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. Furthermore, there have been innovations in chemicals (e.g. flocculants), equipment (e.g. membrane bioreactors), and biological processes (e.g. anaerobic technologies). These innovations have focused on the microbial environment or physically managing the microbial community through solids separation.

Novozymes believes the next round of significant innovations will come from developments in biotechnology. New techniques in DNA sequencing are providing the beginning framework for understanding how to identify microorganisms and evaluate the microbial community, with the ultimate goal of optimizing the microbial community for improved treatment.
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INNOVATIONS IN WASTEWATER: What’s next?

The core of any activated sludge system is the biomass—the community of microorganisms responsible for removing organic pollutants and nutrients from wastewater. It is well understood that the types of microorganisms that make up the biomass are strongly influenced by the influent, treatment scheme, and operational conditions; however, little was known about how to identify, quantify, and assign 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 through 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 more possible due to advances in metagenomics. Metagenomics enables the comprehensive analysis of the microbial community by sampling genetic material directly from an environment and applying advanced DNA sequencing technology. Prior to the development of metagenomics, 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.

Metagenomics allows samples to be taken directly from the environment and allows sequencing of all of the DNA extracted in those samples. The sequences are then compared to known sequences and identified. New species can also be readily determined this way.

Samples are taken directly from the environment. DNA from all microorganisms, even ones that are not culturable in a lab, can be sequenced.

All of the DNA in the sample is sequenced, which allows researchers to identify multiple microorganisms (potentially hundreds or thousands) captured in a simple sample.
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. 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 comprehensively track commercial bioaugmentation products in wastewater (D’Imperio et al., 2013). Using a technique called quantitative PCr (qPCr), Novozymes can track the concentration of targeted strains over time to determine how 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 floc particles or remaining in the bulk water.

Combined, these techniques become a powerful tool, not only to validate bioaugmentation applications, but also to understand which strains are more adaptable in certain environments. For example, a strain that is more active and grows faster than others would be expected to have more of an impact on performance than a strain that does not grow and gets washed out of the system. Information like this is used in product development and for optimizing products to site-specific conditions.

The Human Microbiome Project (HMP) is driving a lot of development work in the field of metagenomics. The Human Microbiome Project (HMP) is one of the key fields of study pushing the boundaries of metagenomics. The Human Microbiome Project (HMP), sponsored by the US National Institutes of Health (NIH). The aim of the HMP is to characterize microbial communities found at multiple human body sites and to look for correlations between changes in the microbiome and human health. Specific goals of the HMP include developing a reference set of microbial genome sequences and preliminary characterization of the human microbiome, and elucidating the relationship between disease and changes to the human microbiome.

At Novozymes, we believe that the same concept could be applied to wastewater treatment. A reference set of microbial genome sequences could be developed under a variety of treatment conditions and configurations. Understanding the relationship between treatment conditions and changes in the microbial community could be the basis for control parameters and/or diagnosing treatment problems. In fact, a better understanding of the microbial community make-up, microbial interactions, and how change the community through bioaugmentation and/or operational changes could influence not only troubleshooting, but also design and process controls.
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Continued innovations in wastewater biotechnology will come from the collaboration of engineers and microbiologists specializing in microbial ecology, microbial physiology and microbial screening. Each brings a different mindset and skillset and all need to be represented to enable the right microorganisms to perform in the right environment, and to treat wastewater in the most efficient and sustainable way. Success will also come from education and eliminating the “Black Box” mentality around biological treatment (“I don’t care what happens, 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.

Case study summary
Novozymes demonstrated the ability to track and identify bioaugmentation strains during an application at a 31,000 cubic meters per day (8.2 MGD) petrochemical facility. The trial lasted for 30 days. During the trial period, the concentration of bioaugmented strains grew nearly 5 logs above the actual dose concentration. Strains identified via RING-FISH were mostly found associated with floc particles. During the treated period, BOD removal improved from 94.8% to 97.4%; a 2.6% improvement compared to the 60-day time period immediately prior to the trial. This increase translates to a 48.4% reduction of residual BOD in the effluent compared to the pre-trial period (272 mg/L vs. 528 mg/L).
As the world leader in industrial enzymes and microorganisms, Novozymes has specialized expertise in developing and applying biotechnology; however, this is not something we can accomplish without partnerships with engineering companies, plant operators, and other stakeholders in the wastewater industry. It is these partnerships that enable us to meet our ambition to change the world together with our customers. We’re excited about Wastewater biotechnology 2.0 and looking forward to creating new opportunities for improvements in wastewater treatment.

**Citations:**
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