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Comparative Analysis of Microbiological and Enzymatic Methods for Strengthening Sandy and Weathered Soils to Mitigate Degradation and Desertification

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Abstract

Soil degradation and desertification represent significant environmental challenges, particularly in arid regions such as Kazakhstan. This research examines the potential of biological soil consolidation techniques to address these issues by enhancing soil strength. The objective of this study was to undertake a comparative analysis of two contemporary methods of calcium carbonate precipitation for the purpose of soil strengthening in sandy and weathered soils: microbially induced calcium carbonate precipitation and enzymatically induced calcium carbonate precipitation. Both methods are based on the precipitation of calcium carbonate, which is intended to enhance soil stability. The study was conducted on sandy soil samples collected from the vicinity of Aktau, Kazakhstan. The enzymatic precipitation method employed the use of urease, whereas the microbial approach involved the utilisation of *Sporosarcina Pasteurii* and bacterial isolates derived from the test site soil. Over the course of a 12-day experiment, the mechanical stability of the samples was assessed under vertical and horizontal loads. The findings demonstrate that both methods enhanced soil strength, with microbially induced calcium carbonate precipitation exhibiting greater efficacy, augmenting biocement stability by 2.5-3 times in comparison to the control, and enzymatically induced calcium carbonate precipitation yielding a 1.5-2 times improvement. The microbial method demonstrated a clear correlation between urease activity, calcium carbonate deposition, and mechanical resistance. The efficiency of soil-isolated bacterial strains was found to be comparable to that of the reference strain, indicating the potential for practical application. The results, validated in both laboratory and field conditions, indicate that these methods can be scaled for wider use in mitigating soil degradation and desertification.

Keywords: biological consolidation, biocement, calcium carbonate deposition, enzymatic deposition, microbially induced deposition.

1. Introduction

Soil degradation and desertification are significant environmental issues, especially in arid regions like Kazakhstan. Biological soil consolidation techniques, such as microbially induced calcium carbonate precipitation (MICP) and enzymatically induced calcium carbonate precipitation (EICP), have gained attention as sustainable solutions. MICP uses uratolytic bacteria to hydrolyze urea into ammonia and carbonate ions, which react with calcium ions to form calcium carbonate. These ions act as a binder for soil particles and serve as nucleation sites for calcium carbonate crystals. EICP, on the other hand, uses the enzyme urease without living organisms, catalyzing the same urea hydrolysis reaction to produce carbonate ions and calcium carbonate. However, EICP lacks the additional nucleation effect provided by bacterial cells in MICP. Both methods are environmentally friendly alternatives to chemical soil stabilization, but MICP generally achieves better results due to bacteria's role in enhancing calcium carbonate deposition. EICP is simpler to implement and may be more cost-effective in certain situations. This study aims to compare the effectiveness of MICP and EICP in consolidating sandy soils, particularly in desertification-prone regions, under laboratory and field conditions (Bulba et al., 2024).

The degradation of soil, driven by both economic activities and climate change, has accelerated the processes of desertification, particularly in arid regions. This has resulted in a notable decline in agricultural output, which directly endangers food security in Central Asia, including Kazakhstan. One of the most significant consequences of soil erosion is wind erosion, which not only reduces soil fertility but also has a considerable impact on biodiversity by destabilising local ecosystems. The loss of biodiversity, in conjunction with declining soil fertility, gives rise to a series of cascading effects, which serve to exacerbate the problem of desertification in these regions (Dagliya et al., 2022). The research conducted by Dagliya and colleagues

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underscores the critical necessity for the implementation of soil stabilisation techniques to address the significant issue of large-scale desertification in Kazakhstan. The expansion of arable land has led to the deterioration of soil quality and a considerable reduction in biodiversity, necessitating the urgent development of effective solutions to combat this alarming phenomenon. This underscores the global significance of addressing soil degradation.

In response to this growing problem, researchers have been investigating a range of technological solutions with the aim of stabilising sandy soils. In their comprehensive review of innovative soil stabilisation methods, Mymrin et al. (2019) present a range of promising techniques. The researchers analysed the use of casein, a protein that serves as a natural binder, as well as chitosan derived from shrimp shells, a biopolymer known for its soil-binding properties. Furthermore, Mymrin et al. investigated the potential of using drainage system sediments to enhance soil structure. One of their most significant contributions was a method involving the use of slag, an industrial by-product of lime production, to strengthen sandy soils. This approach offers an economically viable solution through the repurposing of waste materials, thereby aligning with sustainable environmental practices. The work of Mymrin et al. contributes to the broader search for innovative, cost-effective solutions to improve soil stability in vulnerable regions like Kazakhstan.

In contrast, Bhurtel et al. (2024) adopted an alternative methodology, investigating the potential of biotechnological techniques for enhancing soil strength. The comparative study of biomedical soil stabilisation methods demonstrated the potential of bacteria and fungi in enhancing soil strength. The utilisation of microbial and fungal agents in soil stabilisation has garnered attention due to their intrinsic interactions with the soil environment, facilitating soil cohesion without the necessity for deleterious chemical additives. Bhurtel et al. emphasised the potential of stabilisation as a method that could complement or even replace traditional techniques, thereby creating a more sustainable and eco-friendly approach to improving soil strength. This is particularly pertinent in the context of addressing soil degradation in regions where soil disturbance from agricultural activities is a significant contributing factor to desertification.

