Bio-catalyzed carbetogenesis: a substudy into the effects of trace metals on urease activity

By: Mélanie Robichaud, Undergraduate
Supervisors: Dr. Danielle Fortin, Professor and Justin Whitaker, undergraduate
Department of Earth science, University of Ottawa

Reason for research

Soil liquefaction, the result of seismic activity in regions of erosion-prone soils, is a major threat to infrastructure. The agitation of loose soil particles (i.e. sandy soils) during an earthquake causes the soil to behave as a fluid, greatly compromising the structural integrity of building foundations. Current solutions exist to remedy this problem, but are often hazardous to the environment, not very cost efficient or just impractical for large-scale uses (e.g. jet-grouting, chemical grouts, soil compaction, improved drainage at the site, etc.). For these reasons, it is of vital importance to find a simpler, cost-effective and environmentally-friendly method to provide resilience to the otherwise frail substrate.

Objective

The aim of the project is to explore various methods of optimizing soil solidification through calcium carbonate (CaCO₃) production in particular. It is hypothesized that calcium carbonate production can be induced in urease-positive organisms by introducing trace amounts of various metals such as nickel and iron into the already calcium-rich environment. Through various imaging techniques, the effects of such composites are analysed as to determine whether this method aids in the production of ammonia, and in turn, of calcium carbonate precipitate.

Theory behind the idea

The precipitation of calcium carbonate is a process occurring passively in Calcium-rich environments when maintained at relatively high pH. This high pH environment can be artificially provided for precipitation to occur, with the use of urease-positive bacteria. Part of these strains’ metabolisms encompasses the transformation of urea (provided in the media) into ammonia, which in turn increases the pH. The bacterial cells themselves then act as nucleation sites for the calcification to take place and become encased with the calcium carbonate precipitate. When the cell metabolism is efficient enough to produce a significant amount of ammonia (and as a result a homogenously basic environment), the production of calcium carbonate causes the frail soil particles to bond together in a solid and impact resistant way.

Results analysis

The effects of metal supplementation on the production of calcium carbonate are measured quantitatively through the HACH Salicylate method, and qualitatively through the use of compound light microscopy with a polarized light filter and scanning electron microscopy (SEM). The images allow for the confirmation of CaCO₃ production due to the presence of bacteria.

The quantitative results for the the HACH method are still in the process of being collected and analysed.

Method (1)

In order to determine the best ammonia-producing bacterial strain, characterized by the production of the compound per hour per bacteria, the HACH salicylate method was employed. The four strains tested are 1) Sporosarcina ureae, 2) Bacillus megaterium, 3) Bacillus subtilis and 4) Bacillus spaeicrus. A bacteria inoculate was charged into 450mL of media containing 10g/L Yeast extract, 10g/L Tris (buffer), 0.1M (NH₄)₂SO₄ and 0.125M Urea and was left to grow for 32 hours. Aliquots were removed after 0, 1, 2, 4, 8, 16 and 32 hours and then diluted to 10⁻⁴. These samples were then used to determine the colony forming units (with the growth of these colonies on agar plates containing the media described above) and the levels of ammonia produced in each time trial. The ammonia production, being proportionate to the urease activity and pH change, is therefore a good indicator of the capacity of the strain to cause calcification.

Method (2)

The effects of trace amounts of Nickel and Iron on the proposed optimal strain were analyzed qualitatively through scanning electron microscopy and compound microscopy. In order to account for various possible sources of fluctuation in data, five samples were tested; 1) bacterial strain (10mL inoculate) + 10ppm NiCl₂ + 390mL of media (described above), 2) 10ppm NiCl₂ + 400mL of media, 3) bacterial strain (10mL inoculate) + 10ppm FeSO₄ + 390mL of media, 4) 10ppm FeSO₄ + 400mL of media, and 5) bacterial strain (10mL inoculate) + 390mL of media.

Acknowledgements

This research was made possible by the UROP committee for funding the project, by Dr Danielle Fortin for supplying the laboratory space, materials and guidance throughout the projects as well as by Justin Whitaker for supervising all laboratory sessions.