Bacterial Communities Grow as Biofilms

Resource Type: Curriculum: Laboratory

Publication Date: 1/5/2005

Authors

Barbara Rundell
Natural and Applied Sciences
College of DuPage
Glen Ellyn, IL 60137
USA
Email: Rundell@cdnet.cod.edu

Abstract

Common household biofilms were sampled, grown on slides, observed, and discussed to promote an understanding of biofilms and common locations where they grow. Students observed the organization of the cells in the biofilms and identified the general types of organisms (eucaryotes versus procaryotes) that grew in their biofilms. They designed experiments for possible further study of biofilm growth.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Time.
Three consecutive class periods are required for this activity. The ideal intervals between class periods should be between 2 and 7 days. This lab should be done near the middle or end of the course when students are familiar with the cellular morphology of a variety of microbes.

Learning Objectives.
At the completion of this activity, students will:

1. understand what a biofilm is.
2. recognize that biofilms grow in many common household settings.
3. demonstrate that when cells are removed from a biofilm and suspended in water, they can attach to a new surface to produce a new biofilm even if the new growth conditions are very different from conditions at the original biofilm site.
4. recognize that microbes often grow in complex, diverse aggregates that contain more than one type of organism.
5. recognize that biofilm growth is very different from the growth of pure cultures of floating cells in broth or colonies on agar plates.
6. work in groups to discuss their results and to design possible future experiments to investigate environmental factors that influence biofilm growth.

Background.
Students should know how to use a microscope.

Microorganisms Used.
The microorganisms used were unidentified environmental microbes sampled from biofilms that grow on moist surfaces such as dish drains, shower curtains, bird baths, pond stones, etc.

PROCEDURE

Materials.
(For a class of 24 students arranged in six groups of four students)

Lab or Class Session 1 (This session can be conducted in lecture or lab.)

24 plastic zip-top sandwich bags
24 sterile packaged swabs
24 collection tubes: sterile screw cap tubes containing 5 ml of sterile distilled water

Lab Session 2

24 sterile glass slides: each slide wrapped separately in aluminum foil and autoclaved
24 sterile petri plates
24 sterile screw cap tubes containing 10 ml of sterile dilute trypticase soy broth:

5% trypticase soy broth (BBL Becton Dickinson Co.) in distilled water (5 g of powder/100 ml or 5% of "normal" medium concentration)

2 trays to incubate the petri plates
12 black wax pencil markers

Lab Session 3

12 wash bottles containing tap water
6 beakers (400 ml or larger) containing a small amount of disinfectant
24 pairs of latex or vinyl disposable gloves
Gram crystal violet stain
12 black wax pencil markers
Paper towels
Biohazard waste container
Microscopes
Immersion oil
Lens tissue
24 Biofilm Discussion sheets (authored by the instructor)
Microscope connected to a monitor (optional)
Digital camera (optional)

Student Version.

Lab Session 1

1. Introduction to Biofilms
Biofilms are complex aggregates of microbes that grow on surfaces. They attach to diverse substrates such as soil particles, pipes, and contact lenses. Biofilms perform beneficial activities such as water purification and nutrient cycling, but they also cause problems. For example, they can foul plumbing systems and cause stubborn infections. Scientists are just beginning to understand the nature of biofilms because microbes have been traditionally studied as pure cultures. However, this is not the normal mode of growth for many microbes. This exercise will demonstrate that biofilms are very common and easy to cultivate, and that they have distinctive appearances.

2. You will receive a zip-top bag containing a sterile swab and a collection tube. Collect a biofilm sample from your body or household within 24 hours of your next lab session. Good collection sites that are likely to have biofilms include teeth (dental plaque), contact lenses, sink drain, toilet bowl, flower vase (with old flower water), dog dish, bird bath, and swimming pool filter.

Surfaces that are not submerged will be easier to sample. To collect material from a biofilm that is not submerged, moisten the swab with the sterile water and then rub the biofilm surface. Stir the swab vigorously into the collection tube sterile water and then discard the swab.

To collect material from a biofilm that is submerged, rub the biofilm with the swab. Stir the swab vigorously into the collection tube sterile water and then discard the swab.

Lab Session 2

1. You will receive a petri plate that already contains a sterile slide. Label both the top and bottom of the petri plate, using care not to open the plate.

