Bio-cementation through controlled dissolution and recrystallization of calcium carbonate

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Abstract

We present an approach to bio-cementation of sand where the calcium source is prepared by dissolving powdered limestone (chalk) in lactic acid. Cementation is achieved through enzyme induced carbonate precipitation (EICP) with Jack Bean urease. The real-time nucleation and growth of crystals, crystal morphology and mechanical strength of consolidated samples was studied for dissolved chalk solution as well as calcium chloride (CaCl₂) and calcium lactate solutions. Solutions containing lactate were found to yield spherical calcite crystals. The compressive strengths of consolidated samples ranged from 0.06 to 2.8 MPa, increasing with the amount of precipitated CaCO₃.

Keywords:

Enzyme induced carbonate precipitation (EICP)
Bio-cementation
Calcite precipitation
1. Introduction

Cement is an important component in construction materials. It is used as a binder in concrete and as a component in mortar for masonry. The cement industry accounts for approximately 5% of global anthropogenic carbon dioxide (CO₂) emissions [1, 2]. The calcination of limestone and the combustion of fossil fuels are responsible for about 90% of CO₂ emitted from the cement industry. During the calcination process, limestone (CaCO₃) is thermally decomposed into lime (CaO) at high temperatures. For example, Portland cement and magnesium cement are produced by heating limestone to 1450°C and 750°C, respectively [3]. This process not only releases a lot of CO₂ from the material itself, but also involves high energy consumption.

Due to the increase in public and industrial environmental awareness, several approaches have been introduced in recent years to minimize the environmental impact caused by the cement industry. One of the most effective methods for reducing emissions and energy use in cement production today is to replace a portion of the Portland cement with pozzolanic materials of natural (volcanic) or industrial (fly ash, blast furnace slag) origin [4]. New cement materials such as energetically modified cement [5] and bio-cement [6] have also been introduced over the last few decades, in an effort to reduce the energy consumption and environmental pollution by producing cement via alternative routes.
Bio-cement is a material that is produced via a biological approach. To date, the most commonly reported system of bio-cement is based on ureolytic bacteria that produce the enzyme urease as a metabolic product. In the presence of urea and a calcium source, urease hydrolyzes urea to form ammonia and CO₂. The produced ammonia increases pH and CO₂ is transformed into carbonate ions. This leads to precipitation of calcium carbonate (CaCO₃). The mechanism is known as microbially induced carbonate precipitation (MICP) [7, 8]. When this takes place inside a granular material, such as sand, the formed crystals can act as a binder between grains to achieve cementation. The resulting material is a porous, sandstone-like material that can be used as bricks, for ground stabilization and could be considered for other application where low-strength concrete is used today, but should not be in direct contact with steel reinforcement due to the low pH (around 8.6) of the calcium carbonate binder.

Calcium chloride (CaCl₂) is often used as the calcium source in MICP. However, a major drawback of CaCl₂ is the excessive production of chloride ions that may lead to corrosion of the steel reinforcement used in concrete. MICP can also be achieved using other calcium salts such as calcium lactate [9, 10], calcium nitrate [9, 11, 12] and calcium acetate [10, 12], which reduce the unfavorable effects of chloride ions on concrete durability.

In search for low cost alternatives to pure calcium salts, some studies have used calcium ions from sources such as limestone [13, 14] and eggshells [15], dissolved using organic acids, for the MICP process. Limestone, which is primarily composed of CaCO₃, is one of the major components in conventional cement. Due to its low cost and high global abundance, it would
be a great advantage if it could be used as a major binder in concrete without the need to first
decompose it at high temperatures.

In this paper, we present a two-step process to achieve cementation by dissolution and
recrystallization of limestone, as illustrated in Figure 1. Our final aim is to use bacteria to
both produce organic acids for limestone dissolution and urease enzyme for carbonate
precipitation. However, in order to investigate the feasibility of the chemical processes
involved, we present a simplified setup where we use reagent-grade lactic acid and
commercial plant-derived urease from Jack Bean (*Canavalia ensiformis*).

The use of purified urease to precipitate CaCO₃ is known as enzyme induced carbonate
precipitation (EICP). EICP offers several advantages over MICP. It eliminates the need for
cultivation of, and effort to sustain, the bacteria. The use of plant-derived urease makes the
system less susceptible to bio-plugging, due to its smaller size (~12 nm) [6, 7]. Furthermore,
plant-derived urease is readily available in the market and will degrade after use, while in situ
microbial production of urease will leave the microorganisms behind in the material.

