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Abstract: Microbial-induced calcium carbonate precipitation (MICP) is a novel approach that is already being applied in various areas of construction. The precipitated calcium carbonate can be used to reduce porosity and thus increase the durability of deteriorated building components. This study investigates whether MICP injections are suitable for building rehabilitation. Porous mortar test samples of recycled aggregate and parts of deteriorated masonry were prepared. The MICP injections were performed without pressure and with an injection pump. The treatment effect was investigated after MICP injection by testing the porosity, strength and microscopic evaluation. It can be observed that multiple MICP injections under pressure result in a reduction of the pore volume of porous mortar samples. The produced calcium carbonate precipitates in the pore space of the samples and increases the density by 1.59% and the weight by 7.56%, which also results in a 48.3% reduction of the capillary water absorption. The results of strength tests show an increase of 45.16% in flexural strength and 35.64% in compressive strength compared with the untreated mortar samples. In addition, the MICP process was investigated and the precipitation was characterised. The X-ray diffraction (XRD) of the precipitated calcium carbonate confirms that mainly calcite was formed, which was also found in the pore structure of the MICP-injected masonry after the microscopic analysis. Precipitated calcium carbonate could be detected especially near the injection spots.

**Keywords:** microbial-induced calcium carbonate precipitation (MICP); biocementation; injection method; masonry; building rehabilitation

# 1. Introduction

As a result of multiple causes of deterioration, the demand for new building rehabilitation techniques is constantly increasing. Injection methods are important techniques to prolong the durability of structures. The deterioration of masonry is mainly caused by chemical reactions or physical impact leading to voids and cracks [1]. Lime- or cementbased grout injection is the most common rehabilitation method and is also used for historic masonry [2,3]. For sealings, polymer-based materials such as epoxy resin and acrylates with high groutability are often used. However, these materials have disadvantages such as long hardening periods or are not applicable to large-scale reinforcement [2]. Moreover, the potential of the recycling ability could be affected if non-mineral grout materials were used. Mu et al. [4] summarised further advantages and disadvantages of various conventional grouting materials, i.e., they suggested that mortar-based grouts could cause efflorescence of the masonry due to the high alkalinity.

A sustainable alternative could be the use of the microbial-induced calcium carbonate precipitation (MICP) method. In this process, ureolytic bacteria such as *Sporosarcina pass-teurii* precipitate calcium carbonate in combination with a cementation solution consisting of a solution of a calcium salt and urea (Equations (1) and (2)) [5]:

$$CO(NH_2)_2 + 2H_2O \rightarrow CO_3^{2-} + 2NH_4^+$$
 (1)



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$$\mathrm{CO_3}^{2-} + \mathrm{Ca}^{2+} \to \mathrm{Ca}\mathrm{CO}_3 \tag{2}$$

The precipitated calcium carbonate can be used to reduce porosity and thus increase the durability of deteriorated building components. In addition, MICP injection into the masonry joints could achieve a sealing effect. MICP is considered to be sustainable and offers many advantages. In contrast to conventional polymer-based grouts, only mineral precipitates are transferred into the structure with the MICP method and could therefore also be suitable for use in listed buildings. Another advantage is the high groutability of the liquid MICP reagents, which ensures an optimal distribution of the material in the building. However, the formation of ammonium by-products of the MICP process may have a detrimental effect on the component. A number of studies have been conducted to investigate MICP injection methods for application in soil improvement with soil column experiments [6,7]. However, there is still a huge demand for research on proper injection methods which achieve an effective  $CaCO_3$  distribution [8]. Some studies have already focused on possible MICP applications on bricks, stones or masonry [4,9–12]. Jimenez-Lopez et al. [12] developed biomineralization experiments by activated bacteria inhabiting the stone for consolidation and increasing the resistance to the deterioration of stone pieces. Le Métayer-Levrel et al. [13] were able to achieve a surface improvement of limestone façades which led to a reduction in water absorption. Moreover, Tiano et al. [14] and De Muynck et al. [15] induced a decrease in stone porosity with MICP surface treatment. However, so far, the application of an MICP injection method on structures has hardly been researched. In the studies by Yang et al. [2,16], field tests were presented to reinforce deteriorated historic masonry structures. Bacterial culture and cementation solution were pumped into sand-filled boreholes for more than 2 weeks. A visual evaluation showed that the formed biomediated sandstone could be a promising approach for masonry improvement. The precipitated  $CaCO_3$  is able to close pores and increase the density of the components [17]. The MICP application in cementitious materials, however, still offers research potential [18].

