



Biofilm Growth (2011)

Ken Bateman (Wellesley High School)
Mary Brunson (Brookline High School)
Doug Brown (Wellesley High School)

Summary

Bacterial biofilms are important because they can be helpful (in water purification and nutrient cycling), but also harmful (persistent infections in the human body and build up on medical equipment/prosthetic devices). In this activity, students will explore biofilm formation on glass slides. They will also choose surfaces that they use in their everyday lives (like fabrics, stainless steel, plastic, etc) and test the ability of this same bacteria to form a biofilm on each of these surfaces. This activity is designed for an upper-level biology course, but could be adapted for other levels by adding or removing certain concepts (described in “Extensions” below). It should be noted that this procedure has not been tested as written.

Key Concepts

- Massachusetts Curriculum Frameworks:
 - SIS1. Make observations, raise questions, and formulate hypotheses.
 - SIS2. Design and conduct scientific investigations.
 - SIS3. Analyze and interpret results of scientific investigations.
 - 4.7 Recognize that communication among cells is required for coordination of body functions. The nerves communicate with electrochemical signals, hormones circulate through the blood, and some cells produce signals to communicate only with nearby cells.
 - 6.2 Analyze changes in population size and biodiversity (speciation and extinction) that result from the following: natural causes, changes in climate, human activity, and the introduction of invasive, non-native species.
- AAAS benchmarks:
 - The variation of organisms within a species increases the likelihood that at least some members of the species will survive under changed environmental conditions. 5A/H1a
 - A great diversity of species increases the chance that at least some living things will survive in the face of large changes in the environment. 5A/H1b
 - In addition to the basic cellular functions common to all cells, most cells in multicellular organisms perform some special functions that others do not. 5C/H2b

Objectives

At the end of this activity, students will be able to

- **Describe** a biofilm and *explain* its importance.
- **Identify** surfaces on which biofilms will form.
- **Develop** a controlled experiment to formulate a conclusion about biofilm behavior.

Materials

- Fabric (polyester, cotton, etc)
- Stainless steel (body jewelry)
- Plastics (water bottles, etc)
- Polystyrene (that meat is sold on)
- Sterile Petri dishes (60mm x 15mm)
- Sterile Petri dishes (120mm x 15mm)
- *Pseudomonas fluorescens* (positive control – starter plate)
- *E. coli* (negative control – starter plate)
- Sterile Basal Medium broth (peptone 7g/L, MgSO₄ 2g/L, CaCl₂ 0.05g/L)
- 0.1% Crystal Violet
- Inoculating loop

Procedure

1. Introduce the concept of biofilms. You could show pictures of biofilms. The websites listed below have some pictures that may be helpful. You could also do a search online.
2. Student will complete Procedure Day 1 in small groups of 3 or 4. They will set up the positive and negative control groups so the students can see what biofilm formation looks like. These results will not be ready until the experiment is over. It also gives them an idea of how the procedure works. They will follow the same procedure on Day 2, but with their experimental surface samples. (At this point, you can ask students to bring in their own samples or you can provide samples for Day 2.)
3. Before starting Procedure Day 2, you must sterilize the sample surfaces so that there is no contamination. You can do this with an autoclave or by submerging them in bleach. Students complete Procedure Day 2 in the same groups.
4. After 4-5 days, students will follow the procedure to detect the biofilms. They will use crystal violet to stain the bacteria. They must rinse the surface multiple times to remove all bacteria that are not part of the biofilm.
5. During analysis, the students must be careful to consider the negative bacteria control groups. The stain may stick to some of the surfaces even though there are no bacteria present. The negative bacteria control group will also give them a good indication as to how much rinsing they need to do.
6. Students could answer the questions at the end, or they could write a lab report as the final product.

Extensions

- You could make this inquiry based by giving the students procedure day one to introduce the general procedure, then tell students to develop their own controlled experiment to test a variety of surfaces.
- You could ask students to take the experiment further and try to develop ways to get rid of the biofilms.
- You could remove the data tables from the write-up and have them develop their own meaningful data tables.
- You could isolate bacteria from the human body or from their houses and see which of those can form biofilms.
- You could test biofilm formation in different temperatures or different media.

Assessment

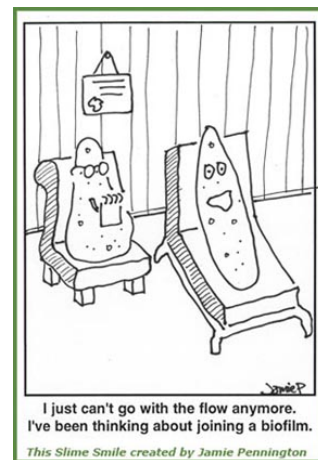
- **Performance**—Walk around and discuss the procedure and hypotheses with the students as they are doing it.
- **Product**—The activity handout with observations and summary questions answered.

