Proceedings of the 17th Pan-American Conference on Soil Mechanics and Geotechnical Engineering (XVII PCSMGE), and 2nd Latin-American Regional Conference of the International Association for Engineering Geology and the Environment (IAEG), La Serena Chile, 2024.

ROCK FRACTURES BIO-SEALING THROUGH MICROBIOLOGICAL INDUCED CALCIUM CARBONATE PRECIPITATION.

Bio-sellado de fracturas en rocas mediante la precipitación de carbonatos inducidos microbiológicamente.

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ABSTRACT:

Microbiologically induced carbonate precipitation (MICP) has been used to improve the mechanical properties of soils, concrete and rocks. The use of microorganisms is proposed as an alternative treatment. Three assemblies were made with fractured rock cores, using X-ray diffraction (XRD) for characterization. *Sporosarcina pasteurii* DSM 323 was used in Van Paasen culture medium, with $CaCl_2$ and urea 0.5 molar (M). Four variables were measured, in addition to quantifying the precipitation of $CaCO_3$ (CC). In test 1, polyvinyl chloride (PVC) pipes and rock cores were used, generating CC precipitation in the spaces between internal PVC diameter and external core diameter, without evidence of sealing of fractures with a efficiency of 70%, in relation to the precipitation of CC (with calcite (78.9%) and vaterite (21.1%)). In trial 2, acetate sheets were used covering the rock cores, renewing $CaCl_2$ and urea on day 3. An efficiency of 75% (with calcite (60%) and vaterite (40%)), evidencing partial filling of the fractures. Finally, test 3 was made with total renewal of the culture medium and the cementation solution on day 3. A depletion of calcium on 3rd and 6th days, with an efficiency of 65% (100% calcite), showing a better sealing of fractures. It can be concluded that CC precipitation was successful, with some differences for the different assemblies.

KEYWORDS: Bio-sealed, Precipitation, *Sporosarcina pasteurii*, Urease.

1. INTRODUCTION.

The massive and systematic fracturing of rocks is a common situation in the geological environment. This condition creates favorable conditions for landslides or instability in geological areas common in various settings such as natural or artificial slopes, considered a geologic risk of significant importance and concern. Rock instability is controlled and defined by various discontinuities such as stratification in sedimentary rocks, foliation in metamorphic rocks, as well as faults, fissures, fractures, among others, resulting from the diverse processes of genesis of different rock masses or the subsequent effects of tectonism on them, leading to additional costs and maintenance expenses (Bear et al., 1993; Berkowitz, 2002; Bonnet et al., 2001; de Dreuzy et al., 2012; Olson et al., 2009; Tsang & Neretnieks, 1998). Therefore, the study, control, and mitigation of these factors are of vital importance for civil and mining engineering. Most fractures and fissures in rock masses are immediately permeable after their formation, providing pathways for fluid flow in formations. Hence, the sealing of porous rocks or soils, as well as their fractures and fissures, is crucial (Bergsaker et al., 2016; Eppes & Keanini, 2017; Olson et al., 2009).

Grouting in fractured rock masses has been one of the primary choices for controlling the entry of groundwater and other phenomena, such as slope instability and tunnel-related issues (Abo-El-Enein et al., 2012; Stanaszek-Tomal, 2020). The purpose is to seal off groundwater ingress, reinforce in-situ soils and rock masses, especially in the implementation of underground mining projects and, more broadly, in open-pit environments. Conventional cement-based processes have been employed, including clay suspensions (bentonite) or chemicals such as soluble glass (water glass), acrylamide, polyurethanes, or epoxies, ensuring no water flow and, consequently, safe excavation and/or mining operations (Choi et al., 2017; Mujah et al., 2019; Portugal et al., 2020)

Thus, in order to develop a safe method to automatically repair fractures in rocks, ensuring their durability, functionality and cost-effectiveness, a technique called *Microbiologically Enhanced Crack Remediation (MECR)* has been proposed (Abo-El-Enein et al., 2012). In this method, ureolytic bacteria are used to produce a biocement (Choi et al., 2017; Mujah et al., 2019), which has the ability to continuously precipitate calcium carbonate.