A noteworthy method of enhancing soil density and mechanical resistance to erosion is through the precipitation of calcium carbonate. Chittoori et al. (2021) conducted an investigation into this process, with a particular focus on its capacity to bind soil particles and enhance soil resistance to mechanical displacement. This process, which is commonly referred to as "biocementation", entails the extraction of calcium from the soil in order to precipitate calcium carbonate, which serves as a natural binding agent. The precipitation of calcium carbonate has gained attention due to its ability to improve soil stability, offering an effective solution to the problem of desertification, particularly in sandy soils, which are highly susceptible to wind and water erosion. This research highlights the potential of biocementation as a promising strategy for soil stabilisation in arid regions such as Kazakhstan.

In recent years, there has been a notable shift in the focus of research towards biological solutions, particularly the utilisation of MICP and EICP. The efficacy of these methods has been explored by studies conducted by Cuccurullo et al. (2022), Zomorodian et al. (2023), and Kim and Youn (2016), with particular emphasis placed on their environmental benefits in comparison to traditional chemical stabilisers. MICP employs the use of urease-producing bacteria, such as *Sporosarcina Pasteurii*, to induce calcium carbonate precipitation, whereas EICP utilises urease enzymes to achieve a similar outcome. Both methods are praised for their non-toxic, environmentally friendly, and relatively straightforward implementation. Furthermore, the scalability of these methods renders them suitable for large-scale applications, such as the mitigation of dust storms and soil displacement, which are prevalent in desert regions. Nevertheless, it is essential to acknowledge that while both techniques have demonstrated efficacy in diverse geographical locations, further investigation is necessary to ascertain their cost-effectiveness and long-term environmental consequences.

Richardson et al. (2016) traced the early developments of MICP, noting that the first studies on microbial calcium deposition mechanisms emerged in the early 2000s. These pioneering studies established the foundations for the accelerated development of biological soil stabilisation techniques, which subsequently attracted significant scientific interest. The research demonstrated the potential of MICP for applications beyond soil stabilisation, such as crack sealing in building materials, thereby opening up further avenues for innovation in biocementation technologies.

Erdmann and Strieth (2022) expanded on the applications of enzymatic and microbial calcium carbonate deposition, noting that these methods are not limited to soil stabilisation but also play a crucial role in the development of sustainable building materials. The researchers demonstrated the potential of biocementation in the construction industry, particularly in the reinforcement of building facades and the enhancement of construction material density. This cross-disciplinary application highlights the versatility of biocementation technologies and their potential integration into broader sustainability initiatives.

In Kazakhstan, initiatives have already been launched to utilise these technologies for the stabilisation of soil. In their respective studies, Kurmanbaev et al. (2017) and Sembaev et al. (2019) have concentrated on the identification of microorganisms that can be employed for MICP, as well as the development of optimal methods for biocementation in Kazakhstan's distinctive environmental circumstances. Their work underscores the necessity of selecting the appropriate microbial strains and biocementation methods that are tailored to specific soil and climate conditions. This is of great consequence, as the process of biocementation is a complex, multi-component phenomenon that is influenced by a multitude of factors, including soil type, climate, and microbial activity. As Zhang et al. (2023) observed, the selection of MICP, EICP, or an alternative method is largely contingent upon the specific environmental conditions prevailing at the site in question. This renders the process a highly intricate engineering undertaking, necessitating meticulous evaluation of a multitude of variables.

While the results of MICP and EICP in laboratory settings have been promising, there is a dearth of large-scale studies that directly compare the effectiveness of

these methods under real-world conditions in Kazakhstan's arid regions. As Zhang et al. (2023) have observed, research into biocementation has, to date, been largely confined to small-scale studies or specific environmental conditions. This has resulted in a notable gap in the existing literature with regard to the performance of these methods in different regional settings. This research aims to address the aforementioned gap in the literature by providing a comparative analysis of calcium carbonate deposition methods in sandy soils, with specific consideration of the environmental conditions prevalent in Kazakhstan.

There is a paucity of research comparing the efficacy of MICP and EICP methods in the unique environmental conditions of Kazakhstan. While both methods show promise, a comprehensive analysis comparing their performance in sandy and weathered soils, especially in arid regions like Kazakhstan, is lacking. This study addresses this gap by assessing the degree of soil consolidation achieved using both microbiological and enzymatic methods, thereby optimising the technology for producing biocement samples resistant to environmental degradation.

Also, this study provides insights into optimizing soil stabilization in arid environments, with potential implications for large-scale efforts to combat desertification, reduce soil erosion, and enhance agricultural productivity. The use of indigenous bacterial strains offers an environmentally friendly and cost-effective alternative to traditional chemical stabilizers, making this research a potential model for other regions facing similar environmental challenges.

The study aims to assess the degree of consolidation of sandy soil during biocementation using the methods of microbiological and enzyme-induced deposition of calcium carbonate and optimise the technology for producing biocement samples that are resistant to external environmental factors.

2. Materials and methods

In the investigation of the physicochemical properties of sandy soils, standard laboratory methods were employed, including pH determination, total dissolved solids measurement, and X-ray fluorescence analysis for mineral composition.

Natural soils contain a wide variety of microorganisms, including strains capable of producing urease. In this regard, the study attempted to isolate isolates from sandy soils. The material for isolates isolation was 3 samples of sandy soils collected in the vicinity of Aktau, Mangistau region of Kazakhstan. Its physicochemical properties were investigated using standard laboratory methods. Three different sand samples were used, taken at three points 500 m apart.