This study aims to develop an injection method using MICP reagents (bacteria and cementation solution) to improve porous building components such as mortar joints or masonry. A proven injection technique with pump and packer was used, which is actually designed for grouting with resins and was filled with MICP reagents instead. For this purpose, preliminary experiments were carried out on porous mortar samples with different dimensions to produce CaCO<sub>3</sub> precipitates in the pore space of the samples by MICP. In the last step, the method was transferred to pieces of deteriorated masonry in order to investigate whether there were any improvement effects due to precipitated CaCO<sub>3</sub> by MICP.

# 2. Materials and Methods

# 2.1. Bacterial Culture and Cementation Solution

A bacterial culture of *Sporosarcina passteurii* (DSM33) was used for all MICP injection experiments provided by the Biotechnology Laboratory of the Department of Engineering and Management. Based on the findings by Lapierre et al. [19], the bacteria were cultivated in a supplemented complex medium. The optical densities of the cultures used were 11.5, 12.2 and 15.2 at a wavelength of 600 nm (OD<sub>600</sub>). The bacterial cultures were stored at 12 °C (for not longer than one week) before use. For the MICP injection tests, the original optical density of the bacterial culture after cultivation was diluted with NaCl solution to an OD of 1. CaCl<sub>2</sub> was used as a calcium source for the MICP process due to its high solubility. The cementation solution was prepared equimolar with 1 mol/L CaCl<sub>2</sub> (110.99 g/mol) and urea (60.06 g/mol). The ratio between bacterial culture and cementation solution was set to 1:1 and stored separately until the beginning of the experiments. The total volume of the MICP reagents used depends on the respective pore volume of the samples. In this concept, one treatment of injection corresponds to the combined volume of both MICP reagents, regardless of the order of application.

# 2.2. Sample Preparation and MICP Injection Method

The MICP experiments in this study consist of three test series with different sample types (Figure 1).



Figure 1. Scheme of the three test series of this study.

For the first test series, mortar samples were used for an initial evaluation of the MICP method. The mortar was made from CEM I and recycled concrete aggregate (1–4 mm) to generate high pore volume to imitate porous masonry mortar. The MICP reagents (bacterial culture and cementation solution) were filled in drilled holes without pressure. Different filling orders of the two MICP components were tested to find the optimum. The interval between MICP treatments was 24 h. This was decided in order to ensure the optimum time period of the precipitation process. After drying at 50 °C, parameters of porosity, bulk density, weight increase, flexural and compressive strength [20] and capillary water absorption [21] were determined.

In order to test a MICP injection with pressure in the second test series, the mortar samples were scaled up. Different sample dimensions were tested: cubes (15 cm) and beams ( $10 \times 10 \times 50$  cm and  $15 \times 15 \times 70$  cm). These sample dimensions enable the drilling of holes for the packers. The cube samples were used to confirm the dosage of the volume of the MICP reagents. The mortar beams were used to investigate the effect of lining up the packers, with various distances between the packers on the degree of CaCO<sub>3</sub> filling after MICP injection. With the larger beams, offset arrangements of packers were also investigated. To prevent the injection reagents from leaking out of the test samples, all sides except the surface for injection were sealed with a polyurethane coating. The injections were carried out with a WEBAC<sup>®</sup> IP 1K-F4 pump (2.2 L/min). The MICP reagents were applied via boreholes with packers. The packers were distributed at regular centres depending on the sample size. Pressures between 25 and 30 bars were measured. After injection, these samples were cut open to determine the MICP effect by microscopic evaluation.

In the last test series, pieces of deteriorated masonry were taken from a demolition site. Due to the high instability of the defective mortar joints, the pieces were encased in concrete to stabilise them for the injection tests. The injections were also carried out with the pump and the packers, placed 10 cm apart and drilled through the mortar joint at a  $45^{\circ}$  angle as recommended in [22].

To investigate the MICP process and further analyses, 50 mL of the MICP reagents from the pump were collected in a beaker. Similar to [16], powdery CaCO<sub>3</sub> was precipitated with 1 mol/L equimolar CaCl<sub>2</sub> and urea. Moreover, it was observed that the precipitation process starts immediately. However, it takes 1–2 h for the precipitation to settle. This precipitate was filtered and analysed by X-ray diffraction (XRD). A PANalytical Aeris diffractometer (Malvern Panalytical, Kassel, Germany) with CuK $\alpha_1$  was used. For this purpose, the CaCO<sub>3</sub> was ground with an XRD Mill (McCrone, Westmont, IL, USA) with the addition of ZnO standard and filtered again with a cellulose nitrate membrane filter. This allows for the determination of the amorphous content of the samples. XRD analysis was evaluated by element restriction determined after XRF element analysis (X-ray fluorescence) of the precipitate. For quantitative results, a Rietveld analysis was carried out. The filtrate was analysed by ion chromatography for the determination of the remaining calcium ions that have not been transformed by MICP. Moreover, the ammonium content could provide information about the urea hydrolysis. The particle density of the powdery precipitate was measured using a helium pycnometer.