2. Pour the entire contents of a tube of dilute (5%) trypticase soy broth into the petri plate.

3. Pour the entire contents of your biofilm collection tube into the petri plate.

4. Carefully place your plate on a tray designated for incubation at the temperature that is closest to the temperature of your biofilm collection site (room temperature or 37°C). Be careful to avoid spilling as you carry your plate.

Lab Session 3

1. Put on disposable gloves. Retrieve your plate from the incubation tray. Gently pour the contents of your plate into a beaker that contains disinfectant. Rinse the plate gently but thoroughly with a tap water wash bottle. Be careful to avoid disturbing the biofilm that has grown on your slide; direct the stream of water to the bottom of the plate but not right onto your slide. Pour off all of the water, using care not to drop the slide out of the plate.

2. Do not heat fix your slide. Add Gram crystal violet stain to the plate so that the entire surface of your slide is covered with dye. Let the dye set for 5 minutes.

3. Pour off the dye into the disinfectant beaker and rinse as in step one. Remove your slide from the plate and wipe the bottom of the slide on a paper towel. Let the slide air dry.

4. Discard the plates, gloves, and paper towels in a biohazard waste container.

5. Your instructor will give you a Biofilm Discussion sheet. Read the sheet before you view your slide so that you can start thinking about your answers as you observe your biofilm.

6. Label your slide and observe it with the microscope at each power of magnification. Rotate the fine focus knob to observe the three-dimensional properties of your biofilm. Most biofilms will show several growth patterns such as scattered microcolonies (small isolated clumps that sometimes grow to considerable thickness) as well as smaller cell clusters. Look for these patterns on your slide. Determine whether eucaryotic cells (larger cells with a nucleus) are present. Observe slides that were prepared by your classmates. Take a picture of your slide with a digital camera if one is available. Aim the camera through the ocular of the microscope.

7. Begin answering the questions on your Biofilm Discussion sheet.

8. Your instructor will place you in a group with three other classmates. Share your responses on the Biofilm Discussion sheet with the other members of your group. After a period of time your instructor will initiate a discussion involving the entire class. Add content to your Biofilm Discussion sheet as you collaborate with your classmates.

9. Your instructor may ask you to display your biofilm slide to the entire class with a microscope connected to a camera and monitor or with digital camera photos. Discuss similarities and differences among biofilms grown from various collection sites.

Instructor Version.

Optional class assignment prior to doing this lab: several weeks before the lab exercise is scheduled, I introduce the concept of biofilms in a brief lecture. I display examples of biofilm growth and provide some references for further study. There are many useful websites with good illustrations including the following:

http://archive.microbelibrary.org/index.asp

http://www.personal.psu.edu/faculty/j/e/jel5/biofilms/

http://helios.bto.ed.ac.uk/bto/microbes/biofilm.htm

http://www.cdc.gov/ncidod/eid/vol8no9/02-0063.htm#est

In my class each student is required to write a brief two-page paper on an aspect of biofilm research that interests him or her using at least three references. Biofilms impact many fields such as environmental studies, ecology, pharmacology, health, and engineering. Therefore, every student should be able to find a biofilm topic of personal interest. If students are already familiar with biofilms before starting the lab exercise, they will be more observant and more interested in the outcome. However, students will learn much from the lab even if they have not been assigned this prior research.

Lab Session 1 (1 hour)
Note: this session can be conducted in lab or lecture.

1. Introduction to Biofilms
Provide a brief introduction to biofilms and suggest some references (cited at the end of this exercise) for further study. Biofilms are complex aggregates of microbes that grow on surfaces. They attach to diverse substrates such as soil particles, pipes, and contact lenses. Biofilms perform beneficial activities such as water purification and nutrient cycling, but they also cause problems. For example, they can foul plumbing systems and cause stubborn infections. Scientists are just beginning to understand the nature of biofilms because microbes have been traditionally studied as pure cultures. However, this is not the normal mode of growth for many microbes. This exercise will demonstrate that biofilms are very common and easy to cultivate, and that they have distinctive appearances.

Explain how the entire lab will be carried out over the next two class periods. Show the class some biofilm slides that were prepared in advance using the techniques described in this lab exercise.

FIG. 1. Petri plates after staining and removal of the biofilm slides.