The bacterial strain *Sporosarcina Pasteurii* was used as a reference strain. The original strain was stored at -80°C. For activation, the cells were thawed following cryopreservation, washed to eliminate the cryoprotective medium, and subsequently cultured in nutrient medium at 30°C for 24 hours. The isolation of natural bacterial isolates was carried out by washing the sand samples with a sterile 0.85% NaCl solution. The isolates were cultured in medium containing 3 g/L nutrient broth, 10 g/L NH_4Cl, 25.2 mM $NaHCO_3$; 3.7 g/L $CaCl_2$, 20 g/L urea. The plates were incubated at 30°C for 5 days. The method of sand biocementation is based on the ability of microorganisms to release urease, a urea-degrading agent, into the environment, which, in the course of further transformations, decomposes into ammonia and carbonic acid. Carbonic acid breaks down easily and can react with free calcium ions in the soil. The resulting calcium carbonate forms crystals that can bind soil particles together and fill in gaps.

Determination of urease-producing bacterial isolates was performed using a selective Kirstensen medium containing the indicator phenol red. A 40% urea solution in a ratio of 1:10 was added to the prepared medium during cooling (to 50°C). After that, the medium was poured into tubes in an inclined position and, after solidification, was used for the cultivation of microorganisms. Incubation was performed at 30°C for 20-24 hours after inoculation. The presence of urease activity was concluded by the presence of a characteristic pink-crimson colour of the medium. Isolates showing a positive reaction were cultured on selective media for 24 hours. Microorganisms were identified by protein profiling using MALDI-TOF Microflex mass spectrometry (Bruker, Germany). The obtained spectra were analysed by comparison with the MBT Compass library. The urease activity was determined by the potentiometric method, and the activity was expressed in mol/L. The measurement results of all indicators were averaged across the samples. The enzymatically induced precipitation of calcium carbonate was carried out by adding a solution containing 1 M urea, 0.76 M calcium chloride ($CaCl_2$) and the enzyme urease at a concentration of 3 g/l to a sand column. A solution containing 1 M urea, 0.76 M calcium chloride ($CaCl^2$) and a bacterial suspension with a cell concentration of 10^6/ml was used for microbially induced calcium carbonate precipitation.

The laboratory test of calcium carbonate deposition was carried out in a cylinder filled with sand, 15 cm high and 5 cm in diameter. The weight of the sand was 500 g. The bacterial suspension was inoculated on days 1 and 6 of the experiment. Every day for 12 days, 10 ml of substrate solutions containing 1 M urea and 0.76 M calcium chloride ($CaCl_2$) were added to the cylinder. At the end of the experiment, they were dried naturally at room temperature. As a control, a solution containing 1 M urea, and 0.76 M calcium chloride ($CaCl_2$), but no urease or bacteria was used. The content of calcium compounds in the samples was determined by X-ray semi-quantitative method. Compressive strength was determined in the laboratory using a hand press, using the vertical loading method. The study was carried out using the KP-9 compression device. The shear strength was determined by conducting shear tests under a horizontal load using a P10-C shear tester.

The field tests were carried out with the reproduction of the laboratory experiment scheme by laying out experimental plots of $1m^2$. The boundaries of the experimental plots were fenced with four metal plates 1 m long and 50 cm high, which prevented sand from moving and mixing within the plot. The solutions were applied by spraying onto the surface. The volume of the sprayed solution was 2 litres/m^2. Bacterial solutions were added on days 1 and 6, and substrates were added daily for 12 days. Compressive and shear strengths were determined in

samples taken from the experimental plots on day 12 of the experiment. The compressive strength was determined in surface layer samples taken from a depth of 10 cm using a hand press. The samples were delivered to the laboratory, where physical and mechanical tests were carried out. All measurements were made 3 times.

The shear strength of the soil is the most important indicator for determining the resistance of sandy soils to weathering. Therefore, the soil shear test was carried out directly in the field using the rotary shear method with a rotary impeller shear meter. The results of the measurements and calculations of the studied indicators are presented in the form of mean (±) standard deviation. The statistical significance of the differences was assessed using the parametric T-test. In instances where discrepancies were identified between the groups, p-values were calculated to ascertain the statistical significance. To illustrate, the comparison between the microbiological and enzymatic methods revealed that the compressive strength differences yielded a p-value of $p<0.05$, indicating statistically significant improvements in the MICP-treated samples in comparison to the control group. Additionally, confidence intervals for the compressive strength values were calculated. The MICP-treated samples exhibited 95% confidence intervals of 740.4 to 784.3 kPa, whereas the control had a CI of 240.2 to 258.4 kPa. Similarly, the EICP method yielded a compressive strength with a 95% confidence interval of 360.3 to 377.0 kPa.

3. Results

The first work characterised sand samples obtained from the sites where further studies were carried out. The samples were analysed for indicators relevant to the study. The physical, chemical, and mechanical characteristics of the soil samples employed in this study are presented in Table 1. These include pH, total dissolved solids, chloride (Cl) content, silicon dioxide (SiO_2), calcium oxide (CaO), and calcium carbonate ($CaCO_3$).

Table 1. Physical, chemical and mechanical characteristics of the soil under study

Indicator	Units of measurement	Value
pH		8.2±0.12
TDS	‰	455±5.7
Cl	‰	40±0.15
SiO_2	%	97±1.1
CaO	%	1.5±0.2
$CaCO_3$	%	1.5±0.2
ρ	mg/cm^3	1.5±0.05
Compressive strength	kPa	227±12.5
Shear strength	kPa	210±10

Source: compiled by the authors.

These parameters were of great importance to establish the baseline condition of the soil before the commencement of the biocementation process. For example, the compression and shear strength values (227 kPa and 210 kPa, respectively) served as a control reference for subsequent experiments.

