Bewitching Biofilms

Teacher Resource

Grade Level **5-12**

Objectives

1. The student will explain the concept of microbes.
2. The student will explain the potential impact these organisms can have on our lives.
3. The student will identify biofilms and explain their significance in human health.
4. The student will identify the requirements for the formation of biofilms.
5. The student will demonstrate the use of 21st century technology with a digital microscope.

National Standards

NS.5-8.1; NS.9-12.1	Science as Inquiry
NS.5-8.3; NS.9-12.3	Life Science
NS.5-8.5; NS.9-12.5	Science & Technology
NT.K-12.1	Basic Operations and Concepts
NT.K-12.3	Technology Productivity Tools
NT.K-12.6	Technology Problem-Solving & Decision-Making Tools

Teacher Background Information

Biofilms are formed by free-floating microorganisms adhering to a surface area in a moist environment. The microorganism, or bacteria, will colonize an area allowing the community to grow and also for protection. Biofilms form a slimy substance to the surface area to allow the colony to attach itself permanently to the site. Biofilms can consist of one species of bacteria to over 500 species of bacteria, fungi, protozoans, algae, etc. Biofilms can form on almost any imaginable surface on Earth; plaque on teeth, wood, plastic, human tissue, hot tubs, swimming pools, etc. Biofilms are usually undetectable to the naked eye and have thick layers of bacteria slime to form a complex community. Biofilms can be hard to treat since they usually are made up of millions of different species of microscopic organisms.

For example, plaque on teeth is a biofilm caused by the buildup of food residue and bacteria. The bacteria release this slimy substance produced by the bacteria as they form a

bacterial community on your teeth. The slimy polysaccharide coating secreted by the bacteria acts like an adhesive that allows the colony of bacteria to thrive and reproduce. The new bacteria will adhere to the surface of the slime and live off the waste of the original colony allowing for the growth of a complex community.

The challenge is to understand how a scientist can defeat a colony of biofilms. Let's stick with our example about plaque on teeth, using mouthwash daily is said to fight off plaque and "millions of bacteria on contact." Now recall that those millions of germs are in a protective biofilm that has developed defense mechanisms to fight off any outside predators. The fact that mouthwashes can kill bacteria on contact, but this is tested by killing the bacteria in a planktonic state, which means the bacteria are floating in a solution individually, not together as a biofilm. The research needs to start testing how to penetrate biofilms in defeating the thick layers of bacteria in able to start really killing off millions of germs in the mouth.

MATERIALS

- Digital Microscope
- Stereo Microscope
- Personal Computer
- Interactive White Board/Projector
- Bucket of Pond Water
- Clear 2-liter Plastic Bottle
- Scissors
- Hole Punch
- 10 Medium Size Washers
- Coating Materials *(Vaseline, Hot Pepper Sauce, Nail Polish, Hand Lotion, Paint)*
- 1 meter long Piece of String
- Small Bucket
- Magnifying Glass
- Newspaper
- Safety Goggles
- 5 Glass Microscope Slides
- Bunsen burner
- Inoculating Loop

PROCEDURE

Activity 1 Make a Biofilm

1. Use your scissors to cut the top and bottom off of your 2-liter plastic bottle.

2. Cut through the cylinder of your plastic bottle so that you form a plastic rectangle.
3. Flatten the plastic rectangle and cut a series of 5 equal 'hot dog' length strips.
4. Punch a hole on both ends of each of the 5 strips in order to loop the string through both holes.
5. Choose four of the five coating materials and coat 4 of the five sides with Vaseline, nail polish, hand lotion, paint, or hot pepper sauce.
6. Take the 1 meter long string, and string it through the right end of the slide from the bottom to the top.
7. Take the string and string it through the left end from the top to the bottom.
8. Then attach a medium size washer on the bottom of each end of the slide.
9. Tie a knot below the washer to hold it in place.

Note: The weights are used to separate each of the slides and to weigh the apparatus down so that it remains submerged in the bucket.

10. Repeat steps 7-9, until all 5 slides have been attached to the string.
11. You should have the two ends of the string hanging from the bottoms of both sides of the 5 rectangle slides.
12. Attach the last medium size washers on each end of the string.
13. Take your small bucket to the teacher to fill with pond water.
14. Place your completed structure in the pond water bucket so that the slides are submerged and the loop is floating on top.
15. Make sure to label your bucket.
16. The slides will be left for a week in the pond water.