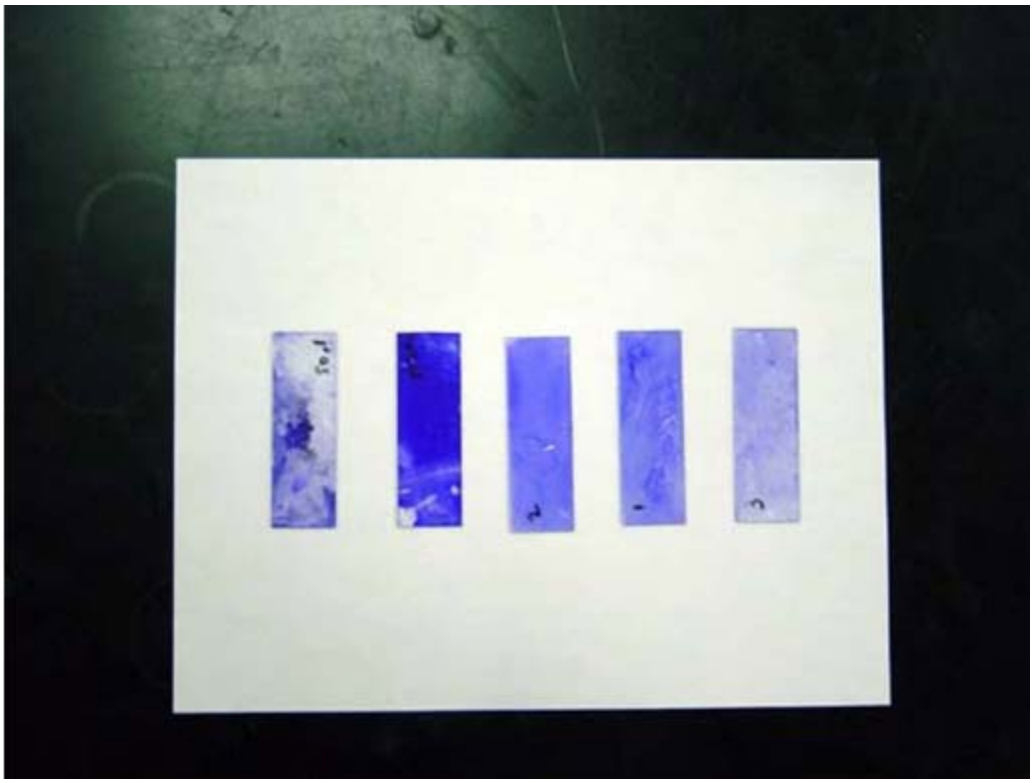

FIG. 2. Stained biofilm slides.