Consequently, fractures can be quickly sealed by bioprecipitation of this mineral, hindering the entry of water and other chemical compounds. In this way, bacteria improve the strength of various composite materials by repairing cracks on the surfaces of their structures (Choi et al., 2017; Mujah et al., 2019; Portugal et al., 2020). This process, known as *Microbiologically Induced Calcite Precipitation (MICP)*, involves the bioprecipitation of calcium carbonate, a phenomenon widely investigated for various technological and scientific applications such as remediation of historical monuments, remediation of soils and rocks (biogeotechnology), synthesis of calcium carbonates, among others (Arias et al., 2019; Phillips et al., 2013). Within biomineralization, calcifying bacteria, of the genus *Sporosarcina*, play a crucial role in modifying the natural environment, generating significant quantities at low environmental and economic cost (Omoregie et al., 2019).

The focus of this work is directed towards finding optimal conditions for the bioprecipitation of calcium carbonate, employing one calcifying microorganism directly on rocks, for the self-remediation of fractures, fissures, and other structures, thereby mitigating the effects on the instability of rock masses.

2. EXPERIMENTAL SETUP AND METHOD.

For the experimentation, *Sporosarcina pasteurii* DSM 323 was cultured in Van Paasen liquid medium (VP) at pH 8.0 (Yeast extract 20 g/L, NH_4Cl 10 g/L, $NiCl_2$ 10 µM), at a temperature of 30°C for 24 hours with an agitation speed of 120 revolution per minute (rpm). In all setups, an inoculum of *S. pasteurii* DSM 323 equivalent to 10% of the final volume was added. For the experimental setup, six pre-fractured core samples were used, and the initial weight of each of them was taken to verify the precipitation of carbonates on the core.

For the first experiment, the cores were placed in PVC tubes with dimensions of 0.2 m in height and 0.0762 m in diameter. One end of each tube was sealed with a standard market cap and the other with a plug made of gauze and cotton as shown in Figure 1. Additionally, the assembly (Batch) was done using the liquid medium VP and the cementation solution composed of calcium chloride ($CaCl_2$) and 0.5 M urea, adding it only once.

For the second and third setups, acetate sheets were used and rolled into a cylinder shape adjusted for each core in a unique way as shown in Figure 2. The acetate sheet was adhered to a plastic base of 0.0762 m diameter. The difference between the second and third experiments was the method of MICP evaluation on the rocks. For the second (addition), the calcium source and 0.5 M urea were added on the third day. In contrast, in the third set-up (medium change), on the third day all VP medium was changed, as well as the cementing solution and a new inoculum of *S. pasteurii* DSM 323. All setups were carried out until eight days of incubation at 30°C.

Four biotic and abiotic variables were measured for all assemblies. For cell growth, a Thermo Scientific Genesis 10 UV-Visible spectrophotometer at a wavelength of 600 nm and an Olympus CX31 optical microscope with Neubauer chamber (Achal et al., 2009) were used to determine cell growth. The specific growth rate was calculated from the exponential phase cell growth data of each microorganism. Urease activity was determined using the phenol-hypochlorite method (Natarajan, 1995). The pH measurements were carried out in a HACH HQ30d multiparametric equipment coupled with a pH probe (PHC101), and calcium was measured by the EDTA titration method, according to the method described in Standard Methods for the Examination of Water and Wastewater (3500-Ca B), using *Eriochrome Blue Black R* as an indicator.

Figure 1. Experimental setup number one using PVC tubes for bio-sealing fractures in rock.

XRD analysis was carried out to identify the main mineralogical phases of the precipitates previously macerated to 200 mesh. A Rigaku Miniflex II X-ray diffractometer with Cu Kα radiation, nickel filter, 30 kV and 15 mA and graphite monochromator were used. The scans were made in an interval of 3 to 80° 2θ, with a step of 0.02° and a counting time per step of 2 s. The results were further processed and analyzed with the X'pert Highscore Plus v. 2.2.9 software. In addition, a semi-quantification of the mineral phases was performed by means of the Rietveld method, using the described software.

