

## Article

# From Waste to Strength: Applying Wastepaper, Fungi and Bacteria for Soil Stabilization

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**Abstract:** Biocementation is a promising soil stabilization technology that relies on microbiologically induced calcite precipitation (MICP). The addition of wastepaper was found to enhance the mechanical strength of biocemented soil. This study examined the effects of incorporating wastepaper into biocemented soil, focusing on the use of the ureolytic bacterium *Bacillus licheniformis* DSMZ 8782 and the yeast-like fungus *Scytalidium candidum* 3C for soil stabilization. The optimal wastepaper content was determined to be 2%, as it did not disrupt the uniform distribution of CaCO<sub>3</sub> and contributed to improved soil strength. The combination of bacteria and fungi significantly increased the unconfined compression strength of samples containing 2% wastepaper (161.1 kPa) compared to untreated soil (61 kPa) and bacteria-only treatments (66.5 kPa), showing improvements of 2.6 and 2.4 times, respectively. Furthermore, we demonstrated that adding fungal biomass without wastepaper significantly improved the compressive strength, achieving a value of 236.6 kPa—nine times higher than untreated soil (26.4 kPa) and four times higher than soil treated with bacteria alone (60.6 kPa). This study identifies the optimal wastepaper content and highlights the potential of combining fungal and bacterial biomass for biocementation in soil stabilization.

**Keywords:** MICP; wastepaper; calcium carbonate; *Bacillus licheniformis*; *Scytalidium candidum*



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## 1. Introduction

Humanity’s destructive activities have led to numerous irreversible changes on Earth, such as environmental pollution and climate change [1]. Additionally, rapid urbanization has fueled the demand for products and services, resulting in the intensive use of fossil resources and the generation of organic waste [2]. To counter these negative trends associated with technological advancement, it is essential to increasingly adopt “green” technologies.

One promising technology that takes an interdisciplinary approach is microbially induced calcium carbonate precipitation (MICP). This process harnesses the ability of microorganisms to induce the precipitation of calcium carbonate as a result of their metabolic activity [3]. Among the various pathways leading to the biomineralization of CaCO<sub>3</sub>, the microbial urea decomposition mechanism is the most widely studied and applied, owing to its high rate of reaction and ease of control [4–6]. One of the most frequently used microorganisms in biomineralization is the aerobic rod-shaped bacterium *Sporosarcina pasteurii*, which has the maximum ureolytic activity and provides the highest yield of minerals [7–9]. Bacteria of the genus *Bacillus* also demonstrate significant urease activity and high carbonate precipitation rates [10–12].

Biogenic calcium carbonate is highly compatible with a wide range of materials, making MICP a promising solution for various engineering and environmental challenges.

These include water conservation, prevention of underground fluid leakage, soil erosion control, concrete structure restoration, earthquake mitigation and addressing issues like fugitive dust and contaminated soils [3,13]. Several companies worldwide, such as «Biomason» in Denmark and «Soletanche Bachy» in France, have already implemented MICP technology for commercial engineering applications. In the Middle East, the Kingdom of Saudi Arabia is the exclusive distributor of bioconcrete products [14]. Additionally, in 1993, the French company «Calcite Biocement» applied a surface coating (biocalcin) produced by microorganisms to a church tower in Paris, covering an area of 50 m<sup>2</sup> [15].

In recent decades, there has been a noticeable increase in the construction industry's adoption of environmentally friendly materials and technologies. In the early stages of building infrastructure, soil strengthening is often required to enhance its mechanical properties. MICP technology offers an eco-friendly alternative to traditional chemical cementation methods, which typically involve the addition of bentonite, silicates or acrylamide to the soil. Biocementation allows for strengthening and stabilizing the soil by increasing the content of the solid phase, reducing the pore size and improving the rigidity of contact between particles [16]. The calcite crystals that form in the process fill voids in the soil, binding loose particles together and improving the overall strength characteristics of the material.

While much of the scientific focus has been on bacterial biomineralization processes, fungi also play a significant role in biogeochemical cycles and are increasingly recognized for their potential in soil improvement [17]. Fungi are capable of precipitating minerals through processes like biomineralization and organomineralization [18,19]. Fungal hyphae contain chitin, which can bind Ca<sup>2+</sup> ions. Both live and dead fungal biomass can capture these ions on their cell walls, leading to the nucleation and deposition of mineral phases such as calcium carbonate [20].

