

Molecular Biology

The utilization of molecular biology is increasingly important in the scope of wastewater treatment. EBS understands the importance of using molecular methods to obtain a better understanding of the inner workings of a wastewater treatment plant. We currently utilize two molecular methods – quantitative polymerase chain reaction (qPCR) and DNA sequencing. The type of method used depends on the goals of the study. Read more about each method below to learn how molecular methods may help you.

Quantitative Polymerase Chain Reaction (qPCR) for fecal source tracking studies

Many facilities have a fecal indicator bacteria (FIB) permit limit, and with stricter receiving water TMDLs more plants are having to test for fecal bacteria than ever before. FIB, which include fecal coliforms, *E. coli*, and *Enterococci*, are ubiquitous in fecal contamination from all animals and therefore cannot tell us anything about their source. When FIB concentrations are too high, environmental managers may begin to implement costly disinfection processes without first understanding the cause of the issue. Test methods for FIB are subject to interferences from industrial wastewater samples that may cause false positives, and not all bacteria included in the FIB groups actually originate from a fecal source. This is where using qPCR becomes useful. This technology can identify unique DNA sequences that tell us exactly where the bacteria come from. We can look for the presence of bacteria that are known gut inhabitants of humans, birds, and deer, which would indicate a fecal source in the system. If a non-human source of fecal contamination is identified, managers can approach the state with evidence that their facility is not the source of the fecal contamination. If no fecal sources of bacteria are identified in the qPCR analysis, the presence of other DNA sequences can tell us whether the FIB measured in the system are from non-fecal, or environmental sources of bacteria. These bacteria are more common in paper mills with lagoon-type systems, as they are often associated with decaying plant material and soil. This data can be used in support of a new permit limit or a different type of FIB on the permit that may be more specific to fecal contamination in that system.

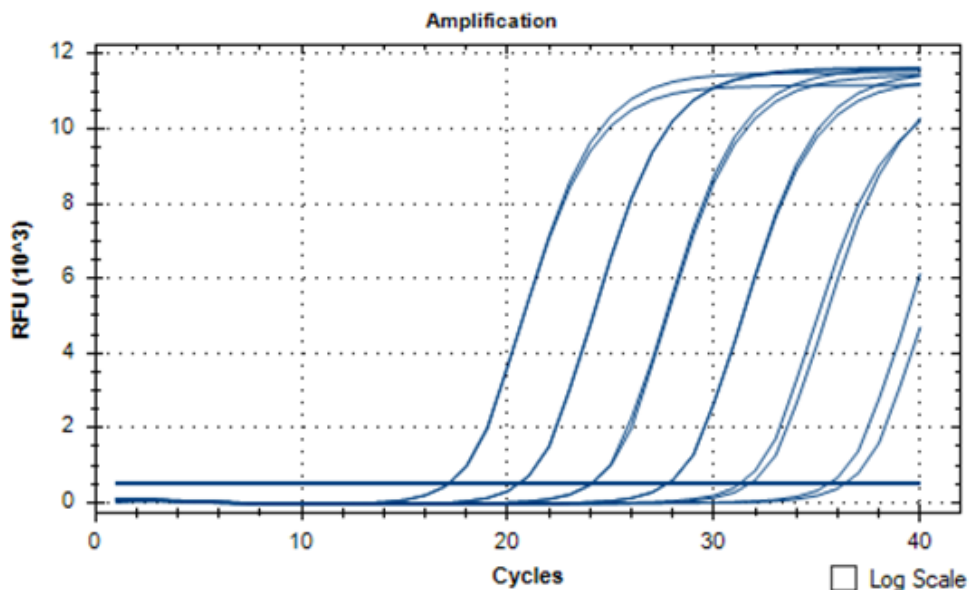


Figure 1: Amplification of a unique DNA sequence using qPCR.

DNA Sequencing

DNA sequencing is most useful for getting a broad overview of the types of bacteria present in your wastewater treatment system. This technology is not as specific as qPCR, but it can identify more types of bacteria at once. Results from DNA sequencing typically sort bacteria into functional groups such as filaments, nitrifying organisms, fermenters, sulfate reducing organisms, phosphorus and glycogen accumulating organisms (PAOs and GAOs), fermenters, etc. This data lets you know who the key players in your treatment system are. Carrying out DNA sequencing on a routine basis allows wastewater treatment plant personnel to track changes in these key populations over time in response to changes in temperature (seasonal), treatment campaigns, operational conditions, and during upset conditions. This information can be vital for systems that rely on a certain type of bacteria for success, for example, nitrifying organisms in a system that must remove ammonia, fermenting bacteria in anaerobic systems, and/or filaments for systems that experience bulking sludge.

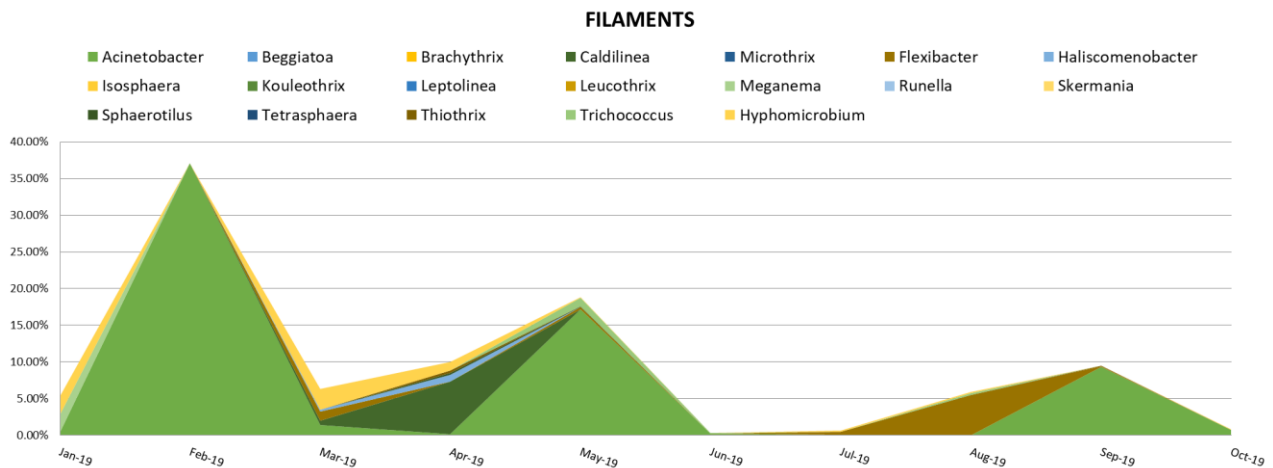


Figure 2: Population distribution of filaments over time.