion of the combined mixture of soil, tap water and glucose solutions. Optional measurements include: turbidity and CFU, which also may be measured daily.
5. Incubate the 2-Liter bottles at room temperature; measure the DO, pH, glucose, and/or COD of the liquid portion daily or every other day for at least five days.

Table 3 lists the parameters for evaluating bioreactor performance and Table 4 provides several questions to be considered by the students.

Table 3. Parameters to evaluate.

1. Plot each of the above parameters (DO, pH, glucose, COD) as a function of time for each reactor.
2. Determine the growth rate of the microorganisms for each reactor. (Optional)
2. Determine the COD or glucose removal rates for each reactor.

Table 4. Questions and data analysis.

Which reactor yielded the highest microbial growth rate and which one had the highest rate of glucose removal?
Did the microorganism concentration in any of the soil bioreactors decrease with time? Explain why this might have happened.
How does the DO concentration affect the microorganism growth rate?
How does pH affect microbial growth rate?
How would you modify your experiment to improve the glucose removal rates?
Using the maximum glucose removal rate determined from the above experiment, how long would it take to reduce an initial glucose concentration of 1000 mg/L?

Sample Runs

Six, 2-L soil bioreactors were operated from December 17, 2001 through December 27, 2001. One hundred grams of indigenous soil from outside the engineering building at Mercer University and 500-mL of tap water were added to each 2-L container. One reactor served as the control and, varying amounts of glucose were added to the five remaining soil bioreactors. Reactors 1, 2, 3, 4, and 5 had theoretical glucose concentrations of 0.5 g/L, 1.0 g/L, 2.0 g/L, 4.0 g/L, and 4.0 g/L of glucose. Bottle #5 was aerated constantly for the first five days of the experiment. Aeration was omitted on day 6 and resumed on day 7. The remaining bioreactors were aerated for approximately 5 minutes daily and the following parameters were measured: DO, pH, COD, and glucose concentration. DO was measure using a Orion Model 810 dissolved oxygen meter and probe. The meter was calibrated in air according to the manufacturer's recommendations. pH was measured with a Fischer Scientific Accumet Model 25 pH meter with plastic electrode. The pH meter was calibrated with pH buffer solutions at pH values of 4, 7, and 10, respectively. COD was performed using a HACH COD Reactor with a colorimetric reading[2]. Colorimetric measurements were made with a HACH DR/2000 direct reading spectrophotometer. Glucose was measured using Keto-Diastix Reagent Strips manufactured by Bayer. The Keto- strips were placed into the supernatant from each soil bioreactor and a reading taken at 30 seconds. Glucose concentrations were measured in terms of dC/mL and converted to mg/L. Results from the sample runs are presented in Table 5.

Discussion

The pH in soil bioreactors #1 through #5 decreased as time increased due to microbial degradation of the glucose. The indigenous microbes degraded the glucose producing carbon

dioxide thereby lowering the pH in the supernatant. The supernatant pH in the control remained virtually the same indicting there was minimal microbial activity occurring in this bioreactor.

Table 5. pH values in bioreactor supernatant as a function of time.

Date	Control	#1	#2	#3	#4	#5
17-Dec	6.5	6.4	6.5	6.5	6.4	5.5
18-Dec	6.2	6.3	6.3	6.3	6.1	7.0
19-Dec	6.5	6.1	6.0	5.8	5.5	6.5
20-Dec	6.7	5.4	5.2	5.0	4.9	5.8
21-Dec	6.6	4.9	4.8	4.7	4.7	5.1
22-Dec	6.4	4.7	4.6	4.6	4.5	4.6
27-Dec	6.4	4.8	4.4	4.3	4.1	5.1

Table 6. DO concentration (mg/L) in bioreactor supernatant as a function of time.

Date	Control	#1	#2	#3	#4	#5
17-Dec	5.16	5.04	4.89	5.01	4.75	4.80
18-Dec	4.75	4.20	4.18	4.03	4.18	6.30
19-Dec	4.40	1.93	1.74	1.70	1.32	5.16
20-Dec	4.62	1.23	1.98	0.96	1.17	6.90
21-Dec	5.86	1.15	0.35	0.93	0.87	1.19
22-Dec	5.04	2.12	1.89	1.48	1.32	5.42
27-Dec	4.31	2.23	1.83	1.96	2.21	6.4

Table 7. Glucose concentration (mg/dL) in bioreactor supernatant as a function of time.

Date	Control	#1	#2	#3	#4	#5
17-Dec	0	40	80	180	300	300
18-Dec	0	100	250	500	1000	500
19-Dec	0	100	350	600	1500	1500
20-Dec	0	100	350	700	1000	1000
21-Dec	0	50	250	500	1000	1000
22-Dec	0	50	200	350	700	900
23-Dec	0	20	150	250	700	700
27-Dec	0	0	0	100	300	500

Table 8. COD concentration (mg/L) in bioreactor supernatant as a function of time.