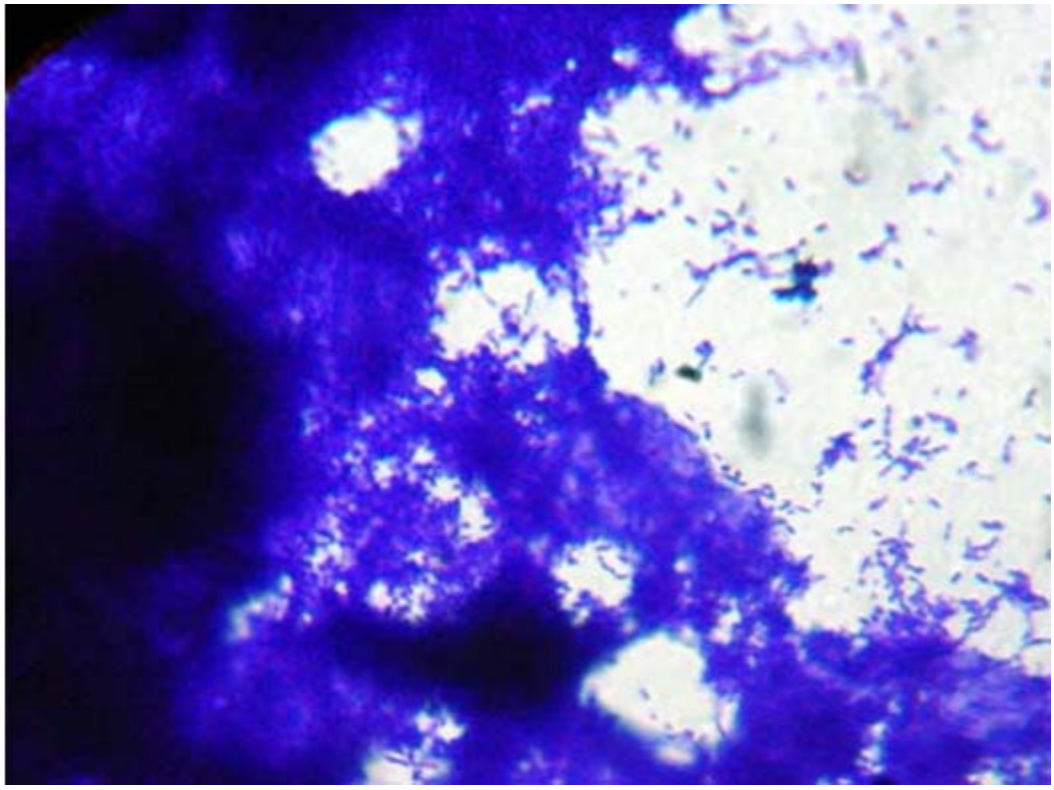

FIG. 3. Biofilm with heavy growth.

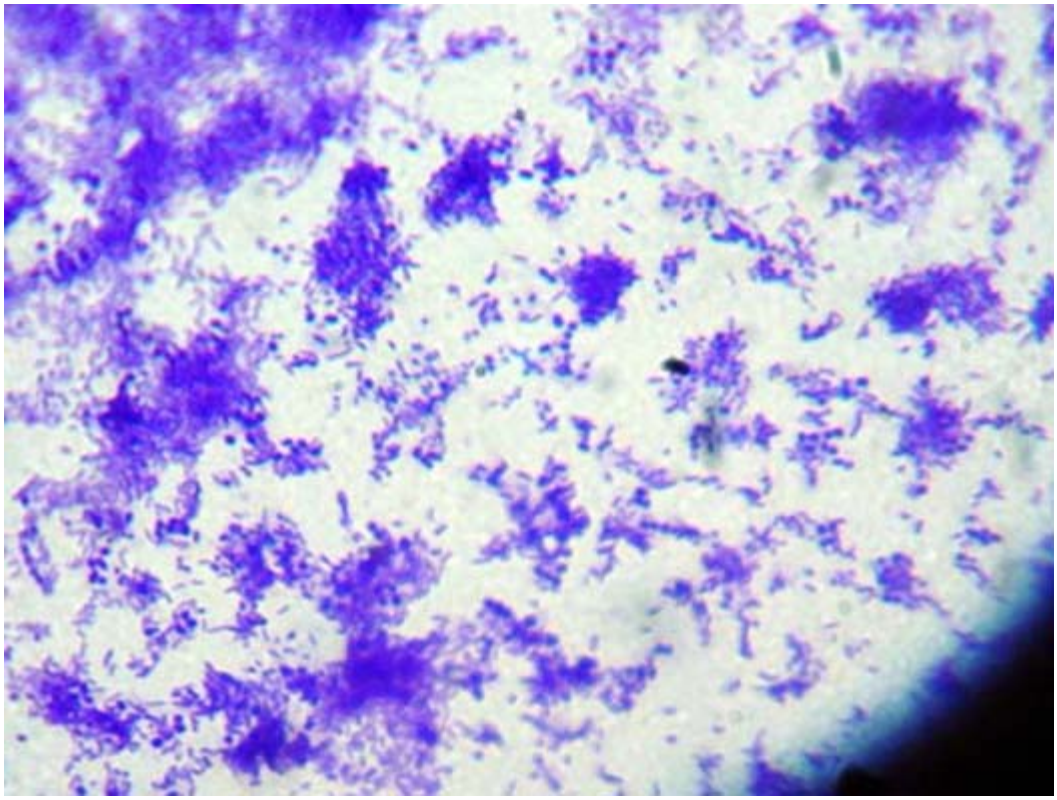

FIG. 4. Biofilm with moderate growth and microcolonies.

Prior to class prepare zip-top bags, each containing one sterile swab and one collection tube.
2. Give each student a zip-top bag that contains a sterile swab and a collection tube. Explain how to collect a biofilm sample within 24 hours of your next lab session. Suggest good collection sites that are likely to have biofilms such as teeth (dental

plaque), contact lenses, sink drain, toilet bowl, flower vase (with old flower water), dog dish, bird bath, and swimming pool filter.

