

# The Potential Of Biostimulating MICP For Soil Stabilization And Geotechnical Risk Mitigation

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**RESUMO:** A demanda por métodos de estabilização de encostas com baixo impacto de carbono tem crescido diante do aumento de rupturas induzidas por chuvas intensas. Este estudo avalia o potencial da Precipitação de Carbonato de Cálcio Induzida por Microrganismos (MICP) via bioestimulação por bio-intemperismo como alternativa sustentável aos métodos tradicionais intensivos em CO<sub>2</sub> e ao MICP convencional baseado em ureia. Diferente da ureólise, o bio-intemperismo evita subprodutos tóxicos e pode contribuir para a captura de CO<sub>2</sub>, configurando-se como uma solução potencialmente carbono-negativa. Experimentos em laboratório compararam os dois caminhos metabólicos, ureólise e hidratação de CO<sub>2</sub>, mostrando que, embora a ureólise leve a precipitação mais rápida, o bio-intemperismo pode superá-la com tempos de cura prolongados. Testes adicionais demonstraram que a técnica melhora a resistência à compressão simples, especialmente em solos arenosos e siltosos. Já em solos argilosos, o desempenho foi limitado, possivelmente devido à menor permeabilidade e à restrição da mobilidade microbiana. Os resultados indicam que a eficácia do tratamento depende da estrutura do solo e é favorecida por maior permeabilidade. A técnica mostra-se especialmente promissora para estabilização superficial de encostas, reforçando a importância de estratégias bio-mediadas adaptadas ao local e avaliações integradas entre geotecnia, química e biologia.

**PALAVRAS-CHAVE:** Biogeotecnia, Biointemperismo, Precipitação de Carbonato de Cálcio Induzida por Microrganismos (MICP), Estabilidade de taludes, Soluções Baseadas na Natureza, Tecnologia de Carbono Negativo.

**ABSTRACT:** The demand for low-carbon slope stabilization methods has increased due to the growing number of rainfall-induced failures. This study evaluates the potential of Microbially Induced Calcium Carbonate Precipitation (MICP) via carbonic anhydrase biostimulation as a sustainable alternative to both traditional CO<sub>2</sub>-intensive methods and conventional urea-based MICP. Unlike ureolysis, carbonic anhydrase avoids the generation of toxic by-products and may contribute to CO<sub>2</sub> sequestration, positioning it as a potentially carbon-negative solution. Laboratory experiments compared the two metabolic pathways—ureolysis and CO<sub>2</sub> hydration—showing that, although ureolysis leads to faster precipitation, carbonic anhydrase can surpass it under extended curing times. Additional tests demonstrated that the technique improves unconfined compressive strength, particularly in sandy and silty soils. In clay-rich soils, however, performance was limited, possibly due to lower permeability and restricted microbial mobility. The results indicate that treatment effectiveness depends on soil fabric and is enhanced by greater permeability. The technique appears particularly promising for near-surface slope stabilization, reinforcing the importance of



site-specific bio-mediated strategies and integrated assessments combining geotechnical, chemical, and biological factors for successful field-scale applications.

**KEYWORDS:** Biogeotechnics, Carbonic anhydrase, Microbially Induced Calcium Carbonate Precipitation (MICP), Slope stabilisation, Nature-based Solutions, Carbon-negative Technology.

## 1 INTRODUCTION

Slope failures triggered by ever-more intense and prolonged rainfall events have become a defining geotechnical threat, driving landslides, erosion, and infrastructure disruption on every inhabited continent (Natalia & Yang, 2024). Effective slope stabilisation begins with a clear understanding of the instability itself. This requires identifying potential failure surfaces, assessing the mechanical behaviour of the soil, and understanding the hydrological and structural factors that contribute to failure. Only with this knowledge can targeted interventions be developed, interventions that act directly on the mechanisms responsible for loss of strength, increased pore pressure, or material detachment.

Traditionally, these interventions have included techniques such as cement grouting, lime treatment, and the use of chemical additives. Such methods aim to improve soil strength, reduce permeability, or provide surface protection against erosion. While often successful from a mechanical standpoint, they are associated with significant environmental drawbacks. High carbon emissions, leachate production, and the irreversible modification of natural soil structure and composition raise concerns regarding their long-term sustainability (Rekha N. & Ramakrishna U. B., 2022). A stabilisation strategy capable of reinforcing slopes without exacerbating the environmental conditions that contribute to their failure is therefore urgently needed.