The process is outlined as follows (Figure 1): First, the calcium source is obtained by
dissolving powdered limestone (chalk) in lactic acid to form a dissolved chalk solution (DCS):

\[
\text{CaCO}_3 + \text{Lactic acid (HLact)} \rightarrow \text{Ca}^{2+} + \text{HCO}_3^- + \text{Lact}^-
\]

(1)
Next, the DCS is mixed with equivalent amounts of urea and added into a column with sand and urease. The urease starts to hydrolyze the urea, producing bicarbonate ions and increasing pH:

\[(\text{NH}_2\text{H})_2\text{CO} + 3 \text{H}_2\text{O} \rightarrow 2 \text{NH}_4^+ + \text{HCO}_3^- + \text{OH}^- \]  

(2)

This causes the dissolved CaCO₃ to re-precipitate:

\[\text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^- \rightarrow \text{CaCO}_3 + \text{H}_2\text{O} \]  

(3)

In order to investigate the performance of the DCS as an alternative calcium source for bio-cementation, we also performed experiments with solutions made by dissolving commercial salts, i.e. CaCl₂ and calcium lactate.

Figure 1: Illustration of cementation by dissolution and recrystallization of limestone.

In the first part of the paper, we describe real-time *in situ* monitoring of CaCO₃ precipitation and crystal growth from different calcium sources using an optical microscope. The structure
and morphology of the CaCO$_3$ crystals were characterized via Raman spectroscopy and scanning electron microscopy (SEM). In the second part of the paper, we report on consolidation of sand using the same approach. Different processing parameters can have substantial impact on the properties of the final consolidated product. Thus, the effects of different calcium sources and number of injections during the consolidation experiment were studied. Powdered limestone was also added in some of the samples to investigate the effects of remaining limestone particles in DCS preparation on bio-cementation. The quality of cementation was evaluated by mechanical tests of the consolidated products.

2. Materials and Methods

2.1 Materials

The powdered limestone was industrial grade chalk powder obtained from Franzefoss Miljøkalk AS (Norway) with a density of 2.7 kg/dm$^3$ and a particle size range of 1 - 200 µm. Sand (50 – 70 mesh particle size) from Sigma-Aldrich (USA) was used in the consolidation experiment. Jack Bean (Canavalia ensiformis) urease, Type IX, from Sigma-Aldrich (USA), with a specific activity of 50,000 – 100,000 units/g solid was used. Calcium chloride dihydrate was supplied by VWR Prolabo (Belgium), while calcium lactate pentahydrate and urea pellets (ReagentPlus®, purity ≥99.5%) were obtained from Sigma-Aldrich (USA).

2.2 Preparation of reagents

The dissolved chalk solution (DCS) was prepared by dissolving 25 g of powdered limestone in one liter of 300 mM lactic acid at room temperature (~21 ± 2 °C) for 24 hours. The mixture was stirred using a magnetic stirrer, and changes in pH and Ca$^{2+}$ ion concentration
during the dissolution process were monitored using pH (ELIT P2011) and calcium ion sensitive electrodes (ISE: ELIT 8041, PVC membrane) produced by Nico2000 (London, UK).

The final Ca\(^{2+}\) concentration was also measured by atomic adsorption spectroscopy (AAS, Perkin Elmer AANALYST400). After completing the dissolution, the solution was filtered through 11 µm pore size filter paper to remove any remaining non-dissolved limestone.

Calcium chloride (CaCl\(_2\)) and calcium lactate (CaLact) solutions were prepared by dissolving the calcium salts in deionized water. Finally, urea was added to the solution, and mixed well. Information about the reagents is presented in Table 1.

<table>
<thead>
<tr>
<th>Solution code</th>
<th>Calcium source</th>
<th>Calcium (mol/l)</th>
<th>Urea (mol/l)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCS</td>
<td>Dissolved chalk</td>
<td>0.065 - 0.12</td>
<td>0.15</td>
<td>6.7 ± 0.03</td>
</tr>
<tr>
<td>CaLact</td>
<td>CaLact</td>
<td>0.1</td>
<td>0.1</td>
<td>7.9</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>CaCl(_2)</td>
<td>0.1</td>
<td>0.1</td>
<td>7.6</td>
</tr>
</tbody>
</table>