To collect material from a biofilm that is submerged, rub the biofilm with the swab. Stir the swab vigorously into the collection tube sterile water and then discard the swab.
To collect material from a biofilm that is not submerged, moisten the swab with the sterile water and then rub the biofilm surface. Stir the swab vigorously into the collection tube sterile water and then discard the swab.
Surfaces that are not submerged will be easier to sample.

Lab Session 2 (1/2 hour)

Prior to class, place a sterile slide in a sterile petri plate for each student. If you partially unwrap the foil from the slide you can gently move the slide from the wrapper into the dish without touching the slide. Place trays that are labeled "room temperature" and "37°C" in a very noticeable site.

1. Ask each student to label both the top and bottom of a petri plate that already contains a sterile slide, using care not to open the plate.

2. Have each student pour the entire contents of a tube of dilute (5%) trypticase soy broth into the petri plate.

3. Have each student pour the entire contents of his or her biofilm collection tube into the petri plate.

4. Have each student carefully place his or her plate on a tray that is labeled for incubation at the temperature that is closest to the temperature of the biofilm collection site (room temperature or 37°C). Caution students to avoid spilling. After all the plates are on the trays, place them in the incubators.

Lab Session 3 (2 hours)
Note: the allotted time is flexible depending on the amount of time that you wish to devote to group discussion.

Prior to class dispense beakers that contain disinfectant, paper towels, disposable gloves, tap water wash bottles, and crystal violet stain. Retrieve the trays of plates from the incubators.

Demonstrate steps 1 through 3 to the class at the beginning of the lab:
1. Put on disposable gloves. Retrieve your plate from the incubation tray. Gently pour the contents of your plate into a beaker that contains disinfectant. Rinse the plate gently but thoroughly with a tap water wash bottle. Be careful to avoid disturbing the biofilm that has grown on your slide; direct the stream of water to the bottom of the plate but not right onto your slide. Pour off all of the water, using care not to drop the slide out of the plate.

Note: remind students that they should not heat fix their slides.

2. Add Gram crystal violet stain to the plate so that the entire surface of your slide is covered with dye. Let the dye set 5 minutes.

3. Pour off the dye into the disinfectant beaker and rinse as in step one. Remove your slide from the plate and wipe the bottom of the slide on a paper towel. Let the slide air dry.

Note: drying does not cause a marked change in the appearance of the biofilms. Many biofilms were examined before and after drying to verify this.

4. Students should discard their plates, gloves, and paper towels in a biohazard waste container while their slides are drying.

5. Distribute the Biofilm Discussion sheets. Urge students to read the sheet before they view their slides so that they can start thinking about their answers as they observe their biofilms.

Each instructor should author his or her own sheet to meet the needs and time constraints of the class. Questions 5 through 10 can be completed during a later class period so that students have more time to do additional research and think about this assignment. A sample sheet is provided below.

_____

Barbara Rundell
Microbiology 220
Spring, 2004
Name_____
Biofilms Discussion sheet

1. What is meant by the term "biofilm"?

2. What site did you sample to obtain biofilm material?

3. Briefly describe your biofilm:
a. Describe each type of procaryote that you see based on cell shape and size.

b. Describe each type of eucaryote that you see based on cell shape and size.

4. Observe several of your classmates' biofilms.
a. Propose some reasons why biofilms that were sampled from diverse sites and then grown here in lab might look similar.

b. Propose some reasons why biofilms that were sampled from diverse sites and then grown here in lab might look different

from each other.

5. Life in a biofilm community provides what advantages and disadvantages to a microbe?
a. Advantages of living in a biofilm community:

b. Disadvantages of living in a biofilm community:

6. Do you think that the biofilm you sampled is beneficial, harmful, a nuisance, or of no consequence for humans? Explain your answer.

7. What are some likely differences between bacteria that grow in biofilms and bacteria that grow as free-floating independent cells. For example, what differences might there be in growth rates, ease of exchanging DNA, resistance to antibiotics, etc.?

8. How could you modify this exercise to obtain additional information about your biofilm? (For example, you could determine whether a bacterium that was isolated from your biofilm can propagate a pure culture biofilm.) Think of some additional possibilities.

9. Devise an experiment that would provide information on how an environmental factor affects the amount of growth in a biofilm. For example, describe an experiment to determine the effect of temperature on biofilm growth.