## 3. Results

#### 3.1. Results of the 1st Test Series: Initial Tests on Mortar Samples

The results of the initial tests of the small mortar samples ( $4 \times 4 \times 16$  mm) showed that multiple MICP injections affected the properties of the samples. Optimum CaCO<sub>3</sub> filling of the pore space by the MICP process was achieved after three treatments. Further treatments showed no significant improvement. The results are shown in Figure 2. It can be seen that the weight of the samples increased due to the CaCO<sub>3</sub> formed and thus the bulk density of the samples increased (Figure 2a,b). However, compared to the untreated samples, the bulk density increased by 1.59% on average. A noticeable increase in strength can be observed (Figure 2c,d). The flexural strength after MICP treatment increased by 45.16% and the compressive strength increased by 35.64%. The results correspond to an average decrease in the pore volume of 7.8%. Moreover, a reduction in capillary water absorption of 48.3% of the small mortar samples was measured.



**Figure 2.** Results of properties of the initial tests on mortar samples before and after three MICP treatments: (**a**) Increasing the weight; (**b**) Bulk density; (**c**) Flexural strength; (**d**) Compressive strength of the samples.

Based on these results, the mortar beams and cubes were injected three times with MICP reagents of the same composition in the second test series. It should be noted, however, that during the treatment, some of the MICP reagents always leaked out of the samples and therefore the full improvement potential was probably not achieved. To avoid this, the samples were sealed with PU for further tests. Due to influences from polyurethane coating, however, no characteristic properties could be determined on samples. Thus, optical evaluation of the MICP treatments has to be carried out.

## 3.2. Results of the 2nd Test Series: MICP Pressure Injection Tests Using Pump

The optical evaluation of the samples from the second test series with the MICP injection method showed that CaCO<sub>3</sub> formed inside the samples. Thus, with the up-scaling of the mortar samples, a successful MICP treatment could also be proven.

Figure 3a shows the cut halves of a beam after the three injections of MICP reagents. It can be seen that most of the CaCO<sub>3</sub> was precipitated in the lower section of the beams. Precipitation can also be clearly found in the boreholes of the packers (Figure 3b). The coating of the samples thus provided optimal conditions for the MICP injection method. Since no liquid of the MICP reagents leaked from the samples, the precipitated CaCO<sub>3</sub> was able to fill the pore space of the samples, as was the case with the samples from the smaller-scale preliminary tests. Although the calculated volume of MICP reagents is based on the complete pore space of the sample, only about half of the pore space of the samples could be filled with precipitated CaCO<sub>3</sub>. Microscopic investigation confirmed that a CaCO<sub>3</sub> layer formed in the borehole of the samples, especially at the outlet of the packer (Figure 3c, red circle). A comparison of the lower (Figure 3d) and upper sections (Figure 3e) shows a clear difference in the filling grade of the pore space.



(a)



(d)

(c)

**Figure 3.** Test series with MICP reagents' injection of coated porous mortar beam samples using an injection pump: (**a**) Cut open beam after MICP injection; (**b**) Packer borehole; (**c**) Microscopic images of the packer borehole; (**d**) Almost filled pore space with precipitated CaCO<sub>3</sub> in the lower section of the beam and (**e**) Partly filled pore space in the upper section of the beam.

(b)

(e)

Although the pore space of the lower area has been clearly filled, some unfilled pore space can still be found here. This suggests that the MICP reagents probably did not form the maximum possible amount of CaCO<sub>3</sub>. It should be noted, however, that with the pump used, it is difficult to dose the volume exactly and that deviations may therefore be possible. No evidence of cracking due to injection with pressure can be detected either.

The results of weighing are consistent with the optical evaluation. The treated mortar beams showed a weight increase of up to 3.6%, which was achieved by the precipitated CaCO<sub>3</sub>. Thus, the weight increase compared to the small samples of the first test series was more than 50% lower.