The potential of fungi to develop soil improvement technologies is discussed in a review by Mountassir [21]. Their ability to precipitate calcium carbonate is actively being investigated to be exploited in concrete structure restoration [19]. Additionally, fungi are widely used in organic waste disposal, owing to their production of enzymes such as cellulases, hemicellulases and ligninases, which catalyze the breakdown of various organic polymers [22,23].

The strength characteristics of MICP-treated soils can also be improved by incorporating reinforcing additives. According to a study [24], the addition of jute fibers enhanced the engineering properties of MICP-treated sand, increasing its durability by more than 50%. Yao showed that adding 0.1% wool fiber to MICP-treated sand increased the compressive strength threefold and significantly improved its flexural strength [25]. Wen reported that MICP treatment combined with bamboo fibers boosted the ductility as well as the flexural strength of sand specimens by several times [26].

To reduce the cost of soil strengthening technology by MICP, it is advisable to incorporate organic waste at various stages of the process [27,28]. In our previous study [29], we demonstrated that replacing expensive components of Luria–Bertani (LB) medium with spent brewer's yeast extract did not reduce the ability of ureolytic bacteria to bio-mineralize. Additionally, we demonstrated that, among the three types of cellulose-containing waste (wastepaper, flax shives and sawdust), the incorporation of wastepaper into MICP-treated soil samples resulted in a 2.1-fold increase in compressive strength and nearly a 1.5-fold increase in the precipitated calcite percentage compared to samples without additives. This finding aligns with another study [27], which reported that adding wastepaper as a reinforcing material to MICP-treated soil significantly improved its mechanical strength.

This study aimed to determine the optimal wastepaper content to be introduced with microbial biomass to enhance soil strength through calcium carbonate deposition by MICP. We studied the yeast-like fungus *Scytalidium candidum* 3C [30], used for the first time for this purpose; the previously selected ureolytic mineralizing bacterial strain *Bacillus licheniformis* DSMZ 8782 [31]; and their combination. We evaluated the impact of these microorganisms

on the microbiota of soil samples and highlighted the potential of the mycelial fungus *Sc. candidum* to enhance soil strength.

## 2. Materials and Methods

Urea,  $\text{CaCl}_2$ , NaCl and HCl were purchased from JSC “VEKTON” (St. Petersburg, Russia), and glucose was obtained from Wuhan Servicebio Technology Co., Ltd. (Wuhan, China). LB medium was sourced from VWR, Life science (Radnor, PA, USA), while other chemicals were from Sigma-Aldrich Corp. (St. Louis, MO, USA) unless otherwise specified.

### 2.1. Soil and Wastepaper

#### 2.1.1. Soil Characteristics

The soil samples used for this study were collected in the Gatchina region of Russia (WSG84 N59.5007, E30.00477). The soil in this area was characterized as sod-podzolic surface-gleied (pH 6.8) according to [32] and as SC (clayey sand) according to [33]. The soil samples were collected from a depth of 0.25 m using a sterile drill (washed with 70% ethanol) and stored at 4 °C until further analysis. Granulometric analysis was carried out using the dry sieving method [33]. The results of the granulometric analysis of the soil are presented in Table 1.

**Table 1.** Granulometric composition of the soil, %.

| >20 mm | 20–10 mm | 10–5 mm | 5–2 mm | 2–1 mm | 1–0.5 mm | 0.5–0.25 mm | 0.25–0.1 mm | 0.1–0.05 mm | 0.05–0.01 mm | 0.01–0.005 mm | <0.005 mm |
|--------|----------|---------|--------|--------|----------|-------------|-------------|-------------|--------------|---------------|-----------|
| 10.5   | 3.5      | 6.0     | 3.7    | 4.0    | 5.7      | 4.4         | 15.0        | 3.8         | 3.2          | 5.5           | 45.2      |

#### 2.1.2. Wastepaper

The shredded wastepaper, with a humidity of 12–14% (pH 7.1), was provided by the wastepaper recycling plant LLC “PTF Rustom”, Russia, Smolensk region, Safonovo.