10. Did you enjoy this lab exercise? Please explain your answer.

(End of Biofilm Discussion sheet) _____

Note: students may have difficulty determining whether there are eucaryotes in their biofilms. Briefly review the microscopic appearance of eucaryotic microbes versus procaryotes before the students view their slides.

6. After the slides have dried, ask the students to label their slides and observe them with the microscope at each power of magnification. Remind them to rotate the fine focus knob to observe the three-dimensional properties of their biofilms. Most biofilms will show several growth patterns such as scattered microcolonies (small isolated clumps that sometimes grow to considerable thickness) and smaller cell clusters. Ask students to look for these various growth patterns. They should determine whether eucaryotic cells are present. Students should also observe the slides that were prepared by the other members of their group. Optional: ask students to take pictures of their slides with a digital camera by aiming the camera through the ocular of the microscope.

7. Students should spend 15 to 20 minutes working independently to begin filling out their Biofilm Discussion sheets.

8. After students have worked independently, place them in groups of four to share their responses on the Biofilm Discussion sheet. Have the groups discuss the questions for 15 to 20 minutes. Finally, initiate a full class discussion. Working in teams fosters the development of interpersonal skills as students become aware of the need to communicate their thoughts very clearly and to reflect on their classmates' contributions in a supportive manner. Encourage students to add content to their Biofilm Discussion sheets as they collaborate. The Biofilm Discussion sheets may be collected at the end of the lab, or you may instruct your students to keep their sheets and do additional research to fully answer their questions.

9. If possible, have several students display their biofilm slides to the entire class with a microscope connected to a camera and monitor or with digital camera photos. Students should discuss similarities and differences among biofilms grown from various collection sites.

Safety Issues.

Unidentified infectious pathogens may be present in the biofilm samples. Therefore, rigorous aseptic technique is essential. Students should be cautioned to read the lab instructions carefully before they begin their work. They should wear gloves, lab coats, and goggles while handling their cultures and discarded materials should be disinfected and autoclaved. Students should report any accidental spills or bodily contact with the biofilm fluid to the instructor.

Use care to avoid splashes and spills while handling the petri plates and pouring off liquid to the disinfectant beaker.

ML Safety Statement regarding Environmental Isolates

The Curriculum Resources Committee recognizes that isolated organisms can be a powerful learning tool as well as a potential biological hazard. We strongly recommend that:

- Environmental enrichment laboratories should only be performed in classes in which students have been trained to work at a BSL2.
- Direct environmental samples (eg. soil, water) which are known to contain infectious organisms should be handled according to the biosafety level of that infectious agent.
- Cultures of enriched microorganisms, derived from environmental samples, should be handled using Biosafety Level 2 precautions.
- Mixed, enriched or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a biosafety cabinet if available.
- Where possible, media used for the enrichment of environmental isolates should contain an appropriate anti-fungal agent.
- Instructors should be aware if they are teaching in regions with endemic fungi capable of causing systemic infections, and should avoid environmental isolations.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
The Biofilm Discussion sheet is the basis for individual reflection as well as group and class discussions. I graded students on the thoroughness of their answers and their participation in the discussions.

Suggested rubric:
Completion of Biofilm Discussion sheet: 50 points
Preparation of stained biofilm slide: 25 points
Participation in class discussion: 25 points

Field Testing.
This lab has been tested with two separate classes of 24 students each. Most of these students had no prior science background before taking microbiology. Most of these students were studying microbiology as a prerequisite for admittance to an allied health program.

Student Data.
I asked my students whether or not they enjoyed this lab. The response from a poll of 20 students was 100% positive. Here are a few of the comments:

- "I didn't have a clue what a biofilm was before this lab."
- "This lab brought microbiology into my life."
- "It was interesting to see things that we collected rather than the usual cultures provided in lab."
- "I didn't know that my drain has that many bacteria."
- "I liked being able to choose what to collect."
- "This lab built on what we learned earlier in the quarter—I feel comfortable with lab techniques now."
- "It was cool to see how many organisms there are in common places."
- "This lab summed up many things that I learned earlier in class."
- "A fun lab—but we need more time to investigate the slides."
- "I was very aware of the three-dimensional character of the films by using the fine focus knob."
- "I was delighted to be able to grow my own biofilm after reading about them."
- "The biofilms formed with so little experimental effort that it seems very likely that bacteria really "want" to grow this way."
- "The big lesson is that these little guys (the microbes) are not only complex and subtle inside the microscopic world, but can exhibit macroscopic features of great subtlety and utility (for themselves)."

