Bioprospecting for Cellulose-Degrading Microbes — Filter Paper Assay Method

Background Reading:

Bioprospecting is the search for diverse organisms that produce or contain genes, enzymes, biochemicals, and other compounds that are of value to humans. Bioprospecting enables scientists to identify useful compounds so that they can be replicated in a lab and used to produce medicines, enzymes, and other useful products.

Well-known products obtained through bioprospecting include antibiotics, Taxol (a cancer-fighting drug), and aspirin. Bioprospecting is now playing a pivotal role in the research and production of sustainable biofuels. A biofuel is any fuel produced from a biological source; examples include biodiesel and ethanol. Ethanol is made from fermenting plant sugars, such as glucose. Most ethanol is made from sugar cane or corn grain, which is in limited supply. Scientists and engineers are looking for efficient ways to produce ethanol from other plant sources, such as plant cellulose, which could have the potential to produce 30% of U.S. transportation fuel needs.

Cellulose is the rigid, long molecule found in the cell walls of plants. It is made when carbon dioxide is absorbed from the atmosphere during photosynthesis and, as a result, is abundant and renewable. While it is very abundant and can be found in every plant, cellulose does have draw...
backs. The biggest problem with cellulose is that it is very hard to break down. In order to be fermented into ethanol, the rigid cellulose must be broken down into a much simpler molecule: glucose. This requires a combination of mechanical grinding and chemical treatments of acids and/or enzymes. The treatment and chemical breakdown (or hydrolysis) of the cellulose molecule is currently the most expensive and difficult step in the process of converting cellulosic biomass into ethanol.

Fortunately, many organisms have evolved in nature to break down cellulose into glucose, which can then be consumed for energy. Examples include the fungi that decompose wood and the microorganisms in the stomach of the cow that allows it to consume cellulosic crops, such as grasses.

These fungi and bacteria are able to break down cellulose into glucose because they produce special enzymes called cellulases. The cellulase enzymes break the chemical bonds between the glucose molecules that make up the cellulose strands. Researchers at the Great Lakes Bioenergy Research Center (GLBRC) are bioprospecting for microorganisms in diverse environments that can break down cellulose in hopes of discovering new cellulase enzymes that can improve the efficiency of producing biofuels. For example, scientists have made important discoveries through investigating the cellulase-degrading microbes found in tropical leaf-cutter ant colonies and studying the bacteria in the stomachs of dairy cows.

Pre-Lab Questions:

1. What is the difference between cellulose and cellulase?

2. Why are researchers trying to figure out how to efficiently convert cellulose into ethanol? What are some of the benefits of using cellulose?
3. What are some of the challenges of using cellulose for producing ethanol?

4. What is the purpose of cellulase enzymes for converting cellose into ethanol?

5. If a microbe was discovered that could rapidly break down cellulose, how would this discovery impact the potential use of cellulose as a biofuel?

6. Think of four environmental characteristics that would be suitable for high concentrations of cellulose-degrading microbes and explain how those characteristics would support a large population of this kind of microorganism.

<table>
<thead>
<tr>
<th>Environmental Characteristic</th>
<th>Explain how it supports cellulose-degrading microbes</th>
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Student Procedure: Bioprospecting for Cellulose-Degrading Microbes — Filter Paper Assay Method

Overview: In this lab you will take on the role of a biofuels researcher bioprospecting for cellulose-degrading microbes in your local environment. The goal of this lab is to collect samples from your environment that contain microbes that can rapidly break down cellulose. To determine whether your microbial samples can degrade cellulose, you will observe whether or not your sample breaks down filter paper in a test tube. The methods that you use are very similar to those used by scientists at the Great Lakes Bioenergy Research Center (GLBRC) and the results could be useful for discovering new enzymes that can efficiently break down cellulose to produce sustainable biofuels.

Part 1: Planning and Bioprospecting

Set-Up Procedure:
1. In groups of 3-4, acquire each of the following:
   a. 4 test tube and caps (aluminum foil or parafilm can be used in lieu of caps)
   b. 25 mL of minimal media solution
   c. 4 pieces of filter paper cut into 1x10 cm strips
   d. Labels or masking tape and pen
   e. Protective gear - gloves, aprons, goggles
2. Serilize your work area and hands.
3. Add one strip of 1x10 cm filter paper to each tube.
4. Using a pipette or eyedropper, add 5 mL of minimal media solution to each tube.
   a. Be careful not to hit the filter paper when adding the media solution.
5. Using a sterilized glass rod, pipette, or other sterile object, push the filter paper flat against the side of the tube. Raise or lower the filter paper so that it is straight and not folded.
   a. If the filter paper strip is not flat against the side of the tube, it may fall into the solution and make the results difficult to interpret.
6. Securely cap the test tube and add a label. On the label, write your group name and date. Leave room so that you can later write the contents of the test tube.
7. Place your group’s test tubes in the space designated by your instructor.