In this context, biogeotechnics emerges as a promising field, offering solutions that integrate principles from biology and geotechnical engineering (DeJong & Kavazanjian, 2019). This discipline aims to mimic or harness natural biological processes to improve the mechanical and hydraulic properties of soils, providing a pathway for stabilization with reduced environmental impact. Biogeotechnics is generally divided into two main approaches: bio-inspired solutions, which are based on observing biological mechanisms to develop new technologies without the use of living organisms, and bio-mediated solutions, which actively employ microorganisms or their metabolic products to induce transformations in the soil. Among the bio-mediated approaches, Microbially Induced Calcium Carbonate Precipitation (MICP) has stood out as a technique with significant potential for soil improvement, enhancing particle bonding and reducing permeability (El Mountassir et al., 2018).

While conventional MICP—typically based on urea hydrolysis or denitrification—has demonstrated effectiveness in improving soil properties, it also presents notable environmental challenges. The release of by-products such as ammonia ( $\text{NH}_3$ ), ammonium ions ( $\text{NH}_4^+$ ), and nitrogen oxides ( $\text{NO}$ ,  $\text{N}_2\text{O}$ ) can be harmful to ecosystems and human health (Erşan et al., 2015; Osinubi et al., 2020; Stallings Young et al., 2021). In response to these limitations, recent research has focused on developing alternative MICP pathways that are more sustainable (César et al., 2024, 2025; Lopes, César, et al., 2025; Lopes, Chrusciak, et al., 2025; Mwandira et al., 2024). The biochemical pathway of carbonic anhydrase has emerged as one such alternative. This approach relies on the enzyme carbonic anhydrase produced by soil bacteria to accelerate  $\text{CO}_2$  hydration, leading to carbonate precipitation without generating toxic by-products. In addition to offering a cleaner biochemical route, this method may support carbon sequestration, providing a dual benefit of soil improvement and climate mitigation.

This study investigates the potential of MICP via carbonic anhydrase as a technique for mitigating geotechnical risks related to slope instability. It compares the biochemical mechanisms and laboratory outcomes of the carbonic anhydrase and urea-based pathways, focusing on differences in performance. Unconfined Compressive Strength (UCS) tests are used to assess mechanical behaviour following treatment, and the influence of soil fabric and composition is explored to understand how soil characteristics affect treatment outcomes. By framing these findings within the broader context of sustainable slope stabilisation, the study offers insights into how carbonic anhydrase MICP can contribute to the development of low-impact, resilient ground improvement strategies.



## 2 UREA-BASED MICP: PROCESS AND BENCHMARK RESULTS

The urea-based MICP process relies on the activity of ureolytic bacteria, such as *Sporosarcina pasteurii*, which catalyze the hydrolysis of urea to produce carbonate and ammonium. This reaction leads to the formation of calcium carbonate ( $\text{CaCO}_3$ ) in an alkaline environment, promoting cementation between soil particles. (Phillips AJ et al., 2013) outlined the fundamental principles underlying the viability of this technique, emphasizing that its effectiveness depends on the balance between reagent transport and reaction rates, urease activity, and control over the location of calcium carbonate precipitation.

The feasibility of the technique under field conditions was demonstrated by (Ghasemi & Montoya, 2020), who applied urea-based MICP on a coastal sandy slope via surface spraying. The results showed the formation of a hardened crust in the upper centimeters of the soil, with increased penetration resistance and a noticeable reduction in erodibility. Although the carbonate precipitation was largely limited to the shallow layers, the treatment significantly enhanced the cohesion of the exposed soil, demonstrating its potential to mitigate erosion on slopes with low initial cohesion.

