Micro-Scale Processes in Microbially Induced Carbonate Precipitation

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Abstract

The concept of using bacteria to control the precipitation of calcium carbonate for engineering purposes, such as increasing soil strength and decreasing permeability, is well established in lab-scale experiments. What is not so clear is how to transition from these experiments to a practical field-scale ground improvement tool.

One difficulty is that soil properties are highly site specific and also vary spatially within a site (e.g. porosity, permeability, particle shape and size distribution, mineralogical composition). Meanwhile, microbially induced carbonate precipitation relies on complex interactions between pore structure, fluid flow pathways, and injection strategies which all influence where the injected bacteria will attach, where CaCO3 will precipitate, and which evolve over time as CaCO3 is progressively precipitated and the pore structure is altered.

To unpick these processes and optimise MICP treatment, we use light microscopy in microfluidic devices offering high time-resolution observations of bacterial attachment and CaCO3 crystal nucleation and growth in 2D systems. This is followed by X-ray µCT of sand packed columns offering more complex and realistic flow conditions in which we observe the evolving pore structure and relate this to changes in the flow fields through reactive-transport modelling with the software OpenFOAM.

Results show that the processes of bacterial attachment and crystal growth are complex and highly dependent on micro-scale conditions, however feedback mechanisms, repeated treatment cycles, and operator controlled parameters such as flow velocity can act to minimise these local variations across a range of soil types.

Introduction

The concept of using bacteria to control the precipitation of calcium carbonate for engineering purposes, such as increasing soil strength [1–4] and decreasing permeability [5–7], is well established in lab-scale experiments. What is not so clear is how to transition from these experiments to a practical field-scale ground improvement technology.

One difficulty is that soil properties are highly site specific and also vary spatially within a site (e.g. porosity, permeability, particle shape and size distribution, mineralogical composition). Meanwhile, microbially induced carbonate precipitation (MICP) relies on complex interactions between pore structure, fluid flow pathways, and injection strategies which all influence where the injected bacteria will attach and where $CaCO_3$ will precipitate. Moreover, these interactions evolve over time as $CaCO_3$ is progressively precipitated and the pore structure is altered.

Currently, the most promising MICP pathway is that of urea hydrolysis using the bacterium *Sporosarcina pasteurii*. The aim of this research is to investigate the factors influencing the transport, attachment, and subsequent mobilisation of *S. pasteurii* within porous media.

Materials and methods

A type culture of bacterium *S. pasteurii* (DSM-33) was grown in yeast extract broth, incubated at 30 °C for 24 hours, centrifuged to separate cells from the broth, and re-suspended in a sterile 9 g/L NaCl solution to an optical density of 1.0 OD_{600}. The cells in broth were stored at 4 °C for up to a week and fresh cell suspensions in NaCl were prepared for each experiment.