SUPPLEMENTARY MATERIALS

Possible Modifications.
The following four modifications were suggested by the students in their responses to Question 8 on the Biofilms Discussion sheet.

1. Isolate some of the biofilm bacteria and identify them using tests to characterize "unknowns." Determine whether similar organisms can be isolated from biofilms of diverse origins. Determine whether an isolated organism can form a biofilm when it is grown as a pure culture.

2. Compare a slide of the microbes that float in the broth above the biofilm slide to the biofilm slide to detect differences in growth patterns. (We consistently found fewer and smaller aggregates among the floating microbes in comparison to the attached microbes in the biofilms.)

3. Prepare several biofilm slide cultures and harvest the slides over a period of days to observe changes as the biofilm matures.

4. Students collaborated to propose experiments to investigate how environmental factors influence biofilm growth in their responses to Question 9 on the Biofilms Discussion sheet. They suggested comparing the extent of growth when biofilms are grown at different pH levels, different temperatures, with different nutrient supplies, or on different subtrates. They also suggested comparing agitation versus stationary growth, and growth in the presence and absence of an antimicrobial agent. The development of these proposals provides opportunities for students to develop critical thinking skills and apply the scientific method of inquiry. It is desirable to have the students work in groups so they gain experience in developing consensus as they share in planning and making strategic decisions about their proposals.

Biofilm growth under some of these test conditions can be quantified by culturing the biofilms in 50 mm plates or on 24-well microtiter plates. Rinse the plates and let them dry thoroughly. Then stain, rinse, and dry the plates as described in the lab exercise. Destain each culture with a fixed volume of 95% ethanol for 10 minutes. Collect the destaining alcohol from each culture and determine its color intensity by measuring the optical density at 595 nm with a spectrophotometer.

References.

1. **Costerton, J. W., and P. S. Stewart.** 2001. Battling biofilms. Sci. Am. **285:**75–81.

2. **Davey, M. E., and G. A. O'Toole.** 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. **64:**847–867.

3. **Djordjevic, D., M. Wiedmann, and L. A. McLandsborough.** 2002. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl. Environ. Microbiol. **68:**2950–2958.

4. **McBain, A. J., R. G. Bartolo, C. E. Catrenich, D. Charbonneau, R. G. Ledder, A. H. Rickard, S. A. Symmons, and P.**

Gilbert. 2003. Microbial characterization of biofilms in domestic drains and the establishment of stable biofim microcosms. Appl. Environ. Microbiol. **69:**177–185.

Barbara Rundell
Microbiology 220
Spring, 2004 Name_____

Biofilms Discussion Sheet

1. What is meant by the term, biofilm?

2. What site did you sample to obtain biofilm material?

3. Briefly describe your biofilm:
a. Describe each type of procaryote that you see based on cell shape and size.

b. Describe each type of eucaryote that you see based on cell shape and size.

4. Observe several of your classmates' biofilms.
a. Propose some reasons why biofilms that were sampled from diverse sites and then grown here in lab might look similar.

b. Propose some reasons why biofilms that were sampled from diverse sites and then grown here in lab might look different from each other.

5. Life in a biofilm community provides what advantages and disadvantages to a microbe?
a. Advantages of living in a biofilm community:

b. Disadvantages of living in a biofilm community:

6. Do you think that the biofilm that you sampled is beneficial, harmful, a nuisance, or of no consequence for humans? Explain your answer.

7. What are some likely differences between bacteria that grow in biofilms versus bacteria that grow as free floating independent cells. For example, what differences might there be in growth rates, ease of exchanging DNA, resistance to antibiotics, etc?

8. How could you modify this exercise to obtain additional information about your biofilm? (For example, you could determine whether a bacterium that was isolated from your biofilm can propagate a pure culture biofilm.) Think of some additional possibilities.

9. Devise an experiment that would provide information on how an environmental factor affects the amount of growth in a biofilm. For example, describe an experiment to determine the effect of temperature on biofilm growth.

10. Did you enjoy this lab exercise? Please explain your answer.