Microbial $CaCO_3$ Precipitation for the production of Biocement

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“Upon touching sand, may it turn to gold”

Anon. (Greek proverb)

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Declaration

I declare that 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.

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Summary

The hydrolysis of urea by the widely distributed enzyme urease is special in that it is one of the few biologically occurring reactions that can generate carbonate ions without an associated production of protons. When this hydrolysis occurs in a calcium-rich environment, calcite (calcium carbonate) precipitates from solution forming a solid-crystalline material. The binding strength of the precipitated crystals is highly dependent on the rate of carbonate formation and under suitable conditions it is possible to control the reaction to generate hard binding calcite cement (or Biocement). The objective of this thesis was to develop an industrially suitable cost-effective microbial process for the production of urease active cells and investigate the potential for urease active cells to act as a catalyst for the production of Biocement.

The biocementation capability of two suitable strains was compared. *Sporosarcina pasteurii* (formally *Bacillus pasteurii*) produced significantly higher levels of urease activity compared to *Proteus vulgaris*, however the level of urease activity was variable with respect to biomass suggesting that the enzyme was not constitutive as indicated by the literature, but subject to regulation. The environmental and physiological conditions for maximum urease activity in *S. pasteurii* were investigated and it was found that the potential urease capacity of the organism was very high (29 mM urea.min^{-1}.OD^{-1}) and sufficient for biocementation without additional processing (e.g. concentration, cell lysis). The regulation mechanism for *S. pasteurii* urease was not fully elucidated in this study, however it was shown that low specific urease activity was not due to depletion of urea nor due to the high concentrations of the main reaction product, ammonium. pH conditions were shown to have a regulatory effect on urease but it was evident that another co-regulating mechanism existed. Despite not fully exploiting the urease capability of *S. pasteurii*, sufficient urease activity to allow direct application of the enzyme without additional processing could still be achieved and the organism was considered suitable for biocementation.

Urease was the most expensive component of the cementation process and cost-efficient production was desired, thus an economic growth procedure was developed for large-scale cultivation of *S. pasteurii*. The organism is a moderate alkaliphile (growth optimum pH 9.25) and it was shown that sufficient activity for biocementation could be cultivated in non-sterile conditions with a minimum of upstream and downstream processing. The cultivation medium was economised and expensive components were replace with a food-grade protein source and acetate, which lowered production costs by 95%. A high level of urease activity (21 mM urea hydrolysed.min^{-1}) was produced in the new medium at a low cost ($0.20 (AUD) per L).

The performance of urease in whole *S. pasteurii* cells was evaluated under biocementation conditions (i.e. presence of high concentrations of urea, Ca^{2+}, NH_4^+/NH_3, NO_3^- and Cl^- ions). It was established that the rate of urea hydrolysis was not constant during cementation, but largely controlled by the external concentrations of urea and calcium, which constantly changed during cementation due to precipitation of solid calcium carbonate from the system. A simple model was generated that predicted the change in urea hydrolysis rate over the course of cementation. It was shown that whole cell *S. pasteurii* urease was tolerant to concentrations of up to 3 M urea and 2 M calcium, and the rate of urea hydrolysis was unaffected up to by 3 M ammonium. This allowed the controlled precipitation of up to 1.5 M $CaCO_3$ within one treatment, and indicated that the enzyme was very stable inspite of extreme chemical conditions.

A cost-efficient cementation procedure for the production of high cementation strength was developed. Several biocementation trials were conducted in order to optimise the imparted cementation strength by determining the effect of urea hydrolysis rate on the development of strength. It was shown that high cementation strength was produced at low urea hydrolysis rates and that the development of cementation strength was not linear over the course of the reaction but mostly occurred in the first few hours of the reaction. In addition, the whole cell bacterial enzyme had capacity to be immobilised in the cementation material and re-used to subsequent applications, offering a significant cost-saving to the process.

An industry-sponsored trial was undertaken to investigate the effectiveness of Biocement for increasing in-situ strength and stiffness of two different sandy soils; (a) Koolschijn sand and (b) 90% Koolschijn sand mixed with 10% peat (Holland Veen). After biocementation treatment, Koolschijn sand indicated a shear strength of 1.8 MPa and a stiffness of 250 MPa, which represents an 8-fold and 3-fold respective improvement in strength compared to unconsolidated sand. Significantly lower strength improvements were observed in sand mixed with peat.

In combination, trials of producing bacteria under economically acceptable conditions and cementation trials support the possibility of on-site production and in-situ application of large field applications.

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