2.3 Microscopy experiments

Figure 2 shows the microscope setup used in this study. Around 10 mg of urease, in solid form, was placed in the center of a glass-bottom petri dish and covered with a cover glass. The gap along the edge of the cover glass was partially sealed with vacuum grease to secure the position of the cover glass. 3 ml of reagents were added to the petri dish and entered the gap between the cover glass and the bottom glass through capillary action. The petri dish was then covered with a plastic lid to minimize evaporation of the solution. Crystallization was observed with an Olympus PMG3 inverted optical microscope (Olympus, Japan) at room
temperature (~25 °C) for approximately 72 hours, and pictures were obtained with a Point Grey Grasshopper3 High Performance USB 3.0 Camera (Canada). Observations were made within the region 1 - 2 mm away from the cluster of the urease powder, which can be seen from the top view of the cover glass in Figure 2. Images were taken in time-lapse mode every minute for the first two hours, and every hour after that. After 72 hours of observations, the cover glass with precipitated crystals was rinsed with deionized water, dried, and imaged with SEM (see below).

Figure 2: Microscope setup to study precipitation of CaCO$_3$

2.4 Consolidation of sand

Bio-cemented samples were prepared in a 25 mm diameter split mold (Figure 3). The two halves of the mold were held tightly together by screws. 3D printed porous flow channels and a filter paper (pore size = 11 µm) were placed in the bottom of the mold. 54 g of grains (only sand or a mixture of sand and powdered limestone; composition given in Table 2) and 0.2 g of urease were thoroughly mixed and fed into the split mold, and another filter paper and
porous flow plug was placed on top. The grains were compacted by tightening a screw and spring assemble on the top of the mold. The split mold was connected to an AL-4000 programmable double syringe pump (World of Precision Instruments, USA) at the inlet, and the outlet tube was placed in a beaker to collect the effluent fluid. The final sample height ranged from 80 to 95 mm.

To achieve cementation, 20 or 40 injections were performed at 5 hour intervals. In each step, 25 ml of reagent was injected into the mold at an injection rate of 0.5 ml/min. The reagent was pumped upwards (against gravity) through the sand in order to allow air to escape. The 5 hour interval was chosen based on the findings of Yasuhara, Neupane [16], who found that the pH of the calcium-urea-urease solutions used in a similar setting reached a steady value after 5-6 hours. In our experiments, the Ca\(^{2+}\) concentration and pH of the effluent fluid from each cycle of injections were recorded. It showed pH from pH 7.9 – pH 8.5 and Ca\(^{2+}\) ion concentration of \(1 \times 10^{-3} \text{ – } 8 \times 10^{-5}\) mol/l, indicating that most of the calcium had been used after 5h.

After finishing the prescribed number of injections, 50 ml of distilled water was injected into the column to wash out any soluble salts that might have formed as byproducts. Finally, the mold was split open, and the consolidated sample was removed from the mold. In order to ensure complete drying before characterization, the sample was dried in an oven at 70 °C overnight. Samples were then characterized using the methods described below. The processing conditions used are listed in Table 2 together with the sample coding. At least two samples were made for each set of processing parameters.
Table 2: Processing conditions and material designation

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Calcium source</th>
<th>Aggregate</th>
<th>Urease (g)</th>
<th>Number of injections</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>sand20</td>
<td>DCS</td>
<td>Sand (wt%)</td>
<td>0.2</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
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<td>DCS</td>
<td>100</td>
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<td>4</td>
</tr>
<tr>
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<td>DCS</td>
<td>90</td>
<td>10</td>
<td>0.2</td>
<td>40</td>
</tr>
<tr>
<td>50%sand20</td>
<td>DCS</td>
<td>50</td>
<td>50</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td>sand20_CaLact</td>
<td>CaLact</td>
<td>100</td>
<td>0</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>sand20_CaCl2</td>
<td>CaCl2</td>
<td>100</td>
<td>0</td>
<td>0.2</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 3: Illustration of the split mold setup

2.5 Scanning Electron Microscopy (SEM)
Samples from the microscopy experiments were imaged using a Hitachi SU5000 Schottky field-emission scanning electron microscope (FESEM) at an accelerating voltage of 10 kV. Prior to the observations, the samples were sputter-coated with a thin layer of gold to avoid electrical charging during examination. Consolidated samples were imaged using a TM3000 tabletop microscope (bench-top SEM) from Hitachi High-Technologies. Elements present in the samples were identified using Quantax70 energy dispersive spectroscopy (EDS), which is an attachment to the SEM.

2.6 Raman spectroscopy

After the microscopy experiment, the cover glass with precipitated crystals was analyzed using a multichannel Jobin Yvon Horiba T64000 Raman spectrometer. A Milennia Pro, frequency doubled, diode-pumped (Nd:YVO₄ crystal) 532 nm laser from Spectra-Physics (Model J 40) was used. The light was collected through a confocal microscope with an Olympus 20x objective, which enabled analysis of the selected crystals. The reported spectra are an average of three scans with an acquisition time of 60 seconds.