Figure 2. Experimental setup number two and three using acetate sheets for bio-sealing fractures in rock using addition and change of media as different assemblies.

3. RESULTS AND DISCUSSION

The pH behavior of *S. pasteurii* DSM 323 is presented in Figure 3. For all the assays, it is observed that in general, the pH decreases after the first 24 hours due to the metabolism of the urea and calcium chloride source, then it begins to increase after 24 hours, being variable in each set-up. In the Batch type assembly, it is observed that this behavior is increasing, due to the generation of ammonium by the breaking of the urea molecule, making the pH

become more basic and having an increase of 1.25 units with respect to the initial pH. However, in the addition and medium change assemblies, it can be observed at 48 hours in both pH and biological treatment, the decrease in pH due to the new sources of the cementation solution. Subsequently, it is observed that the pH of the addition set-up increases 0.37 units with respect to the initial pH, on the contrary, the pH of the medium set-up increased 1.96 units reaching a value of 10.11, a value so alkaline that it is not favorable for cell growth for microbial metabolism. In addition, no increase in the pH value of the controls was observed, which were evaluated at the same conditions in all experiments, but without the addition of the biological agent, which in our case was the bacterium *Sporosarcina pasteurii* DSM 323, indicating the safety of these rock cores due to the non-formation of $CaCO_3$. The pH is a key factor that governs the MICP process, pH conditions higher than 8.0, favor the formation of carbonates from bicarbonate (Anbu et al., 2016; De Muynck et al., 2013; Németh et al., 2018), in addition, pH influences the solubility and bioavailability of nutrient, being more efficient the process in a semi-alkaline medium (pH 7.5 - 8.5) (Hashim et al., 2011). On the contrary, native microorganisms have been reported to have the capacity to grow in very alkaline pH values higher than 10 (J. L. Arias & Fernández, 2008; Kawaguchi & Decho, 2002; Lian et al., 2006; Warthmann et al., 2000; Wei et al., 2015), however, in our case it is not possible to visualize an improvement in cell growth but rather a decrease in it by slowing down carbonate precipitation.

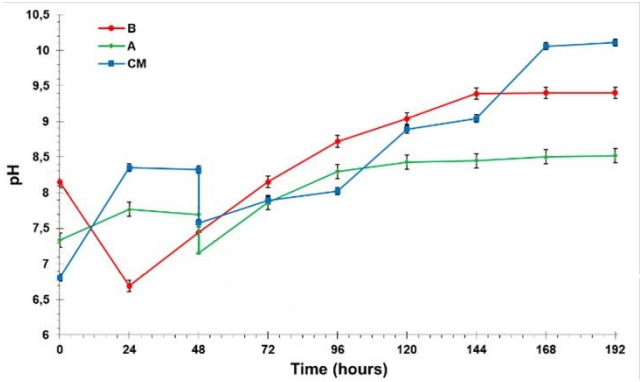

Figure 3. Behavior of pH with respect to time in three distinct types of setups. Letter B indicates Batch type set-up, letter A, addition set-up, and letters CM, the third medium change set-up.

Cell growth is indicative of the metabolic capacity of *S. pasteurii* DSM 323 to generate metabolites and compounds of interest such as calcium carbonate. In general, in Figure 4., it can be observed that cell growth in the Batch type set-up has a period of cell adaptation during the first 24 hours, after which the microorganism grows exponentially until it remains constant for the rest of the incubation time. On the contrary, in the addition and medium change assemblies it can be observed that the growth starts being exponential, being higher in the addition than in the medium change at 48 hours, at which point the population decreases due to the calcium and urea sources added, after this, a tendency of increase in the biomass and subsequent decay can be observed, possibly indicating a cell death, at which point the precipitation of carbonates stops and the metabolic process comes to an end.