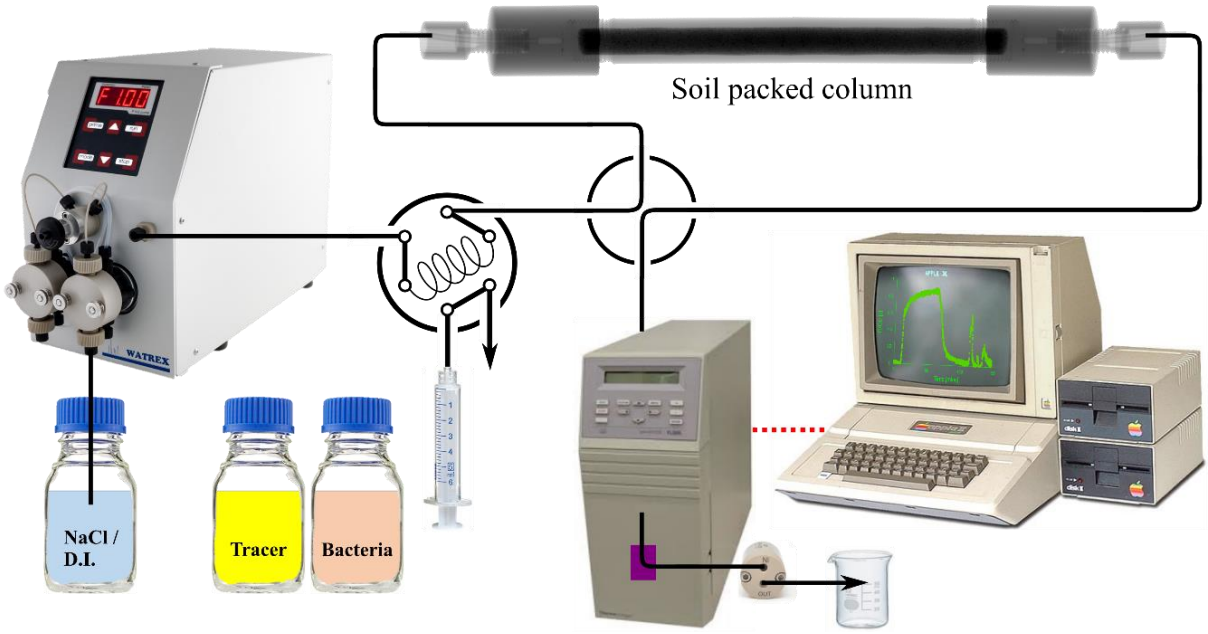

Figure 1. Experimental setup allowing continuous injection of salt solutions and controlled pulses of conservative tracer and bacteria through soil packed columns. Effluent breakthrough curves are recorded based on fluorescence. A six-way valve allows switching between injection and flushing, a four-way diagonal valve allows measurement of bacteria and tracer stock concentration, and a back pressure regulator set to 100 kPa prevents bubble formation within the system.

The experimental setup is shown in Figure 1. X-ray transparent columns 150 mm long and 4 mm diameter were packed with two different soils and fully saturated with a 9 g/L NaCl solution. The two soils were Sand 1: a lab grade quartz sand and Sand 2: a washed 'building sand' from a builder's merchant. Details of the two sands are shown in Figure 2 with the main difference being that the washed building sand had a greater range in particle size distribution and particle shape, contained finer particles and thus had a lower porosity and a greater number of grain contact points.

A conservative fluorescein tracer was injected to characterise fluid flow in each soil using a six-way valve with sample injection loop to control tracer volume. Effluent breakthrough curves were recorded using a scanning fluorescence detector. A four-way valve was used to direct tracer either through the column, or directly to the detector for an influent reading. Bacteria were injected and detected in the same manner.

Results and discussion

Results are presented in the form of breakthrough curves which plot the concentration of tracer at the outlet of the column (as a ratio of the inlet concentration) against time (shown as the number of pore volumes injected). Transport of the conservative tracer was distinctly different in the two soil types (Figure 3A). In Sand 1 the effluent breakthrough curve could be fitted by a simple convection-dispersion model, whereas for Sand 2, the break-through curve could only be fitted using a two-region non-equilibrium model. This is thought to be due to the more irregular particle shape creating stagnant regions of the pore network into which the tracer could diffuse.