#### 3.3. Results of the 3rd Test Series: Masonry MICP Injection

In this test series, an application of the MICP injection method to masonry was investigated. Two pieces of deteriorated masonry with defective mortar joints (Figure 4a) were reinforced with concrete and prepared for injection with packers. During the first pressure injection using a pump and packers, it was observed that some amount of MICP reagent leaked out from the masonry sample. Moreover, parts of the masonry around the boreholes were broken by the injection pressure. However, after the second and third injections of the first masonry sample (Figure 4b), a lower amount of MICP reagents seemed to leak out. A similar effect was observed in the second piece of masonry (Figure 4c). Since the masonry samples were not sealed, it must be assumed that a higher quantity of MICP reagents leaked out. To prevent the MICP reagents from leaking out of the packers' boreholes, they were sealed with mortar during the injection test of the second masonry section. The mortar also provided strong holding of the packers, allowing for multiple injections.





Figure 4. Test series with deteriorated masonry samples: (a) Detail of untreated masonry before MICP injection; (b,c) Pieces of masonry sample embedded in concrete with drill packers for MICP injection.

Due to the sample size, the masonry samples were dried at room temperature for several weeks. After drying, cores were taken from the masonry (Figure 5d). At first sight, the drilled core samples did not show a typical CaCO<sub>3</sub> layer, as was the case with the concrete samples. However, detailed microscopic analysis showed slight traces of precipitated  $CaCO_3$  on the inner surface of the packer wells (Figure 5a). These  $CaCO_3$ particles were not found in other parts of the sample. In detail, it was observed that the precipitated CaCO<sub>3</sub> mainly formed in the areas close to the boreholes (Figure 5b), whereas in more distant areas, no  $CaCO_3$  precipitated in the pores of the masonry (Figure 5c). Due to the deteriorated condition of the masonry samples, a strength test cannot be carried out. It seems that the encasing concrete layer contributed to the stability of the sample but did not affect the primary weathered and deteriorated structure of the masonry. Therefore, pieces of the masonry were measured with the helium pycnometer in order to measure the density. The results showed that samples from the injected areas had a lower density compared to untreated masonry samples. The capillary water absorption of the treated masonry pieces also did not show any significant improvement compared to untreated samples. Although precipitated CaCO<sub>3</sub> was found in the treated samples and in the beaker (Figure 5e), it cannot be excluded that the pumping process resulted in increased pore



**Figure 5.** Microscopic evaluation of drilled core samples: (a) Detail from the inside surface of the packer borehole after the MICP injection; (b) Detail of the masonry pores partly filled with MICP; (c) Detail of the outer surface of the drilled core sample with masonry pores without traces of MICP. Drilled core sample from a MICP-injected area (d) and MICP reagents collected in beaker with precipitated  $CaCO_3$  (e).

Figure 6 shows the result of the XRD analysis of the precipitate formed from the extracted MICP reagents that flowed through the pump during the injection experiments. The quantitative results of the CaCO<sub>3</sub> polymorphs showed a calcite content of 76.5%, vaterite content of 2.4% and an amorphous content of 20.8%.

The particle density measured with a helium pycnometer resulted in 2.85 g/cm<sup>3</sup>. The density corresponded to values from previous MICP experiments of CaCO<sub>3</sub> using similar conditions of optical density of the bacterial dilution and cementation solution. The ion chromatographic investigation of the filtrate showed that a low concentration of calcium ions was detected. In contrast, a higher ammonium content was measured, indicating a successful MICP process. Moreover, a comparison of the measured weight of the CaCO<sub>3</sub> powder and the calculated theoretical amount that can be precipitated from the MICP reagents confirmed a high precipitation rate. However, due to a large amount of MICP reagents leaking from the samples, it can be assumed that the CaCO<sub>3</sub> did not totally precipitate in the masonry.

space, which reduced the density. Compared to the mortar samples, in this case, some small cracks could be detected after injection.

(**d**)

(e)



Figure 6. Results of the XRD analysis of the precipitation (C = Calcite, V = Vaterite).