In addition, Rahman et al. (2020) reviewed multiple laboratory studies reporting substantial increases in shear strength of treated soils, with values up to five times higher than those of untreated samples. A marked reduction in permeability was also observed, attributed to pore filling by carbonate crystals, which directly contributes to controlling undesired water flow on slopes. Gowthaman et al. (2023) further reinforced the sustainable potential of the technique by demonstrating that alternative, low-cost inputs such as fertilizer-grade urea and brewer's yeast could still achieve surface strengths exceeding 1 MPa, and biocement penetration up to 10 cm.

Despite its promising ability to improve soil strength and reduce permeability, urea-based MICP generates high concentrations of ammonia, which may harm local microbiota and the environment. Nevertheless, it remains a promising technique, and alternative MICP pathways are being explored to address these limitation and expand its range of applications.

## 3 COMPARISON BETWEEN UREA-BASED BIOSTIMULATION (UREOLYSIS) AND CARBONIC ANIDRASE (CA) BIOSTIMULATION ( $\text{CO}_2$ HYDRATION)

First of all, it is essential to benchmark the performance of carbonic anidrase against the more established urea-based MICP methods. To this end, direct comparisons were conducted between the two metabolic pathways—ureolysis and  $\text{CO}_2$  hydration—focusing on their respective abilities to precipitate calcium carbonate over time. By analysing treatments under identical environmental conditions, it becomes possible to isolate the influence of the microbial pathway itself on carbonate formation and, ultimately, the potential of carbonic anidrase as a viable alternative to conventional MICP.

Before conducting the comparison, appropriate nutrient formulations were selected for each pathway. For urea-based MICP, a well-established formulation commonly found in the literature and reported by Roth; Caslake; MacGuire (2022) was used. In contrast, César et al., (2024) conducted bench-scale tests to optimise the nutrient composition for carbonic anidrase, evaluating calcium source and concentration, glucose type, and incubation time. Their findings highlight the importance of tailored nutrient strategies to enhance  $\text{CaCO}_3$  precipitation and support the comparative analysis that follows.

The performance of specimens treated by carbonic anidrase biostimulation was compared to that of specimens treated by conventional urea-based biostimulation, based on the amount of  $\text{CaCO}_3$  precipitated. All treatments were applied to clean quartz sand to ensure that any carbonate precipitation could be attributed solely to the microbial process. The amount of  $\text{CaCO}_3$  precipitated was quantified by loss of ignition (LOI) analysis, and control samples treated only with distilled water were prepared to establish a baseline. For each treatment condition, duplicate specimens were produced to investigate reproducibility and assess variability.

The application protocols differed according to the metabolic pathway targeted. For carbonic anidrase biostimulation, specimens received 10 applications of a nutrient solution composed of 1.5 % w/v Ca-acetate, 0.4 % w/v yeast extract, and 0.5 % w/v dextrose, administered over a 10-day period, followed by one additional week (totaling 21 days) and 7.5 weeks (totaling 67 days) of incubation to allow mineralisation to progress. For urea-based biostimulation, the protocol followed the approach described by Roth; Caslake; MacGuire (2022) specimens were first treated for 5 days with a stimulation solution containing 0.1 % w/v yeast extract, 0.5 % w/v ammonium chloride, 0.35 % w/v sodium acetate, and 21 % w/v urea, and then for 5



days with a cementation solution composed of the same components plus 28 % w/v calcium chloride, specimens were tested also after 21 and 67 days of the first stimulation application.

As shown in Figure 1 after 21 days, specimens treated with urea-based biostimulation exhibited significantly higher  $\text{CaCO}_3$  contents than those treated by carbonic anhydrase (CA), reflecting the rapid precipitation kinetics associated with the ureolysis pathway. However, over the extended curing period of 67 days, the amount of  $\text{CaCO}_3$  increased for both treatments, with carbonic anhydrase showing a greater relative gain. By the end of 67 days, carbonic anhydrase biostimulation achieved higher  $\text{CaCO}_3$  contents than urea-based biostimulation, indicating that although the  $\text{CO}_2$ -hydration pathway progresses more slowly, it can ultimately result in greater overall mineralisation given sufficient incubation time.