Although the biochemical reactions contributing to soil strengthening have been outlined, it is essential to explain how these reactions specifically enhance soil structure. The precipitation of calcium carbonate plays a pivotal role in the binding of soil particles, forming crystals that fill voids and thereby enhance the overall strength of the soil matrix. This process of crystallisation increases the density of the soil and improves its resistance to mechanical stress. The precipitation of calcium carbonate acts as a cementing agent between particles, leading to increased compressive strength and improved stability under both vertical and horizontal loads. These processes contribute to the mitigation of soil degradation and desertification, particularly in sandy soils (Aliu et al., 2020; Kpoda et al., 2024).

Thirty cultures of microorganisms were inoculated from 3 sites, 11 of which showed urease activity based on changes in the colour of the medium. The colour of the medium in the negative control, which did not contain bacteria, was straw-coloured, while in the media containing urolithic bacteria and urea, the latter decomposed to form ammonia, which, when interacting with the indicator, gave a red-raspberry colour to the medium. The more pronounced the proteolytic activity of the bacteria was, the more intense the crimson colour of the medium was. The reaction equation is shown below:

$$CH_4N_2O + H_2O \leftrightarrow CH_2NO_2+, \quad (1)$$

$$CH_4NO_2 + NH_4 \rightarrow HCO_3 + NH_3. \quad (2)$$

Cultures that showed the most pronounced qualitative reaction (bright crimson colour of the medium) were marked and assigned numbers 1, 2, and 3, and the remaining cultures were also sequentially numbered. The measurement of urease activity showed that the cultures that gave the most pronounced qualitative reaction had the highest urease activity in the quantitative measurement (Figure 1). *Sporosarcina Pasteurii* was used as a control strain, the activity of which was the highest among the cultures studied, and one of the isolates (No. 2) had comparable activity. As a positive control, a urease solution with known activity was used, which was further used in the course of the study.

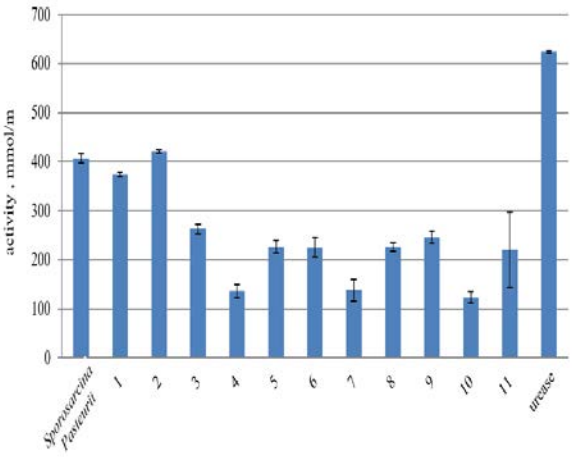

Figure 1. Urease activity in different bacterial isolates from soil samples
Source: compiled by the authors.

Isolates 1, 2, and 3 exhibited the highest urease activity, which is directly correlated with their capacity to induce calcium carbonate precipitation. It is noteworthy that

isolate 2 exhibited urease activity that was comparable to that of the control strain, *Sporosarcina Pasteurii*. This suggests that it has the potential to be an effective biocementation agent.

Despite the urease activity in the control sample being the highest, it did not result in the greatest calcium fixation. This suggests that factors other than urease activity play a role in calcium carbonate precipitation. The distribution and retention of calcium ions in the soil, environmental conditions such as temperature and moisture, and the presence of other nucleation sites or competing reactions within the soil matrix are all potential factors that may influence the process of calcium carbonate precipitation. Furthermore, the efficacy of precipitation may be contingent upon the interplay between microbial activity and soil attributes, including particle size and porosity.

However, the duration of the planned experiment was 12 days, and the urease measurement was performed on day 6 so that in real conditions the predicted enzyme activity could have increased while maintaining the bacterial production activity. Mass spectrometric analysis of proteins allowed us to determine the systematic affiliation of the three isolates. The determination was made to the genus, and the isolates obtained were identified as 1 – *Aeromonas sp*., 2 – *Bacsllus sp*., and 3 – *Staphilococcus sp*. Literature analysis confirms the presence of urease-producing capacity in many species of microorganisms belonging to these genera. However, no species-level analysis was carried out in this study. The addition of urea to the medium containing bacteria led to an increase in the pH of the medium, which creates optimal conditions for the growth of these microorganisms and contributes to the supersaturation of the solution with free calcium ions. When urea was added to the medium containing urease, reaction cascades occurred:

$$CO(NH_2)_2 + H_2O + \text{urease} \rightarrow NH_2COOH + NH_3, \quad (3)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3, \quad (4)$$

$$H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (5)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-, \quad (6)$$

$$HCO_3^- + H^+ + 2OH^- \rightarrow CO_3^{2-} + 2H_2O, \quad (7)$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3. \quad (8)$$

Formulas 7 and 8 indicate the balance of CO_3^{2-} and HCO_3^-, H_2CO_3 – the balance of ions depends on the pH of the medium, with an increase in pH, dissociation to carbonate ions prevails and $CaCO_3$ precipitation occurs. When observing laboratory samples, this process looks like sample curing. After the laboratory experiment on cementation, the parameters of the biocement were studied. Among the laboratory parameters studied was the density, which was expected to increase in all experimental variants compared to the control. Figure 2 illustrates the density of the biocement samples obtained through microbial and enzymatic methods.

