Chapter 10 Microscope

The microscope is a great tool to use in a activated sludge wastewater laboratory. Frequent microscopic examination can provide valuable information about the condition of the microorganisms in the activated sludge. Observing and recording the different types of microorganisms can help the wastewater treatment plant operator identify changes that may cause a plant upset.

Parts of a Microscope

The eyepiece or ocular lens is located at the top of the microscope. It may be monocular or binocular. If it is binocular, there is a slide adjustment to move the eyepieces so the lab tech can see clearly. The eyepiece usually magnifies the object 10x.

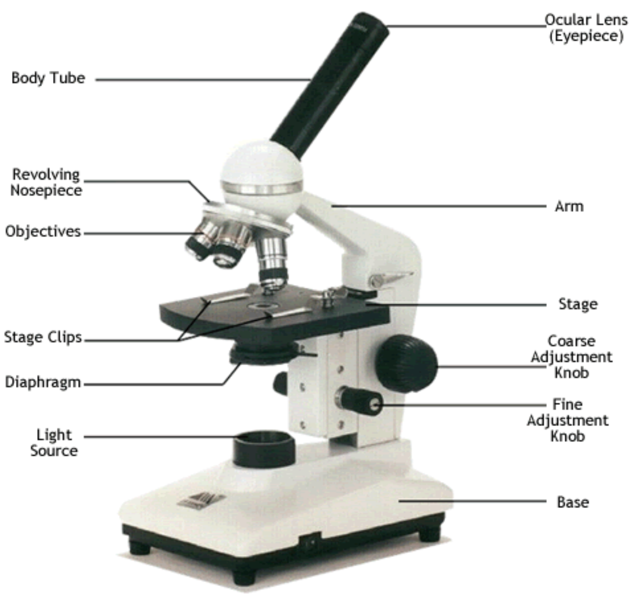


Figure: Parts of Light Microscope

The next set of lenses called objective lenses are mounted on a turret or nosepiece. There will be either 3 or 4 objective lenses. The objective lenses are rotated and come in close contact with the microscope stage. Each objective lens will have a different magnification, commonly 4x,10x, 20x, 40x or 100x. The 4x lens is a considered a scanning lens, magnifying the object only a total of 40 times. This lens is often used to locate an object of interest but shows little detail. Once identified, the nosepiece can be rotated to a higher magnification for more detail.

A good microscope will be parfocal and parcentered. Parfocal means that once the object is focused on the slide, the next more powerful objective lens can be swung into place with only a minimum of fine focus adjustment. Parcentered means the object in the center of the viewing area will still be in the center when the turret is rotated to the next more powerful objective lens with minimum adjustment. The 10x, 20x or 40x objective are all used to see the object in more detail. The 100x objective lens is called an oil immersion lens. This lens provides a magnification of 1000 times. Oil is used to reduce light diffraction and improve the resolution, the ability to distinguish between objects that are close together. As light passes upward through the slide, it is diffracted (bent). This bent light is lost and makes visualizing the object more difficult. By using oil, the light is not bent as much, allowing more light to reach the lens and improve the object resolution. The oil should always be cleaned from the lens after each use to reduce the accumulation of dirt on the lens.

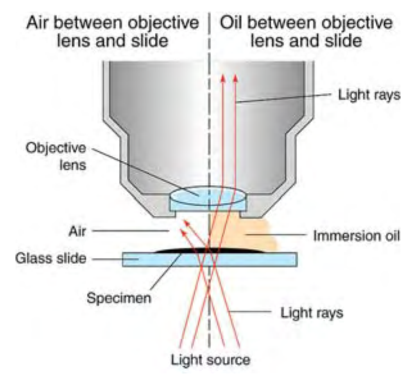


Figure: Oil immersion lens refraction

The microscope stage is the platform that holds the slide. A good microscope will have two slide movement knobs. These knobs allow the slide to be slowly moved back and forth on the stage. Below the stage are the diaphragm lever and the condenser. The condenser consists of a series of lenses which focus the light on the slide. The condenser is almost always kept close to the stage for maximum image sharpness. The iris diaphragm lever is located between the condenser and the light source. Moving the lever causes the iris to open and close, thus controlling the amount of light reaching the condenser. If too much light is allowed to reach the condenser, the object on the slide gets "burnt out" by decreasing the image contrast. Focusing the object occurs by turning the two knobs on the body of the microscope. The larger knob is the coarse adjustment and should be used to bring the objective lens close to the slide. The fine adjustment knob is used to bring the object into focus. Care should be taken not to bring the objective lenses into contact with the slide. Rapid adjustment using the coarse adjustment knob may crack the slide or worse may crack the objective lens. The safest procedure is to watch the objective lens from the side of the microscope as the lens is lowered to the slide, then look through the microscope as the lens is lifted off the slide using the fine focus knob. Finally, at the bottom of the microscope is the light source. Some microscopes have a knob which can adjust the intensity of the light. The on/off switch is either on the cord or the base.