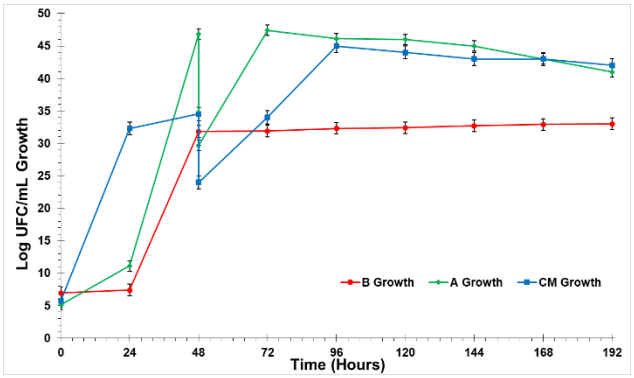

Figure 4. Cell growth of *Sporosarcina pasteurii* DSM 323 in three different setups with respect to time. The letter B indicates the Batch type configuration, the letter A, the addition configuration, and the letters CM, the third medium change configuration.

For MICP-mediated calcium carbonate precipitation, several metabolic pathways of bacterial growth have been reported. Among all of these, urea hydrolysis, ammonification, denitrification, sulfate reduction, mediated by extracellular polymeric substances and photosynthesis (Lian et al., 2006; Warthmann et al., 2000; Wei et al., 2015), however, of all the mentioned metabolisms, the least complex, most reported and where higher percentages of carbonate precipitation have been obtained, is by urea hydrolysis, catalyzed by the enzyme urease (Rajasekar et al., 2017; Umar et al., 2016; Whiffin et al., 2007). In enzymatic hydrolysis, one mole of hydrolyzed urea generates one mole of ammonium and through various biochemical reactions, carbonate ion is synthesized to allow precipitation of calcium carbonate when this metal is added or in the environment. However, the generation of the ammonium ion increases the pH of the medium and the reaction continues spontaneously favoring the formation of calcium carbonate. It is at this point, where the urease activity can be measured, that the ammonium ions make it possible to quantify the amount of enzyme synthesized by *S. pasteurii* DSM 323. In our results, it was determined that the highest urease activity is at 24 hours of cell growth. Likewise, it was evidenced that the highest urease activity of *S. pasteurii* DSM 323 in the three assemblies obtained was a value of 530 $U.ml^{-1}$ for the medium change assembly, followed by the addition assembly with an enzymatic value of 138 $U.mL^{-1}$ and 73 $U.mL^{-1}$ in the Batch type assembly, allowing to determine that the renewal of VP culture medium, cementing solution and inoculum allows to improve the conditions of microbiologically induced carbonate precipitation.

In addition to the above, it should be noted that carbonate precipitation is obtained by the reaction between the carbonate ion generated by urease activity and the addition of a calcium source. In our experimentation we used $CaCl_2$, since it has been the calcium source with the highest reports and yields in works using MICP (Cheng et al., 2017; Cheng & Shahin, 2016; Mwandira et al., 2017; Portugal et al., 2020). In Figure 5, it can be seen that in a Batch system process or without adding nutritional sources to the assembly, the calcium source is exhausted after the third day (72 hours), indicating the end of the reaction, causing the precipitation of calcium carbonate stop. On the contrary, when there is a change of medium or an addition of cementing solution, it is possible to

provide a longer chemical and enzymatic reaction time to increase the amount of this mineral in the cracks of the rocks evaluated, thus allowing sealing these fractures.

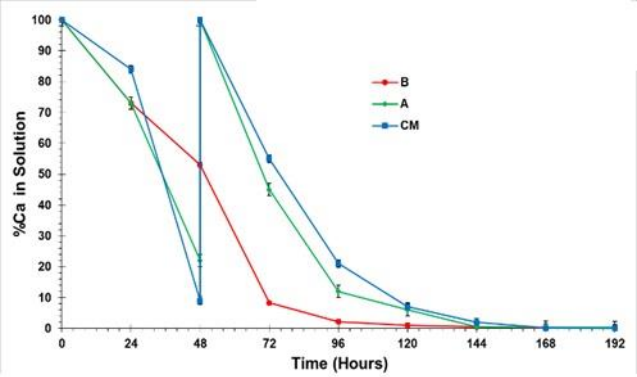

Figure 5. Calcium behavior in solution in three different setups. Letter B indicates Batch type set-up, letter A, addition set-up, and letters CM, the third medium change set-up.