The Purpose of Media Solution & Filter Paper: The media solution is necessary to provide non-energy related nutrients for microbial cell growth. This media mainly provides three key nutrients essential for any cell growth – nitrogen, phosphorus, and potassium. Note that these nutrients by themselves cannot be used as energy – they only provide the raw materials for building a cell. The only source of energy in the test tube is the cellulose in the filter paper. If the community of microbes cannot break down cellulose, its members will die.

Controls: Your instructor will set-up test tubes that will serve as the “control” in this experiment. No environmental sample will be added to the control tubes. The control should not show any signs of filter paper degradation and will serve as a baseline for comparing any changes you see in your samples.
Bioprospecting: Planning and Bioprospecting

Planning: In your group, discuss some potential locations to collect environmental samples with cellulose-degrading microbes. In deciding where you want to collect samples, think about the environmental characteristics that would support large, diverse populations of these microbes. Brainstorm a list of potential locations to collect samples.

<table>
<thead>
<tr>
<th>Location</th>
<th>Why this is a good place to find cellulose-degrading microbes:</th>
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<tbody>
<tr>
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</table>

Collecting Samples:

1. In your group, acquire the following:
   4 plastic sealable sandwich bags
2. To collect your samples:
   a. Invert the bag so that the outside is on the inside (i.e. so that the bag is inside out)
   b. Reach your hand inside the bag and grab the sample through the bag
   c. Re-invert the bag so that the sample is inside the bag. (Your hands should never actually come in contact with the sample.)
   d. Seal the bag and date and label it.
   e. Collect four samples and bring them to the classroom.

*Figure 1: a proper collection method—the bag is flipped inside-out so the hand never comes in contact with the sample.*
Planning and Comprehension Questions:

You will need to select two of the four samples that you collected to test for cellulase activity. As a group, discuss which sample you predict will have the highest activity.

1. List the four samples that your group collected:

2. Why did you choose each of your two samples? What made you think that the microbes in these samples would have the most cellulase activity? For each sample, describe it and defend your choice with an explanation.

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Why was it chosen?</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td></td>
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<tr>
<td>2.</td>
<td></td>
</tr>
</tbody>
</table>

3. How will you know if the microbes in your bioprospected samples are effective in producing cellulase enzymes? How will your test tubes change if your samples are effective?

4. If the microbes in your samples are not effective at producing cellulase enzymes or at breaking down cellulose, how will you be able to tell?

5. Could a microbe that does not produce cellulase enzymes survive in these test tubes? Why or why not?
Planning and Comprehension Questions (cont’d)

6. What role does the liquid media solution play? Why was it necessary?

7. What role does the filter paper play? Why was it necessary?

Predictions and Explanations:

8. What differences do you think you will see between your control and your test tubes with your bioprospected samples? *I predict that*...

9. Explain your reasoning for all predictions made above: *I think these predictions will be proven correct because*...

10. What differences, if any, do you think you will see between two test tubes with your bioprospected samples? *I predict that*...

11. Explain your reasoning for all predictions made above: *I think these predictions will be proven correct because*...
PART 2: Sample Inoculation and Observation

Innoculation:

1. As a group, acquire the following: your samples, your test tubes (that you prepared earlier).
   a. Check to make sure the filter paper is still flat against the tube; if it is not, use a glass
      rod or other sterile long object to re-flatten it against the side of the test tube.
2. As a group, select two of the samples that you wish to add to
   your test tubes. Select your two samples based on which
   you think will have the most cellulose-degrading activity.
   a. Be prepared to defend your choice of samples.
3. With a gloved hand, acquire a pea-sized amount of one of the
   samples you chose from its bag.
4. Take one of the test tubes you prepared; slightly tip it so that the
   filter paper is on top.
   a. With a tweezers (or a gloved hand) add your pea-sized
      sample so that it does not hit the sides or the filter paper.
   b. If you do hit the side of the tube, use a sterile glass rod or
      similar object to push it all the way down to the media
      solution.
5. Cap the tube loosely and add the inoculated sample to the label.
6. Repeat this once more for the same sample in a second test tube.
7. Repeat this for the second sample using the remaining two tubes
   you prepared. For replicates of the same sample use “A” or “B”
   to distinguish them, ex: “Soil A,” “Soil B.”
8. When you have inoculated all four tubes, make sure they are
   labeled correctly with the substance and place them in a lab
   shaker as specified by your instructor.
9. Make sure tubes are not completely sealed. Without oxygen
   many microbes will not be able to survive.