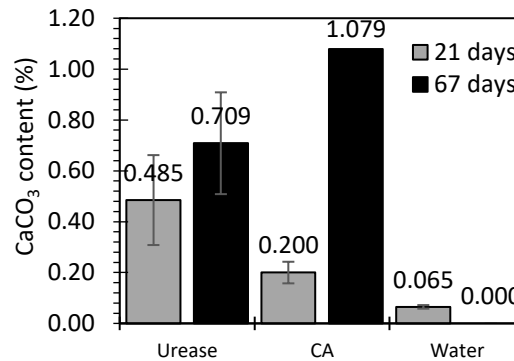


Figure 1. Comparison between urea-based and carbonic anhydrase (CA) biostimulation

#### 4 CARBONIC ANIDRASE BIOSTIMULATION: MECHANICAL BEHAVIOUR

Having established that carbonic anhydrase biostimulation can match or even surpass urea-based MICP in terms of carbonate precipitation under extended curing, the next step is to assess the mechanical benefits this pathway can offer on its own. While comparisons with ureolytic treatments help validate its potential, practical application ultimately depends on how effectively it improves the strength and stiffness of treated soils.

Compressive strength, in particular, serves as a key indicator of soil reinforcement, especially in shallow failure scenarios where surface crusting and particle bonding can enhance resistance to detachment and deformation. This chapter presents the results of Unconfined Compressive Strength (UCS) tests conducted on carbonic anhydrase biostimulation treated specimens, aiming to evaluate the structural performance of the technique in conditions relevant to slope stabilisation.

The soil used in this experiment was a silty sand collected from an experimental embankment site in Boa Vista, Brazil. The compaction curve was previously determined in the laboratory, and all specimens were remoulded at the optimum moisture content and compacted to the corresponding maximum dry density to ensure uniformity and reproducibility across test conditions.

The nutrient solution used in the carbonic anhydrase treatment was modified to include glucose, yeast extract, calcium acetate and sodium bicarbonate. The addition of sodium bicarbonate was a deliberate adjustment of the medium, aimed at enhancing the availability of inorganic carbon, acting as an indirect  $\text{CO}_2$  source in the process involving carbonic anhydrase, as proposed by Mwandira et al., (2024). This modification helps accelerate the carbonate precipitation process.

Prior to treatment, all samples were saturated with saline solution. This pre-treatment step was designed to simulate the ionic conditions typically found in natural soils and to improve ion mobility within the pore space, thereby facilitating microbial activity and promoting faster and more uniform mineral precipitation. Each specimen then received the nutrient solution twice a week for four weeks, followed by two additional weeks of incubation, resulting in a total treatment period of six weeks.

Figure 2 presents the results of the UCS test, conducted on both control and carbonic anhydrase biostimulated samples. As shown in Figure 2a, the biostimulated samples achieved an average compressive strength of 1137.54 kPa, compared to 541.71 kPa from the control group, an increase of over 100%. This substantial gain demonstrates the effectiveness of the carbonic anhydrase treatment in enhancing soil strength. The higher standard deviation observed among the biostimulated samples reflects the natural heterogeneity



of the MICP process, likely resulting from variations in microbial activity and carbonate precipitation within the soil matrix.