Lens Cleaning

It is critical to keep all lenses clean, including the condenser. Frequent wiping with lens paper or Kimwipes is necessary to keep all surfaces free of dust and oily substances. Dust and oil will drastically reduce the microscope resolution. Resolution is defined as the ability to distinguish between 2 objects that are close together. Use only lint-free cleaning tissues and store in a dust free environment hopefully with the dust cover on.

Objective lenses should be cleaned using manufacturer approved solvents. The most common approved solvent is xylene. Apply xylene to the lenses using a cotton swab, then wipe off with lens tissue, and finally blow off any remaining lint with an air syringe (pipet bulb).

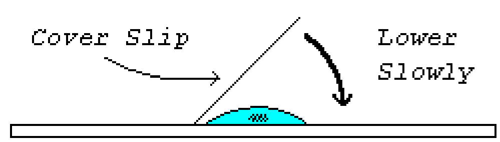
The eyepiece lens gets the dirtiest because it is always contacted by eyelashes and makeup. It should be cleaned frequently. Using a dust cover will help reduce the cleaning frequency.

Better Viewing Hints

1. Adjust the condenser so it is close to the stage
2. Close the diaphragm lever to almost shut to block out most of the light and prevent glare. As the magnification is increased, slowly open the lever to allow more light in.
3. Always clean the lenses. Always use lens paper to clean the lenses and begin at the lowest objective to avoid contamination with the oil immersion lens.
4. Start scanning for objects of interest using the 4x or 10x lens.

Preparing and Viewing a Wet Mount

1. Use a clean dry oil-free slide. Dry the slide using lens paper if necessary to avoid scratching the slide.
2. Obtain a freshly collected sample, aerate if needed to keep aerobic microorganisms alive.
3. Place a drop of sample on the slide using a pipet or eyedropper. Place a clean, dry cover slip onto the slide at a 45^o angle so that one edge touches the droplet and the water spreads along the edge of the cover slip.



Gently lower the cover slip, squeezing out air bubbles as you go.

4. Carefully blot any moisture off the bottom of the slide. If the bottom of the slide is wet, it will not move freely across the stage.
5. Place the slide on the stage and make sure it is securely held in place.
6. Start at one corner of the cover slip. Use the 4x or 10x objective and work your way up one edge until you reach the top then move over one complete field of view. Start back down until you reach the bottom. Continue until the entire area is viewed. Center any object of interest, then turn the turret to magnify the object.

Oil-Immersion Objective

1. Focus the object with the high dry objective (20x or 40x). Be sure to focus on an object that is not near the edge of the cover slip because some of the oil may get under the cover slip.
2. Rotate the 20x or 40x objective out of the way but stop before the 100x has swung into place.
3. Take a dropper of immersion oil and allow a drop of oil to fall onto the slide at the point where the light is shinning up from below.
4. Rotate the oil immersion lens (100x) into place. Check the image focus before adjustment using the fine focus knob.
5. Clean the lens with lens paper when finished. Do not use paper towels.

Safety

1. Always carry the microscope using two hands.
2. Slides and cover slips can break and cause cuts and infection.

Application

In wastewater analysis, the microscope is a valuable tool in activated sludge process control. Frequent microscopic examination of activated sludge can determine floc structure and identify, count, and check on the activity of various types of protozoans and metazoans.

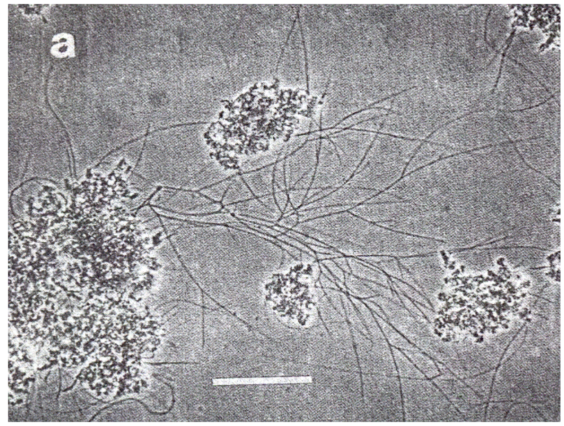


Figure: Compact floc with filamentous interbridging

Floc Structure

Daily microscopic examination of floc by the laboratory technician can alert the operator to changes in the activated sludge treatment process. Floc types include:

a. Compact, well-formed floc which settles easily, leaving a clear supernatant.
b. Pinfoc -- tiny very compact floc, often with few bacteria that may or may not settle well, but leaves floc suspended in the supernatant.
c. Straggler floc -- loose floc structure, which is characterized by large, amorphous floc which may or may not have filamentous bacteria. Generally settles slowly.
d. Bulking floc -- usually characterized by filamentous bacteria. Filamentous bacteria extend between floc particles, connecting them together, and thus preventing them from compacting and settling well.
e. Dispersed floc-- loose small floc structure, very little organization. Poor settling usually has a very turbid supernatant.

Microorganism classification

Besides examining the floc characteristics, the types of microorganisms present in activated sludge can also be identified. The quality of the activated sludge can be monitored by identifying, counting, and describing the activity of the organisms present. By doing this, the operator/lab technician can:

a. determine which organisms are present when the treatment system is running well
b. determine if a toxic shock has taken place in the activated sludge system
c. provide data for trend charts relating protozoan numbers to treatment parameters.
d. identify filamentous bacteria types to determine cause of bulking or foaming
e. count filamentous bacteria numbers to predict effective or ineffective treatment.

Common Microorganisms

Safety:
While microorganisms are commonly taken for granted in wastewater treatment plants, they are opportunistic which means they can use the laboratory technician as a food source. Always disinfect the lab bench and wear gloves when handling wastewater samples. Wear gloves and a mask if there is significant overspray during sampling.

Bacteria
Bacteria are the major work force in waste treatment. There have many different strange names but most fit in the category called heterotrophs.

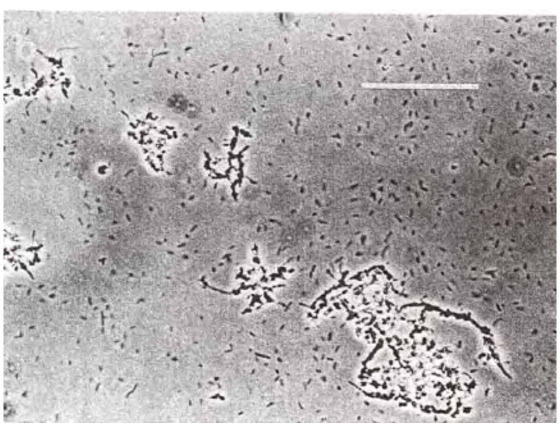


Figure: Bacteria

Under the microscope, bacteria will appear as very small black dots some will be stationary others will be motile. The presence of lots of bacteria indicate very early treatment and high Food:Microorganism ratio (BOD). Bacteria can barely be seen under the 400x magnification (40x objective).

There is a second type of bacteria called filamentous bacteria. They provide similar waste stabilization as normal bacteria except they create problems because they don't settle well. Filamentous bacteria begin to predominate when the environment is not "happy" such as low pH or low dissolved oxygen.

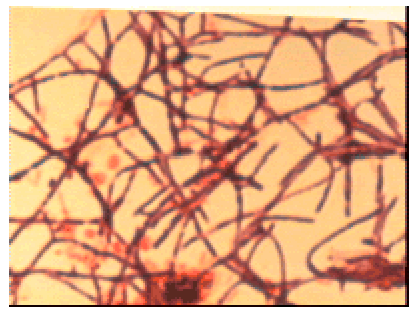


Figure: Filamentous Bacteria

Protozoans
Protozoans are one-celled, animal like organisms which include amoeba, flagellates, ciliates, and sporozoans. Protozoans can be free-living or parasitic. Common parasitic protozoans found in water cause amoebic dysentery, Giardiasis, and Cryptosporidasis.

Amoeba
Amoeba are very flexible one-celled protozoans that have a cytoplasmic membrane but no cell wall although some are encased in shells. They have pseudopodia (false feet) for both locomotion and food capturing. They feed upon complex organics by surrounding the food with their pseudopodia. Enzymes are used to break down the organics which are then ingested by absorption through the cell membrane. They are quite numerous during plant start-up or during a

plant upset. Their presence indicates a very young activated sludge with a high F:M ratio.

Flagellates

These one-celled organisms vary in size and have one or more thread-like flagella attached to their cells at either the posterior or anterior end. The flagella are used to move the organism and if a flagellate has two or more flagella, it tends to move in a corkscrew fashion.

