Contribution ID: **716** Type: **Oral 20 Minutes**

High Phylogenetic and Physiological Diversity of Ureolytic Bacteria in Native Soils Bio-stimulated for MICP

Monday, 14 May 2018 12:12 (15 minutes)

Worldwide demand for new and sustainable approaches to geotechnical engineering problems has generated novel research opportunities in the emerging field of bio-mediated soil improvement. The most widely researched of these processes is microbially induced calcite precipitation (MICP), which has shown promise for a wide variety of engineering applications. Initially MICP was accomplished by bio-augmentation with a high density of the constitutively ureolytic bacterium, Sporosarcina pasteurii (Stocks Fischer et al., 1999). The amended soil was then supplemented with liquid medium containing calcium salts, urea, and sometimes growth-promoting organic compounds. Bacterial hydrolysis of urea generates a molecule of carbonic acid and two of ammonia. The resulting ammonia, a weak base, equilibrates with water and tends to form ammonium and hydroxide ions. This shifts the carbonic acid-bicarbonate-carbonate equilibrium toward carbonate, which will precipitate as calcium carbonate in the presence of sufficient calcium, ideally in the immediate vicinity of ureolytic bacteria, thereby cementing adjacent soil particles and increasing soil strength and stiffness. More recently bio-stimulated MICP has been fully demonstrated in native sands with prospects for eliminating costs and environmental impacts of propagating and transporting large quantities of bacteria. In our recent column experiments, completed on 14 different sandy soils from different depositional environments – including several samples obtained from natural deposits as deep as 12 meters – bio-stimulated MICP was always successful (Gomez et al. 2014; 2017; 2018).

Over 300 bacterial pure cultures were obtained from the most recently bio-stimulated soils and were stored (-80℃) to enable future physiological and genetic studies. A study of the urease kinetics of 8 randomly selected bacteria enriched in a meter-scale stimulated MICP demonstration (Gomez et al., 2017) showed that whole cell rates of urea hydrolysis follow Michaelis-Menten kinetics, with half-maximum values achieved at urea concentrations ranging from 56 to 837 mM and maximum rates varying from strain to strain by 100-fold. In progress 16S rRNA sequencing of the culture collection shows it includes a wide variety of ureolytic strains closely related to, but not identical to, the S. pasteurii (ATCC strain 11859), which has been employed almost universally in bio-augmentation experiments. Certain strains were found repeatedly in all or most of our bio-stimulated MICP experiments. Physiological differences between these strains will be discussed along with their surprisingly high diversity at the end of bio-stimulated MICP treatments. We have also begun to link pure culture physiology with relative abundance of these same strains in bio-stimulation treatments by extracting and amplifying bulk DNA from dilute aqueous bacterial suspension. In progress sequencing of 400 full-length clones is expected to provide a higher resolution but lower density sampling of diversity versus time for a single bio-stimulated MICP column. In parallel, high throughput 16S amplicon sequencing will provide much higher depth but lower resolution snapshots of changes in bacterial diversity throughout this same progression.

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Session Classification: Parallel 1-B

Track Classification: MS 4.03: Applications of biochemical modification of porous media