2.7 X-Ray diffraction (XRD) analysis

Crystals present in the consolidated samples were identified using Rigaku MiniFlex600 X-Ray diffractometry, with a scan range from 10° - 90° and 10°/min scanning rate. The X-Ray source was Cu-Kα radiation with a wavelength of 0.154 nm.

2.8 Mechanical tests
The consolidated samples were cut into half with similar height using a Discoplan-TS cutting machine (Struers Inc., USA) to compare the mechanical properties of the top and bottom parts of the samples. The first set of samples was subjected to uniaxial compression tests using a Zwicki-line testing machine (Zwick/Roell, Germany) with a 1 kN load cell and a cross-head speed of 10 mm/min. A round rubber pad with 1 mm thickness and 35 mm diameter was placed on the top and the bottom of the samples assuring evenly distribution of the applied forces across the sample surface. The second set of consolidated samples was capped with gypsum on both ends to ensure smooth, parallel, uniform bearing surfaces that were perpendicular to the applied axial load during the uniaxial compression test. The specimens were subject to uniaxial compression tests using an Instron 3345 universal testing machine (Instron, USA) with 5 kN load cell and a cross-head speed of 10 mm/min.

2.9 CaCO₃ content measurement

Portions of the consolidated samples (11 - 20 g) were dried in an oven at 70 °C for 24 hours. The samples were repeatedly weighed to ensure that constant weight had been reached. Then, the samples were digested in 1 M hydrochloric acid (HCl) at 40 °C under continuous stirring. To ensure a full dissolution of the solid CaCO₃, changes in pH and Ca²⁺ ion concentration was monitored by pH meter and ISE until constant values had been reached. The remaining solids were filtered through filter paper, washed several times with distilled water, followed by drying and re-weighing. The CaCO₃ content was determined as the ratio of the sample weight before and after acid digestion. In samples with added limestone, the amount of precipitated CaCO₃ was found by subtracting the initial CaCO₃ concentration.
3. Results and discussion

3.1 Dissolution of powdered limestone

During the preparation of DCS, changes in pH and Ca\(^{2+}\) ion concentration were monitored throughout the dissolution process using a pH/ISE meter. Representative curves from three different batches of DCS are presented in Figure 4. All three batches showed an increase in pH and Ca\(^{2+}\) concentration with time, but the values differed slightly from batch to batch. pH increased rapidly from ~pH 2 to ~pH 5.8 within the first 10 min after the addition of limestone to the lactic acid indicating a rapid dissolution of limestone in the initial phase. The dissolution rate then slowed down and reached pH 6.6 – pH 6.9 after 24 hours of stirring.

Also, the Ca\(^{2+}\) concentration increased rapidly during the first 10 min to around 0.01 – 0.03 mol/l, and then increased at a slower rate. The measured Ca\(^{2+}\) concentrations were slightly different in the three batches. A final Ca\(^{2+}\) ion concentration of 0.02 – 0.06 mol/l was recorded when the dissolution process was stopped after 24 hours of stirring. The final solution was also measured by atomic absorption spectroscopy (AAS) and showed a Ca\(^{2+}\) concentration ranging from 0.065 to 0.12 mol/l. The reason why the Ca\(^{2+}\) concentration measured by ISE is lower than that measured by AAS, is that lactate ions in solution complex with and chelate Ca\(^{2+}\) ions [17, 18], and the chelated calcium is no longer detectable by ISE measurement.

Calculations using PhreeqC geochemical software [19] with the minteq database and a \(K_{sp}\) of 3.86 for lactic acid shows that the theoretical solubility of calcium carbonate in 300 mM lactic acid is between 0.15 and 0.18 mM, depending on the amount of dissolved carbonate.
This is higher than the final Ca\textsuperscript{2+} concentrations we measure, indicating that the dissolution process had not reached thermodynamic equilibrium after 24 hours.

Figure 4: Changes in pH and Ca\textsuperscript{2+} ion concentration with time for three different dissolution batches.

3.2 Crystal growth and morphology investigations

Real-time monitoring of crystal growth was performed for up to 72 hours using the optical microscope setup shown in Figure 2. Figure 5 shows representative time-resolved microscope images of CaCO\textsubscript{3} crystal growth in CaCl\textsubscript{2}-solution, CaLact-solution and DCS. Based on the collected images, the crystal growth rate was assessed by analyzing the fraction of the field of view covered by crystals every 2 hours, as presented in Figure 6.