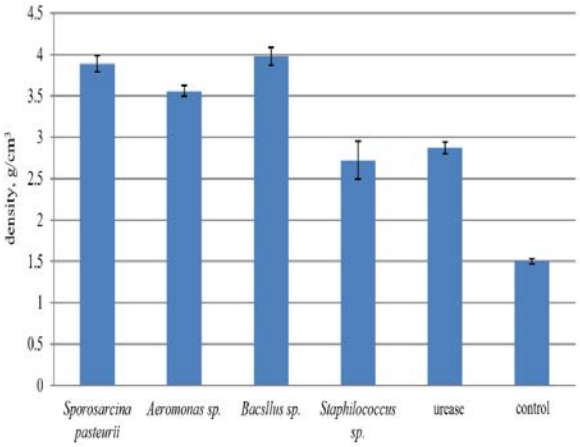

Figure 2. Density of biocement samples obtained by microbiological and enzymatic methods in the laboratory and the field

Source: compiled by the authors.

The density of soil in the control was 1.5g/cm^3. The diagram shows that the highest increase in density was observed in samples obtained using cultures of *Bacsllus sp*. isolate (3.98±0.1 g/cm^3), *Sporosarcina Pasteurii* (3.85±0.1 g/cm^3) and *Aeromonas sp*. Despite the high activity of native urease, the soil density was lower than in the above variants and amounted to 2.9±0.05 g/cm^3. When using an isolate of *Staphilococcus sp*., the density of the material obtained was 0.37 ± 0.05 g/cm^3. One of the most significant direct indicators of cementation efficiency is the calcium carbonate content of biocement. The study demonstrated a distribution pattern that was fully correlated with the ability to produce urease. The highest calcium carbonate content was observed in the medium containing *Bacsllus sp*. (7.2±0.4 mg/cm3), and the second highest calcium carbonate content was recorded in the sample obtained using culture of *Sporosarcina Pasteurii (6.6±0.*4 mg/cm^3), and the third highest – *Staphilococcus sp*. (6.1±0.7 mg/cm^3). The elevated urease activity of *Bacsllus sp*. facilitates accelerated urea hydrolysis, which in turn precipitates calcium carbonate at a faster rate. Furthermore, the robust cell walls and enhanced metabolic efficiency of *Bacsllus sp*. facilitate more efficacious calcium ion capture and crystal formation. The superior biocementation performance of this strain in comparison to other strains used in the study is likely attributable to its ability to thrive in the specific pH and temperature conditions of the soil (Suerbaev et al., 2009).

When using the *Aeromonas sp.* isolate, the content was 5.3±0.7mg/cm^3. The content of calcium carbonate in biocement obtained by the enzymatic method using urease solution was lower than in all variants of microbial deposition and amounted to 3.67±0.7 mg/cm^3, which is higher than in the control, but less effective than when using microorganisms. The concentration of calcium carbonate in the biocement samples is presented in Figure 3. As can be seen from these results, the urease concentration is not a decisive factor in the formation of calcium carbonate, as the urease activity in the control was the highest among the samples studied, which did not ensure maximum calcium fixation from the soil.

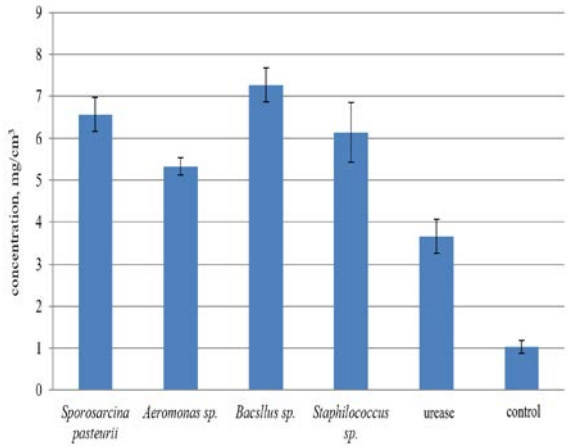

Figure 3. Calcium carbonate concentration in microbiologically and enzymatically produced biocement samples under laboratory and field conditions
Source: compiled by the authors.

As anticipated, microbial treatment resulted in elevated calcium carbonate content, with Bacillus sp. demonstrating the greatest yield (7.2 mg/cm³). The enzymatic method yielded a lower calcium carbonate content (3.67 mg/cm³), indicating that microbial methods are more efficacious in inducing calcium carbonate deposition.

The most important indicators are the mechanical properties of the samples obtained since the goal of the experiments is to produce biocement that is resistant to mechanical stress. To verify the reproducibility of the test results in the field, strength measurements were performed on both laboratory and field samples on a larger scale. This scaling makes it possible to move from laboratory conditions to field conditions, including exposure to natural weather and climate conditions, including large volumes of soil. The compressive and shear strengths of the biocement samples are shown in Figures 4 and 5 respectively. Figure 4 shows the compressive strength results. Several patterns can be observed from the chart above: firstly, in all variants of the experiment, the MICP technique showed the best results in increasing the strength characteristics of sandy soil.

In all the results of the experiment, there was a clear tendency for a more significant increase in strength indicators in the laboratory than in the field. It seems plausible to suggest that environmental factors, including temperature fluctuations, soil moisture content and wind conditions, may have contributed to the discrepancies observed between the laboratory and field results. In the field, the presence of natural elements such as precipitation and wind may have affected the consistency and distribution of calcium carbonate precipitation. For example, precipitation may have resulted in the dilution or washing away of some of the applied solutions, thereby reducing the concentration of active substances in the soil (Tonkha et al., 2024; Floqi et al., 2009). Furthermore, the fluctuations in temperature between day and night in the field may have resulted in a reduction in bacterial and enzymatic activity, consequently leading to a slower deposition rate of calcium carbonate. It can be reasonably deduced that the aforementioned factors resulted in a reduction in shear and compressive strength values in the field when compared to the more controlled conditions of the laboratory, where temperature and moisture were maintained at optimal levels for the biocementation process.

