Using the Microscope to evaluate your System

A wastewater treatment plan is a biological "bug" factory. You need to grow bacteria to successfully meet final effluent permits. There are no replacements for the biological activity. You can build more equipment, you can use all the chemicals you want, but basically, it comes down to how well you can grow bacteria! So if you are running a "bug" factory, it would be a pretty good idea to check the status on your product – the bugs.

In order to view the bacteria present in the wastewater system for monitor and control purposes, samples must be collected. Where, how and when you sample the MLSS or bacteria can make a total difference in how accurate the analyses; one thing to note if you have a municipal wastewater treatment plant. We have run analyses in the morning vs. the afternoon. There can be major changes in your biomass, depending upon the time of day, whether it is just after a weekend, or even if it is just after a major rain. Keep notes on when you take the sample. Take multiple samples during a normal day just once to see if there are changes in your system. Remember, the bacteria life span is 20 minutes to 2 hours, so 8-10 hours can sometimes make a difference in how the mlss looks, mainly the types of higher life forms. If you get hit with a high loading, 4 hours later, the amount of amoebae, flagellates and higher life forms will change, so keep that in mind. This also can go for food plants, where the night shift is completely different from the day shift. In order to keep from chasing your tail, try to standardize your sampling. Take the same place the same time of day and keep track of trends, unless you know you just got hit by a big spill.

Always make sure to take the sample at the back of the aeration basin, lagoon, or whatever piece of equipment you are using for the biological portion near the outfall, below the surface of the water. Do not collect the sample in dead corners where scum has built up.

Preparation of a wastewater sample can be as simple as placing a drop of the sample on a glass slide, covering the droplet with a cover slip and examining it under the microscope. Unfortunately, if the procedure is done carelessly you will miss many important clues the microscope can yield, or even worse, add some artifacts that could lead you to incorrect conclusions!

The following method is a brief, step by step, procedure that can help you make good quality slide preps.

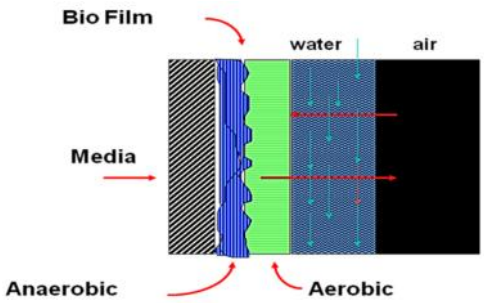
Equipment:
Clean glass slides, cover slips, transfer pipettes, and microscope.

Method for a wastewater sample:
Shake or swirl the wastewater sample to get an even mixture before sampling. Place a drop of the wastewater on the glass slide. Carefully tilt a cover slip at a 45º angle against the droplet of water. Try to avoid trapping air bubbles under the glass. Lower the cover slip into place. Wipe off excess water around the edges of cover slip. If you don't, it will look like a whirlpool is under the cover slip. Place the slide on the microscope stage for examination.
*Note: Tiny air bubbles will look perfectly round with very dark edges.

Method for making an India Ink Slide
Follow the same procedure previously mentioned; only add a drop of India ink to the drop of wastewater before lowering the cover slip. The India ink stain is made of carbon black particles and is used to determine the level of exopolymer (polysaccharide coating). The polysaccharide will look like bright, clear areas. The areas that do not contain polysaccharide will stain dark brown/black. Excessive levels of polysaccharide coating can indicate a nutrient deficiency. *Note: Air bubbles and oil will also appear as a clear area in the India Ink Stain.

Method for making a Lactophenol Cotton Blue Slide
Follow the same procedure previously mentioned; only add a drop of Lactophenol Cotton Blue to the drop of wastewater before lowering the cover slip. The Lactophenol cotton blue stain will stain the specimen blue and allow it to be easily visualized by bright field microscopy. This will somewhat simulate phase contrast microscopy.

Case History of a plant where the location of the sample, can make a huge difference in what you see and how you interpret how well the plant is running! If the analysis does not match with plant effluent data, such as BOD and TSS, N and P levels, then pull more samples in various locations around the plant and try to figure out just where the problems or causes are occurring.