Observations:

9. Check your tubes periodically (if not daily) for any signs of
   growth and cellulose degradation.
   a. If your tubes have the same consistency and cloudiness as the
      day you added the sample, you likely do not have a sample
      that can degrade cellulose.
   b. If you see any increased cloudiness, yellowing or definite color change on the filter paper
      strip (ignoring the color change caused by the color of the sample itself), or microbial
      growth on the filter paper, you have possible cellulose degradation.
   c. If you observe any ripping, tearing, or dissolving of the filter paper, you have definite
      cellulose degradation, an indication of cellulase activity.
10. Record each observation on the accompanying table and describe, draw and/or photograph
    your visual observations for each tube (see attached table to record observations). When the
    experiment is finished, complete the attached questions.
Table 1: Filter Paper Test Tube Results

<table>
<thead>
<tr>
<th>Description of Sample</th>
<th>Test Tube 1</th>
<th>Test Tube 2</th>
<th>Test Tube 3</th>
<th>Test Tube 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observations (circle which description best fits each sample for each date)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Date:</strong></td>
<td>No Growth</td>
<td>No Growth</td>
<td>No Growth</td>
<td>No Growth</td>
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<td></td>
<td>Possible Growth</td>
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<tr>
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<td>Definite Growth</td>
<td>Definite Growth</td>
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<td>Definite Growth</td>
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<tr>
<td><strong>Observations</strong></td>
<td>No Growth</td>
<td>No Growth</td>
<td>No Growth</td>
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<tr>
<td><strong>(you may write a description or draw it)</strong></td>
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“No Growth” = sample remains largely clear and mostly unchanged. Little or no indication of microbial growth.

“Possible Growth” = media solution is cloudier than when the sample was added. Visible color change has occurred on the filter paper (often with a clear line on the paper). Signs of microbial growth and reproduction are observable.

“Definite Growth” = the filter paper has ripped, torn, or dissolved.
Table 2: Filter Paper Test Tube Results (second page, if needed)

<table>
<thead>
<tr>
<th>Description of Sample</th>
<th>Test Tube 1</th>
<th>Test Tube 2</th>
<th>Test Tube 3</th>
<th>Test Tube 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date:</strong></td>
<td>Test Tube 1</td>
<td>Test Tube 2</td>
<td>Test Tube 3</td>
<td>Test Tube 4</td>
</tr>
<tr>
<td><strong>Observations</strong></td>
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<td>Test Tube 4</td>
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“Definite Growth” = the filter paper has ripped, torn, or dissolved.
Name: _______________________________  Hour: ___________  Date: ______________

Lab Analysis and Comprehension:

For each sample, describe how it changed from the first day of incubation to your final day of results:

<table>
<thead>
<tr>
<th>Test Tube</th>
<th>Observed Changes</th>
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<tbody>
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</tbody>
</table>

1. Which tubes showed evidence of cellulase enzymes? Explain what happened to the microbes and filter paper in these tubes.

2. Which tubes showed little or no evidence of cellulase enzymes? Explain what happened to the microbes and filter paper in these tubes.
Lab Analysis and Comprehension (cont’d)

3. Review your initial predictions about the differences you expected to observe between the control and the samples you collected. Did your observations match your predictions? Propose an explanation for differences between what you predicted and what you observed.

4. Review your initial predictions about the differences you expected to observe between the two samples you collected. Did your observations match your predictions? Propose an explanation for differences between what you predicted and what you observed.

5. Compare your results with the samples collected by the rest of the class. Which samples showed the most cellulase activity? Which showed the least?

