High Strength *In-Situ* Biocementation of Soil by Calcite Precipitating Locally Isolated Ureolytic Bacteria

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Declaration

I declare that, except where specific reference is made in the text to the work conducted by other authors, this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any university.

Salwa M. Al-Thawadi

Abstract

This study has contributed to the patented technology of biocement (Microbial Biocementation, WO/2006/066326). Biocementation or biogrout is a sand consolidation technology, in which the carbonate released from microbial urea hydrolysis precipitates with an excess of calcium ions to form *in-situ* calcite ($CaCO_3$) precipitation. Under the right conditions this can result in soil solidification and has found significant commercial interest.

This study has enriched and isolated highly urease active bacteria, particularly suitable for the fermentation process. Six strains with different properties relevant for biocementation were isolated. The most urease active strain (strain MCP11) produced sufficient urease to allow the use of the non-concentrated cell suspension for biocementation experiments. Activities and specific activities were 11-28 mM urea hydrolysed.min^{-1} and 2.2-5.6 mM urea hydrolysed.min^{-1}.OD^{-1} respectively. A separate strain (strain MCP4) showed spontaneous flocculation at the end of the batch growth, showing its increased tendency to attach to surfaces. This can be useful for effective cell concentration and for improved attachment during the cementation process.

The possibility of causing cementation by using enrichments rather than pure strains has been documented. This may allow a cheaper production of the urease than by traditional pure culture processes.

Urease production was optimised by increasing the concentration of yeast extract and the addition of Ni^{2+} ions to the growth media, resulting in increasing urease activity as the reproducible urease yield. This was accomplished by the addition of 10 μM Ni^{2+} ions and increasing the level of yeast extract to 20 g.L^{-1}

Some of the isolated strains were suitable for biocementation process producing mechanical strength (≥0.6 MPa) within several hours depending on the rate of urea conversion. This mechanical strength enhancement of the cemented columns was performed without a large decrease in the permeability.

The formation of $CaCO_3$ crystals in the presence of high concentration of calcium and urea was monitored. This crystal growth was monitored over time by video recording the ureolytic reaction on a microscopic slide. The crystals also were examined through SEM. It was found that two types of $CaCO_3$ precipitates were formed; these precipitates were calcite rhombohedral crystals and spheroids. Video clips showed that the rhombohedral crystals originated from the spheroids. These spheroids were fragile, not stable and were considered to be vaterite.

This study suggested that the strength of the cemented column was caused mostly due to the point-to-point contacts of rhombohedral $CaCO_3$ crystals and adjacent sand grains.

A method of producing high strength cemented samples from sand was developed. This method first attaches the cells into the sand-column by growing them in the presence of calcium ions as little as 6 mM. Then, the cells were incubated *in-situ* for about 48 hours to enable attachment to the surface of the sand granules. Then the cells were reused over 20-times by continuous supply of cementation solution (equi-molar concentration of calcium and urea). This method produced a mechanical strength of up to 30 MPa, which is equivalent to construction cement.

The mechanical strength could be increased by supplying the bacteria *in-situ* with a food source and 10 μM Ni^{2+} ions, allowing some measures of reaction rate control *in-situ*. To our knowledge, this study was the first study to use biological cementation to produce strength comparable to that

of traditional cemented construction materials such as sandstone and concrete.

The key factors for the optimal $CaCO_3$ precipitation (strength production) *in-situ* were examined. It was found that *in-situ* urease activity was the key factor for strength production. The maximum *in-situ* urease activity was achieved by supplementing the cementation solution with growth media, and the use of 0.5 M urea and Ca^{2+} as cementation solution. The *in-situ* urease activity differed according to the different bacterial strains which tolerated the cementation conditions differently.

One of the advantages of the present study was that cementation of porous media could be achieved without clogging the injection end. The injection end could be clogged by $CaCO_3$ precipitation due to cementation reaction (cells, calcium and urea). By sequentially flushing the cells and cementation solution, clogging of the injection end could be avoided and high penetration depth was achieved as long as there was sufficient passage of cementation solution. Uniform cementation along 1 m packed sand-column was obtained. This uniformity was confirmed by the urease activity measurement, calcite precipitation and mechanical strength production. For finer sand, homogenous cementation proved more difficult.

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