

## Combined Effect of Relative Density and Calcium Carbonate Content on Drained Triaxial Behaviour of Bio-cemented Sand

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### ABSTRACT

Bio-cementation of soil is a promising technique recently used to address a wide range of geotechnical engineering problems. In this study, bio-cementation in sand samples has been initiated through the bacteria *Sporosarcina pasteurii* using the premixing and injection methods. Monotonic drained triaxial tests (CD) have been performed to study the effect of initial sand density on the strength improvement due to bio-cementation. The loose, medium dense, and dense samples were also investigated with respect to the homogeneity of the calcium carbonate content distribution. The results showed that, regardless of the initial density, peak deviatoric stress increased with calcium carbonate content. Below a cementation degree of about 7%, peak stress was higher for the dense initial state, while above that cementation level, it was higher in case of a loose initial state. In addition, the repeatability of the resulting strength under similar bio-cementation conditions was demonstrated.

### INTRODUCTION

Microbially Induced Calcium Carbonate Precipitation (MICP) is a soil improvement technique that uses bacteria producing urease enzyme. In this bio-cementation process, the reaction is facilitated by the urease enzyme, which catalyzes the hydrolysis of urea, producing ammonia and carbonic acid. As a result, the pH of the solution raises, enabling aqueous carbonate to precipitate as calcite upon the availability of adequate amounts of calcium ions in the treatment solution (DeJong et al. 2006). While the mechanical properties of bio-cemented sand have been extensively studied using various soil mechanical laboratory tests (Nafisi et al. 2021, Qing et al. 2023), there is a lack of studies on reproducibility of those properties. For instance, Konstantinou et al. (2021) studied the repeatability of producing calcium carbonate level as a function of the number of cementation solution injections. They found that the repeatability of the cementation level was

better when using fine sand compared to coarse sand. However, the repeatability of the mechanical properties under similar treatment and boundary conditions has not been thoroughly explored. An assessment of the combined effect of relative density and MICP treatment on the dynamic response of sand by Xiao et al. (2019) demonstrated the improvement of a loose sand by bio-cementation led to higher liquefaction resistance than that of both untreated loose sand and untreated dense sand. Similarly, the effect of relative density and bio-cementation on the monotonic response has been investigated by Gao et al. (2019). They demonstrated that even with a low level of bio-cementation comparable or higher levels of strength were achieved than with densification. For example, the shear strength of a loose sample ( $I_D=30\%$ ) bio-cemented with 1% calcium carbonate was higher than the shear strength of clean sand compacted to a dense state ( $I_D=90\%$ ). The study, however, was limited to only low cementation levels up to 1.2%. Therefore, more research is needed to identify a relationship between relative density, calcium carbonate content resulting from bio-cementation and shear strength.

In the present study, bio-cementation experiments have been performed on uniform Karlsruhe fine sand, which has intensively been studied both experimentally and numerically with respect to the drained and undrained behavior under monotonic and cyclic loading (Wichtmann & Triantafyllidis 2016, Wichtmann 2016, Wichtmann et al. 2020). The aim of the current study is to establish a relationship between the initial relative density, the degree of cementation and the mechanical properties. Moreover, the reproducibility of the strength under comparable bio-cementation conditions has been examined.

The specimens have been compacted to different initial relative densities and treated by MICP for different durations. Treatment injections have been applied every twelve hours. After the completion of treatment, bio-cemented sand samples have been subjected to drained triaxial compression tests. After the tests, the distribution of the calcium carbonate content within the samples has been determined.

## **MATERIALS & METHODS**

### **Bacteria and Cementation Solution**

*Sporosarcina pasteurii* (strain DSM 33) was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). The liquid bacterial culture consists of peptone from casein (15 g/l), peptone from soy meal (5 g/l), and sodium chloride (5 g/l). The pH of the solution is raised to a final value of 7.3 before being autoclaved. After cooling down the solution urea (20 g/l) is filtered using a sterilized syringe filter with pore size 0.2  $\mu\text{m}$ . Inoculant solution is aerobically incubated overnight at 30°C. The final optical density of the bacterial solution  $OD_{620}$  ranges between 0.8 and 1.2. The cementation solution has a molarity of 0.25 M and consists of urea (15 g/l), calcium chloride dihydrate (36.8 g/l), ammonium chloride (10 g/l), sodium carbonate (2.12 g/l), and nutrient broth (3 g/l).

