Biotechnology techniques reveal microbial activities

Advances in biotechnology are launching the next round of innovations in the field of wastewater treatment. Matt Livingston of Novozymes explains how two molecular techniques track and identify bioaugmentation strains in treatment facilities in order to better understand microbial processing for the purpose of improving operations.

Since the activated sludge process was invented approximately 100 years ago, the wastewater industry has been continuously innovating and improving. Considering that the first activated sludge experiments were precursors of sequencing batch reactors, there have been many operational innovations to the conventional activated sludge process – the addition of secondary clarifiers and selector technologies, for example. In addition, there have been innovations in chemicals (e.g. flocculants), equipment (e.g. membrane bioreactors), and biological processes (e.g. biological nutrient removal technologies). These innovations have mostly focused on managing the naturally prevailing microbial community through engineering changes.

The core of any activated sludge system is the biomass – the community of microorganisms responsible for removing organic pollutants and nutrients from wastewater. The types of microorganisms that make up biomass are strongly influenced by the influent characteristics, treatment scheme, and operational conditions; however, little was known about how to identify, quantify, and assign levels of importance to individual species. Void of this specific knowledge, plant data can be collected and analyzed to understand plant performance. Classical wastewater troubleshooting methods have been developed that can be used to improve treatment through operational modifications. Further understanding of the biology can come from performing microexams, which can also help guide operational strategies by understanding floc quality, filamentous growth, and higher life forms. While the goal of operational changes is to improve plant performance, it is actually the changes in the microbial community that ultimately make the difference.

Knowing what the ideal community should look like and understanding the best means to achieve it are becoming possible due to advances in the available molecular biology tools such as "metagenomics." Metagenomics enables the indepth analysis of the microbial community by sampling genetic material directly from an environment and applying next-generation DNA sequencing technology. Prior to the development of such culture independent techniques, researchers were limited to isolating organisms one at a time and studying each isolate independently. Limiting researchers using these methods even further was the fact that most organisms are not culturable in a laboratory environment, which creates a bias toward only those organisms that were capable of growing in a lab.

One of the key fields of study pushing the boundaries of metagenomics is the Human Microbiome Project (HMP), sponsored by the US National Institutes of Health (NIH). The HMP is a focused attempt to characterize microbial communities found at multiple sites on the human body with the aim of developing a reference set of microbial genome sequences and elucidating the relationship between disease and changes to the human microbiome.

The same concept can be applied to wastewater treatment. Understanding the relationship between treatment conditions and changes in the microbial community could someday be a basis for diagnosing and remediating treatment problems. A better understanding of the microbial community make-up, microbial interactions, and how to change the community through bioaugmentation and/or operational changes could influence not only troubleshooting, but also design and process controls.

As the goal of the HMP is to better understand microbial ecology in the human body and to gain perspective on human diseases, one result is that ingestible and topical probiotics are receiving greater attention as potential medical treatments. Likewise, bioaugmentation is receiving greater attention as a means for changing the microbial community and improving wastewater treatment.

New tools for wastewater bioaugmentation

Plant operators are continuously optimizing wastewater treatment systems in order to stabilize biological activity, meet regulations, and improve efficiency. Many are turning to bioaugmentation methods to achieve those objectives; however, the efficacy of commercial bioaugmentation products are difficult to confirm because the specific role that exogenous microbial strains play in these changes has yet to be fully understood.

Questions remain regarding application strategy, product formulation optimization, and the direct link between bioaugmentation and plant performance. Compounding this issue, most bioaugmentation products contain a mixture of micro-biological strains, many of which are phylogenetically related to those found endogenously in wastewater treatment systems.

Furthermore, decisions regarding the application of these products are generally based on operational parameters that may not accurately represent the state of microbial activity in the system. The Danish biotech firm Novozymes uses whole genome sequences of specific bioaugmentation strains in order to employ two molecular techniques – quantitative polymerase chain reaction (qPCR) and Recognition of Individual Gene Fluorescence In Situ Hybridization (RING-FISH) – that allow for improved understanding of their activity in the treatment process. The combination of these techniques helps determine the presence, quantity, and spatial distribution of several bioaugmentation strains in full-scale wastewater treatment plants. Application of these advanced tools enabled Novozymes to establish the missing link between the treatment performance improvement and the persistence of the bioaugmented microbes at a full-scale wastewater treatment facility.