At the end of the experimentation, disassembly and drying of the rock samples, it could be determined that depending on the type of assembly, the sealing of the fractures in the rocks in the biological treatment varied from one another. For the Batch type assemblies, 70% of the carbonates were precipitated according to the initial concentration of the calcium source (0.0105 kg), of which only 0.002 kg remained resting on and between some cracks of the evaluated core (Figure 6a-b.), the above mentioned can be verified in Table 1., where it is evident that the difference between the final and initial mass of the core corresponds to two units. This allows determining that the type of assembly with PVC tubes needs to be modified in such a way as to guarantee that the calcium precipitates in the form of calcium carbonate in the fractures of the core and not at the bottom of the vessel as occurred in this case. This could be achieved by adjusting the tubes or cylinders to be used to the dimensions of the cores to be treated.

Table 1. Quantification of precipitated $CaCO_3$ adhered or not adhered to the rock cores evaluated in three types of assemblies.

Mounting type	Initial mass (kg)	Final mass (kg)
Batch	0.458	0.460
Addition	0.434	0.448
Medium Change	0.411	0.416

On the contrary, in the addition type assemblies, it was possible to determine that there was a 6% increase in precipitation with respect to the Batch type assembly, in this assembly a change was made to acetate sheets, which allowed half of the total carbonate precipitated to adhere to the core and not at the bottom as in the Batch assembly. In total, 0.014 kg of carbonate was precipitated on the core (Table 1), thus allowing for improved sealing of the rock fractures (Figure 7a-b). Likewise, in the medium change set-up, having a greater nutrient source, it would be expected that the precipitation of carbonates on and between the fractures would increase, however, this is not observed in this set-up, only the fractures were sealed with an amount of 0.005 kg (Figure 8a-b), this may be due to the fact that by decanting to remove the culture medium it was possible to wash the calcium carbonate superimposed on or in the rock fractures, thus allowing its removal and precipitation in the external zone. However, despite this occurrence, the media change set-up was the best among the three for sealing the fractures in the rocks evaluated.

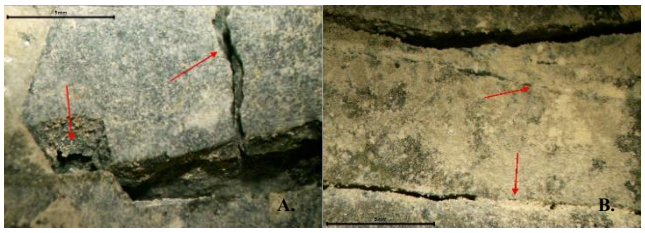

Figure 6. Rock core used in batch-type assembly (in 5 mm). a. Control core evaluated. b. Core evaluated with bioprecipitated carbonate. The red arrows indicate the empty (a) and calcium carbonate-filled (b) cracks.

Figure 7. Rock core used in Addition-type assembly (in 5 mm). a. Control core evaluated. b. Core evaluated with the presence of *S. pasteurii* DSM 323 and the bioprecipitated carbonate. The red arrows indicate the empty (a) and calcium carbonate-filled (b) cracks.

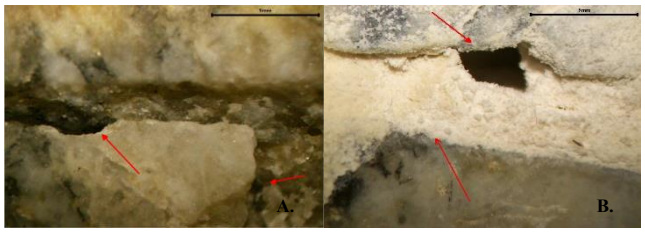

Figure 8. Rock core used in Medium Change-type assembly (in 5 mm). a. Control core evaluated. b. Core after exposure to a biological MICP treatment. The red arrows indicate the empty (a) and calcium carbonate-filled (b) cracks.