However, all the observed trends identified in the laboratory could be translated to open-field conditions with great accuracy. Under laboratory conditions, the biocement obtained using *Sporosarcina Pasteurii* culture had the best strength characteristics: the compressive strength of the soil was 762.7±28.3 kPa and the shear strength was 420.3±7.23 kPa compared to the control 249.3±9 kPa and 118.6±6.29 kPa, respectively. The characteristics of the soil treated with *Bacsllus sp.* culture were close – 765±10.69 kPa in compression and 394.67±8.29 kPa in shear.

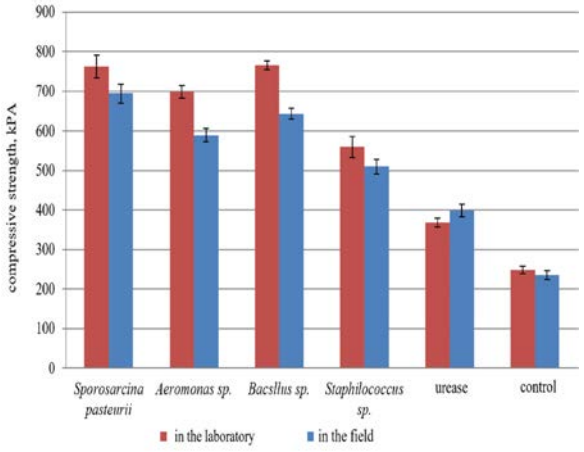

Figure 4. Compressive strength of microbiologically and enzymatically produced biocement samples under laboratory and field conditions
Source: compiled by the authors.

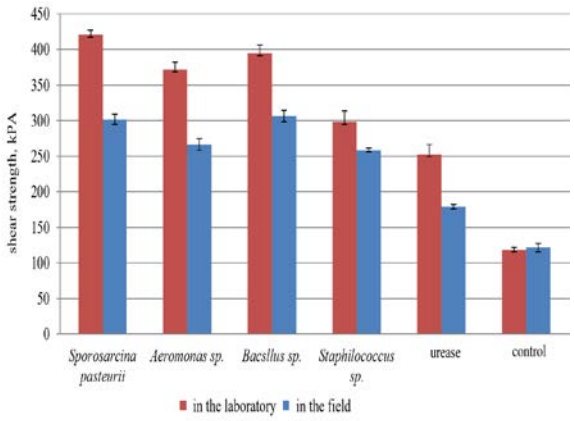

Figure 5. Shear strength of microbiologically and enzymatically produced biocement samples under laboratory and field conditions
Source: compiled by the authors.

The biocement samples obtained using the *Aeromonas sp.* culture withstood a pressure of 698±16.8 kPa in compression and 371.33±8.25 kPa in shear. The isolate of *Staphilococcus sp.* provided a sand strength of 559.3±26.1 kPa in compression and 298.33±5.68 kPa in lateral shear. Thus, the use of ureolytic bacteria cultures contributed to an increase in the strength of biocement by 2.5-3 times compared to the control. The use of the enzymatic method of calcium carbonate deposition, using a urea solution, increased the vertical compressive strength to 368.67±10.5 kPa and the lateral shear strength to 252.2±7.02 kPa. Thus, the strength indicators increased by 2 times compared to the control, but this method is inferior to the

microbiological method. Thus, the strength indicators increased by 2 times compared to the control, but this method is inferior to the microbiological method (Figure 5).

The study of samples obtained in the field showed a similar pattern of sample density distribution. It should be noted that all samples obtained in the laboratory using the same strains demonstrated a 10-20% higher efficiency in terms of increasing the strength of biocement. At the same time, the results of the evaluation of the mechanical properties of biocement obtained in different variants of microbiological precipitation of calcium carbonate were more homogeneous. This may be due to the peculiarities of the conditions that have a limiting effect on the growth and secretory activity of bacteria. The highest values of compression resistance were found in the sand sample treated with *Sporosarcina Pasteurii* culture – *603.67±24 kPa, close to the values of Bacillus sp.* – 643.3±13 *kPa*. This was followed by a sample of soil treated with *Aeromonas sp.* at 589.3±18.9 kPa and *Staphilococcus sp.* at 510±18.5 kPa. The compressive strength using the enzymatic method was 398.67±15.85 kPa, which is higher than in the control variant (235.33±11.93 kPa), but lower than in all microbiological samples (Figure 4). The most important for this study are the shear strength tests carried out in the field, as they characterise the ability of the soil to resist wind erosion to the greatest extent. The strength of the control sample was 121.63±6.29 kPa. The shear strength values after the microbiological treatment were as follows: soil samples obtained using *Bacsllus sp.* and *Sporosarcina Pasteurii had similar values – 6123306.17±11*.37 kPa and 301.83±7.08 kPa, respectively. Samples obtained from the areas treated with cultures of *Aeromonas sp.* and *Staphilococcus sp.* isolates were slightly lower – 266.17±10.98 kPa and 258.33±15.6 kPa, respectively. Enzymatic treatment provided a resistance of 179.17±14.21 kPa.

The results of the field tests for rotational shear resistance showed trends close to those in the laboratory. The maximum resistance at 50 cm is shown in Figure 6. The highest resistance was observed in the soil treated with a solution containing *Bacsllus sp.* – 299.5±12.97 kPa. The soil strengthened with the laboratory strain was practically not inferior in terms of rotational shear strength – 256.33±15.78 kPa, no significant differences in the indicators were observed. The strength of the sand treated with *Aeromonas sp.* was 51267311.66±31514.51 kPa and *Staphilococcus sp.* was 299.5±12.97 kPa. The strength of the soil in the urease-treated area was 162.5±6.61 kPa – the differences are significant in comparison with both the microbiological treatment and the control (97.16±9.25 kPa).