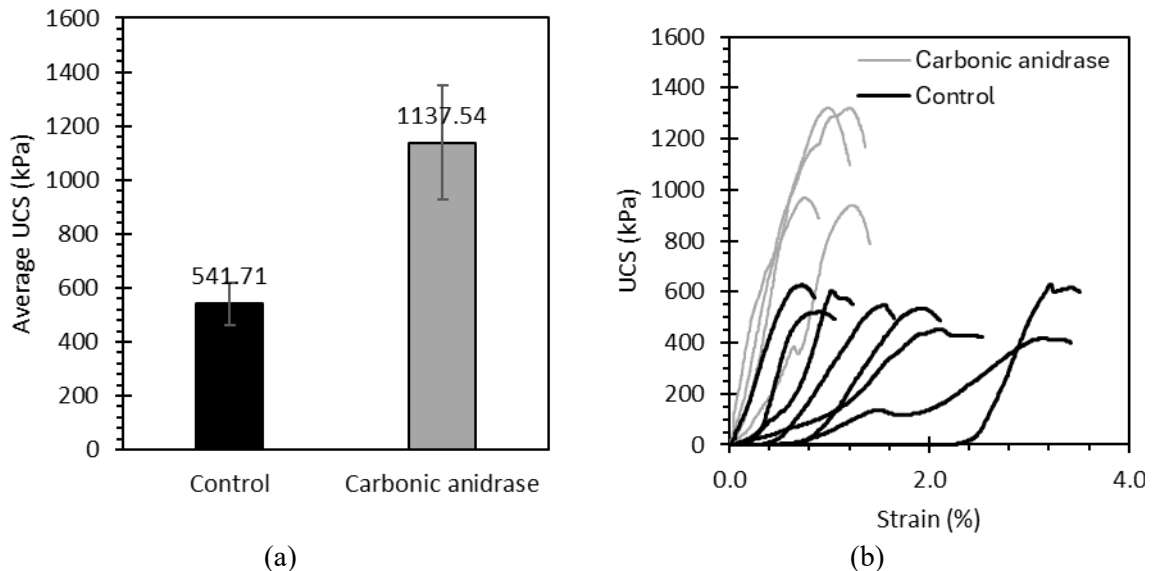


Figure 2. UCS Results: (a) Average UCS values (b) stress-strain curves for control and carbonic anidrase samples.

This strength gain is even more evident in Figure 2b, which shows the individual stress–strain curves. The biostimulated samples exhibited a stiffer behaviour, with higher peak strength and more abrupt failure, which is consistent with the formation of cementing bonds between soil particles. In contrast, the control group showed greater deformability and lower strength, reflecting a more ductile and less cohesive behavior.

In addition to confirming the occurrence of  $\text{CaCO}_3$  precipitation, the results showed maximum compressive strength values exceeding 1300 kPa, which are consistent with those reported by Gowthaman et al. (2023) in field-scale experiments of urea-based MICP using alternative inputs for slope stabilization. These findings suggest that carbonic anidrase biostimulation holds promise as a slope stabilisation technique, particularly given its reliance on native microorganisms and its environmental advantages over urea-based methods.

## 5 CARBONIC ANIDRASE BIOSTIMULATION: INFLUENCE OF SOIL FABRIC

Thus far, the results have demonstrated that carbonic anidrase biostimulation can induce substantial calcium carbonate precipitation and notable compressive strength gains under optimised laboratory conditions. However, the success of this technique in real-world applications depends on how the treatment interacts with the intrinsic properties of the soil. Factors such as particle size distribution, clay content, bulk density, and overall permeability can strongly influence microbial mobility, nutrient transport, and carbonate precipitation. This chapter presents findings from César et al. (2025) which investigated how variations in soil fabric affect the mechanical response to carbonic anidrase treatment, offering insight into the conditions that may enhance or constrain performance in practical geotechnical settings.

To explore the influence of soil fabric on carbonic anidrase performance, César et al. (2025) tested three soil types varying in particle size distribution and clay content. Each sample was treated with a bio-stimulation solution based on calcium acetate (1.5% w/v, 0.5% w/v dextrose and 4% w/v yeast extract) for 2 weeks daily, following 10 weeks of incubation. Control samples received distilled water only, under the same handling and curing conditions. Additionally, natural undisturbed specimens were collected to serve as reference points for baseline strength.

For each condition, duplicate specimens were produced to assess reproducibility and variability. To preserve the original soil structure as much as possible, all specimens—treated, control, and natural—were collected undisturbed or with minimal disturbance during sampling. After incubation, samples were removed from the mould and dried in an oven at 105°C for 24 hours. Subsequently, they were subjected to Unconfined Compressive Strength (UCS) testing at a strain rate of 1 mm/min.

UCS tests revealed that soils with lower clay content and sand-dominated textures exhibited the highest gains in strength following treatment, exceeding 400 kPa in some cases. In contrast, the soil with over 50% clay content showed limited improvement, with UCS values similar to or lower than those of the untreated controls (Figure 3a). This reduction in treatment effectiveness at high clay contents is consistent with reduced permeability, which can limit microbial mobility and nutrient transport—key factors in successful carbonate precipitation.