Date	Control	#1	#2	#3	#4	#5
17-Dec	10	440	910	1790	3100	3820
18-Dec	540	500	940	2220	4290	4830
19-Dec	90	410	840	1740	3470	3190
20-Dec	0	220	790	1400	3250	3630
21-Dec	80	440	850	1960	3760	4470
22-Dec	20	310	690	1730	3330	3820
23-Dec	60	260	700	1430	3010	3440
27-Dec	10	280	530	1450	2680	3200

Dissolved oxygen concentration for soil bioreactors #1 through #4 decreased as time increased due to microbial degradation. Soil bioreactor #5 was continuously aerated with exception to 21 December 2001, which resulted in a lower DO reading on that particular day. The DO in the control was relatively constant such that it varied between 4.3 and 5.0 indicating minimal microbial activity was occurring.

The glucose concentration as measured using the Keto- strips increased the first day and then decreased during the duration of the monitoring period. The initial glucose readings on December 17 that were lower than the theoretical values may have been due to incomplete dissolution of the glucose. On day 2 (December 18), the glucose concentrations were higher in all bottles and then a decrease was observed in all bottles (except the control. The Keto- strips provide a pseudo-quantitative measurement, but do indicate the concentration decreased. The Keto- strips confirmed there was no glucose in the control.

The observed increase in COD of the supernatant in each bottle from December 17 to December 18 is thought to have been caused by the release of organic materials attached to the soil particles. After December 18, the COD of the supernatant decreased with an increase in time. As a result of soil bioreactors being continuously aerated, we had anticipated a faster COD/glucose degradation rate to occur as compared to the other reactors. Also note that there was a higher release of organic materials resulting in the highest COD of any of the bioreators. Table 9 presents the COD removal rates for each of the soil bioreactors. These rates were calculated starting with day two of the monitoring period (December 18). The COD removal rates generally decreased from soil bioreactor #1 to #5. The bioreactors with the higher initial COD values (higher glucose concentrations) had the lower COD removal rates. The control had the highest removal rate, which is unusual. Perhaps this can be attributed to the indigenous microorganisms being acclimated to the organic material in the soil whereas, the other bioreactors contained microbes that were not acclimated to the new substrate, glucose.

Table 9. COD removal rates ($days^{-1}$).

Control	#1	#2	#3	#4	#5
0.375	0.053	0.061	0.035	0.044	0.028

Summary and Conclusions

Six, 2-L soil bioreactors were operated from December 17, 2001 through December 27, 2001 for demonstrating how soil microorganisms can be used for treating waste. In this study, glucose was utilized as the contaminant and was degraded to carbon dioxide and water. The materials that were required for performing the laboratory exercise and step-by-step procedures were presented. A list of parameters to evaluate bioreactor performance along with a set of questions for students to consider was provided. Sample data were presented followed by an analysis and discussion of the data.

Major conclusions developed from this study are:

1. Glucose degradation occurred in soil bioreactors #1 through #5.

2. The pH of the supernatant in each soil bioreactor generally decreased from the start to the end of the monitoring period due to the production of carbon dioxide.

3. DO concentration decreased in each soil bioreactor during the monitoring period with exception to the control which averaged 4.88 mg/L indicating minimal microbial activity.

4. The glucose and COD concentrations in the supernatant decreased during the monitoring period due to the indigenous soil microorganisms degrading the glucose in solution.

Bibliographic Information

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Biographical Information

RICHARD O. MINES, JR. is the Program Director of Environmental Engineering at Mercer University and has taught for 14 years at the graduate and undergraduate levels. He worked in consulting with CH2M Hill and Black & Veatch for 6.5 years. Dr. Mines holds a BS, ME, and Ph.D. in Civil Engineering from Virginia Military Institute, University of Virginia, and Virginia Tech. He's a registered PE in Florida, New Mexico, and Virginia.

JANET CARLSON POWELL is the Associate Director at the Biological Sciences Curriculum Study, a non-profit dedicated to providing leadership in science education through curriculum and professional development, and research in these areas. She has a BA in environmental biology, an MS in curriculum and instruction, and a Ph.D. in science education. She has taught and conducted research in elementary, secondary, and higher education settings.

LAURA W. LACKEY has four years of experience as an assistant professor of Environmental Engineering at Mercer University. She has six years of industrial experience at the Tennessee Valley Authority as research Environmental/Chemical Engineer. She earned a BS, MS, and Ph.D. in Chemical Engineering from the University of Tennessee. The terminal degree was awarded in 1992. She is a registered professional engineer.