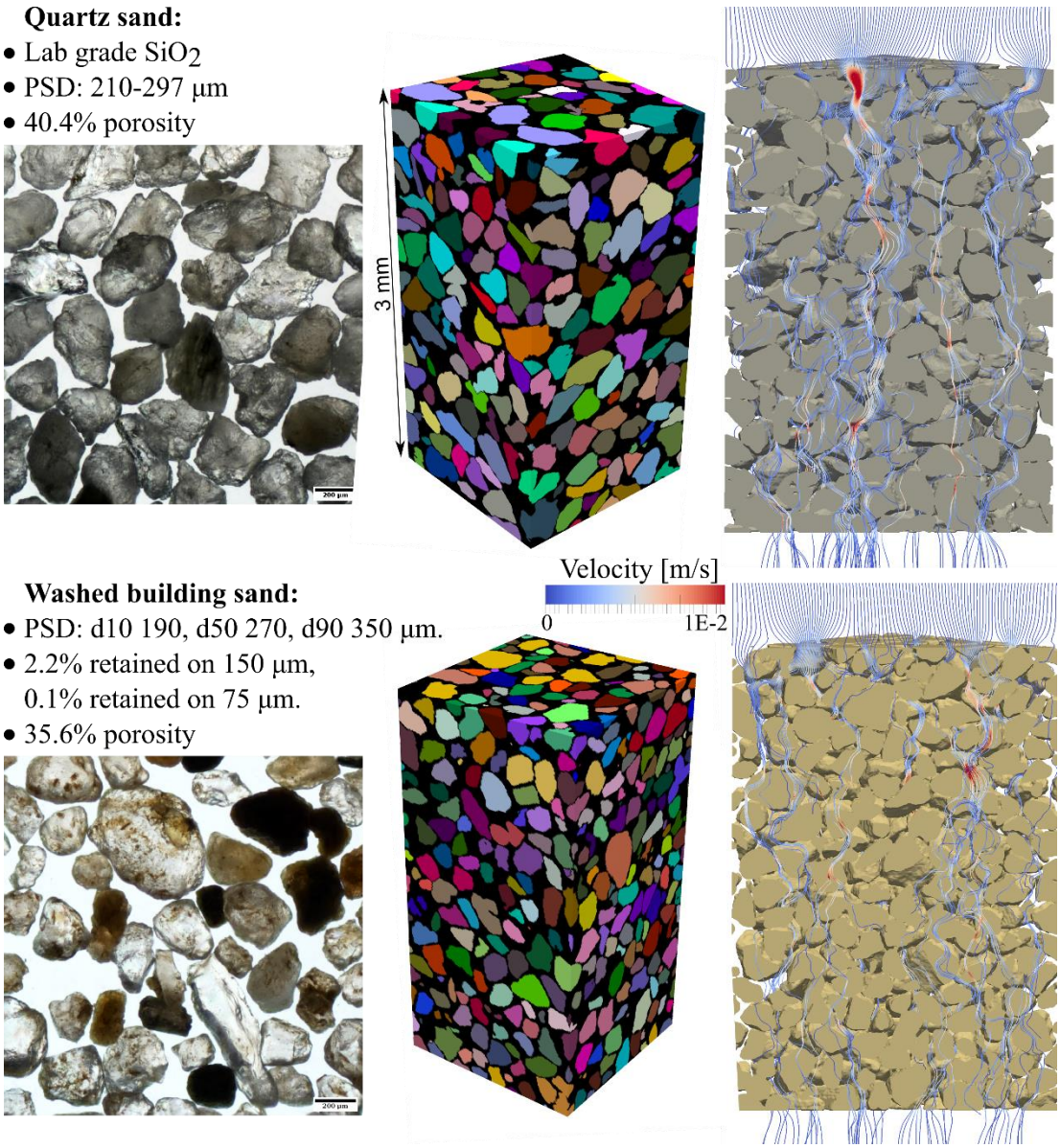

Figure 2. Top to bottom: lab grade quartz sand with near uniform particle size distribution; washed 'building sand' with greater range in particle size and shape and containing finer particles. Left to right: Optical microscope images of sand shape; X-ray µCT images of packed sand columns with individual grains separated and labelled by colour in post-processing; results of numerical flow simulation showing flow paths through the sand packs using code developed by Minto et al. [8].

Transport of the bacteria was affected by fluid velocity with higher velocities resulting in less attachment (Figure 3B), which has also been observed in sandstone cores by Tobler et al. [9]. Significantly more attachment occurrs in Sand 2 (the building sand) than in Sand 1, moreover the threshold at which flow velocity affects attachment appears to be soil dependent. Finally, the absolute magnitude of attachment also depends on soil type with significantly more attachment occurring in Sand 2 than Sand 1. This is thought to be due to the irregular particles creating more contact points for physical removal of cells (filtration) and stagnant regions which promote physico-chemical removal (adsorption).