Interestingly, a trend was observed linking lower bulk density to higher UCS values in the treated samples (Figure 3b). While counterintuitive at first, this observation suggests that moderately porous structures may facilitate better microbial activity and carbonate nucleation by allowing easier gas exchange and nutrient penetration. Soils with lower clay content and moderate bulk densities appeared to provide the most favourable conditions for bio-cementation.

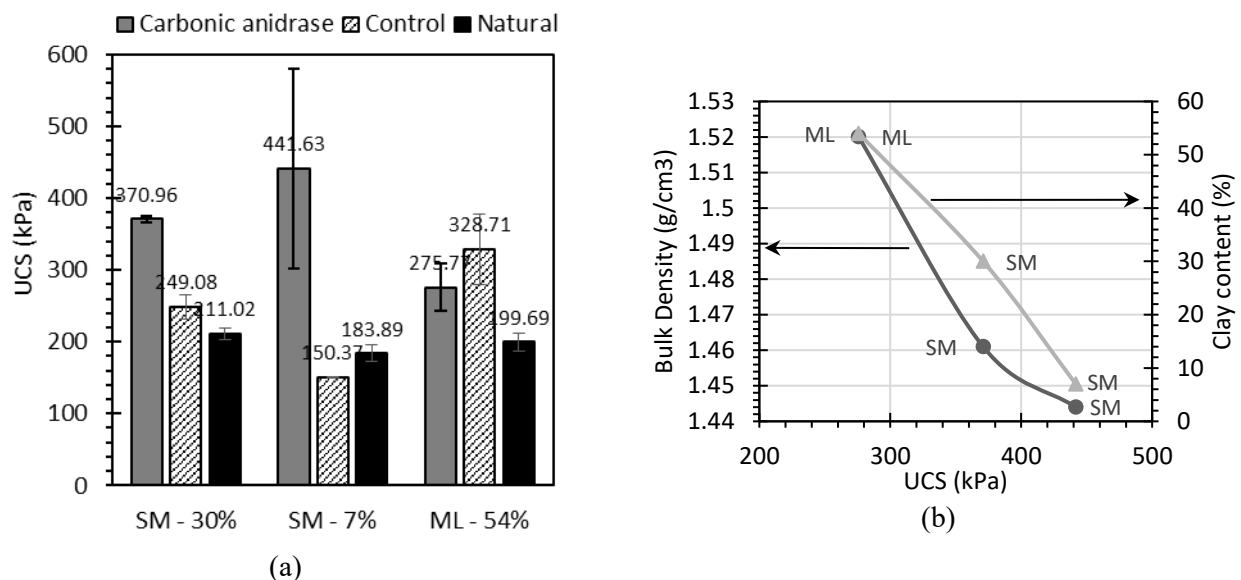


Figure 3. (a) UCS of treated and untreated samples, where SM: Silty Sand, ML: Low plasticity Clay and percentages represent clay content, (b) Correlation between UCS, bulk density, and clay content.

Stress–strain curves (Figure 4) further support these findings, showing stiffer, more brittle failure patterns in treated sandy and silty soils, and more variable, localised failure in the clay-rich specimen. These behaviours suggest less uniform improvement in finer-grained materials and highlight challenges for consistent performance in clay-rich slopes.

The findings suggest that physical soil characteristics, particularly particle size distribution, clay content, and bulk density, play a decisive role in the success of carbonic anidrase biostimulation. Soils with moderate fines content, higher permeability, and lower plasticity supported greater carbonate precipitation and strength gain. Conversely, clay-rich soils with high plasticity and low permeability presented clear challenges. Although direct biogeochemical measurements were not conducted, it is plausible that variations in native microbial populations, enzymatic activity (e.g., carbonic anhydrase production), and local carbon availability also influenced treatment outcomes. Future studies integrating physical, chemical, and biological data will be essential to fully optimise carbonic anidrase strategies for field use.

These findings also suggest that the effectiveness of carbonic anidrase biostimulation should be considered in light of the specific failure mechanism being addressed. In cases where slope instability is governed primarily by surface processes, such as erosion, shallow slip, or weathering-driven degradation, treatment can be focused on the near-surface zone, where carbonate precipitation is more readily achieved and microbial access is less restricted. In such scenarios, limited permeability and clay content may not pose significant constraints, as strengthening the uppermost soil layers may be sufficient to enhance stability. This highlights the importance of aligning treatment strategies with the depth and nature of instability when designing bio-mediated interventions for slope stabilisation.

