Courtesy Microbe Detectives

Industrial treatment troubleshooting

DNA can help you

Trevor Ghylin and Steve Leach

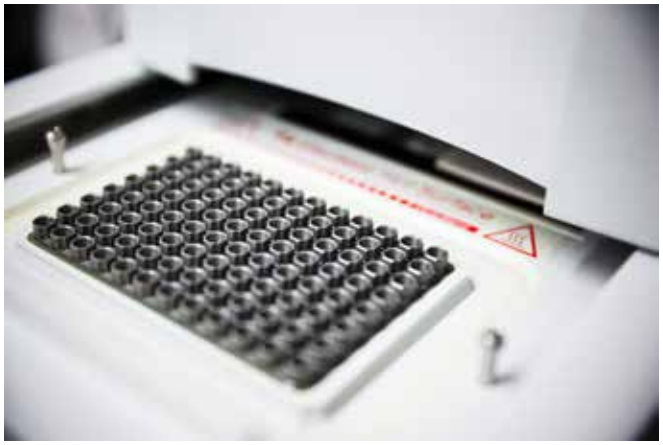
Industrial wastewater facilities tend to treat wastes with highly variable flows and concentrations. As a result, these facilities tend to be difficult to operate and may have issues with settling, toxicity, and ammonia removal. Operators struggle to understand operational issues resulting from a lack of information on the bacteria responsible for these processes. Troubleshooting issues may consist of microscopy as well as checking pH, alkalinity, mixed liquor suspended solids (MLSS) and a lot of head scratching.

Fortunately new DNA technology enables users to see what is going on with the bacteria directly, rather than looking only at indicators and surrogate measurements. DNA technology finally can answer the most basic question: Do I have the right bacteria growing?

Moving beyond traditional monitoring

Traditionally, biological monitoring and troubleshooting of activated sludge systems has been performed using a microscope to observe protozoans, metazoans, and filaments. A large body of knowledge has been built up based on microscopy to troubleshoot settling issues. However, this method requires a trained observer and is limited only to organisms that have unique shapes and staining features.

The vast majority of microbes in activated sludge do not have unique shapes or staining features, including some of the most critical bacteria, such as nitrifiers (ammonia removal) and phosphate-accumulating organisms. As a result, microscopy provides essentially no information for troubleshooting issues with ammonia or phosphorus removal.



DNA analysis can help reveal more details about the bacteria at work within wastewater treatment systems. Courtesy Microbe Detectives

DNA technologies are revolutionizing the way operators analyze activated sludge. These technologies have been decreasing in cost exponentially since the first human genome was published in 2001 at a cost of $3B USD. Figure 1 (below) shows how the cost of sequencing a human genome has dramatically fallen to just a couple thousand dollars today and will likely be $100 in a couple of years.

This same technology can be used to analyze activated sludge. And the price is similar to sending a sample for microscopic analysis of filaments. These technologies are economically feasible to identify and quantify nearly every microbe in a sample of wastewater using DNA.

Microbiological problems and troubleshooting

Wastewater treatment relies on such microbiological processes as activated sludge with little knowledge of the microbial community present. Instead, these systems typically are operated based on empirical monitoring data such as dissolved oxygen and effluent biochemical oxygen demand (BOD).

This simple operational strategy generally works for many facilities most of the time. However, problems can arise when facilities are stressed because of high loading, low temperatures, toxic influent, and growth of filaments and foam-producing organisms. These problems are especially prevalent in industrial treatment facilities where influent can be variable and difficult to treat.

Microscopic characterization and staining

The traditional method of troubleshooting activated sludge consists of analyzing a droplet of sludge under a microscope. This technique is easy and cheap and can provide some information very quickly. However, as was stated earlier, it has significant limitations because it cannot identify most microbes that are important to treatment, such as nitrifiers and phosphorus-accumulating organisms (PAOs).