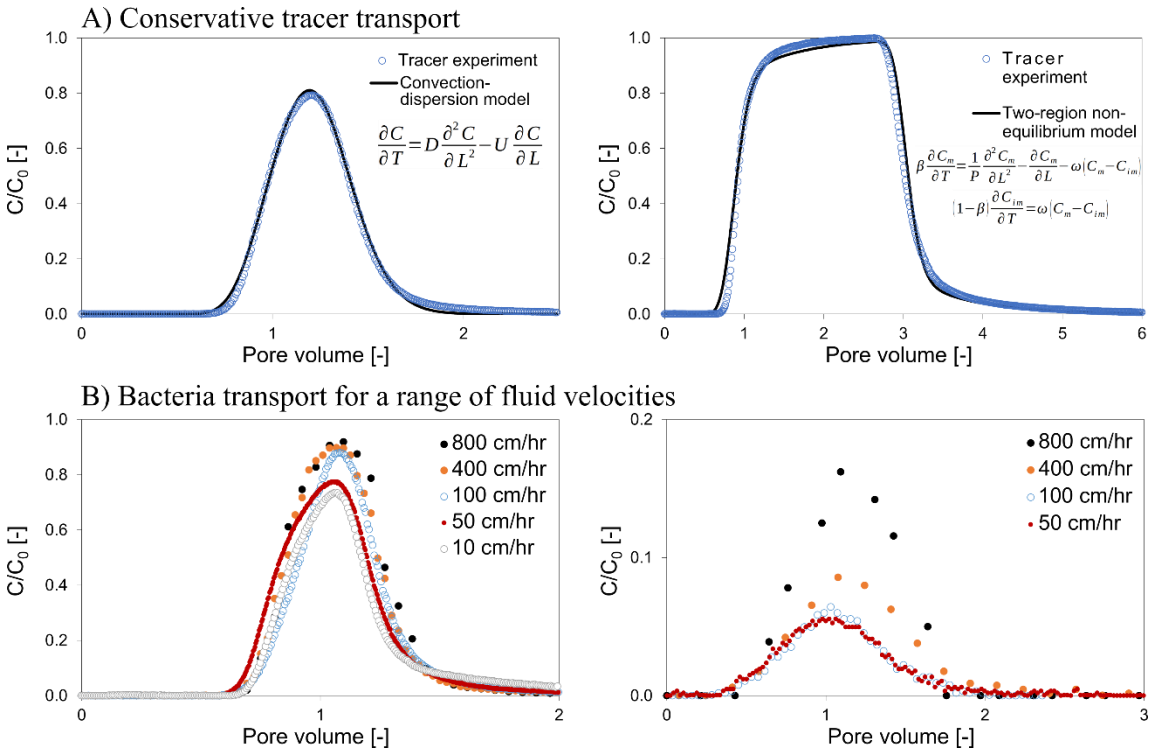

Figure 3. Effluent breakthrough curves for the quartz sand (left) and building sand (right) for A) a conservative tracer, and B) 1.0 OD_{600} *S. pasteurii* in 9 g/L NaCl cell suspensions injected at different flow rates, all at 0.75 pore volumes.

Given that the columns were 150 mm long, the high retention of bacteria in Sand 2 (the building sand) could suggest that the extent of treatment around any one injection point is limited, with the result that many injection points would be required to provide uniform MICP treatment over a large area. However, this 150 mm length represents the region immediately adjacent to an injection point and, in order to access the wider volume around the injection point, it would be necessary for multiple pore volumes of bacteria to pass through this same region. By increasing the volume of bacteria injected (Figure 4), we see that retention diminishes significantly and that the soil does not continue to inhibit transport of the bacteria.

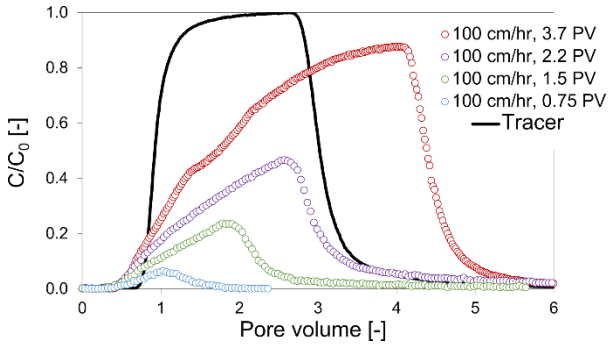

Figure 4. Effluent breakthrough curves for the building sand. 1.0 OD_{600} *S. pasteurii* in 9 g/L NaCl cell suspensions injected at a constant flow rates and different durations resulting in different total pore volumes injected.

This observation is consistent with a retention mechanism of filter-blocking in which attachment sites within the porous media become occupied by bacteria and hence removal efficiency decreases over time, enabling the bacteria to be transported away from the injection point and access a greater soil volume. We hypothesise that this mechanism allows for more even distribution of bacteria within a given injection period, however the precipitation of

$CaCO_3$ that follows the bacterial injection would provide fresh attachment points for each subsequent treatment cycle.

Conclusion

For microbially induced carbonate precipitation to be a successful ground improvement technology, it is necessary for treatment to be uniform and predictable over large areas. This research has shown that soil properties and flow velocity must be taken into account when designing a treatment strategy. We have shown that attachment of bacteria is greatly influenced by soil properties, with more bacteria being retained within a well-graded sand compared to a uniform sand. We have demonstrated that an increase in flow velocity reduces the amount of bacteria retained and that blocking of attachment sites can help to minimise blocking of injection points.

Acknowledgment

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