Note: Make sure the slides never come in contact with each other and are submerged in the water the entire time.

Activity 2 Data Analysis of Biofilms

1. You will now analyze your biofilm slides to determine which one had the most growth.
2. Develop a data table to rate "1" through "10" to indicate the complexity of the biofilm on each slide.

3. A rating of 1 will indicate no biofilm. A rating of 10 will indicate the presence of a significant biofilm covering.

   The bacteria colonies may be green, red, brown, or black. The number of colonies on the slide will indicate the complexity of the colony.

4. Use the stereo microscope to observe each slide.

   Remind students to keep the slides wet during this process as they make their observations.

5. Design a data table which will include:
   a. Use graphing paper to design a histogram to indicate which covering provided the most conducive medium for the development of a biofilm.

      A histogram is a statistical representation of the data that has been collected. It can be represented as columns/bars without gaps.

Activity 3 *Bacterial Smears*

Make sure the students adhere to all safety procedures and wear goggles and gloves through the entire activity.

1. Carefully wash the 5 glass slides with water.

   A clean slide is a necessity in bacterial smearing.

2. Label each of your slides #1-5.
3. Light the Bunsen burner and sterilize your inoculating loop, making sure the loop becomes red hot before each smear.

   An inoculating loop is used to retrieve the bacteria to smear on the slide for staining.

4. Smear biofilm sample #1 over the center of the slide making a thin film.

   The thinner the film the easier it will be for the students to see the microorganisms.

5. Allow the slide to air dry.
6. Pass the slide slowly through the Bunsen burner 3 times to heat fix the microorganisms to the slide.

   This keeps the bacterial samples from being washed away as you work with the smear.

7. Allow the slide to cool. *60 to 90 seconds*
8. Place 3 drops of methylene blue stain over the surface of the smear.

9. Allow the methylene blue stain to remain on the smear for 30 seconds, and then use tap water to wash away the stain.
10. Repeat steps 3-10, for slides 2-5.
11. Have students view the slides under a digital microscope.

Or have each group prepare 1 slide to view under the digital microscope on the interactive white board for the entire class to observe and record.

This is a great lab to introduce oil immersion with microscopes. This site is a great introduction on how to do oil immersion if you need some refreshing! ☺

http://biology.clc.uc.edu/fankhauser/labs/microscope/Oil_Immersion.htm

DISCUSSION QUESTIONS

1. What is a bacterial smear?
2. How do bacterial smears relate to human/animal health?
3. Can biofilms be harmful to humans/animals?
4. Which of the 5 biofilm samples provided an environment most favorable to the formation of a successful bacteria colony?
5. Why was pond water used for this activity?
6. Would tap water have been a better choice of a medium in which to place the plastic slides?
7. How are biofilms related to dental health? Make sure to discuss the ways in which bacteria can be related to biofilms associated with teeth and dental caries (tooth decay).
8. Do bacterial microorganisms only exist as pure colonies? Provide evidence to support your viewpoint.

PRESENTATION

Have students prepare a lab report including the data and images to share on the interactive white board for the class. To aid students in their presentations, share the following websites and also a scoring guide.

Below is a list of great websites to let your students' research about biofilms, bacteria, and human health issues to help them with the lab report.

www.microbeworld.org

- *Information about microbes and the environment*

http://commtechlab.msu.edu/sites/dlc-me/news/news.html

- *Current information about microbes in the news*

http://www.biofilm.montana.edu/

- *Information about biofilms*

http://www.essology.com/Biofilms.html

- *Site that relates biofilms to dental caries.*

Example Scoring Guide

WORD BANK	CONCEPT MAP	DISCUSSION ?'S	DATA ANALYSIS
Word Bank is complete and includes terms that are related to the lab. Organized, Spelling, and Legible	Includes all Word Bank words. Appropriate connecting terms. Clear relationship between map and biofilms. Nature of biofilms and bacteria are clearly addressed in map.	3 step method used to answer all ?'s accurately ✓ Each ? accurately restated ✓ Each ? clearly & accurately answered ✓ Support for each answer is included, clear and accurate. Very few grammatical and spelling errors.	All tables, charts, graphs are accurate. ✓ Title ✓ Labels ✓ Units ✓ Dependent Variable on Y axis ✓ Independent Variable on X axis ✓ Clear & Accurate
/25	**/25**	**/25**	**/25**