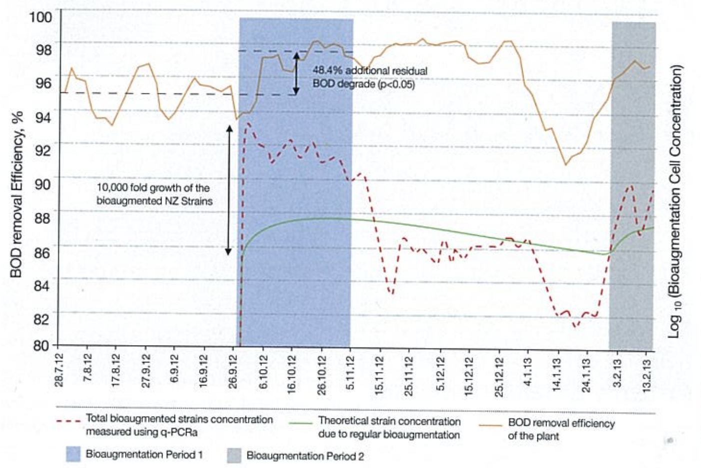
Tracking bioaugmentation

Bioaugmentation is the practice of adding specialized microbial strains in an effort to enhance the ability of the community to degrade certain compounds or respond to process fluctuations. Applications include plant start-ups, increasing treatment efficiency, improving plant stability, and facilitating upset recovery. While the success of any application is determined by the operational data collected from the plant, the best way to fully understand and optimize bioaugmentation products is by monitoring the growth and persistence of the added organisms.

> NOVOZYMES IS THE FIRST COMPANY TO TRACK COMMERCIAL BIOAUGMENTATION PRODUCTS IN WASTEWATER.

Novozymes is the first company to comprehensively track commercial bioaugmentation products in wastewater. Using a technique called quantitative polymerase chain reaction (qPCR), Novozymes can track the concentration of targeted strains over time to determine how

Graph shows the results of a trial that tested the performance of bioaugmentation strains at a petrochemical wastewater treatment facility.



they are growing and/or washing out of the system. Using a different technique called Recognition of Individual Gene Fluorescent In-Situ Hybridization (RING-FISH), Novozymes can visually identify strains using fluorescent probes to understand whether they are becoming incorporated into sludge floc particles or exhibiting planktonic growth.

Combined, these techniques become a powerful tool, not only to validate bioaugmentation applications, but also to understand which strains are best suited to application in certain environments. Information like this is used in product development and for optimizing products to site-specific conditions.

Novozymes demonstrated the ability to track and identify bioaugmentation strains during a 30-day trial to improve treatment efficiency at a 31,000-cubic-meters-per-day petrochemical wastewater treatment facility. During the trial period, the concentration of bioaugmented strains grew nearly 10,000 times above the actual dose concentration. During the treated period, there was a 48.4 percent additional reduction of residual biochemical oxygen demand (BOD) in the effluent compared to the 60-day time period immediately prior to the trial (272 mg/L vs. 528 mg/L) and BOD removal efficiency increased from 94.8 percent to 97.4 percent. Strains identified via RING-FISH were mostly found associated with floc particles.

Leading the way

Continued innovations in wastewater biotechnology will come from the collaboration of engineers and scientists specializing in microbial ecology, microbial physiology, and microbial screening. Such collaboration can enable selection of the right microorganisms for treating wastewater in the most efficient and sustainable way. Success will also come from education and eliminating the "Black Box" mentality around biological treatment ("It doesn't matter what happens or what's in there, as long as what comes out meets permit.").

It is these inner workings of the microbial community that are becoming better understood and more accessible from an operational standpoint. Industry influencers and early adopters need to understand the technology in order to embrace it, and new information needs to be shared openly to allow for independent validation.

Author's Note

Matt Livingston is a regional marketing manager for Novozymes Wastewater Solutions division (North and South America) and is based in Salem, Virginia, USA. Novozymes, a biotech company specializing in the development and application of industrial enzymes and microorganisms, is looking for partnerships with engineering companies, plant operators, and other stakeholders to create new opportunities for improvements in wastewater treatment. For more information, contact the author by email at mliv@novozymes.com.

Reference

Citations: S. D'Imperio, S. Leach, M. R. Livingston, V.P. Tale, C. T. Edwards, J. Terra, and G.L. Lucas (2013). High-specificity tracking of bioaugmentation strains in full-scale wastewater treatment facilities. Proceedings of the 86th Annual Water Environment Federation Technical Exhibition and Conference.

For further information please see the Advertiser Contact List on page 50