## Specimen Preparation & Treatment

The bio-cementation method applied has been developed during intensive research conducted by Zeitouny et al. (2023). The bio-cementation treatment procedure can be summarized as follows:

- Karlsruhe fine sand (mean grain size  $D_{50} = 0.14$  mm, uniformity coefficient  $C_u = 1.5$ , maximum void ratio  $e_{\max} = 1.054$ , minimum void ratio  $e_{\min} = 0.677$ ) is mixed with 0.6 of the sample pore volume of bacteria solution in addition to 0.04 pore volume of cementation solution for fixation reasons.
- The sand is compacted in a split mold (diameter = 5 cm, height = 10 cm) in ten layers using the under-compaction method (Ladd 1978). The bio-cementation mold is shown in Figure 1.
- Additional 0.4 pore volume of bacteria solution is percolated upon the compaction of sand.
- Samples are kept at rest for about 12 hours.
- The cementation solution is injected through the sample using a pump twice per day, every 12 hours, with an amount of 1.2 pore volume.
- Upon the desired treatment duration, the specimen is flushed with de-ionized water to stop the precipitation process and remove the dissolved salts.
- Samples are oven-dried at 60°C for four days.

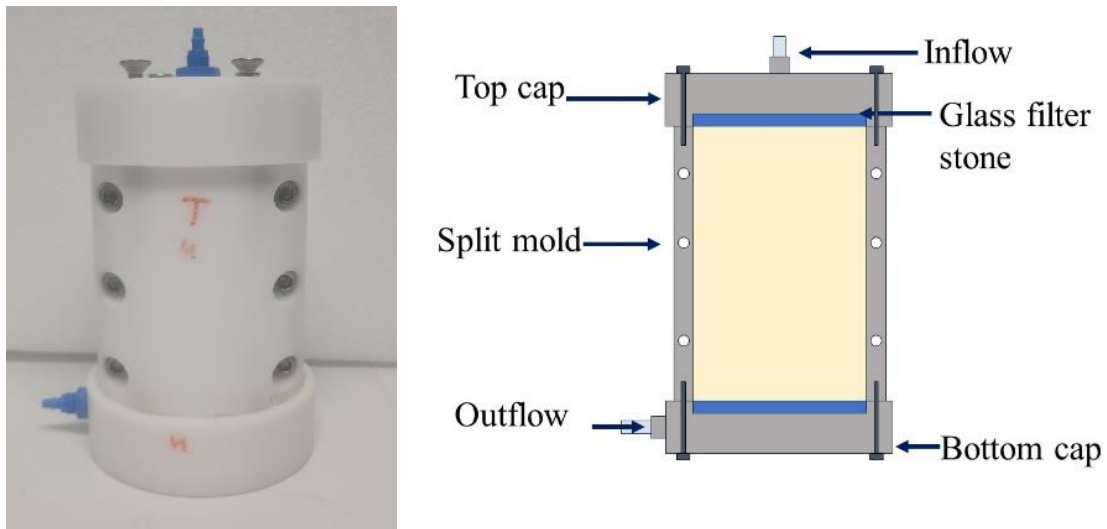


Figure 1. Bio-cementation mold.

## Drained Triaxial Testing

The specimen is taken out of the mold, placed within the triaxial apparatus and subjected to a cell pressure of 50 kPa. The sample is then saturated using deaired and de-ionized water. Afterwards, both the back and cell pressure are increased stepwise to 200 kPa and 240 kPa, respectively, and maintained overnight to enable the compression of any remaining air bubbles under this pressure. To reach the required minimum B-value of 0.96, the sample is finally flushed with pressurized water under 200 kPa back pressure. If the required B-value is not reached after those steps, the back and cell pressure are further increased to 500 and 540 kPa. In the consolidation step, the cell pressure is raised to reach an isotropic effective confining pressure of 100 kPa or 400 kPa,

respectively. The shearing is then commenced by axial compression with a rate of 0.045 mm/min at open drainage.