Flagellates dominate when bacterial populations are low and the organic loading (BOD) is high. Their presence also indicates a young activated sludge with a high F:M ratio. The floc usually is light and dispersed. (straggler floc). Flagellates are very small but can be seen under 100x magnification (10X objective)

Ciliates

Ciliates are protozoans having hair-like structures (cilia) covering all or part of their cell membrane. The beating cilia either move the cell or cause currents in the water which aid in food gathering. They use bacteria or particulate organic matter as food. The movement of the cilia force the particles into the cell's gullet. Ciliates can be divided into free-swimmng, grazers, and stalked ciliates.

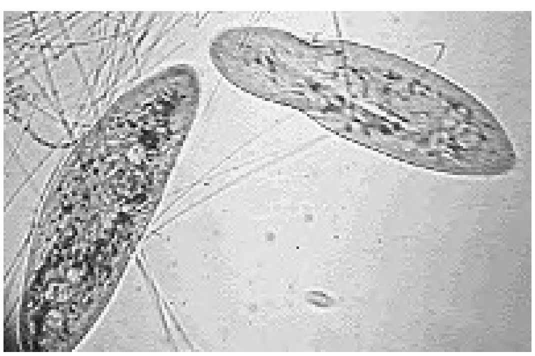


Figure: Free swimming Ciliates

Free-swimming ciliates are present in high numbers when the bacteria population is large. Two types of free swimmers include those that cruise between floc eating whatever particulate matter is available; and grazers, those that usually stay within or on the floc and feed on the floc as cows would munch on grass in a field.

Figure: Grazing ciliate

The presence of free-swimmers indicate the activated sludge system is approaching optimum treatment, although lots of grazers seem to indicate a better treatment than the presence of the cruisers.

Stalked ciliates use a stalk to hold onto the floc. Because they do not have to propel themselves through the water, they do not expend as much energy as the free-swimmers and therefore live in water with lower organic loadings. Since they are attached to the floc, they cannot move to more oxygenated areas, so the dissolved oxygen must be fairly high for them to survive. The rhythmic beating of the cilia at the anterior end (head) produce currents which drive bacteria and organic particles into their gullet. Some stalked ciliates have a contractile protein in their stalk that routinely contracts into a tightly wound coil, then springs out. This "spring action" stirs the water and helps the stalked ciliates gather food.

Figure: Stalked ciliate

Stalked ciliates may occur individually or form colonies. They begin to predominate when free-swimming ciliates are unable to compete for food because the bacteria population is small.

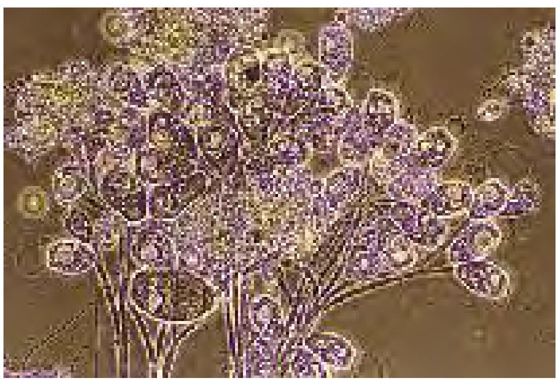


Figure: Colonized Stalked Ciliates

When stalked ciliates are present, floc formation is usually good and the effluent is clear. Ciliates are easily visible under 100x and 200x magnification (10x and 20x objective)

Metazoans
Metazoans are the largest organisms in the activated sludge system. They include rotifers, nematodes, and bristleworms.

Rotifers
Rotifers are found mainly in older activated sludges. At the head end, most rotifers have a conspicuous corona with cilia used both for locomotion and food gathering. Just below the corona is the mouth which is connected to a grinding structure called the mastax. The mastax is usually easily seen in rotifers and can be used to differentiate rotifers from other microorganisms. The foot, at the posterior end has spurs and retractile toes which allow the rotifer to attach to floc.

Figure: Rotifer

Rotifers have a high DO requirement and will die when the DO is too low. The presence of stalked ciliates and rotifers indicates a good activated sludge floc and produce a clear effluent.

Nematodes
Nematodes commonly found in wastewater are non-segmented roundworms. They have a long, slender body with one end usually sharply pointed while the other end tapers to a blunt tip (little fire hoses). They feed on bacteria, protozoans, ciliates, rotifers, and floc. The presence of nematodes indicates an older sludge. Sludge worms are easily seen under the 4X objective.

Bristleworms
Aeolosoma is the segmented worm commonly found in very old activated sludge. It has bristles which extend from its sides and burnt-orange dots on its surface. Bristleworms consume organic sludge.

Once again, the microscope can be a valuable tool to help the lab technician identify types of microorganisms and relate them to the effluent quality (BOD and TSS).