

## Microbial Diversity of Produced Water Sources for Oil and Gas Development

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**Background/Objectives.** The use of recycled water for hydraulic fracturing operations is becoming more common as tougher regulations on water use are established and as production wells are drilled in areas prone to drought. However, high salinity brines can be problematic for reuse. Produced water generally contains high concentrations of dissolved ions and some organics, which can serve as electron acceptors and donors for bacterial growth reactions in both aerobic and anaerobic waters. Bacterial growth can cause a wide range of problems for oil and gas operations, including microbial induced corrosion, well souring from hydrogen sulfide production, formation of biofilms in product pipelines, and reduced downhole formation production. Understanding the microbial composition of produced waters, along with associated geochemical parameters, is critical for produced water management with respect to well production optimization, evaluation of produced water end uses, and selection of effective treatment techniques for reuse.

Metagenomics can be a valuable tool for oil and gas professionals to tailor site specific treatment strategies and biocide applications according to the microbial populations present in the produced waters. In this talk, next generation sequencing (NGS) results from field studies are presented and compared to a larger microbial laboratory database to identify dominant bacterial groups and highlight the degree of microbial diversity that exists among various sources of produced water.

**Approach/Activities.** Produced water samples from two field studies (Upper Green River Basin, WY; Midland, TX) were analyzed using 16S rRNA tag-encoded pyrosequencing to identify dominant classes and genera within the microbial community and their respective relative abundances. One sample (WY) was collected from a large storage pond at a water recycling facility as part of a larger water/air quality research study. The second sample (TX) was part of a comprehensive microbial survey, including samples from formation core material, drilling muds, fracturing fluid source waters, and production wells. The Microbial Database, developed by Microbial Insights (MI), is an internally designed and maintained laboratory information management system containing qPCR results for more than 48,000 unique field samples from all 50 states and 33 countries. While the majority are from environmental remediation projects, samples are also submitted from source, produced and process waters from oil and gas operations to investigate microbial induced corrosion and other microbial processes.

**Results/Lessons Learned.** Overall, results from the field studies and microbial database demonstrate the extraordinarily broad range of microbial densities and the functional diversity of microbial populations in produced and process waters. For the two field studies (WY; TX), *Gammaproteobacteria* (51%; 37%) represented the dominant bacterial class in the microbial populations; however, vastly different genera were identified. Proportions of other major bacterial classes (*Deltaproteobacteria*, *Epsilonproteobacteria*, *Bacilli*, *Fusobacteria*) and associated genera were also different between the two field studies reflecting varying

environmental and site conditions supporting bacterial growth. Within the MI qPCR database, total bacterial populations ranged from near detection limits to more typical values on the order of  $10^5 - 10^7$  cells/mL. In terms of evaluating potential microbial processes, nitrate reducers, sulfate reducers, acetogens and methanogens were frequently detected in produced water samples.