### 2.2. Microorganisms and Cultivation Conditions

#### 2.2.1. Bacterial Strain

The previously selected and characterized mineralizing strain *Bacillus licheniformis* DSMZ 8782 [31] was used. To stabilize the soil, bacteria were grown in 100 mL of brewer’s yeast (BY) medium (extract from spent brewer’s yeast—5 g/L, glucose—10 g/L, NaCl—1 g/L) [29] for 2 days at 37 °C and aerated to an optical density of  $\text{OD}_{600} = 8$ . The precipitate was then separated by centrifugation at 10,000 rpm for 10 min and resuspended in 0.9% NaCl solution.

#### 2.2.2. Fungal Strain

The previously characterized [30] strain *Scytalidium candidum* comb. nov. 3C (RCAM04653, in the Collection of cultures of agricultural microorganisms (ARCAM)) was pre-grown in 2.5 L of a nutrient medium (wheat bran, 40 g/L;  $(\text{NH}_4)_2\text{SO}_4$ , 1.5 g/L;  $\text{KH}_2\text{PO}_4$ , 1 g/L;  $\text{NaNO}_3$ , 1.5 g/L  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ , 0.5 g/L; filter paper, 10 g/L) for 5 days at 28 °C with aeration. Afterward, the biomass was separated by centrifugation at 3900 rpm for 40 min. The wet fungal sediment, which contained no bran, was added to the soil.

### 2.3. Soil Stabilization Using Microorganisms

The design of the experiment is shown in Figure 1.

#### 2.3.1. Sample Preparation

Prior to filling the molds with soil, filter paper ( $\varnothing$  3 cm) was placed at the bottom to prevent soil leaching during treatment with experimental solutions. Soil samples (50 g) were previously mixed with wastepaper in concentration of 2, 4 and 6 wt. %, and then the molds were filled with them using disposable 50 mL syringes (SFM Hospital Products

GmbH, Berlin, Germany). For the fungus-containing samples, the biomass (prepared as described in Section 2.2.2) was added simultaneously with the wastepaper.