6. Based upon the class results, what generalizations can you make about the patterns you observed regarding environmental characteristics that support more cellulose degrading microbes?
Lab Analysis and Comprehension (cont’d)

7. Propose an explanation for any general patterns you observed in samples in your class’s samples.

8. What additional information would you need to determine whether your explanation is accurate? Propose a new investigation that could provide information to evaluate your explanation.
Overview: Students collect samples that they predict will contain communities of cellulose-degrading microbes and test for the ability of microorganisms in their samples to break down pure cellulose (filter paper). In the process, groups collect evidence to test predictions about which environmental microbial samples will be the most effective for degrading cellulose. By comparing results across groups, students can begin to uncover patterns and develop explanations about the types of environments that support cellulose-degrading microbes. This lab method is nearly identical to that used by GLBRC researchers, and student results could help scientists discover new enzymes for efficient cellulosic biofuel production.
For Teachers: Bioprospecting for Cellulose-Degrading Microbes — Filter Paper Assay Method

Overview:

Students collect samples that they predict will contain communities of cellulose-degrading microbes and test for the ability of microorganisms in their samples to break down pure cellulose (filter paper). In the process, groups collect evidence to test predictions about which environmental microbial samples will be the most effective for degrading cellulose. By comparing results across groups, students can begin to uncover patterns and develop explanations about the types of environments that support cellulose-degrading microbes. This lab method is nearly identical to that used by GLBRC researchers, and student results could help scientists discover new enzymes for efficient cellulosic biofuel production.

This lesson is designed to span three to five 50-minute class periods over 10 to 14 calendar days depending upon student familiarity with cellulosic biofuels. The majority of class time is required to provide background information, plan investigations and interpret results. Samples should incubate for 7-14 days while students record periodic observations. This activity provides for flexibility depending upon time constraints, student prior knowledge, equipment and funding.

Learning Outcomes: Students will…

- Explain the relationship between plant cellulose and cellulase enzymes
- Describe the general process for converting cellulosic biomass into ethanol
- Explain the function of cellulase enzymes for converting cellulose into biofuel
- Describe the role of cellulose-degrading microbes in biofuel production
- Predict, test, analyze, and explain the effectiveness of different microbial populations for degrading cellulose
- Describe and explain general patterns in environmental characteristics that harbor cellulose-degrading microorganisms

This lesson assumes some prior knowledge of types of carbohydrates, basic enzyme function, the process of decomposition, the role of microorganisms in ecosystems, and matter and energy transformations.
Bioprospecting — Filter Paper Assay Method

Master Materials List

<table>
<thead>
<tr>
<th>Item</th>
<th>Suggested Quantity</th>
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<tbody>
<tr>
<td>Test tubes w/ stoppers, parafilm, or Falcon 50mL tubes</td>
<td>4/group</td>
</tr>
<tr>
<td>Miracle Gro 20:20:20 Fertilizer</td>
<td>20g/class</td>
</tr>
<tr>
<td>Whatman Filter Paper (cellulose)¹</td>
<td>4 1x10cm strips/group</td>
</tr>
<tr>
<td>Test tube rack</td>
<td>1/class</td>
</tr>
<tr>
<td>Test tube shaker²</td>
<td>1/class</td>
</tr>
<tr>
<td>Graduated cylinders</td>
<td>1/group</td>
</tr>
<tr>
<td>Ziploc bags (snack sized)</td>
<td>4/group</td>
</tr>
<tr>
<td>Markers</td>
<td>1/group</td>
</tr>
<tr>
<td>Labeling tape</td>
<td>1/class</td>
</tr>
</tbody>
</table>

1. High-grade cellulose filteraper circles can be ordered from Ward Scientific for approximately $12. https://www.wardsci.com/store/catalog/product.jsp?catalog_number=153701&sk=1

Sequence:

**Part 1. Background Information: Framing the Problem (Pre-Reading Assignment, Videos) (1-2 50-minute periods)**

Bioprospecting and Biofuels for Beginners: A TED-Ed flip classroom lesson was created with Craig Kohn to accompany this lab activity. Students can watch the online video to introduce the important challenge of making cellulosic ethanol and the larger context for this lab activity. View the video and lesson here: http://ed.ted.com/lessons/biofuels-and-bioprospecting-for-beginners-craig-a-kohn

OPTIONAL: Have students complete online flip lesson with questions and discuss student responses to the online questions during class (http://ed.ted.com/lessons/biofuels-and-bioprospecting-for-beginners-craig-a-kohn#review).