Additional Resources

- <http://biofilmbook.hypertextbookshop.com/v004/r003/index.html> - this is a textbook that is a great resource for finding out about biofilms. It includes lab experiments as well.
- <http://www.cs.montana.edu/ross/personal/intro-biofilms-s1.htm> - this is a great resource on what biofilms are and how they are formed.
- <http://www.bioaliment.ugal.ro/revista/5/Paper%2053.pdf> (Constantin, Oana E. “Bacterial Biofilms Formation at Air Liquid Interfaces.” *Innovative Romanian Food Biotechnology* 5. Dec (2009): 18-22.) – This article describes the procedure with the basal medium.
- <http://www.dartmouth.edu/~gotoole/>
- <http://gasp.med.harvard.edu/>
- <http://www.molbio1.princeton.edu/labs/bassler/>

Biofilm growth

Introduction: A biofilm is a group of microorganisms that stick to each other on a surface. A biofilm community can be made of a single species, but many biofilms include a mixture of different species, including bacteria, fungi, yeast, protozoa, and other microorganisms. These biofilms are held together because these cells produced a sticky extracellular matrix made of proteins and polysaccharides. Biofilms can be found almost anywhere and they can form on living or nonliving substances, such as metal pipes, contact lenses, medical equipment, your shower, and even human teeth. They can have a positive effect, during water purification or nutrient cycling, but they can also clog pipes and lead to stubborn infections.



Biofilms can be especially dangerous in certain situations. *Pseudomonas aeruginosa* is a bacterial species that is the main cause of chronic infection in cystic fibrosis patients. It causes problems because it gets stuck in the thick mucus present in the lungs of cystic fibrosis patients. *P. aeruginosa* can then quickly form a biofilm, which is very difficult to remove. Because of the thick extracellular matrix, the infection is unresponsive to normal methods of treatment and can often be fatal. In this lab exercise, you will use a bacterial species that is closely related to *P. aeruginosa* that is not infectious - *Pseudomonas fluorescens*. *P. fluorescens* is useful to us because even though it is not harmful, it is also known to form a biofilm. You will test different surfaces to see the likelihood of biofilm formation. There are a number of surfaces listed in the materials list, but you can pick something else with the approval of your teacher.

Materials:

Fabric (polyester, cotton, etc)
Stainless steel (body jewelry)
Plastic (water bottles, etc)
Polystyrene (meat container from grocery store)
Sterile Petri dishes (60mm x 15mm)
Sterile Petri dishes (120mm x 15mm)
Pseudomonas fluorescens (positive control – starter plate)
E. coli (negative control – starter plate)
Sterile Basal Medium broth (peptone 7g/L, MgSO₄ 2g/L, CaCl₂ 0.05g/L)
0.1% Crystal Violet
Inoculating loop
Autoclave or 10% bleach

Procedure Day 1: (See table 1 for more instructions)

1. Label three small Petri dishes - one as your positive control (+), one as your negative biofilm control (-f), and one as your negative bacteria control (-b). The positive control will contain *Pseudomonas fluorescens*, the negative biofilm control will contain *Escherichia coli*, and the negative bacteria control will contain no bacteria.
2. Pour 10 mL of sterile basal medium into each small sterile Petri dish.
3. Using an inoculating loop, select one colony from the *P. fluorescens* starter plate and mix it with the basal medium in the positive control Petri dish.
4. Repeat step 3 for your negative biofilm control using the *E. coli* starter plate.
5. The third plate (-b) will contain no bacteria – only the basal medium.
6. Label three glass slides – one as a positive control (+), one as a negative biofilm control (-f), and one as the negative bacteria control (-b).
7. Make observations of your slides in the data table below.
8. Carefully add each sterile glass slide to the appropriate Petri dish. The slide will be diagonal (as shown in Figure 1), partially submerged in the broth to create an air-liquid interface.
9. Place each small Petri dish in a larger Petri dish and cover the setup with the large Petri dish top to prevent contamination from the air. The cover will probably not fit correctly, but it's ok if it leans on the microscope slide (see Figure 2).
10. Store all three dishes at room temperature and leave undisturbed for 4 to 5 days.
11. Read over the procedure for day 2 so you can pick your three surfaces. The teacher must sterilize the materials you choose, or submerge them in 10% bleach before using them.



