

Microbial Biofilms - “Getting By With A Little Help From Our Friends”

Microorganisms are all around us. They are found in soil, saltwater, freshwater. They are found in solid rock deep within the earth and in a variety of extreme environments that no human could live in. They are found on and in our bodies and in the air. Using tools of microbiology such as the microscope and pure culture techniques we have made great strides in understanding microorganisms. As a matter of convenience, we often study microorganisms as single cells or small clusters of cells, and we will be doing that in our lab course as well. However in natural environments, microorganisms often are not found as single cells or small clusters. They often are found in extensive, occasionally “slimy”, layers of growth called biofilms.

Biofilms are made up of microorganisms attached to a solid surface - usually at the boundary of a solid object and liquid water. While many microorganisms are motile as single cells, once they are attached to and a part of a surface biofilm they may be “stuck” in place by a variety of extracellular polymer substances excreted by the microorganisms themselves. Extracellular polymer substances can include polysaccharides, proteins and various other biologically produced polymers. Motile cells can move to find nutrients but cells immobilized in a biofilm need to have the nutrients come to them as water flows by.

A biofilm can consist of one type of microorganism. More commonly, several different types of microorganisms, including bacteria, fungi, algae and protozoa will become associated with a biofilm. You are going to submerge a glass slide in pond water and allow a biofilm to develop on the slide over several days. Later you will examine one side of the biofilm slide under the microscope and see how many different types of microorganisms are present. This is a good way to get some idea about the diversity of microorganisms as you learn proper use of the microscope.

While you will be using pond water to establish your biofilm, please realize that biofilms can develop in several other situations as well. Below are listed just a few cases where microbial biofilms can form:

Teeth (dental plaque)	Heart Valves	Urinary Tract
Water Pipes	Implanted Medical Devices	Catheters
Cooling Towers	Wastewater Treatment Facilities	Hulls of Ships
Artificial Heart Valves	Plant Roots	Submerged Plants
Rocks and Mud in Streams	Rumen of a Cow	Bioreactors

Please check the index of your textbook, for more information on microbial biofilms.

One interesting aspect about microbial biofilms is that the microorganisms living in a biofilm may be more resistant to antibiotics and other chemical agents than their free-living, single-celled counterparts. In such cases we might have to use several times the concentration of antibiotics or disinfectants than we would need to kill or inactivate free-living microorganisms. Another interesting point is that various protozoa are found associated with many environmental biofilms. The protozoa can ingest parts of the extracellular polymer substances as well as some of the smaller microorganisms, such as bacteria. In the area of just one microscope slide you may be able to see an entire microbial community with several diverse life forms.

Setting up the Biofilm:

1. Place a microscope slide in a slide rack. Tie a piece of string to the handle of the rack. Label the string with a piece of tape and place the rack in the aquarium tank (center room).
2. The first stages of biofilm formation can begin almost right away, although this may not be readily apparent.
3. Leave the microscope slide suspended in the pond water for a few days.

4. When it is time to examine the biofilms, retrieve your slide and wipe off one side (the bottom) with a Kimwipe tissue or a paper towel. Do not wipe off the other side (the top).
 5. Gently lower a cover slip on top of the biofilm and examine the slide under the microscope. Your instructor will show you how to set up and adjust your microscope for optimal viewing.
 6. Observe several different areas on your slide. You may also want to look at your neighbors' slides. There should be plenty to see!
- Record the length of time the biofilm was allowed to develop.
 - Estimate the number of different types of organisms you observe and record this in your lab manual.
 - What different sizes and shapes do you see? Estimate size by figuring how many of one organism it would take to fill the microscope field of view.
 - Make drawings of various organisms and if they are motile record how they are moving. If certain organisms are colored - record the color.
 - Do you see any protozoa "feasting" on parts of the biofilm?
 - Estimate the relative number of bacteria, protozoa, algae, and fungi associated with the biofilm?

Thought Questions:

Can you think of another situation other than those listed on this handout where biofilms might form?

Why would microorganisms in general be more resistant to antibiotics, disinfectants, and sterilizing agents while associated with a biofilm than if they were living as single, solitary cells?

If nutrient conditions were really poor in a given environment (stream or lake), would it be more advantageous for an organism to become associated with a biofilm or remain as a motile single cell? Why?

Why are microbial biofilms often involved with the treatment of wastewater, sewage and natural clean up of polluted streams?