Pre-Reading and Pre-Lab Questions: Students can complete the reading and accompanying questions as homework or in their lab groups. Have students discuss answers in their lab groups and then review responses in a class discussion. The accompanying PowerPoint slides can be used to review the structure of cellulose, the function of cellulase enzymes and the process of making cellulosic ethanol. In particular, question #6 is valuable to discuss in lab groups and as a class.
As students consider environmental and microbial characteristics that would be associated with effective cellulose breakdown, they will begin planning where to search for environmental samples and develop reasoned predictions about which samples will be the most effective.

**Bioprospecting and Biofuels - Connecting Classrooms with GLBRC Research:** The video interview with Gina Lewin, microbiology PhD student at UW-Madison, provides a context for how this lab was developed and how the techniques used by the students are very similar to those used by GLBRC scientists. Gina worked with Craig Kohn to develop this activity as part of the GLBRC Research Experience for Teachers program. The leaf-cutter ant example provides a useful case-study for how cellulose-degrading microorganisms, such as fungi, play an important role in ecosystems but also produce cellulase enzymes which could be valuable for biofuel production. View the video here: [https://vimeo.com/72999628](https://vimeo.com/72999628)

**Bioprospecting Lab Introduction with Craig Kohn:** This video can be used as a teacher reference or shown to students to provide a description of the lab protocol. The video is divided into sections that describe each portion of the lab sequence so you can play specific segments to your class before starting a lab step. View the video online: [https://vimeo.com/73143224](https://vimeo.com/73143224)

**Part 2. Planning and Collecting Samples (1-2 50 minute class periods) (pages 1-4)**

**Set-up:**

The process of reviewing the lab protocol and setting up test tubes helps students learn how this method will allow for detection of cellulase activity. As you describe the lab set-up, discuss the purpose of the filter paper and liquid media. Have students discuss what evidence they might see that would indicate the presence or absence of high numbers of cellulose-degrading microbes in their samples.

Demonstrate the proper test tube set-up as described in the student lab and shown in the supplementary video. Emphasize the importance of sterile procedure and avoiding contamination of the test tubes and filter paper. We want to be sure that any signs of cellulase activity are resulting from microbes in the environmental sample and not microbial contamination. Even so, it is unlikely that small amounts of contamination coming from students hands and the classroom will contain sufficient numbers of cellulose-degrading microbes to produce positive results.
It is important that the filter paper strips lie flat against the side of the test tube so that they don’t fall into the fertilizer media making difficult to detect the degree of filter paper degradation. If necessary, a strip of tape can be used to secure the filter to the side of the tubes.

If possible, have students set up four test tubes so that they will have a replicate of each sample. To save materials and space, groups can set up only one tube of each sample, but as a result, students will not see the variation in results between tubes with the same environmental sample. It is common in this lab to see variable results (positive and negative) for replicates of the same environmental sample.

**Control test tubes:** Set up several test tubes to serve as controls. Either do not inoculate these tubes or, preferably, add an environmental sample that has been autoclaved to kill all microbes. The controls will serve as a point of comparison so students can gauge the degree to which their filter paper degraded.

**Media Solution:** The media solution is necessary to provide non-energy related nutrients for microbial cell growth. Either have students mix quantities of the solution or prepare a batch for them. The fertilizer recipe is chemically similar to the media used by GLBRC scientists.

**Miracle Grow Media Recipe:** (developed by Craig Kohn, 2011 – GLBRC) – add 20 g of 20:20:20 Miracle Grow to 1 liter of tap water (pure water is not recommended because it has fewer minerals that can aid microbial growth). This solution will settle if not agitated, so be sure to swirl and re-suspend before adding samples. 20:20:20 Miracle Grow contains 20% Nitrogen, 20% Phosphorus, and 20% Potassium. Other formulations of Miracle Grow will have different concentrations of these nutrients and are therefore not recommended. Other brand names of 20:20:20 fertilizer should work equally well assuming that they have no organic ingredients. If a fertilizer does have organic ingredients, it cannot be used because it will provide a source of energy outside of the cellulose, preventing the test from providing accurate results.

**Planning:**

Remind students of the goal of the investigation: to find samples that have high concentrations of microbes that can rapidly degrade cellulose. To begin the planning process, students can discuss in small groups some of the environmental characteristics that would contribute to samples being effective for degrading cellulose (question #6 in pre-reading activity). Follow-up small group discussion with class discussion in which students share and explain proposed characteristics. These characteristics can be summarized on the board.
Below are some of the characteristics groups might consider. There is no single set of correct answers to this prompt. The goal is for students to reason from basic principles about conditions for active decomposition in the environment.