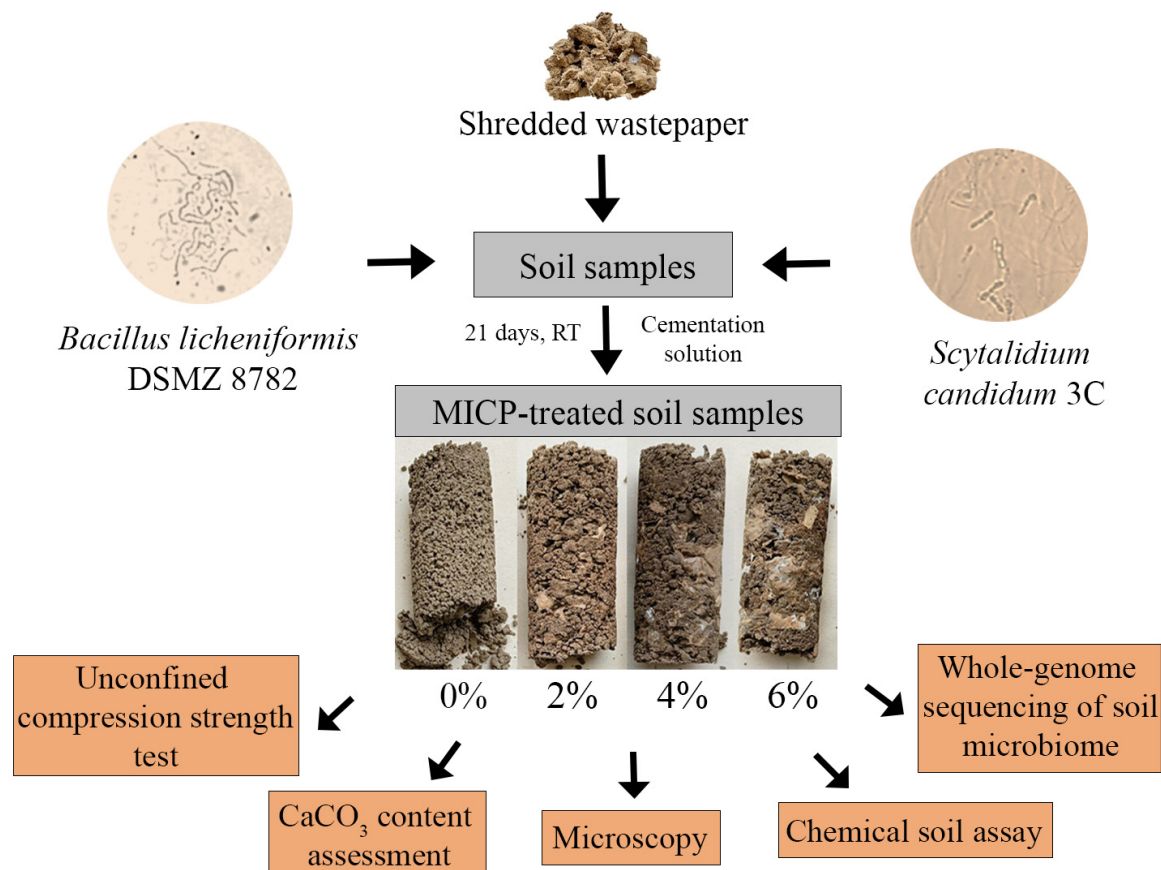


**Figure 1.** The design of the experiment.

Microorganisms were introduced into the soil samples in the following variants:

1. Bacterial suspension (4 mL)—sample Bact;
2. Fungal biomass (10 g wet biomass)—sample Fng;
3. Bacterial suspension (4 mL) u fungal wet biomass (10 g)—sample Mix.

The control soil sample (sample Control), containing 0, 2, 4 and 6 wt. % of wastepaper, was treated only with urea and calcium solutions. The bacterial suspension (prepared as described in Section 2.2.1) was injected into the samples the day after preparation. For samples without bacteria, 4 mL of distilled water was injected instead. After 24 h, 6 mL of the cementing solution (CS), consisting of 1 M urea and 0.5 M  $\text{CaCl}_2$ , was injected into the soil samples. Treatment with the cementing solution was repeated on the 4th and 8th days. The samples were then kept at room temperature for 14 days. After this period, all samples were dried at 50 °C for 3 days, removed from the molds and further dried at room temperature for 2 days until they reached a constant mass. The samples were then cut to a uniform size ( $\varnothing$  3 cm,  $6 \pm 0.5$  cm) for subsequent analysis. All experiments were performed in triplicate.

### 2.3.2. Sample Composition Analysis

The composition analysis of soil samples with varying wastepaper contents was performed using X-ray microfluorometry on an Oxford Instruments (Abingdon, UK) INCAx-act X-ray energy dispersion microanalyzer coupled with a Tescan Vega 3 SBH scanning electron microscope (TESCAN, Brno, Czech Republic) (Table 2).

**Table 2.** Composition analysis results for dry weight, %.

| Chemical Element | Wastepaper | Soil with Wastepaper     |       |       |       |
|------------------|------------|--------------------------|-------|-------|-------|
|                  |            | Content of Wastepaper, % |       |       |       |
|                  |            | 0                        | 2     | 4     | 6     |
| C                | 49.07      | 4.57                     | 5.46  | 6.35  | 7.24  |
| O                | 50.19      | 52.52                    | 52.48 | 52.43 | 52.38 |
| Na               | 0.28       | 0.84                     | 0.83  | 0.82  | 0.81  |
| Mg               | 0.03       | 0.86                     | 0.84  | 0.83  | 0.81  |
| Al               | 0.05       | 5.68                     | 5.57  | 5.45  | 5.34  |
| Si               | 0.03       | 27.92                    | 27.36 | 26.80 | 26.25 |
| P                | — *        | 0.10                     | 0.10  | 0.10  | 0.09  |
| S                | 0.13       | 0.04                     | 0.04  | 0.04  | 0.05  |
| Cl               | 0.01       | —                        | 0.00  | 0.00  | 0.00  |
| K                | 0.03       | 2.40                     | 2.35  | 2.31  | 2.26  |
| Ca               | 0.18       | 1.00                     | 0.98  | 0.97  | 0.95  |
| Ti               | —          | 0.60                     | 0.59  | 0.58  | 0.56  |
| Mn               | —          | 0.09                     | 0.09  | 0.09  | 0.08  |
| Fe               | —          | 3.33                     | 3.26  | 3.20  | 3.13  |
| Cu               | —          | 0.02                     | 0.02  | 0.02  | 0.02  |
| Zn               | —          | 0.03                     | 0.03  | 0.03  | 0.03  |
| Pb               | —          | —                        | —     | —     | —     |

\* less than 0.01%.