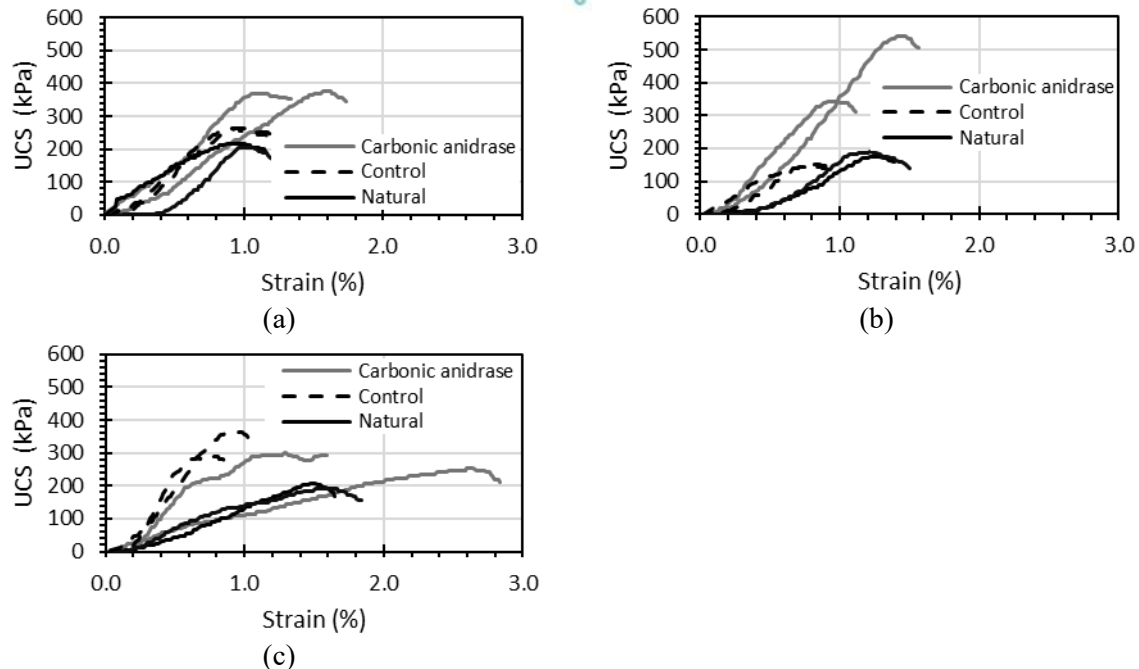


Figure 4. Stress-strain curves for: (a) SM, 30% clay content, (b) SM, 7% clay content, and (c) ML, 54% clay content, where SM: Silty Sand, ML: Low plasticity Clay.

## 6 CONCLUSIONS

This study investigated the potential of Microbially Induced Calcium Carbonate Precipitation (MICP) via carbonic anidrase biostimulation as a sustainable alternative to both traditional CO<sub>2</sub>-intensive soil stabilisation techniques and conventional urea-based MICP for slope stabilisation.

Through comparative experiments evaluating carbonate precipitation over time, carbonic anidrase was shown to match or surpass the performance of urea-based biostimulation, particularly under extended curing periods. Unconfined Compressive Strength tests further demonstrated that carbonic anidrase biostimulation can significantly enhance soil strength, especially in sandy and silty materials. Additional testing across soils with varying textures revealed that soil fabric, particularly clay content and bulk density, plays a decisive role in treatment effectiveness, possibly by influencing microbial mobility, nutrient transport, and the distribution of carbonate precipitation.

These findings support the use of carbonic anidrase biostimulation as a low-impact and potentially carbon-negative strategy for slope stabilisation, particularly where surface instability is the primary concern. In such cases, treatment efforts can be focused on the uppermost soil layers, where permeability is less restrictive and carbonate formation is more readily achieved. The results also highlight the importance of tailoring treatment strategies to specific soil conditions and failure mechanisms, reinforcing the need for integrated geotechnical, biological, and chemical characterisation when designing field-scale applications.

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