### **Calcium Carbonate content**

After the completion of the triaxial test, the calcium carbonate content (CCC) is determined using the acid washing method as outlined by Rebata-Landa (2007). The bio-cemented soil is collected from nine distinct points from each sample with a mass ranging from 3 to 5 g for each point. The nine points are chosen to be located at three height levels (top, middle, and bottom) and within three axes for each level (center and both sides). The collected soil is oven-dried for 24 hours at 105 °C and weighed. After that, HCl is added to solve the precipitated calcium carbonate. The process can be facilitated by shaking the mixture. After ensuring that there are no more sand crumbles and no more generated bubbles upon the addition of HCl, the mixture is filtered and washed several times with distilled water. The filtered soil is again oven-dried for 24 hours at 105 °C and weighed. The weight of calcium carbonate is the difference between those two weight measures. The content of calcium carbonate is the calcium carbonate weight divided by the weight of clean sand without cementation.

## **RESULTS AND DISCUSSION**

### **Effect of Relative Density**

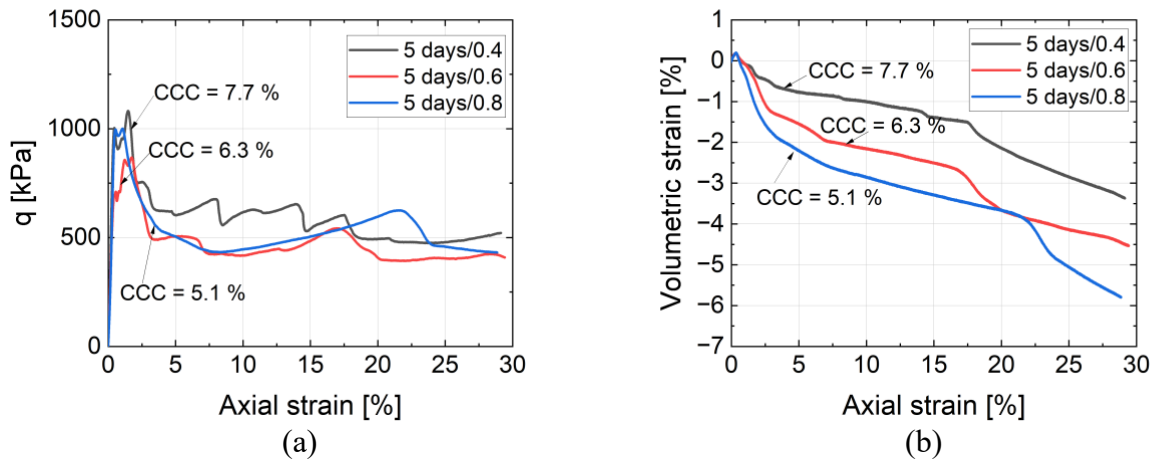
Figure 2 shows the results of the drained triaxial tests conducted under a confining pressure of 100 kPa on samples with three different initial relative densities. The diagrams present the curves of deviatoric stress  $q$  and volumetric strain  $\varepsilon_v$  versus axial strain  $\varepsilon_1$ . All samples were subjected to the same bio-cementation treatment for a duration of 5 days. Peak deviatoric stresses of 1,083 kPa, 869 kPa, and 1,001 kPa have been measured for samples with initial relative densities of 0.4, 0.6, and 0.8, respectively. It is well known for clean sand that the peak stress increases with the increase of relative density. In contrast, this is not the case for bio-cemented sand for which the sample with the lowest initial relative density exhibits the highest peak as well as residual deviatoric stresses, alongside the highest calcium carbonate content (CCC = 7.7%). In terms of volumetric behavior, dilation was observed to increase with higher initial relative density.

Despite undergoing identical treatment conditions and durations, the samples demonstrated varying levels of calcium carbonate. This phenomenon can be attributed to the initial pore volume available for calcium carbonate crystal formation. The sample with the largest pore volume in its loose state provided more space for calcium carbonate precipitation compared to the denser samples, explaining the observed decrease in cementation levels of 7.7%, 6.3%, and 5.1% with increasing initial relative density. In addition, the higher permeability of loose sand facilitates the flow of the cementation solution through the specimen, which may also enhance the precipitation of calcium carbonate. Moreover, the microbial activity can affect the kinetic characteristic of calcium crystal formation by the alteration of the environment chemistry. In this study, the optical density  $OD_{620}$  is chosen to be within a specific range rather than at a distinct value. This can cause variation in the supersaturation level, affecting the crystallization stages, the nucleation and crystal growth.