We were asked to audit a plant up in the Alberta Oil sands. This plant had a small RBC unit. The main difference in a RBC unit vs. activated sludge is that you are trying to grow the bacteria on a fixed film media as opposed to suspended. All other principles of wastewater still apply.

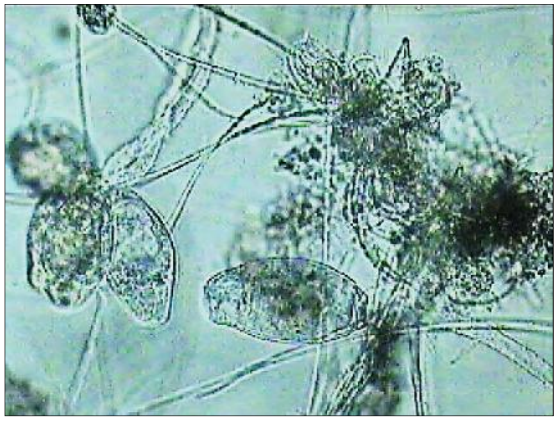
Typically for most RBC units, you would look at the biofilm that sloughs off the disks and winds up in the clarifiers.

This RBC plant had issues with tiny pin floc in the clarifier going over the weirs and impacting TSS and fecal coliform counts. We pulled samples from two different spots, since this plant does not have screening upfront of the disks, but actually after the disks and prior to the clarifier.

Screening is critical as the next few paragraphs will show. Screening also should be at the head of the plant, prior to primaries, EQ tanks, biological portions, etc. Screening, especially in food or municipal plants can take out large solids and debris that can not only impact the efficiency of the biological portion, but help to protect pumps and pipes from clogging, septicity, solids build-up, etc.

Back to our plant. Due to the fact it had a screen, we examined the screen after the RBC and prior to the clarifier. We pulled a "typical" sample of the effluent prior to the clarifier.

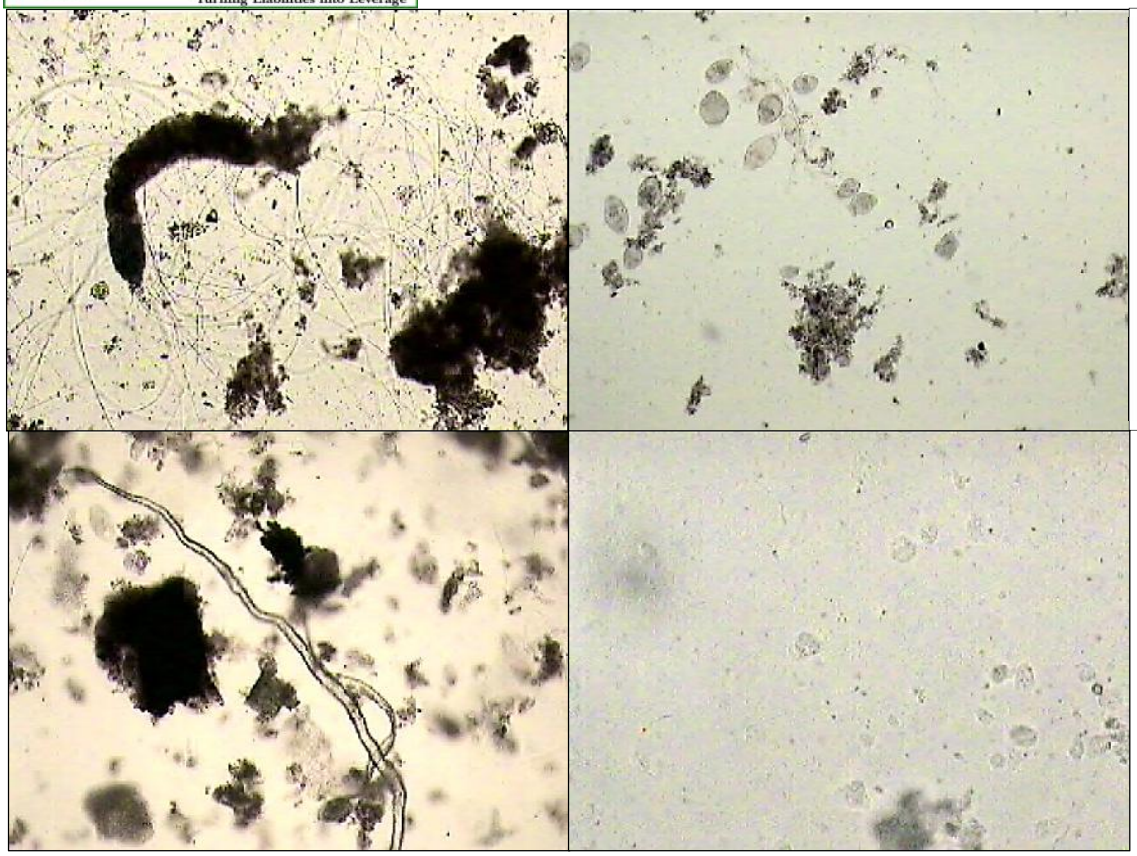
It was loaded with fiber, and excessive levels of filaments and spirillum. The floc structures were large, compact and with some very dark brown and black spots. Stalked ciliates were dominant, with a few rotifers and worms. As far as the higher life forms go, this is what you would expect from a RBC unit, relatively older sludge. The fiber was due to no prescreening and the filaments and spirillum indicates septic conditions. Some fungi was also present, indicating the septicity was lowering the pH.



