Exercise 21

EXERCISE 21

Biofilms

Objectives

After completing this exercise, you should be able to:

1. Define biofilm.
2. Explain the importance of biofilms in clinical medicine.
3. Demonstrate biofilm growth.

Background

Biofilms are populations or communities of microorganisms that attach and grow on a solid surface that has been exposed to water. These microorganisms are usually encased in an extracellular polysaccharide that they synthesize. As the polysaccharide enlarges, other microbes may adhere to it so several different species of bacteria and fungi may be in one biofilm. Biofilms may be found on essentially any environmental surface in which sufficient moisture is present. Biofilms may also float. The pellicle that you've seen on some liquid culture media is an example (see Color Plate X.1). This biofilm at the air–water interface allows the bacteria to float near the surface and get oxygen while getting nutrients from the liquid. Individual cells would have to expend too much energy swimming to stay near oxygen.

As bacterial cells grow, gene expression in bacterial cells changes in a process called quorum sensing. **Quorum sensing** is the ability of bacteria to communicate and coordinate behavior. Bacteria that use quorum sensing produce and secrete a signaling chemical called an *inducer*. As the inducer diffuses into the surrounding medium, other bacterial cells move toward the source and begin producing inducer. The concentration of inducer increases with increasing cell numbers. This, in turn, brings more cells and causes synthesis of more inducer.

Biofilms are not just layers of cells. A biofilm is usually composed of pillars and channels through which water can flow, bringing nutrients and taking away wastes (Figure 21.1).

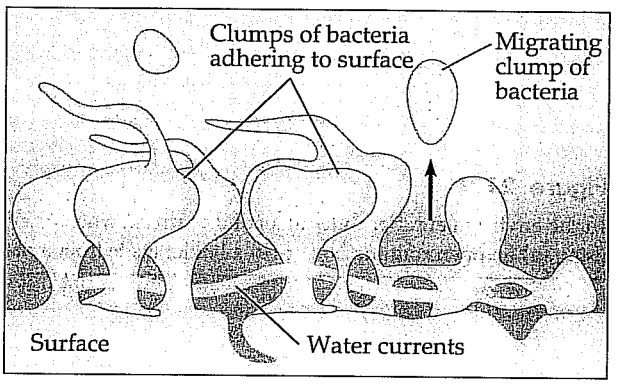


Figure 21.1

Biofilm. Water currents move through the pillars of slime formed by the growth of bacteria attached to a solid surface.

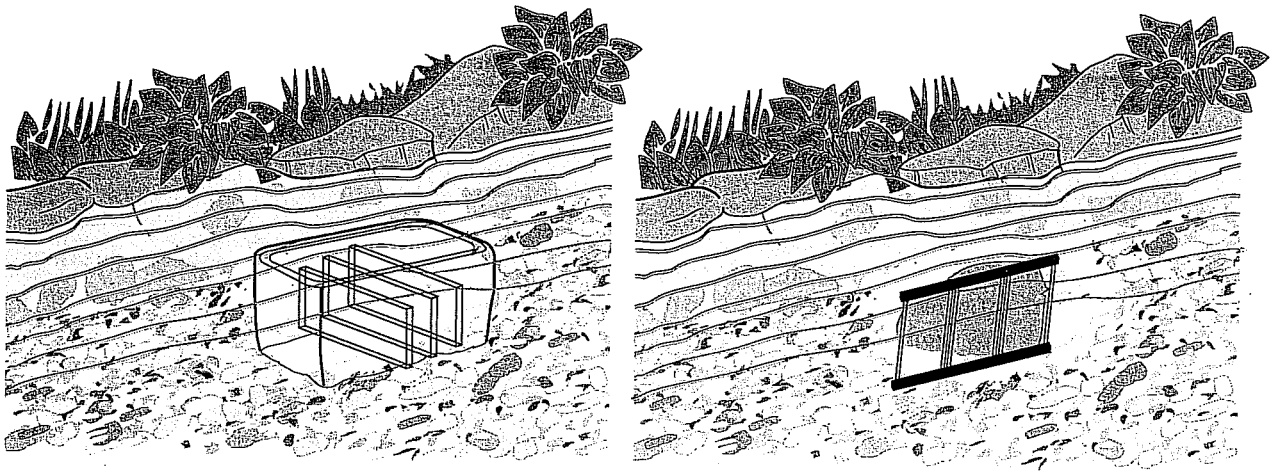
Increasing evidence indicates that biofilms play a key role in disease. Healthy bronchioles are usually sterile; however, *Pseudomonas aeruginosa* establishes a permanent infection in cystic fibrosis patients by forming a biofilm. In healthy individuals, bacteria can form biofilms on implanted prostheses. Bacteria that break off biofilms of indwelling medical devices such as catheters or protheses are a continuous source of infection in patients. The microbes in biofilms are generally more resistant to antibiotics and more difficult for the immune system to destroy.