In all solutions, precipitation of CaCO\textsubscript{3} occurred rapidly. Visible crystals appeared in the solution around 10 minutes after the reagents were added (not shown in the figures). In the
CaCl$_2$ solution, numerous rhombohedral calcite crystals precipitated in the initial stage and grew rapidly during the first 4 hours. After that, the growth slowed down to a very low, almost steady rate for the remaining hours.

In the CaLact and DCS solutions, nucleation appeared to be slower. There were fewer crystals present after 2 hours, and new crystals still appeared after 4 hours. The decrease in growth rate with time was slower than in the CaCl$_2$ solution. In the CaLact solution, a mixture of rhombohedral and spherical shaped crystals was formed, while there were mainly spherical shaped crystals in the DCS solution.
Figure 5: Representative time-resolved microscope images of CaCO$_3$ crystals formed in CaCl$_2$- and CaLact solutions and DCS.

Figure 6: Fraction of area seen in the microscope that is covered by crystals as a function of time and calcium solution. The curves are normalized to the final value.

The precipitated CaCO$_3$ crystals were studied by SEM. Using CaCl$_2$ as calcium source the precipitate consisted of 20 - 80 µm rhombohedral CaCO$_3$ crystals (Figure 7), which is the typical morphology of calcite. Some of the crystals exhibited smooth crystal faces with sharply defined edges, but most of the crystals had only partly developed smooth faces and incompletely formed edges, and appeared as clusters of inter-grown crystals (Figure 7b). CaCl$_2$ is the most commonly used calcium source in MICP and EICP studies [8, 16]. Similar observations were reported in those studies, where rhombohedral calcite precipitated [16, 20, 21].
On the other hand, in the CaLact sample, spherical shaped CaCO₃ crystals with a diameter of 100 - 250 µm was the dominant morphology (Figure 8). A close-up of the spherical crystals at higher magnification (Figure 8b) showed that the spheres appeared to be spherical aggregates of smaller rhombohedral sub-units of 10 - 50 µm size. Other researchers have reported similar CaCO₃ crystal morphology, which is known as calcite spherulites [22-24]. These studies used CaCl₂ as calcium source, but had bacteria or other impurities present in the system. In our experiments, the precipitated calcite spherulites had a larger overall size than the rhombohedral calcite crystals that were precipitated from the CaCl₂ solution. Similar calcite spherulites were found in the sample using DCS as calcium source, the diameter was similar to those precipitated from the CaLact solution (100 - 250 µm). Rhombohedral calcite crystals were also detected in the sample, but calcite spherulite was the dominating morphology of the precipitated crystals.

Several mechanisms have been proposed to explain the formation of calcite spherulites. Al-Thawadi and Cord-Ruwisch [23] studied the formation of calcite spherulites by ureolytic bacteria in an MICP process. They suggested that, initially, spherical shaped vaterite crystals were formed, these however, gradually disintegrated and transformed into rhombohedral calcite (after 9 hours), inheriting the spherical shape of the initial vaterite spheres. In our study, no signs of vaterite formation were observed under the microscope nor in SEM and XRD analysis (see later). Instead, as seen in Figure 5, calcite spherulites appeared from the beginning of crystal formation, and increased their size radially with time. Hence, the concept of spherulitic growth of CaCO₃ as described by Beck and Andreassen [25] is a more likely mechanism to explain the formation of calcite spherulites in our system. An array of
crystalline subunits (here: rhombohedral calcite) grows multidirectionally from a common precursor, leading to the formation of calcite spherulites. Spherulitic growth of calcite can be due to the presence of impurities, organic molecules, or other static heterogeneities like phase separation in the system [22, 25, 26]. Presumably, the presence of lactate is the main reason of calcite spherulite formation in our system.

Figure 7: SEM images of CaCO₃ crystals formed in 0.1M CaCl₂ solution, a) at 100 x magnification and b) at 400 x magnification.
Figure 8: SEM images of CaCO$_3$ crystals formed in 0.1M CaLact solution, a) at 100 x magnification and b) at 350 x magnification.

Figure 9: SEM images of CaCO$_3$ crystals formed in DCS, a) at 130 x magnification and b) at 600 x magnification.
In order to confirm the identity of the precipitated crystals, Raman spectroscopy was performed (Figure 10). Both the rhombohedral calcite obtained by precipitation from CaCl$_2$-solution and the calcite spherulites obtained by precipitation from CaLact-solution and DCS showed identical Raman spectra that corresponded to the characteristic peaks of calcite. The peak at 288 cm$^{-1}$ arises from the external vibrations of the CO$_3^{2-}$ groups that involve rotatory oscillations of those groups. A weak peak at 716 cm$^{-1}$ is attributed to symmetric deformation of CO$_3$ groups. The symmetric stretching of CO$_3$ groups show a strong Raman band at 1092 cm$^{-1}$ [27]. This verifies that all crystals were calcite but with different morphologies.