<table>
<thead>
<tr>
<th>Environmental Characteristic</th>
<th>Reasoning/Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>High concentrations of cellulosic biomass</td>
<td>Serves as food (energy) source for cellulose-degrading microbes</td>
</tr>
<tr>
<td>Evidence of decomposing cellulosic biomass</td>
<td>Cellulose-degrading microbes are actively consuming this biomass</td>
</tr>
<tr>
<td>Moist conditions</td>
<td>Water is required for organism growth and development</td>
</tr>
<tr>
<td>Warm conditions</td>
<td>Microbial growth and reproduction increases in warm conditions</td>
</tr>
</tbody>
</table>

Considering the shared list of environmental characteristics, students should brainstorm with their groups a list of potential locations to bioprospect.

### Bioprospecting for Samples:

Refer to the accompanying video for additional instructions on how to collect samples. Students can collect their four samples in groups during class or individually for homework. Have students consider how to care for their collected samples so that the microbes remain active. For instance, if a sample were collected in a cool, moist location and then left in a hot car over the weekend, many of the microorganisms might not survive to the inoculation stage. Samples can be stored in the fridge if inoculation is delayed.

### Choosing a Sample and Making Predictions:

Lab groups should decide which of the four samples collected will show the most cellulase activity. Have groups discuss and develop explanations for their choice and then share their reasoning with the class. Next, students can complete the planning and comprehension questions on pages 3-4. These questions can serve as a formative assessment to determine how well they understand the experimental design. Students should make reasoned predictions that they can revisit and reevaluate as they collect and interpret data.
Part 3: Sample Inoculation and Observations (one 50-minute class period and brief observations over 7-14 days) (pages 7-10)

Use the accompanying video and demonstrations to illustrate proper inoculation technique and remind students to follow sterile procedures to avoid contamination. Also remind students to keep the tubes upright. Try to avoid hitting the filter paper with the sample. In this way, any filter paper degradation should start in the media solution and work upward.

Before adding tubes to the shaker, have students record initial observations. If possible, take photos with observations to document any changes. Review the potential indicators of cellulase activity in the samples and the causes for those changes.

**Observations:** This lab can be ongoing for 1-2 weeks while doing other activities. Students should make brief observations every 1-2 days and can periodically share results through the course of the experiment. For samples with active cellulase-degrading microbes, expect to see evidence of filter paper tearing within 5-10 days.

One method to quantify the amount of cellulase activity is in a sample is to count the number days until a complete tear is observed in the filter paper. Students should record the date that partial and complete tears in the filter paper are seen so that the degree of cellulase activity can be compared between samples.

**Test Tube Shaker:** A shaker 1) increases microbial contact with the filter paper and 2) aerates the media. This increases the rate of filter paper decomposition. It is possible to use this assay without a shaker but degradation could take a month or more. GLBRC scientists put the test tubes on an angle in the shaker which increases the liquid-air surface area, thus improving aeration. This can be done to speed up the degradation process, but is not necessary.

**Aerobic vs. Anaerobic Conditions:** Note that if the test tubes are completely sealed, anaerobic conditions will exist. Conditions in a tube will select for different subsets of the microbes in the samples based on whether they are aerobic or anaerobic. In general, cellulose decomposition will occur more rapidly in aerobic conditions, but it also occurs in anaerobic conditions (i.e. cow’s rumen). If test tubes are left partially open, students should monitor media solution levels because some of the water can evaporate.
Part 4: Data analysis, Discussion and Conclusions (pages 8-10)

At the end of the observation period, groups can summarize their observations and conclusions by completing the tables and questions in the Lab Analysis and Comprehension section of the student handout. Students should be able to identify which samples showed evidence of cellulose-degrading microbe activity and what caused any observed disintegration in filter paper, i.e. microbes in the sample were able to produce cellulase enzymes, break down the cellulose into glucose and consume the glucose for energy. Likewise, they should be able to explain what causes other test tubes to show no evidence of cellulase activity.

Groups should review their initial predictions and determine how evidence collected in the lab either supports their predictions, refutes them, or is inconclusive. Students should draw on prior knowledge, background readings and supplementary research to propose reasonable explanations for inconsistencies between what was predicted and what was observed. There are numerous factors that affect results in this lab (i.e. microbe pH and temperature tolerance, unpredictable competitive interactions and population dynamics in microbe communities, community variation between replicates of the same environmental sample). It is less important for students to come up the correct explanation for observations than to ask questions, seek answers, reason from evidence and draw on plausible biological principles.