Materials

Microscope slides (3)

Coplin jar or beaker and slide rack

Methylene blue (third day)



(a) (b)

Figure 21.2

In the environment. Set slides in a pond or stream where they won't be disturbed by using (a) an open staining box or (b) a holder made from a plastic report holder.

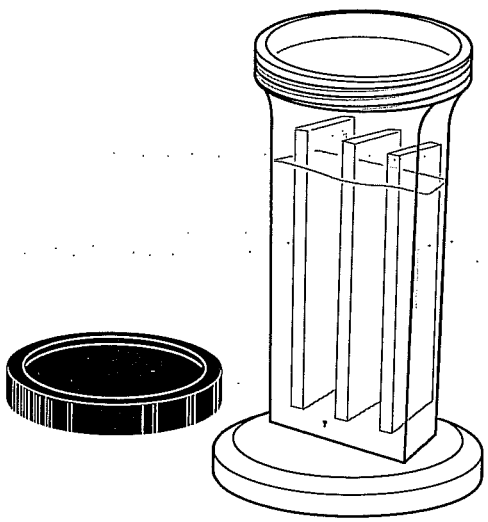


Figure 21.3

In the laboratory. Set slides in the Coplin jar. Fill the jar with liquid, leaving a few millimeters of the slides exposed above the liquid.

Cultures

Hay infusion incubated 3–5 days in the light *or*

Pond or stream water

Techniques Required

Simple staining, Exercise 3

Procedure

1. Establish a biofilm by using one of the following methods:
   a. In the environment
      1. Place three slides in a horizontal staining dish or a plastic slide rack.
      2. Place the slide dish or rack in a pond or stream in an area that is easily accessible and where the slides will not be displaced (Figure 21.2).
      3. Mark the location and note the location in your lab report.
   b. In the laboratory
      1. Place the three slides in a Coplin jar (Figure 21.3).
      2. Add enough of the culture to the jar to nearly cover the slides; leave a few millimeters.

3. Place the cap loosely on the jar and incubate the jar at room temperature.

2. Collect your biofilm.
   a. At the next laboratory period, remove one slide and label it "1."
   b. Wipe one side of the slide clean, leaving the biofilm on the other side.
   c. Air-dry and heat-fix the slide. Save the slide in your drawer for staining in step 3.
   d. Repeat steps 2a–c at the next two laboratory periods, labeling the next slide "2" and the last slide "3."
3. Observe your biofilm.
   a. On the third laboratory period, compare the appearance of the slides without a microscope. Then stain the three slides with methylene blue (Figure 3.3).
   b. Examine the stained slides microscopically using the oil immersion objective. Record your observations.

Exercise 21

Biofilms

LABORATORY REPORT

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Where did you set your slides? _____

_____

	Slide 1	Slide 2	Slide 3
Incubated (days)			
Appearance of the slide with an unaided eye			
Microscopic appearance of the slide			

Conclusions

Compare the slides. Did any changes occur over successive days? _____

_____

_____

What different types of microorganisms did you see? What was the most abundant? _____

_____

_____

Questions

1. Are the cells evenly distributed on your slides? Provide an explanation for their distribution? _____

2. Is a natural biofilm a pure culture? _____

3. How is a biofilm beneficial to bacteria? _____

4. What cell structures help bacteria attached to a solid surface? _____

5. What is a quorum in law? _____

   How is this related to quorum sensing in bacteria? _____

6. Why is quorum sensing important for biofilm formation? _____

Critical Thinking

1. Bacteria are the second most common cause of artificial implant failures in humans. In these cases, why isn't an infection usually detected from cultures of blood or tissue?

2. Suggest a reason why dental researchers were the first to become aware of the importance of biofilms to disease.