



# Wastewater and Feedstock Analysis

Through CENSUS® qPCR analysis, Microbial Insights provides the quantification of microorganisms and functional genes related to wastewater management including anaerobic digestion, biological hydrogen production, pathogens for feedstock analysis, causes of bulking and biofilm formation, microbial fuel cells, metal precipitation, and the nitrogen cycle (see “Closing the Nitrogen Cycle with Molecular Biological Tools”). Many of these organisms are difficult to grow in a laboratory or bottle test, making the use of molecular biological tools (MBTs) for quantification highly preferred over cultivation approaches.

Microbial Insights also offers additional MBTs that provide useful insights into wastewater management and treatment. Next Generation Sequencing (NGS) provides a genus-level description of the total microbial community within a single sample through an analysis known as metagenomics. This can be used to predict problem situations such as bulking or foaming. Metatranscriptomics is an analysis that profiles gene expression within a microbial community – a useful tool for narrowing in on the activity of the microorganisms present.

## Quantifiable Gene Targets Related to Wastewater Management and Feedstock Analysis

### Methanogenic Anaerobic Digestion

There are 4 steps related to Methanogenic Anaerobic Digestion in wastewater treatment. First, fermenters hydrolyze proteins, polysaccharides, and lipids in the wastewater to amino acids, sugars, and fatty acids. Next, the monomers are fermented to intermediates which includes organic alcohols and acids. After this, hydrogen producing acetogens oxidize the intermediates to acetic acid, hydrogen, and carbon dioxide. Last, methanogens convert the acetate into methane and carbon dioxide. Microbial Insights offers quantification of microbial groups related to these processes. There is also evidence that some lactic acid bacteria are present and forming lactate from lactose. Further, fermenters are an important target to monitor for biological hydrogen production.

Target	MI Code	Description
Acetogens	<b>AGN</b>	Targets acetogens which can oxidize organic alcohols and acids to form acetic acid, hydrogen, and carbon dioxide.
Fermenters	<b>FER</b>	Targets several genera of fermenting bacteria that produce organic alcohols and acids from the fermentation of amino acids, sugars, and fatty acids. Additionally, an important target for biological hydrogen production.
Methanogens	<b>MGN</b>	Targets methanogens which utilize fermentation products formed by other anaerobes as electron donors (H <sub>2</sub> , formate, and alcohols) and acceptors (acetate, CO <sub>2</sub> , methanol, methylamines, and methylsulfides) to produce methane.
Acetoclastic Methanogens	<b>AMGN</b>	Targets acetoclastic methanogens which convert acetate into methane and carbon dioxide.
Lactic Acid Bacteria	<b>LAB</b>	Targets the lactate dehydrogenase (ldhL) gene of lactic acid bacteria which form lactate from lactose.



### Pathogens

Pathogen targets for feedstock analysis.

Target	MI Code	Description
Total E. coli	<b>TECOLI</b>	Targets E. coli bacteria which are a potential pathogen that can be found in wastewater.
Total Enterococcus	<b>TENT</b>	Targets Enterococcus bacteria which are a potential pathogen that can be found in wastewater.
Legionella	<b>LEG</b>	Targets Legionella bacteria which are a potential pathogen that can be found in wastewater.

### Bulking and Biofilms

Deinococcus and Meiothermus assays target two genera of slime producing bacteria. Burkholderia cepacian exopolysaccharide (BCE) is an exopolysaccharide that can be found in biofilms. The surfactant assays (SurG, SurL, SurP, and SurT) are also likely to play a role in bulking and foaming because Pseudomonas, Acinetobacter and Mycobacterium are commonly found in wastewater.

Target	MI Code	Description
Deinococcus	<b>DCS</b>	Targets the genus Deinococcus which are slime producing bacteria.
Meiothermus	<b>MTS</b>	Targets the genus Meiothermus which are slime producing bacteria.
Burkholderia Cepacian Exopolysaccharide	<b>BCE</b>	Targets the gene for the production of the cepacian exopolysaccharide from Burkholderia cepacia which can be found in biofilms.
Glycolipid Surfactants	<b>SurG</b>	Targets the rhlA and rhlC genes involved in the production of mono- and di-rhamnolipids in Pseudomonas.
Liposaccharide Surfactants	<b>SurL</b>	Targets the weeA and alnB genes involved in the production of emulsan and alasin in Acinetobacter.
Lipopeptide Surfactants	<b>SurP</b>	Targets the SrfAC, licC, aprE genes involved in the production of surfactin, lichenysin, and subtilisin in Bacillus as well as the visC gene involved in the production of viscosin in Pseudomonas.
Trehalose Surfactants	<b>SurT</b>	Target the treS and treY genes involved in the production of trehalose in Mycobacterium and Rhodococcus.

### Metal Precipitation

Hydrogen sulfide produced by sulfate reducers is known to precipitate metals from wastewater as metal sulfides.

Target	MI Code	Description
Sulfate Reducing Bacteria	<b>APS</b>	Targets the APS gene found in sulfate reducing bacteria. The hydrogen sulfide produced by these bacteria can be utilized to precipitate metals in wastewater.

### Microbial Fuel Cells

Wastewater can also be used in microbial fuel cells (MFCs). In MFCs, pollutants in the wastewater are degraded by anaerobic respiring bacteria at an anode which produces carbon dioxide and generates electrons and protons. The produced electrons are then transferred to the anode by bacteria through an external circuit. Other microorganisms within the MFC are used for the removal of the possible pollutants: the removal of nitrogen is performed through ammonia oxidation, nitrite oxidation, and denitrification by AOB, NOB, and denitrifying bacteria respectively. Please reference the Microbial Insights document on the nitrogen cycle for tools related to nitrogen removal.