Figure 1. Petri dish

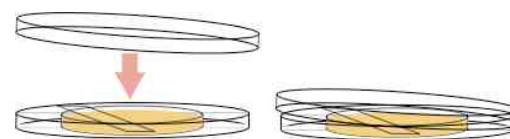


Figure 2. Small Petri dish in Large one

Table 1: Day 1 Procedure

Sample	Positive Control (+)	Negative Biofilm Control (-f)	Negative Bacteria Control (-b)
Media	10ml Basal Medium	10ml Basal Medium	10ml Basal Medium
Bacteria (single colony)	<i>P. fluorescens</i>	<i>E. coli</i>	none
Slide	Clean glass slide	Clean glass slide	Clean glass slide

Procedure Day 2:

12. Pick 3 different study surfaces from the list (or get approval from your teacher for a material of your choice). Cut the material into 3" x 1" pieces – the same size as the microscope slide. You will need two of each sample – one to use as a negative bacteria control and one as an experimental group.
13. Record Observations of the materials you chose in the data tables below.
14. Label 6 small Petri dishes – 2 for each study surface. For example, if you choose cotton you should label one dish as cotton and the other as cotton (-).
15. Add 10 mL of sterile basal medium to each dish.
16. Using an inoculating loop, select one colony from the *P. fluorescens* starter plate and mix it with the basal medium in the experimental dishes.
17. The negative bacteria dishes will contain no bacteria. The media should remain sterile for the duration of the experiment.
18. Add your study surfaces to each Petri dish, making sure that the material is only partially submerged in the basal medium. For the cloth samples, you may hang part of the sample out of the dish.
19. Place each small Petri dish in a larger Petri dish and cover the dish (as you did yesterday and show in Figure 2).
20. Leave the Petri dishes undisturbed for 4 days.

Detecting the Biofilm, Day 5:

1. Using sterile forceps remove the glass slide (and your samples) from each Petri dish and place each slide in a new clean Petri dish.
2. Make observations of your samples in your data tables below.
3. Cover samples with Crystal Violet stain and let sit for 10 minutes.
4. DO NOT POUR excess crystal violet down the drain. After 10 minutes, pour the crystal violet into a separate container for disposal by the teacher.
5. Hold your sample with forceps and dunk your sample into a beaker of distilled water. Repeat these rinsing on each sample **at least** 3 times. You should rinse as many times as necessary until the color of the sample does not change.
6. Record the presence or absence of color on each surface. Compare the level of color to the negative and positive controls.

Data

Day 1 Controls:

	Positive Control (+)	Negative Biofilm Control (-f)	Negative Bacteria Control (-b)
Appearance of slide prior to experiment			
Appearance of slide before staining			
Appearance of slide after staining			

Staining Scale: 3 = heavy purple present on slide, 2 = some purple present on slide, 1 = a little purple on slide, more than negative bacteria control (-b), 0 = no purple on slide (looks like negative bacteria control)

Sample 1: _____

	Experimental (1s)	Negative Control (-1s)
Appearance of material prior to experiment		
Appearance of material before staining		
Appearance of material after staining		

Staining Scale: 3 = heavy purple present on sample, 2 = some purple present on sample, 1 = a little purple on sample, more than negative control (-1s), 0 = no purple on sample looks like negative control (-1s)

Sample 2: _____

	Experimental (2s)	Negative Control (-2s)
Appearance of material prior to experiment		
Appearance of material before staining		
Appearance of material after staining		

Staining Scale: 3 = heavy purple present on sample, 2 = some purple present on sample, 1 = a little purple on sample, more than negative control (-2s), 0 = no purple on sample looks like negative control (-2s)

Sample 3: _____

	Experimental (3s)	Negative Control (-3s)
Appearance of material prior to experiment		
Appearance of material before staining		
Appearance of material after staining		

Staining Scale: 3 = heavy purple present on sample, 2 = some purple present on sample, 1 = a little purple on sample, more than negative control (-3s), 0 = no purple on sample looks like negative control (-3s)

Analysis questions:

1. In your experiment, what was the independent variable? The dependent variable? The controlled variables?
2. Did you see a difference in the ability of the bacteria to form a biofilm on different surfaces? Rank the surfaces in terms of the amount of biofilm formed.
3. Why was it important to include the negative bacteria control for each sample?
4. Why was it important to sterilize all materials before running the experiment?
5. Why might it benefit a bacterial cell to grow as a biofilm as opposed to growing as an individual free floating cell?
6. Why might it benefit a bacterial cell to grow as a free-floating cell as opposed to growing as a biofilm?
7. Why does a biofilm make it harder to eradicate a bacterial infection?
8. Based on your experimental results, how might biofilms impact the everyday use of the materials that you tested?