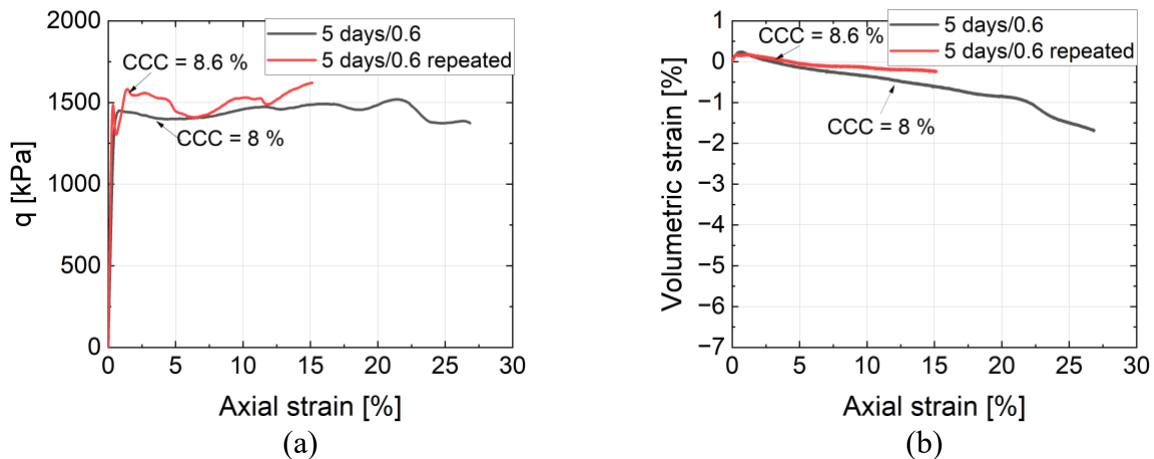
In contrast to the results of this study, Li et al. (2024) found that calcium carbonate content increased with increasing density for bio-cementation with both microbially induced calcium carbonate precipitation (MICP) and Enzyme induced calcium carbonate precipitation (EICP). However, they provided no information regarding the grain size distribution and argued that the formed calcium carbonate in the loose state could be washed away due to repetitive injections. In the current study, however, a fixation solution was applied during mixing the sandy soil with bacteria to prepare the samples. This can promote the adsorption and attachment of the bacterial cells to sand particles, and consequently, promote the fixation of the formed calcium carbonate. Additionally, van Passen et al. (2009) analyzed the relationship between unconfined compressive strength, density and calcium carbonate content. They stated that lower relative density resulted in higher calcium carbonate content but lower UCS. This can be related to the efficiency of the calcite in bonding the host sand.

### Repeatability

To test the repeatability of the mechanical properties of bio-cemented sand, two tests with medium initial density under a confining pressure of 400 kPa were conducted. Figure 3 shows the stress-strain relationship and the volumetric strain for both tests. The overall behavior confirms the repeatability of the tests. The samples had a difference of 0.6% in calcium carbonate content. The sample with the higher cementation level (8.6%) resulted in higher peak stress with a difference of around 61 kPa and less dilation with a difference of volumetric strain at 15% of axial strain of 0.37%. However, the scattering can be related to the distribution of calcium carbonate within the sample as well as the spatial variation in the bonding generation and destruction, which affect the load transfer mechanism through the cemented sand and the formation of shear bands.



**Figure 2. Drained triaxial tests under a confining pressure of 100 kPa: Effect of initial relative density of sand samples bio-cemented for 5 days. a) Deviatoric stress and b) volumetric strain versus axial strain.**

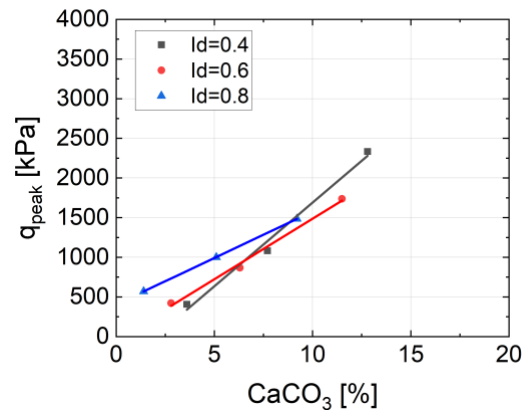


**Figure 3. Drained triaxial tests under a confining pressure of 400 kPa: Repeatability of bio-cemented sand samples with medium initial relative density treated for 5 days. (a) Deviatoric stress and (b) volumetric strain versus axial strain.**