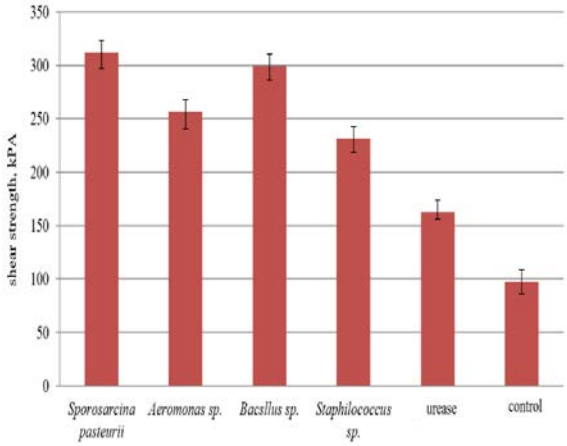

Figure 6. Strength of soil treated in different ways under rotational shear in the field
Source: compiled by the authors.

It is worth noting that the results of these tests show a lower resistance. One of the reasons may be that the resistance was studied in deeper layers, while the cementitious mortar was applied superficially, and samples were taken from more superficial layers for laboratory tests. As can be seen from the results of all the tests, both enzymatic and microbiological treatments proved to be effective in increasing the mechanical strength of sandy soils, both in the laboratory and in the field. However, the effectiveness of the bacteria is higher in all variants of the cultures used in the experiment – both the reference strain and local isolates. The laboratory determination of urease, calcium carbonate and all physical and mechanical strength parameters are directly correlated when using the microbial method of calcium precipitation.

4. Discussion

The main result of the work carried out to compare the microbiological and enzymatic methods of biocementation of sandy soil is the effectiveness of both methods in terms of strengthening soil strength. Increase of various physical and mechanical parameters of its stability under different types of mechanical load. These findings are consistent with numerous other studies, many of which have been reviewed in meta-analyses.

Iqbal et al. (2021) addressed the use of biocementation for strengthening building materials; the method is effective in terms of increasing strength, preventing and reducing cracking of materials, and increasing moisture resistance. Saif et al. (2022) and Ahenkorah et al (2021) analysed the results of enzyme-induced calcium deposition for soil strengthening. The authors point out that the results obtained in experiments on the precipitation of calcium carbonate with the addition of urease depend on many factors of the experiment. The study detected a difference in the effectiveness of the enzymatic method in different conditions – when treated in laboratory conditions, the shear strength of the soil was 252.2±7.02 kPa, and in field conditions – 179.16±14.21 kPa. This value is 35% lower than in the control. This difference is statistically significant, and a similar trend was also found in all variants of microbiological soil treatment. It is possible to assume that these changes are associated with the peculiarities of climatic conditions – temperature, moisture (precipitation can wash the solution into deeper layers).

This is determined by the relative volume of the applied solution that seeped into the soil depth, which requires larger volumes of active substances to achieve higher density. Researchers agree that the effectiveness of ureolytic reactions depends on many factors: method of enzyme application, concentration of urea, calcium ions and urease, urease activity, temperature of the applied solution, initial pH of the cementitious solution and soil pH, soil composition, curing time and chemical composition of pore water (Erdmann and Strieth, 2022; Ahenkorah et al., 2021).

Li et al. (2022) investigated the ability of soil to withstand vertical load under different microbial bioconcretion protocols. It is shown that there is a certain optimum temperature (25°C), solution concentration (2 mol/l), and concentration of dispersed particles above which the mechanical properties do not improve or deteriorate. Due to the many factors that can affect the result, a direct assessment of the mechanical properties of the soil is necessary to assess the effectiveness of the biocementation process. This study shows that the vertical compressive strength of the soil increases by 2-3 times compared to the control, and the shear strength increases by 2.5-4 times compared to the control when using MICP and by 1.5-2 times when using EICP. There is ample evidence of the effectiveness of both methods, with which the results of this study are consistent. Mo et al. (2021) cited the results of studies that show that the shear strength of the soil increased by up to 1080% (52-65 kPa) seven days after treatment. Another study showed that the use of bacterial calcium precipitation reduced calcium compression by up to 158%. Zamani and Montoya (2016), comparing MICP and EICP methods, found that the bacteria-catalysed process increased the unconfined compressive strength to 2.04 MPa, which is about five times higher than the soil strength after the enzymatic process of 0.43 MPa. Sun et al. (2018) studied the effect of a suspension of urease-producing Bacillus sp. bacterial cells and culture fluid containing the enzyme without bacterial cells on the ability to precipitate calcium. The results showed that the unconfined compressive strength of the sand treated with the cell suspension is 1.7 times higher than that of the sand treated with the urea-$CaCl_2$ liquid.