To revisit the discussion about which environmental characteristics should support cellulose-degrading microbes, have groups share results. This can be summarized on a table on the board or in a shared online document. One method would be to have the class report how their samples fall into the three categories:

<table>
<thead>
<tr>
<th>Definite Growth</th>
<th>Possible Growth</th>
<th>No Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For more quantitative comparison to determine which samples had the greatest cellulase activity, a table might be organized as shown below. The samples that showed a complete tear in the filter paper the soonest would be the most active.

<table>
<thead>
<tr>
<th>Group Name</th>
<th>Sample Description</th>
<th>Definite, Possible, or No Growth</th>
<th># Days to Complete Tear in Paper</th>
<th>Length of Paper Decomposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In groups, have students consider what the samples in each category (definite growth, possible growth, no growth) have in common. In particular, they should consider differences in environmental characteristics that could account for the patterns seen in which samples show evidence of cellulase activity. Have groups share their observed patterns and proposed explanations with the class. Discuss how understanding these patterns could help researchers discover new cellulase enzymes that could be used in biofuel production.

Note on interpreting results: When comparing cellulase activity between samples, the best overall indicator is number of days until a complete tear is observed. The fewer the days, the more activity. The position or length of the tear does not necessarily indicate more or less cellulase activity. Available oxygen and nutrients vary along the length of the filter paper which can cause certain microbes to more active and create a tear at different positions.

It is important to remember that you are observing the effects of a community of microbes growing in the test tube. There are numerous species of microbes interacting the sample. More effective filter paper degradation could be caused by either by a larger, more diverse community of microbes that can produce cellulase enzymes or it could be caused by relatively smaller number of microbes that are highly effective at degrading cellulose.

Expected results: Based on conducted lab and classroom trials, filter paper decomposition is frequently observed from soil samples, decomposing wood, compost, and ruminant manure. In very active samples, filter paper decomposition can occur in less than a week. However, as mentioned previously, there can be a great deal of variation in observed cellulase activity between similar samples.

Share your results with GLBRC: GLBRC researchers would be interested in learning about samples in which you observed cellulase activity, especially those environmental samples that can degrade filter paper in less than seven days. In addition, by looking at results from samples collected across a geographic range, scientists might be able uncover broader patterns about how cellulase activity changes with soils or latitude.

Share your results with this online form: https://glbrc.wufoo.com/forms/share-your-bioprospecting-results-with-us/
Extensions and Options:

This lab protocol can be simplified or expanded to include more or less complexity depending upon the goals, classroom environment and audience. Ideas to simplify include:

- Conduct the lab as a demonstration and have students predict and explain results.
- If a shaker is not available, it is possible to set up samples in tubes and let them sit for a month or more and make observations every week. This experiment could be going on in the background of a class for a semester.
- Provide a variety of environmental samples for students to choose from rather than allowing them to collect their own.

Possible extensions:

- Isolate microbes from active samples using the GLBRC Isolation Method protocol: [http://www.glbrc.org/education/educationalmaterials/bioprospecting](http://www.glbrc.org/education/educationalmaterials/bioprospecting)
- In samples that are proven to be effective at cellulose breakdown, conduct trials to evaluate the effect of variables such as oxygen availability, temperature, and inoculation sample size on the results.
- Organize as cooperative class or smaller group investigation of a research question. In this scenario, students could develop and evaluate a scientific claim through the course of the investigation. Here are some example questions:
  - How does cellulase activity change with soil type?
  - Which fungus species show more cellulase activity?
  - Do samples from decomposing wood show more cellulase activity than herbaceous plants?