### Relationship between Calcium Carbonate Content and Peak Deviatoric Stress

Figure 4 shows the relationship between calcium carbonate content (average value for each sample) and peak deviatoric stress derived from the drained triaxial tests conducted under a confining pressure of 100 kPa and varying initial relative densities. The range of calcium carbonate content resulted from the variation of the duration of cementation treatment (ranging from 2 to 10 days). For all relative densities, peak deviatoric stress increases with calcium carbonate content. This is in accordance with other studies, for example Dejong et al. (2022) and Muhammed et al. (2020). In case of calcium carbonate contents less than about 7%, the samples with dense initial state exhibit higher peak stress than the medium dense and loose ones. However, at calcium carbonate contents higher than 7%, the loose initial state demonstrates higher peak deviatoric stress. Similarly, Xiao et al. (2019) studied the effect of relative density on the cyclic response and concluded that the loose bio-cemented samples had higher improvement factor than the dense ones. In their study, this conclusion was drawn for a calcite increment up to 10 g, however, the calcium carbonate content as a percentage was not determined.

The relationship between calcium carbonate content and peak deviatoric stress could be explained by the fact that at low cementation level the interparticle friction of sand is mainly controlling the shear strength. Conversely, the cohesion caused by cementation becomes more prominent with higher cementation level, causing higher peak stress for the loose initial density. It is possible that larger pore spaces facilitate the formation of larger calcium carbonate crystals, which are more effective at bridging sand particles. In other words, since the sand particles may be connected by these larger calcium carbonate crystals, the peak stress increased. However, if the pore size is large but the amount of calcium carbonate is insufficient, the sand particles may not be adequately bridged, and as a result, no significant strength gain would be observed.