Konstantinou et al. (2021) demonstrated that high urease activity has no direct correlation with the quality of sand cementation; the lowest urease concentration (10 mmol/h/l) was more effective for larger particles. Microscopic analysis showed that at lower microbial urease activity, larger crystals are formed, which can fill the voids between the large sand particles. This may explain the obtained results when comparing the urease activity produced by the bacterial isolates with the reference solution. Even though the activity of the urease solution was the highest (624.3±2.51 mmol/l), the efficiency of calcium carbonate deposition and the increase in soil strength were the least significant compared to microbiological samples. Chen et al. (2022) conducted a large-scale study on the dynamics of calcium carbonate crystal formation in the process of biocementation. As such, MICP consists of many chemical and hydrodynamic processes. Bacterial cells play not only the role of crystal formation centres, which is well known but also provide the transport flow of substances in the soil system (Yang et al., 2022; Kuvatova et al., 2024). For this reason, microbial deposition is often more efficient than enzymatic deposition. Shirakawa et al. (2021), in a study of two types of ureolytic bacteria, concluded that the dynamics and growth pattern of calcium carbonate crystals may differ when they are used, but the mechanical properties of sand, such as compressive and shear strength, are directly related to the concentration of calcium carbonate. Similar data were obtained in the present study when comparing urease production and the concentration of calcium carbonate formed.

As for the study of individual bacterial species and the comparison of their calcium-fixing and urease activity, two species proved to be the best: reference *Sporosarcina Pasteurii* and *Bacillus sp.*, where statistically significant differences in the samples obtained with them were absent in all test variants. Many studies confirmed the high urease and calcium-fixing capacity of *Sporosarcina Pasteurii*, similar to that of the *Bacsllus* genus (Hammad et al., 2013; Jiang et al., 2016). However, the other two isolates used in the study, assigned to the genera *Aeromonas sp.* and *Staphilococcus sp.* also showed high rates of sandy soil bioconsolidation ability in this experiment. These microorganisms were isolated from the soil at the location of the experiment. In addition, 11 of the 30 samples tested contained microorganisms that tested positive for urease. This indicates the presence of urease-producing microorganisms in the soil. It has been shown that laboratory selection and cultivation can produce biomass of isolates that are successfully used for the bioconsolidation of sandy soil.

Gomez et al. (2019) studied the comparative effect of native microorganisms and culture *Sp. Pasteurii on the biocementation. As such, the precipitation of calcium carbonate crystals occurs even under the influence of natural soil microorganisms when a solution containing calcium and urea is added, but at a much slower rate than when a bacterial culture is added. It also changes the nature of crystal growth, which can be useful for some types of coarse-grained soils. Similar conclusions were reached by* Heveran et al. (2019) when studying strains with different urease activity, the authors propose to create strains aimed at a lower rate of enzyme release. *Isolation of urease-producing isolates from natural soils was also carried out in Kazakhstan.* Kurmanbaev et al. (2017) isolated 21 natural urease-producing strains, based on the results of quantitative measurement of urease, 4 strains were selected for MICP. Sembaev et al. (2019) described the isolation of a strain with a pronounced ability to biocementation.

Chen et al. (2021) review existing studies comparing different methods of biocementation (using exogenous, local bacteria and enzymes) and conclude that the effectiveness of the methods depends on many factors discussed above. In particular, the introduction of external bacterial strains can slow down the growth of the native bacterial biocoenosis, if present, and, if applied superficially, cause the formation of a dense crust that prevents further penetration of solutions and deeper cementation. In this study, to avoid such effects, gradual daily moistening with small amounts of the solution was used to ensure better penetration. All three methods described were compared, and the microbial method proved to be the most effective. Alotaibi et al. (2022)

compared the traditional method of soil strengthening with Portland cement with EICP and MICP methods, addressing efficiency, environmental friendliness and economic feasibility of application. The authors conclude that both biological methods are superior to the traditional ones in terms of environmental friendliness. Biocement produced by the EICP method is inferior in mechanical properties to microbial cement, but it has fewer metabolites and is, therefore, more environmentally friendly, and, most importantly for large-scale implementation, is much easier and more economical to produce (Chen et al., 2023; Dorvil et al., 2023).

There are few field studies on the effectiveness of sand biocementation in natural conditions. Meng et al. (2021) conducted similar studies using the MICP method in the Ulan Bukh Desert in China. The research has shown the formation of a dense crust 12.5 mm thick, which ensured the resistance of the mounds' soil to weathering. As a result, the soil strength was 459.9 kPa, while in the current study, the maximum vertical load strength was *603.67±24 kPa, and the minimum* was 510±18.5 kPa, which is a high value. Dagliya et al. (2022) conducted a study of MICP on sandy soil in India. A similar scheme of surface spraying of cementitious solutions was used. The study determined that the compressive strength and resistance to wind erosion appeared already from 5 days after the start of treatment and increased by 20 days. The thickness of the surface crust continued to increase, and the soil density increased by 85% compared to 0 days of the experiment. These statements coincide with the results of this study on the effectiveness of spray treatment in the field.

In general, the results obtained during the experiments are in good agreement with the literature data, and the developed protocols can be recommended for further testing and application for soil consolidation.

5. Conclusions

This study compared two contemporary calcium carbonate deposition techniques, namely MICP and EICP, to enhance the strength of sandy soils. Both methods demonstrated efficacy in improving soil stability under mechanical load, with MICP consistently exhibiting superior performance. In field tests, MICP achieved a maximum vertical load strength of 694.33 kPa, in comparison to 394 kPa for EICP, both of which surpassed the control's 235.33 kPa. Similarly, MICP yielded superior shear strength, reaching 301.83 kPa in comparison to EICP's 179.17 kPa and the control's 121 kPa.

It is noteworthy that the study demonstrated that indigenous bacterial strains can achieve biocementation results that are comparable to those of the reference strain, *Sporosarcina Pasteurii*. This highlights the potential for practical application of these strains. The findings highlight that MICP has an additional contribution beyond urease activity, namely the provision of enhanced soil consolidation due to bacterial interactions during the biocementation process.

Further research should concentrate on optimising the scalability and application methods of these technologies in order to enhance their practical implementation.

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