![Figure 10: Raman spectra of crystals precipitated from DCS and CaCl$_2$- and CaLact-solutions.](image)

3.3 Consolidation of sand
Consolidated, cylindrical samples were obtained using the preparation procedure described above. A typical image of a consolidated sample (before cutting) is shown in Figure 11. The samples were all well cemented, and did not disintegrate in water. This shows that consolidation of sand can be achieved through EICP using different calcium sources and processing parameters.

Figure 11: Typical image of a consolidated sand sample made from DCS.

3.4 Crystal morphologies of the consolidated samples

SEM images (Figure 12) of the consolidated sand samples prepared from different processing conditions showed that calcite crystals precipitated on free surfaces and between the sand grains. The latter acted as a cement to bind the sand grains together. The precipitated calcite crystals in the consolidated sand samples had the same morphology as observed in the microscope experiments. The CaCl$_2$ solution yielded typical rhombohedral calcite crystals, while a large fraction of the precipitated calcite crystals from DCS and calcium lactate solution were calcite spherulites.
For the 90\%sand20 and 50\%sand20 samples (Table 2), 10 and 50 weight\% of sand was replaced by powdered limestone, respectively. A similar cementation effect is observed from the SEM images (Figures 12e and 12f), in which the precipitated calcite crystals act as a binder to bind the grains together. Both the precipitated crystals and the added limestone are calcite, and they can therefore be difficult to distinguish. In Figure 12e, two distinct morphologies of calcite were detected. Powdered limestone is known to have a rhombohedral morphology and often with faceted surfaces (as labeled in the figure). Meanwhile, the precipitated crystals appear as a cluster of smaller crystals similar to those observed in sand20 and sand40 samples, but not in a spherical orientation. This can be confirmed by a visible hole that is created by a pulled-out sand grain in Figure 12e, where the precipitated small calcite crystals formed an aggregate and covered the sand grain. For 50\%sand20 sample (Figure 12f), the calcite crystals are difficult to distinguish, because the amount of added limestone is much higher than the amount of precipitated calcite.

In the samples with added limestone, the presence of calcite spherulites were not detected. Differences in calcite morphology between sand and sand-limestone systems are probably due to differences in the number of potential nucleation sites. It is likely that limestone possesses more favorable nucleation sites for calcite precipitation, allowing nucleation to occur simultaneously at multiple sites. This results in the formation of many small calcite crystals in different places, subsequently prohibiting the spherulitic growth of calcite.
Figure 12: SEM images of consolidated sand samples: (a) sand20_CaCl$_2$, (b) sand20_CaLact and (c) sand20, (d) sand40, (e) 90%sand20 and (f) 50%sand20 (note that the magnifications of the images are different).

3.5 Elemental analysis and phase identification

Elements present in the sand20 sample were identified using EDS mapping. The distribution of the different elements in the framed area is illustrated in Figure 13. Large grains that contained silicone (Si) and oxygen (O) correspond to sand (quartz, SiO$_2$). The spherical shape crystal aggregates contained calcium (Ca), carbon (C) and O and assumed to be CaCO$_3$. This was confirmed by XRD analysis (Figure 14). Other polymorphs of CaCO$_3$, such as vaterite and aragonite, were not detected.

Figure 13: Elemental mapping of sand20 sample using EDS.
3.6 CaCO$_3$ content

The consolidated samples were cut horizontally into half along the middle plane, to compare their properties in the top and bottom portions. As described in the experimental procedures, reagents were pumped upward into the mold during the production. Thus, the bottom half is closest to the injection inlet and the top half furthest from the injection inlet. Results (Table 3) show that the bottom samples contain a higher amount of precipitated CaCO$_3$ than the top samples, suggesting that more precipitation occurred near the inlet region.
The precipitation of CaCO$_3$ is influenced by the processing parameters, including the concentration of Ca$^{2+}$ and urea, the amount of urease, and the number of injections [8, 28]. As expected, the sand40 samples subjected to 40 injections, contained more precipitated CaCO$_3$ than samples with 20 injections (Table 3). However, the amount of precipitated CaCO$_3$ in the sand40 samples was less than twice as much as in the sand20 samples, indicating that the urease activity in the system declined with time, probably due to enzyme inactivation or washout. There is also a large spread in the average CaCO$_3$ content in sand40 samples due to leakage of the mold during the production of two of the samples. The two sand40 samples that did not experience leakage had an average precipitated CaCO$_3$ content as high as 16 – 17 %. The same leakage problem also occurred for two of the 50%sand20 samples, and led to high standard deviation in the results.