XRD spectra of the bioprecipitated carbonates (Figure 9a, b, c) showed the presence of calcite and vaterite as the main mineral phases formed in all treatments. As can be seen in Table 2, in all treatments the most abundant mineral was calcite (on average in all tests 79.6%). The occurrence of vaterite also showed a similar trend to that of calcite, its proportion being 20.4% in all experiments using 0.5 M calcium. It should be noted that vaterite was not determined in the Change of Medium setup, due to the elevation of pH above 10, which allows all the carbonate chemical species to rearrange the crystal cell parameters, allowing the more stable and energetically favorable polymorph to form which is calcite. This also allows us to determine that in the addition experiment, vaterite is in a higher proportion, being 40.2%, and that in relation to the pH of the medium, this treatment had a final

pH of approximately 8.5, allowing different crystalline species to form among the polymorphs of calcium carbonate, being more equitable the proportion of vaterite with respect to that of calcite.

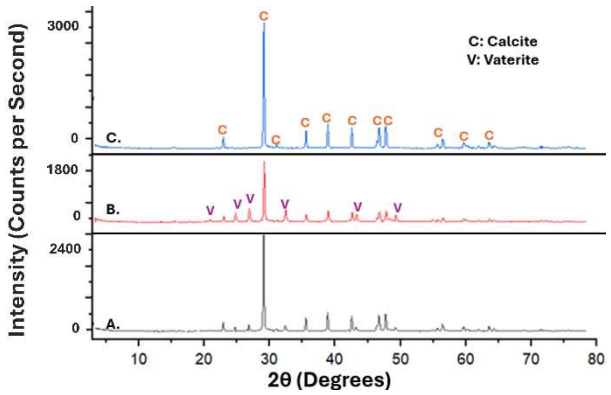

Figure 9. XRD spectra of precipitates produced by *S. pasteurii* DSM 323 in three distinct types of setups. A. Batch type. B. Addition. C. Change of medium.

Table 2. Semi-quantification of phases in XRD by the Rietveld method of precipitates obtained by *S. pasteurii* DSM 323 in three distinct types of assemblies.

Mounting type	Reference code	Mineralogical Fase and Percentage
Batch	ICSD 98-004-0107	Calcite (78.9%)
	ICSD 98-001-5879	Vaterite (21.1%)
Addition	ICSD 98-003-7241	Calcite (59.8%)
	ICSD 98-001-5879	Vaterite (40.2%)
Medium Change	ICSD 98-004-0107	Calcite (100%)

4. CONCLUSIONS

The overall efficiency of $CaCO_3$ precipitation is 70%, being the addition assembly with the highest efficiency with 76%, in fact, it was the one with the highest adhesion of $CaCO_3$, indicating that the method of addition of culture medium and cementation solution is the most optimal condition for *Sporosarcina pasteurii* DSM 323 to achieve growth without difficulty and bioprecipitate a greater amount of calcium carbonate. This is affected by biological agents and chemical composition of the medium in which it is carried out, on the other hand, the rock cores behave in an inert manner in the treatment allowing to determine that they may have some electrochemical affinity when they bioprecipitate $CaCO_3$. It was observed that in the three different assemblies $CaCO_3$ precipitation is in the form of calcite and vaterite, with calcite being the polymorph with the highest proportion in all three assemblies.

5. ACKNOWLEDGEMENTS

The authors acknowledges the financial support from Universidad Nacional de Colombia and the Project *"Biocalcificación como método de remediación microbiológica de fracturas en rocas - Hermes: 57344"*. The authors also thank the Biomineralogy and Biohydrometallurgy Laboratory (Universidad Nacional de Colombia, Medellín). As well as to the company Tecnisuelos S.A.S. for the donation of the drill cores.