Appendix

Video Resources:

- *Bioprospecting and Biofuels for Beginners*. This short video, developed by H.S. Teacher Craig Kohn and TED-Ed animators, provides an excellent introduction to the concept of bioprospecting and potential use for developing sustainable biofuels: [http://ed.ted.com/lessons/biofuels-and-bioprospecting-for-beginners-craig-a-kohn](http://ed.ted.com/lessons/biofuels-and-bioprospecting-for-beginners-craig-a-kohn)
• **Bioprospecting and Biofuels - Connecting Classrooms with GLBRC Research.** This interview with microbiologist and biofuels scientist, Gina Lewin, describes how this lab activity connects to the bioprospecting investigations of leaf-cutter ant communities that could lead to discoveries of new enzymes for more efficient biofuels: [https://vimeo.com/7299628](https://vimeo.com/7299628)

• **Introduction to Bioprospecting Lab and Protocol.** Craig Kohn, the teacher who worked with GLBRC to create this activity, describes and demonstrates the steps of the lab protocol. This video can used as a teacher reference or can be shown to students in sections to introduce the steps of the lab protocol: [https://vimeo.com/73143224](https://vimeo.com/73143224)

• **Bioenergy in the High School Classroom.** Bioprospecting Lab. In this narrated slideshow, Craig Kohn describes the process of developing the lab and his experiences using it with his students: [http://vimeo.com/43991600](http://vimeo.com/43991600)

Text/Online Resources:

• **Why is it so difficult to make cellulosic ethanol?** This short reading introduces the challenges and opportunities associated with producing ethanol from cellulosic biomass. The reading includes details about the chemical composition of plant cell walls: [http://www.glbrc.org/sites/default/files/Cellulosic_Ethanol.pdf](http://www.glbrc.org/sites/default/files/Cellulosic_Ethanol.pdf)

• **Researchers Unearth Bioenergy Potential in Leaf-cutter Ant Communities.** This GLBRC research news article describes how GLBRC scientists are identifying new enzymes produced by fungi in leaf-cutter ant colonies that might used to make cellulosic biofuels: [http://www.glbrc.org/?q=node/2042](http://www.glbrc.org/?q=node/2042)

• **The Ant Man: UW scientist Cameron Currie looks to the insect world for insights on how to save the planet.** This entertaining news article describes GLBRC scientists’ work in unraveling the complex symbiotic relationships in fungus-farming ant communities that could offer clues for developing cellulosic biofuels: [http://www.isthmus.com/isthmus/article.php?article=28844](http://www.isthmus.com/isthmus/article.php?article=28844)

• **I-MOLD Animated Lessons on Leaf Decomposition.** The animations allow you to visualize the matter transformations associated with decomposition at the cellular and molecular scale: [http://imold.utoledo.edu/lessons.html](http://imold.utoledo.edu/lessons.html)
Standards:

NEXT GENERATION SCIENCE FRAMEWORK:

Science and engineering Practices:
• Planning and carrying out investigations
• Analyzing and interpreting data
• Constructing explanations
• Engaging in argument from evidence

Crosscutting Concepts:
• Patterns
• Cause and effect: Mechanism and explanation
• Energy and matter: Flows, cycles, and conservation
• Structure and function

Disciplinary Core Ideas:
• LS1.A: Structure and Function
• LS1.B: Growth and Development of Organisms
• LS1C: Organization for Matter and Energy Flow in Organisms
• LS2.A: Interdependent Relationships in Ecosystems
• LS2.B: Cycles of Matter and Energy Transfer in Ecosystems
• LS4.B: Natural Selection
• ESS3.A: Natural Resources

WI MODEL ACADEMIC STANDARDS (SCIENCE GRADE 12):
• B.12.4 Show how basic research and applied research contribute to new discoveries, inventions, and applications
• C.12.3 Evaluate the data collected during an investigation, critique the data-collection procedures and results, and suggest ways to make any needed improvements
• C.12.5 Use the explanations and models found in the earth, space, life, environmental, and physical sciences to develop likely explanations for the results of their investigations
• F.12.7 Investigate how organisms both cooperate and compete in ecosystems
• F.12.10 Understand the impact of energy on organisms in living systems
Standards:

AGRICULTURE, FOOD AND NATURAL RESOURCE STANDARDS:

- BS.02.05.06.a. Explain reasons for detecting microbes and identify sources of microbes
- BS.03.03.08.c. Creation of biofuels from biomass
- BS.03.03.09.c. Biotechnology processes & molecule synthesis
- ESS.01.01.01.a. Explain the importance of unbiased sampling and collect samples
- ESS.01.01.01.c. Analyze/interpret results of samples
- EES.02.05.06.c. Design and perform an assay to detect a target microorganism in food, water or the environment
Activity was developed with Craig Kohn, Waterford Union High School, Waterford, WI while working with Gina Lewin in Dr. Cameron Currie’s Lab at the University of Wisconsin-Madison. Funding and additional support provided by the Great Lakes Bioenergy Research Center