#### 4. Discussion

#### 4.1. Effect of MICP Treatment on Mortar Samples

The results of the first two test series showed that the MICP injection method could be a promising approach for the rehabilitation of porous building components. On a small scale, it was demonstrated that the properties of the samples could be improved. The improvement effect of the MICP treatment was especially noticeable in the increased flexural and compressive strength of the mortar samples. The optical evaluation confirmed the partially filled pore space with precipitated  $CaCO_3$ . In their study, Mu et al. [4] were able to significantly increase the compressive strength of wall bricks through MICP treatment compared to untreated cracked bricks. Moreover, Manzur et al. [23] reported an improvement of masonry concrete samples using brick aggregates modified by MICP. Thereby, the compressive strength could be increased by 8% or 15%, depending on the MICP treatment of the samples. In this study, the optimum of three treatments for the injection of mortar samples was found, which led to a weight increase and an increase in density. De Muynck et al. [24] also noticed an increase in weight after a second treatment compared to the first treatment. They indicated that the presence of a  $CaCO_3$  layer on the sample surface probably supports the growth of new crystals. In this study, the boreholes of the second test series especially showed noticeable CaCO<sub>3</sub> layers and filled pore space similar to [4] that could visualise the precipitated CaCO<sub>3</sub> well. According to [8], the content of precipitated CaCO<sub>3</sub> correlates with the compressive strength. Therefore, it is beneficial to achieve a high rate of  $CaCO_3$  filling of the pore space. However, Yang et al. [2] stated that the required amount of CaCO<sub>3</sub> produced only by MICP is very high and would take a long time to fill the entire pore space. They therefore used sand in addition as a filling material.

A reduction in capillary water absorption can also be observed in the initial test series. Similar results were found in [4,10,13,14,25] on surface-treated samples. This confirms the assumption of an effective pore closure of the sample, preventing water from permeating further into the sample [10]. However, biomass residues could also be the factor responsible for this [14].

#### 4.2. Effect of MICP Injection on Masonry Samples

Based on the findings of the test series with mortar samples, a MICP injection method was applied to deteriorated masonry samples. However, it seems that the material influences the MICP process. The  $CaCO_3$  layer in the borehole of the masonry samples is not as pronounced as is the case in the mortar samples. One reason for this could be the leaking of the MICP reagents. Leakage could leave too little MICP reagent in the sample

to produce enough  $CaCO_3$ . In contrast to the masonry, the mortar samples were sealed, which probably allowed the MICP reagents to concentrate in the sample. Moreover, the different pore structures of the masonry could be the reason for the different results. It is important that the injection material is compatible with the sample, which depends on a number of factors such as the characteristics of the pores [26]. The pore size plays a role and determines the capacity of water absorption [27]. This property could also be relevant for the absorption of liquid MICP reagents. In the microscopic evaluation, precipitated CaCO<sub>3</sub> was detected in the brick material in areas close to the injection sites. Similar observations are also described in [8], and they suggest that the CaCO<sub>3</sub> formation in areas decreases the further away they are from the injection point. With multiple injections, a homogeneous distribution of CaCO<sub>3</sub> precipitation in the sample can be achieved to reduce permeability [8]. Results from Manzur et al. [23] showed that it is possible to precipitate CaCO<sub>3</sub> on brick material. Moreover, Sarda et al. [9] performed MICP deposition tests on bricks and were able to detect  $CaCO_3$  on the surface. In addition, a blockage of the pores on the surface could be achieved through calcite deposition, thus preventing the penetration of water into the brick. Reduced or retarded water absorption of the bricks may result in increased durability and the weathering process of the masonry [9,10]. In this study, however, no significant reduction in water absorption was observed.

Yang et al. [16] showed the MICP application on sandstone in their study. It can be concluded that the MICP reagents worked properly as the results of the collected reagents in the beaker showed clear CaCO<sub>3</sub> precipitation. The results of the XRD analysis showed similar CaCO<sub>3</sub> polymorphs as those shown in De Muynck et al. [25]. However, there is no indication of how the material properties of the masonry influence the MICP process. In further research, the sample preparation and the properties of masonry should be investigated to develop the MICP injection method. In order to evaluate the durability of the treated masonry, in addition to the investigations on the filling of the pore space by precipitated CaCO3, a comprehensive determination of mechanical parameters is also necessary [2,10]. The injection material should be able to create a bond between the different components of the masonry to increase resistance [26].

### 5. Conclusions and Outlook

This study showed that MICP could also be suitable as an application for the rehabilitation of porous building components. The MICP injection method could thus offer an alternative to conventional techniques. On a small scale, promising results were seen in the improved strength of 45.16% in flexural strength and 35.64% in compressive strength, as well as in the reduction of 48.3% of water absorption of porous mortar samples. Microscopic analysis confirmed that CaCO<sub>3</sub> was precipitated in the pores of the sample. However, there are still research and development steps necessary to achieve similar results with masonry MICP injections. Although precipitated CaCO<sub>3</sub> was also observed, it is difficult to identify the improvement effect. For that reason, investigations on the mechanical properties of the masonry are also necessary to determine the durability of the MICP injection method. Field tests on the walls of historic masonry and investigations of interactions between MICP and the brick material are also planned as the next step. Research is also being conducted into ways of sealing the mortar joints to prevent the problem of MICP reagents leaking out.

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