6. REFERENCES

Abo-El-Enein, S. A., Ali, A. H., Talkhan, F. N., & Abdel-Gawwad, H. A. (2012). Utilization of microbial induced calcite precipitation for sand consolidation and mortar crack remediation. HBRC Journal, 8(3), 185–192. https://doi.org/10.1016/j.hbrcj.2013.02.001

Anbu, P., Kang, C. H., Shin, Y. J., & So, J. S. (2016). Formations of calcium carbonate minerals by bacteria and its multiple applications. SpringerPlus, 5(1), 1–26. https://doi.org/10.1186/s40064-016-1869-2

Arias, D., Cisternas, L. A., Miranda, C., & Rivas, M. (2019). Bioprospecting of ureolytic bacteria from Laguna Salada for biomineralization applications. Frontiers in Bioengineering and Biotechnology, 6. https://doi.org/10.3389/fbioe.2018.00209

Arias, J. L., & Fernández, M. S. (2008). Polysaccharides and proteoglycans in calcium carbonate-based Biomineralization. Chemical Reviews, 108(11), 4475–4482. https://doi.org/10.1021/cr078269p

Bear, J., Tsang, C. F., & De Marsily, G. (1993). Flow and contaminant transport in fractured rock. San Diego, CA: Academic Press.

Bergsaker, A. S., Røyne, A., Ougier-Simonin, A., Aubry, J., & Renard, F. (2016). The effect of fluid composition, salinity, and acidity on subcritical crack growth in calcite crystals. Journal of Geophysical Research: Solid Earth, 121, 1631–1651. https://doi.org/10.1002/2015JB012723

Berkowitz, B. (2002). Characterizing flow and transport in fractured geological media: A review. Advances in Water Resources, 25(8-12), 861–884. https://doi.org/10.1016/S0309-1708(02)00042-8

Bonnet, E., Bour, O., Odling, N. E., Davy, P., Main, I., Cowie, P., & Berkowitz, B. (2001). Scaling of fracture systems in geologic media. Reviews of Geophysics, 39(3), 347–383. https://doi.org/10.1029/1999rg000074

Cheng, L., & Shahin, M. A. (2016). Urease active bioslurry: A novel soil improvement approach based on microbially induced carbonate precipitation. Canadian Geotechnical Journal, 53(9), 1376–1385. https://doi.org/10.1139/cgj-2015-0635

Cheng, L., Shahin, M. A., & Mujah, D. (2017). Influence of key environmental conditions on microbially induced cementation for soil stabilization. Journal of Geotechnical and Geoenvironmental Engineering, 143(1), 1–11. https://doi.org/10.1061/(ASCE)GT.1943-5606.000158

Choi, S. G., Park, S. S., Wu, S., & Chu, J. (2017). Methods for calcium carbonate content measurement of biocemented soils. Journal of Materials in Civil Engineering, 29(11), 1–4. https://doi.org/10.1061/(ASCE)MT.1943-5533.0002064

De Dreuzy, J.-R., Méheust, Y., & Pichot, G. (2012). Influence of fracture scale heterogeneity on the flow properties of three-dimensional discrete fracture networks (DFN). Journal of Geophysical Research, 117, B11207. https://doi.org/10.1029/2012JB009461

De Muynck, W., Verbeken, K., De Belie, N., & Verstraete, W. (2013). Influence of temperature on the effectiveness of a biogenic carbonate surface treatment for limestone conservation. Applied Microbiology and Biotechnology, 97(3), 1335–1347. https://doi.org/10.1007/s00253-012-3997-0

Eppes, M.-C., & Keanini, R. (2017). Mechanical weathering and rock erosion by climate-dependent subcritical cracking. Reviews of Geophysics, 55, 470–508. https://doi.org/10.1002/2017rg000557

Hashim, M. A., Mukhopadhyay, S., Sahu, J. N., & Sengupta, B. (2011). Remediation technologies for heavy metal contaminated groundwater. In Journal of Environmental Management (Vol. 92, Issue 10, pp. 2355–2388). https://doi.org/10.1016/j.jenvman.2011.06.009

Kawaguchi, T., & Decho, A. W. (2002). A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing CaCO3 polymorphism. Journal of Crystal Growth, 240(1–2), 230–235.

https://doi.org/10.1016/S0022-0248(02)00918-1

Lian, B., Hu, Q., Chen, J., Ji, J., & Teng, H. H. (2006). Carbonate biomineralization induced by soil bacterium Bacillus megaterium. Geochimica et Cosmochimica Acta, 70(22), 5522–5535. https://doi.org/10.1016/j.gca.2006.08.044