Microscope users attempt to identify problematic filaments and foaming organisms; however, this task may be impossible as the problem organisms can be hidden inside flocs and because many different species of filaments and foaming organisms have identical morphologies. Additionally, morphologies can change under varied nutrient conditions, according to the July 1994 article, "Molecular identification of activated sludge foaming bacteria," by L.L. Blackfall in *Water Science and Technology*.

Because of the difficulties in microscopic characterization, many studies have focused on creating fluorescent molecular probes that would bind to the DNA of the target organism and fluoresce to provide a visual identification under a microscope. In this way, organisms with identical morphology could be distinguished based on their DNA.

Fluorescent *in situ* hybridization (FISH)

Many probes have been developed to target common filaments, nitrifiers, and PAOs. However, there are so many organisms that create filaments and foaming that it is difficult to identify problem organisms in a sample with this method. A March 2002 article, "*In situ* studies of the phylogeny and physiology of filamentous bacteria with attached growth," published in *Environmental Microbiology* by T. Thomsen *et al.*, showed that FISH probes were only able to identify 15% of organisms in a foam sample. Further, these organisms only were identified at a very high taxonomic level (phylum).

Also, the FISH method can suffer from inefficient permeabilization of cells, inefficient binding to DNA, and lack of specificity. The FISH method also can be somewhat complex, sometimes requiring multiple helper probes or special methods to increase the fluorescent signal.

Figure 1. Historical cost of DNA sequencing ($ per human genome)

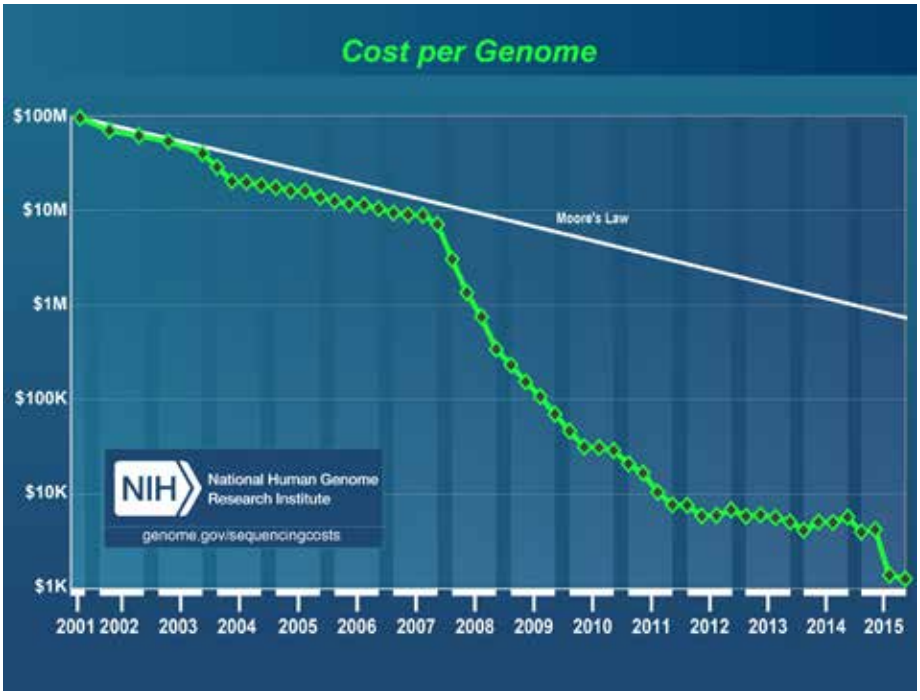
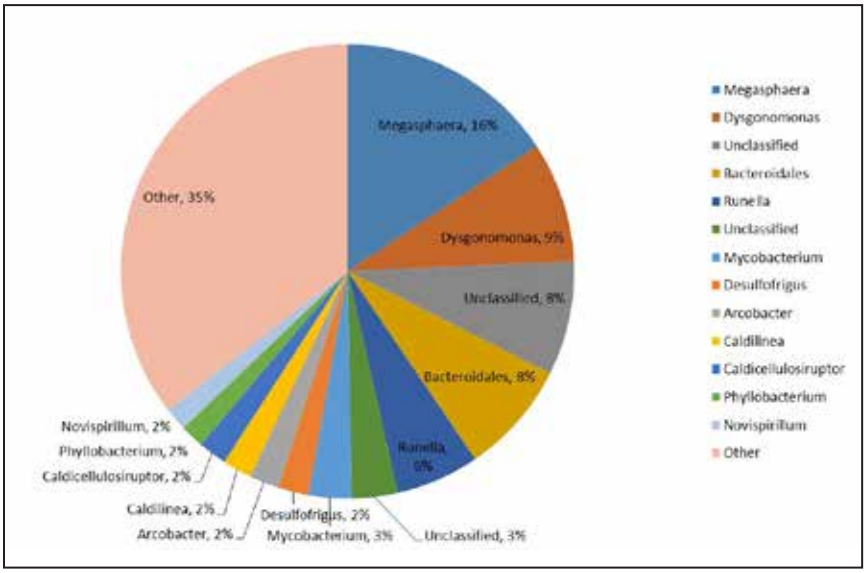