### 2.3.3. Assessment of Unconfined Compression Strength

The unconfined strength test was performed according to [34] using the KP-9 compression testing device. Pressure was applied gradually at a rate of 0.5 MPa/s until the sample structure failed. The unconfined compressive strength was calculated using the following Equation (1):

$$S = 10 * \frac{F}{A}, \quad (1)$$

where  $S$  is the calculated compression strength, MPa;  $F$  is the compressive force applied until a sample failure, N; and  $A$  is the cross-sectional area of the biocemented soil column, (7.065),  $\text{cm}^2$ .

### 2.3.4. Assessment of the Calcium Carbonate Content

To determine the amount of calcium carbonate formed, 1 g of each sample was ground in a mortar to a homogeneous consistency and placed in a carbonometer (KM-HT with an MIT 2.5/0.5 pressure gauge). Then, 10 mL of 10% HCl was added following the procedure outlined in [35]. The amount of calcium carbonate was measured in the different layers of the soil samples (top, bottom and middle). The top and bottom were taken from the corresponding edges of the sample when it was leveled to a length of 6 cm before the compression strength test. The middle sample was taken from the central portion of the column, approximately 3 cm from the edge. The percentage of carbonate formed in 1 g of the sample was calculated using the Equation (2):

$$\text{CaCO}_3 = \frac{(P \times 100)}{(m \times a)} \quad (2)$$

where  $P$  is the pressure, bar;  $m$  is the sample mass, g; and  $a$  is the correction factor (1.4116). All experiments were conducted in triplicate.

### 2.3.5. DNA Extraction, Preparation and Sequencing of Whole-Genome Libraries

DNA was extracted from 200 mg of soil using the DNeasy PowerSoil Pro Kit from QIAGEN (Hilden, Germany), according to the manufacturer's protocol. For genomic library preparation, 60 ng of DNA was enzymatically fragmented using the FTP protocol

described in a previous study [36]. Universal adapters were then ligated using the T4 DNA Ligase Kit from Eurogen (St. Petersburg, Russia), and library amplification was performed with barcoded primers from the NadPrep Universal Adapter (MDI) Module Kit from Nanodigmbio (Singapore), according to the respective manufacturers' protocols. The completed libraries were sequenced on a DNBSEQ-G50 device from MGI (Wuhan, China) in the paired-end 150 (PE150) mode.

#### 2.3.6. Bioinformatics Analysis

The quality of paired-end reads was evaluated using FastQC 0.12.0. Raw reads were trimmed and filtered with TrimGalore 0.6.10, applying the additional parameter  $-1100$ . Metagenomic reads were classified into taxonomic groups using Kraken2 [37] with the Kraken2 PlusPF database, which includes RefSeq entries for archaea, bacteria, viruses, plasmids, humans, protozoa and fungi (12 January 2024). Bracken was employed to refine the quantitative estimates generated by Kraken2. The classification results were further processed using the KrakenTools toolkit, and taxonomic data were visualized with KronaTools.

#### 2.3.7. Microscopy

The microstructure of the samples was examined using optical microscopy (Bresser Advance ICD 10 $\times$ –160 $\times$ , Rhede, Germany), and scanning electron microscopy (SEM) at an accelerating voltage of 1 kV using a Tescan Amber GMH microscope (Brno, Czech Republic).

#### 2.4. Statistics

Statistical data processing and chart plotting were performed using Excel 2010 (Microsoft, Redmond, WA, USA) and OriginPro 16 (Microcal, San Diego, CA, USA). The data are presented as the mean values from at least three independent experiments, with error bars representing the standard error. Statistical significance of differences was assessed using Student's *t*-test.

### 3. Results and Discussion

Previously, we studied the biomineralizing properties of different ureolytic microorganisms [29,31,38]: *Bacillus subtilis* 168, *B. cereus* 4b, *B. subtilis* K51, *Micrococcus luteus* 6 and *B. licheniformis* DSMZ 8782. In the presented work, we used a strain of *B. licheniformis* DSMZ 8782, a non-pathogenic, spore-forming bacterium that has demonstrated good effectiveness in calcium carbonate precipitation, improved soil strength and resistance to extreme environmental conditions. The strain of the fungus *Scytalidium candidum* 3C was chosen due to the presence of a complex of cellulolytic enzymes capable of decomposing wastepaper added to the soil.