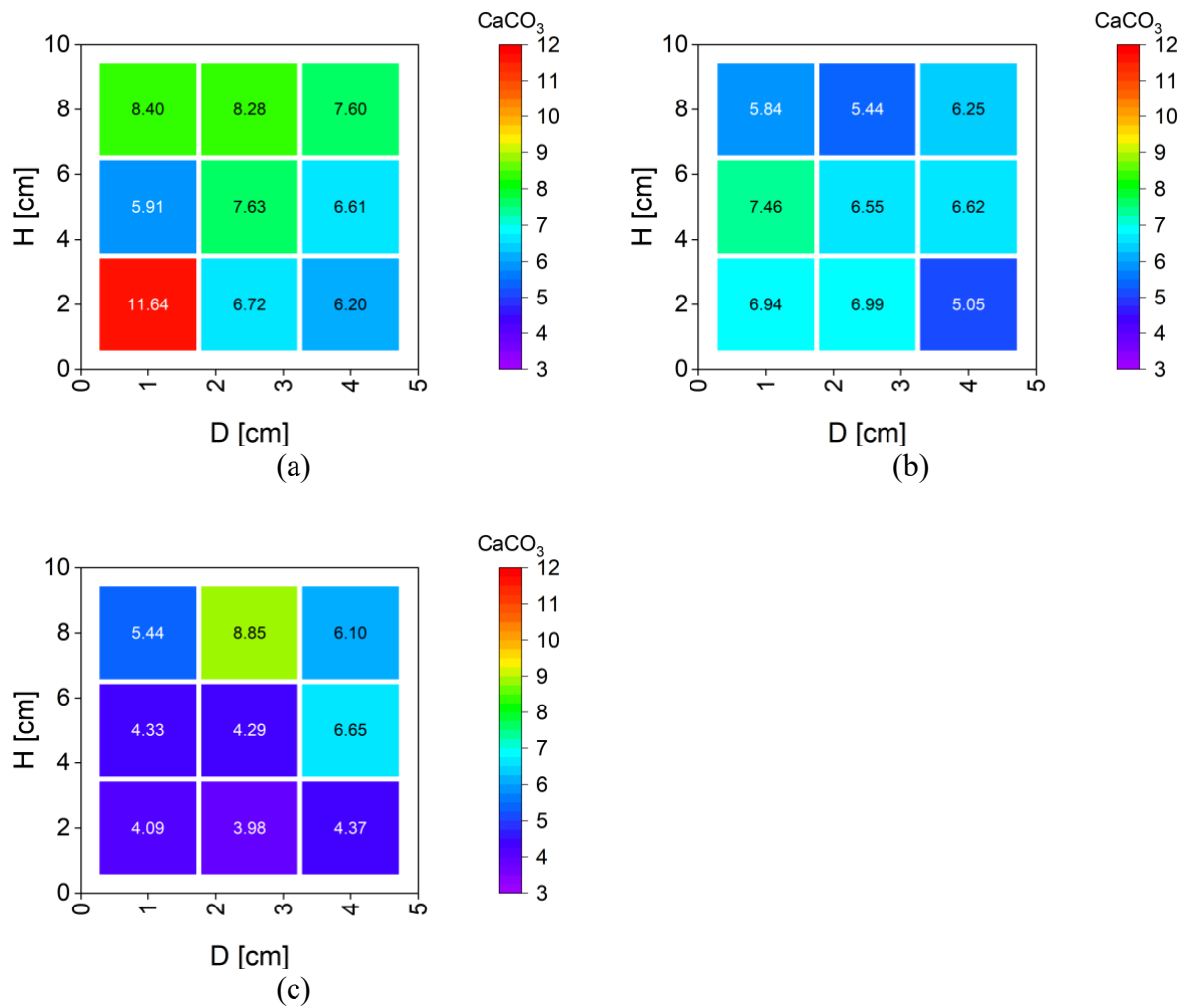


**Figure 4. Drained triaxial tests under a confining pressure of 100 kPa: Relationship between calcium carbonate content and peak deviatoric stress.**

### Distribution of Calcium Carbonate Content

Figure 5 shows the distribution of calcium carbonate content determined after the drained triaxial tests conducted on samples with three different initial relative densities, treated with bio-cementation for 5 days. Their mechanical responses are shown in Figure 2. The average calcium carbonate contents for the loose, medium dense, and dense samples are 7.7%, 6.3%, and 5.1%, respectively. This reveals a decrease in cementation levels as density increases. A similar trend was obtained by Gao et al. 2019.

For the loose sample, shown in Figure 5(a), the CCC is ranging between about 6% and 8%, with the exception of a higher concentration at the bottom left corner (11.6%). In the medium dense sample shown in Figure 5(b), the lowest levels of cementation are observed at the top center, near the injection inlet. In the dense sample (Figure 5(c)), the bottom part exhibited lower cementation. The results can be explained by the cementation solution passing through the larger pore space of the loose sample more freely, leading to a more uniform distribution of the solution in the sample, as opposed to its flow being impeded in the dense state with smaller pores. However, the higher cementation level at the bottom-left corner of Figure 5(a) for the loose sample can be probably explained by the transportation of calcium carbonate particles formed within each injection due to the larger pore spaces and their accumulation near the outlet during the drainage process.



**Figure 5. Calcium carbonate distribution in samples bio-cemented for 5 days, categorized by varied initial relative densities: (a) 0.4, (b) 0.6, and (c) 0.8.**

## CONCLUSION

A series of drained triaxial compression tests was conducted to investigate the impact of the initial relative density on the mechanical behaviour of bio-cemented sand samples. The repeatability of the sand improvement by bio-cementation was also a matter of this study. Bio-cemented samples were produced by mixing *Sporsarcina paasteurii* with sand, followed by compaction of the sand at three different initial relative densities within a mould, and multiple injection of the cementation solution. The samples were afterwards subjected to mechanical testing and calcium carbonate content analysis. The main findings of the experimental investigations were as follows:

- For the same bio-cementation treatment, the calcium carbonate content was found higher in the initially loose samples compared to the dense ones.
- For all tested densities the peak deviatoric stress increased with increasing calcium carbonate content.

- At low cementation levels denser samples showed higher shear strength than the looser samples, while it was the other way around for higher cementation degrees.
- The repetition of triaxial tests on sand with similar bio-cementation treatment resulted in overall comparable findings, demonstrating a good repeatability.

## ACKNOWLEDGEMENTS

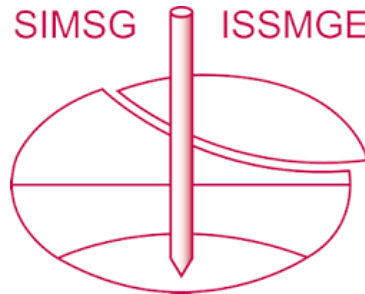
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