The samples with added limestone had a slightly lower precipitated CaCO$_3$ content than the corresponding pure sand samples. This was not as expected, as the presence of limestone increases the nucleation of calcite crystals in the system. As none of the molds were perfectly sealed, we suspect that the lower amount of precipitation in the samples containing limestone was due to a lower initial permeability of these samples, which resulted in a higher injection pressure and therefore more leakage.

The amounts of precipitated CaCO$_3$ using CaLact and CaCl$_2$ solutions were almost equal, presumably because they contained identical concentrations of calcium and urea. This shows
that the calcium source in this case did not significantly affect the rate or amount of precipitation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average CaCO₃ content (wt%)</th>
<th>Theoretically expected CaCO₃ precipitation (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top sample</td>
<td>Bottom sample</td>
</tr>
<tr>
<td>sand20</td>
<td>11.1 ± 1.0</td>
<td>13.7 ± 1.9</td>
</tr>
<tr>
<td>sand40</td>
<td>13.5 ± 3.5</td>
<td>14.1 ± 4.5</td>
</tr>
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<td>90%sand20</td>
<td>9.4 ± 1.3</td>
<td>11.6 ± 0.7</td>
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<tr>
<td>50%sand20</td>
<td>6.9 ± 3.9</td>
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</tbody>
</table>

The theoretically expected CaCO₃ content, based on the amount of Ca²⁺-ions added during the consolidation process, was calculated (Table 3). Twenty injections correspond to a total injected volume of 500 ml calcium solution containing 0.05 moles of Ca²⁺ ions and other dissolved Ca-species for the CaCl₂ and the CaLact solutions, and 0.0325 moles of Ca²⁺ ions for the DCS. Theoretically, DCS may have contained up to 0.15 moles of dissolved Ca-species per liter, corresponding to an addition of 0.075 moles of dissolved Ca-species during the consolidation process. Precipitation of 1 mole of CaCO₃ requires 1 mole of Ca²⁺ ions, and the theoretical maximum CaCO₃ content in the consolidated sand columns is given by 

\[ nM_c/(m_a+nM_c) \]

where \( n \) is the expected number of moles of precipitated CaCO₃, \( M_c \) is the
molar mass of CaCO$_3$ (100.09 g/mol), and $m_a$ is the mass of the aggregate (sand + limestone) in each prototype (54 g).

For the CaCl$_2$ and CaLact samples, the CaCO$_3$ contents of the bottom samples were in good agreement with the theoretical prediction, while the top samples were slightly lower. However, all of the DCS samples had a higher CaCO$_3$ content than expected based on the Ca$^{2+}$ concentrations measured by AAS. If the dissolution process had reached thermodynamic equilibrium, then assuming a calcite solubility of 0.18 M Ca$^{2+}$, the expected CaCO$_3$ content should have been 14.3%. This is higher than most observed values. One possible reason for the discrepancy between the Ca$^{2+}$ content measured by AAS and the amount of precipitated material is that tiny particles of powdered limestone remain in the DCS after filtration, and continue to be dissolved until the solution reaches thermodynamic equilibrium, so that the actual Ca$^{2+}$ content in the injected solution is higher than what was measured.

3.7 Mechanical properties

The compressive strengths of the consolidated samples were evaluated by a uniaxial compression test, and the results are presented in Figure 15. In this test, it is essential to assure that the applied force is evenly distributed to the sample. The first batch of samples were tested using 1 mm-thick rubber pads that were placed on top and bottom of the samples to ensure uniform force distribution (Figure 16a). Another set of consolidated samples made from DCS were subjected to another testing method, in which gypsum capping was applied at
both ends of the samples to create smooth and parallel surfaces that were perpendicular to the applied axial load during the uniaxial compression test (Figure 16b).

The observed compressive strength ranged from 0.06 to 2.8 MPa. The higher values were comparable to soft rocks such as sandstone and siltstone [29]. In general, the bottom samples displayed higher compressive strengths than the top samples. This can be attributed to the greater amount of CaCO₃ precipitation near the inlet, as shown by the CaCO₃ content measurements (Table 3). The trend for all samples was that the compressive strength increased strongly with the content of precipitated CaCO₃ (Figure 15). This is in agreement with how the compressive strength of porous concrete depends on the porosity and on the total area of solid-solid contact [30, 31].