Figure 2. Microbial community in an activated sludge facility treating petrochemical waste (Genus shown)



The FISH method is not something that a typical wastewater treatment lab can perform routinely as it requires some biotechnology expertise and special equipment such as an epifluorescent microscope. Additionally, there are no companies currently offering this as a commercial service. As a result, this method is only appropriate for an academic laboratory with experience in DNA methods.

DNA sequencing

A more recent advancement is next-generation DNA sequencing. DNA sequencing can characterize entire microbial communities based on the DNA of individual organisms with much less complexity and potential problems than FISH. This method can provide quantification of nitrifiers, PAOs, filaments, foamers, and other organisms in a sample.

Figure 2 (above) presents microbial community data from an activated sludge system treating petrochemical wastewater. The most abundant bacteria are *Megasphaera* and *Dysgonomonas*, both anaerobic or facultatively anaerobic bacteria, indicating potential issues with oxygen supply.

Figure 3 (right) provides a time series of filament abundance in a sequencing batch reactor activated sludge system. This figure demonstrates the quantitative nature of DNA data, which enables operators to monitor a system over time and better understand causes of such operational issues as bulking or foaming or problems with phosphorus or ammonia removal.

An example of industrial wastewater bulking and settling issues

A wastewater treatment facility at a large food manufacturer experienced frequent upsets that caused sludge volume indexes (SVIs) to soar higher than 300, resulting in poor solids compaction in the clarifier and very high effluent total suspended solids (TSS) concentrations – greater than 3000 mg/L.

This facility discharged to a local sewer, so there was no concern about violating permits. However, the excessive discharge load resulted in very high sewer charges – greater than $50,000 per month.

Operators collected four samples to begin to diagnose the issue. They collected samples from

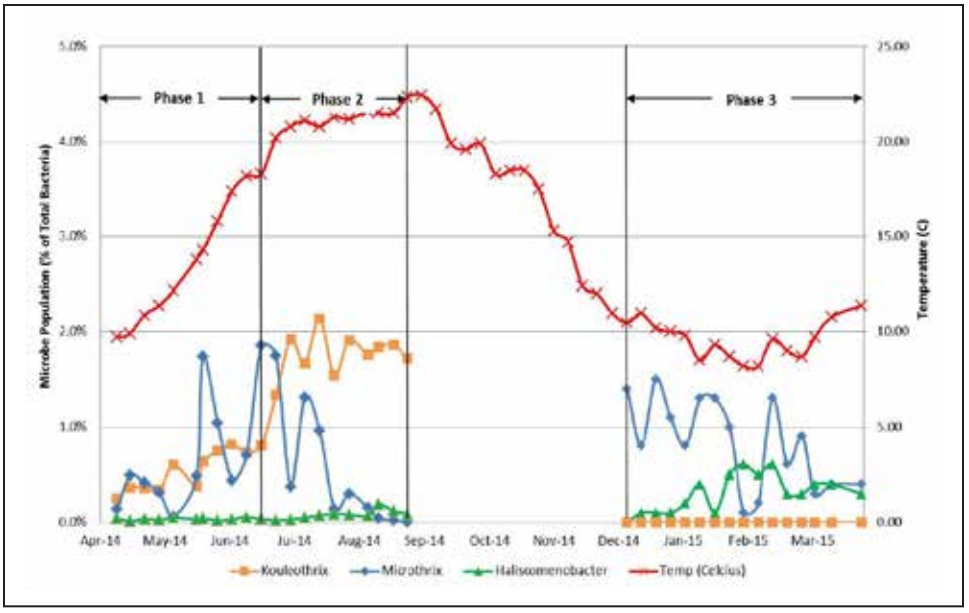
- the moving bed bioreactor just upstream of the activated sludge system,
- the activated sludge basin,
- the clarifier effluent, and
- the clarifier return activated sludge (RAS).