Mujah, D., Cheng, L., & Shahin, M. A. (2019). Microstructural and geomechanical study on biocemented sand for optimization of MICP process. Journal of Materials in Civil Engineering, 31(4), 1–10. https://doi.org/10.1061/(ASCE)MT.1943-5533.0002660

Mwandira, W., Nakashima, K., & Kawasaki, S. (2017). Bioremediation of lead-contaminated mine waste by *Pararhodobacter* sp. based on the microbially induced calcium carbonate precipitation technique and its effects on strength of coarse and fine grained sand. Ecological Engineering, 109(July), 57–64. https://doi.org/10.1016/j.ecoleng.2017.09.011

Németh, P., Mugnaioli, E., Gemmi, M., Czuppon, G., Demény, A., & Spötl, C. (2018). A nanocrystalline monoclinic CaCO3 precursor of metastable aragonite. Science Advances, 4(12). https://doi.org/10.1126/sciadv.aau6178

Olson, J. E., Laubach, S. E., & Lander, R. H. (2009). Natural fracture characterization in tight gas sandstones: Integrating mechanics and diagenesis. AAPG Bulletin, 93(11), 1535–1549. https://doi.org/10.1306/08110909100

Omoregie, A. I., Ngu, L. H., Ong, D. E. L., & Nissom, P. M. (2019). Low-cost cultivation of *Sporosarcina pasteurii* strain in food-grade yeast extract medium for microbially induced carbonate precipitation (MICP) application. Biocatalysis and Agricultural Biotechnology, 17, 247–255. https://doi.org/10.1016/j.bcab.2018.11.030

Phillips, A. J., Gerlach, R., Lauchnor, E., Mitchell, A. C., Cunningham, A. B., & Spangler, L. (2013). Engineered applications of ureolytic biomineralization: A review. Biofouling, 29(6), 715–733. https://doi.org/10.1080/08927014.2013.796550

Portugal, C. R. M. e., Fonyo, C., Machado, C. C., Meganck, R., & Jarvis, T. (2020). Microbiologically Induced Calcite Precipitation biocementation, green alternative for roads – is this the breakthrough? A critical review. Journal of Cleaner Production, 262, 121372. https://doi.org/10.1016/j.jclepro.2020.121372

Rajasekar, A., Moy, C. K. S., & Wilkinson, S. (2017). MICP and Advances towards Eco-Friendly and Economical Applications. IOP Conference Series: Earth and Environmental Science, 78(1), 1–6. https://doi.org/10.1088/1755-1315/78/1/012016

Stanaszek-Tomal, E. (2020). Bacterial Concrete as a Sustainable Building Material? Sustainability, 12(2), 696. https://doi.org/10.3390/su12020696

Tsang, C.-F., & Neretnieks, I. (1998). Flow channeling in heterogeneous fractured rocks. Reviews of Geophysics, 36(2), 275–298. https://doi.org/10.1029/97RG03319

Umar, M., Kassim, K. A., & Ping Chiet, K. T. (2016). Biological process of soil improvement in civil engineering: A review. Journal of Rock Mechanics and Geotechnical Engineering, 8(5), 1–8. https://doi.org/10.1016/j.jrmge.2016.02.004

Warthmann, R., van Lith, Y., Vasconcelos, C., Mckenzie, J. A., & Karpoff, A. M. (2000). Bacterially induced dolomite precipitation in anoxic culture experiments. Geology, 28(12), 1091–1094.

Wei, S., Cui, H., Jiang, Z., Liu, H., He, H., & Fang, N. (2015). Biomineralization processes of calcite induced by bacteria isolated from marine sediments. Brazilian Journal of Microbiology, 46(2), 455–464. https://doi.org/10.1590/S1517-838246220140533

Whiffin, V. S., van Paassen, L. A., & Harkes, M. P. (2007). Microbial carbonate precipitation as a soil improvement technique. Geomicrobiology Journal, 24(5), 417–423. https://doi.org/10.1080/01490450701436505