The consolidated samples produced with DCS had a higher CaCO₃ content and higher strength than those made from CaCl₂ and CaLact solutions. However, when comparing strength as a function of CaCO₃ content, the CaCl₂ and CaLact samples seem to perform slightly better than the DCS samples.

Addition of chalk did not significantly alter the mechanical strength of the consolidated samples. The average compressive strength of the 90%sand20 and the 50%sand20 samples were lower than that of the sand20 sample. The degree of CaCO₃ precipitation was also lower in the samples with added limestone (as shown in Table 3), probably due to leaks. In the mixtures of sand and chalk, the grain size distribution was wider than for pure sand. One could expect that the presence of small chalk grains that can fill up the space between the
sand grains would increase the area of solid-solid contact and therefore result in a higher compressive strength for a given amount of precipitated material. However, the morphology of the precipitated CaCO$_3$ was also different in the samples with chalk. The samples with only sand contained precipitated calcite spherulites, while the samples with chalk contained smaller, more dispersed aggregates of calcite crystals (Figure 12). The strength of biocemented sand is expected to depend on to what degree the precipitated calcite crystals are able to form bridges between the adjacent sand grains [32]. The lack of increased strength in chalk containing samples could be due to a better binding efficiency of the calcite spherulites, through creating a larger contact area between sand grains, compared with multiple small crystals scattered on the grain surfaces.

![Graph showing compressive strength vs precipitated CaCO$_3$ content](image)
Figure 15: Compressive strength of all samples as a function of precipitated CaCO$_3$ content.

Data for gypsum capping are the results of single measurements, while data for the rubber pad are averages over several replicates.

Figure 16: Axial splitting in a rubber pad test (a) and shear failure in a gypsum-capped sample (b) observed on sand20 samples under uniaxial compression.

In order to investigate the effect of the testing method, we can compare the mechanical test results for the sand20 and the 90% sand20 samples. These samples had very similar CaCO$_3$ content (Table 3) and were therefore expected to have similar mechanical strength. However, the strength of the gypsum-capped samples was higher than that measured for the rubber pad samples. The lower strength obtained with the rubber pad was probably a result of tensile stresses normal to the loading direction, set up by lateral extension of the rubber, which resulted in axial splitting of the samples (Figure 16a). The stress-strain curves of the selected samples (Figure 17) showed a significant effect of the presence of the rubber pads both on the measured elastic modulus and the failure behavior. The gypsum capped samples displayed shear failure (Figure 16b) at a higher measured load. The latter is probably a more
representative measure of the compressive strength of the samples, and we expect that the actual strength of the samples tested using rubber pads is around 50% higher than reported in Figure 15.

Figure 17: Typical stress-strain curves of sand20 (black) and 90%sand20 (red) bottom samples that were tested using gypsum capping (solid lines) and rubber pad (dashed lines).

4. Conclusions

Enzymatically induced precipitation (EICP) of CaCO₃ was achieved using solutions of calcium chloride, calcium lactate and powdered limestone dissolved in lactic acid (DCS) as calcium sources. The morphology of the precipitated CaCO₃ crystals depended on the calcium source. Rhombohedral calcite crystals were formed in the CaCl₂ solution, while calcite spherulites were formed in the DCS and the Ca-Lactate solutions. Real-time monitoring of crystal growth showed that the spherulites were formed at the initial stages of crystallization and grew radially with time, suggesting that they were not formed by a
transformation from spherical vaterite to calcite. Presumably, the presence of lactate induced
the formation of calcite spherulites.

Cylindrical samples of consolidated sand, or sand mixed with limestone powder, were
produced using EICP and the different calcium solutions. The morphology of the precipitated
CaCO$_3$ crystals in the consolidated samples were similar to those observed under the
microscope when CaCO$_3$ was precipitated from the same calcium solutions, but the presence
of limestone powder gave rise to smaller, more scattered precipitates. The compressive
strength of the consolidated samples increased with increasing content of precipitated CaCO$_3$.
More CaCO$_3$ precipitation was detected in the region closest to the injection inlet and yielded
a higher strength. The presence of limestone powder did not significantly affect the
compressive strength of the samples, probably due to competing effects of a wider grain size
distribution and smaller, more scattered precipitated crystals.

In general, our results demonstrate the feasibility of using limestone powder dissolved in
lactic acid as a calcium source for bio-cementation purposes.

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**References**