Analysis compared the bacteria in the clarifier effluent to those found in the RAS. This testing can determine which bacteria are settling well and which are settling poorly. Tables 1 and 2 (p. 55) show the bacteria counts.

Surprisingly, two well-known filaments, *Haliscomenobacter* and *Sphaerotilus*, were some of the best settling bacteria. The poor settling bacteria included *Aureispira*, of which little is known, and *Zoogloea*, a well-known bulking bacteria usually present because of nutrient limitations.

Also surprisingly, the differences between the clarifier and the RAS were not as great as expected. This indicates that the MLSS is relatively homogenous and all of it settles poorly.

Figure 3. Filament abundance over time (based on DNA data) vs. temperature



Looking to the most abundant bacteria in the MLSS helps to find the culprits for the high SVI. Table 3 (below) shows that the most abundant bacteria include *Aureispira* as well as several known filaments, *Sphaerotilus, Thiothrix,* and *Haliscomenobacter.* The test also shows *Zoogloea*, which is known to cause bulking.

Based on the DNA data and the presence of *Zoogloea*, the facility will further investigate potential nutrient deficiency (nitrogen or phosphorus) that could be contributing to bulking, causing high SVI, high effluent TSS, and high sewer bills.

Putting DNA to use

DNA sequencing is especially powerful when operators have baseline samples analyzed during healthy operation to provide a comparison with poor operation.

The spreadsheet format of this data makes it very easy to analyze and compare across historical data. DNA data can be obtained by simply shipping small samples of activated sludge and may provide the final piece of the puzzle to solve longstanding, recurring issues with foaming, filaments, nitrifiers, or PAOs.

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As costs have declined, DNA sequencing has become a feasible means to assess which organisms within wastewater could be contributing to operational problems. Courtesy Microbe Detectives

Table 1. Poor settling bacteria (more abundant in effluent than in RAS)

Kingdom	Phylum	Class	Order	Family	Genus	SC Effluent - RAS
Bacteria	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	Aureispira	6.65%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter	1.91%
Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	1.51%
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea	1.01%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	0.63%

Table 2. Good settling bacteria (more abundant in RAS than in effluent)

Kingdom	Phylum	Class	Order	Family	Genus	SC-RAS
Bacteria	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	Haliscomenobacter	–1.68%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Sphaerotilus	–1.26%
Bacteria	Chloroflexi	Caldilineae	Caldilineales	Caldilineaceae	Caldilinea	–1.12%

Table 3. Most abundant bacteria in mixed liquor suspended solids

Kingdom	Phylum	Class	Order	Family	Genus	MLSS
Bacteria	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	Aureispira	22.66%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Sphaerotilus	6.31%
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Thiothrix	5.87%
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	4.68%
Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Cloacibacterium	3.55%
Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	3.19%
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea	3.18%
Bacteria	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Filimonas	3.12%
Bacteria	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Saprospiraceae	Haliscomenobacter	2.46%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter	2.36%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Malikia	2.15%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Fulvimonas	1.65%