We pulled a sample prior to the screen, and found less fiber, and filaments. The RBC unit had a design flaw. The bottom collection system was rounded under the disks, yet the overflow to the clarifier was taken at a point relatively high up on the system, so solids built up on the bottom of the rounded collection pit. We ran a sludge judge and found quite a bit of solids. Again, we looked under the microscope. High levels of Type 021N filaments, spirillum, Thiothrix, Beggiatoa, fiber and black floc. Solids were sitting on the bottom and turning septic.

Sludge judge of solids on the bottom of the collection pit in the RBC- Lactophenol Cotton blue stains 400x Type 021n.

We decided to scrape samples off the RBC disk directly to see if the filaments were growing on the disks, or just in the screen and collection pit. We were quite surprised. Except for the areas where toilet paper and rags had collected on the disk and caused the biofilm to be trapped and turn older and septic, the majority of the biofilm was extremely young; amoebae and flagellates dominant. Floc was young, Zooglea was present.



Section 1-Some filaments in areas, probably where fiber or debris and rags built up solids. Some areas with floc and stalks. Fiber and floc in section two Amoebae and more single celled bacteria in section 3.

As you went across the system from the influent to the back end, the biofilm actually got younger in most normal spots. We looked at the clarifier, primary holding tank and aerated EQ tanks prior to the RBC unit to make sure the septicity was not just in the bottom of the collection pit and screen of the RBC unit.

Black spots on floc, spirillum, tons of single celled bacteria, even Thiothrix II was present in the solids that were built up in the EQ and primary collection Tank. This plant did not have screens or a real primary clarifier, so no solids removal methods were in place. Instead solids were building up in the collections system upstream turning septic, EQ and primary were turning septic, screens and collections pit on the RBC were turning septic and the clarifier itself has solids build up on the slanted sidewalls that was turning septic, causing ashing and gassing and pin floc carryover.

Technically, if you look overall at all the samples and the results, although the final effluent shows pin floc, old sludge and septicity, the RBC disks themselves were actually very young. Changes to the operations were recommended based upon the sampling. Had we only tested one spot, we would never have found out the conditions present in all these spots.

Use your microscope. Since you cannot jump in, see under the water, and really know what is going on in the bottom, pull samples instead and use the microscope to tell you what is going on. Then with a little detective work, you can really pull together what each piece of equipment in your system is doing. This shows how each piece of equipment ties all together and little things here and there can make a big impact.