#### 3.1. Properties of MICP-Treated Soil Samples with Wastepaper Additives

In accordance with the experimental design (Figure 1), soil samples were prepared with varying wastepaper contents and treated with bacterial or fungal biomass, along with the cementing solution. After 14 days of treatment, the resulting soil samples were thoroughly characterized.

##### 3.1.1. Compressive Strength of Soil Samples with Different Wastepaper Contents Treated with Bacterial and Fungal Biomasses

As shown in Figure 2, increasing the wastepaper content in the soil resulted in a corresponding increase in strength for both the control and the samples treated with the bacterial suspension. The highest strength (277.2 kPa) was observed in the Bact soil sample containing 6% wastepaper. Among the Fng samples, the highest strength was recorded in the sample without wastepaper (236.6 kPa). However, the addition of wastepaper reduced the strength: a sample with 2% wastepaper had a strength of 140.1 kPa, while further increases to 4% and 6% wastepaper reduced the strength to 97.6 kPa and 62.2 kPa,

respectively. The strength of soil samples treated with a mixture of bacterial and fungal biomasses increased compared to the control only at 0% and 2% wastepaper contents. Notably, the Mix sample with 2% wastepaper exhibited the highest strength among all samples with the same wastepaper content.

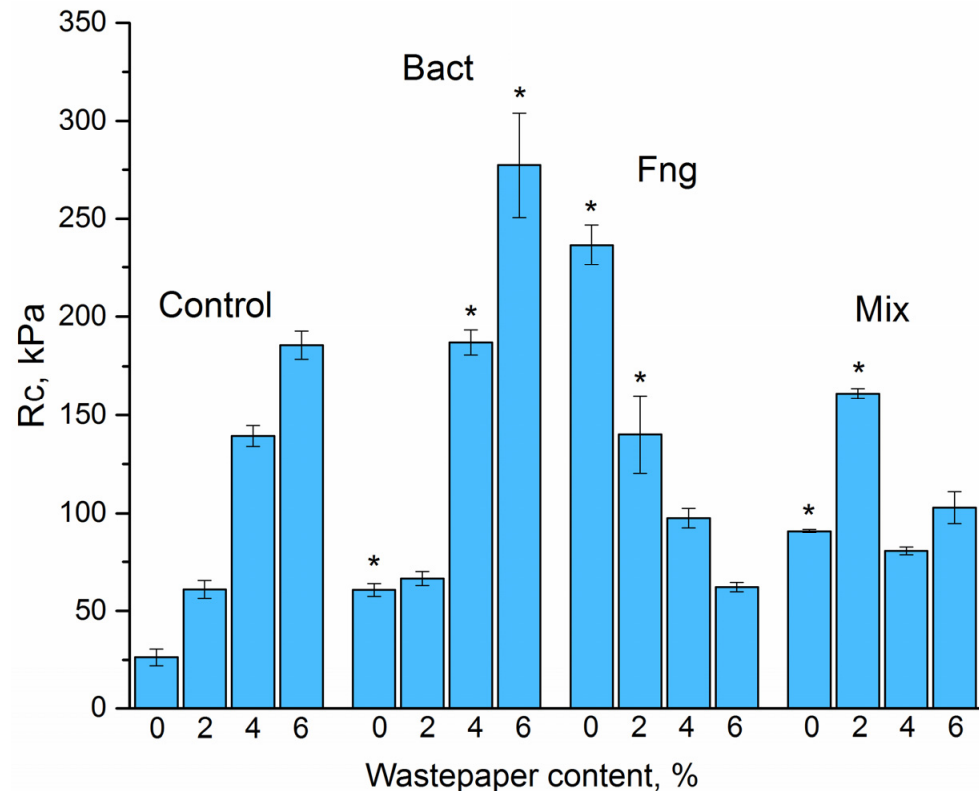


**Figure 2.** Values for compressive strength ( $R_c$ ) of MICP-treated soil samples with wastepaper additives. Soil sample treated with cementation solution (without MICP) was used as control. \* Significantly different ( $p < 0.05$ ).

### 3.1.2. Formation of Calcium Carbonate in Soil Samples with Different Wastepaper Contents, Treated with Bacterial and Fungal Biomasses

The calcium carbonate content was measured at different soil samples layers (top, bottom and middle). The addition of wastepaper did not increase the total  $\text{CaCO}_3$  content (Figure 3A), but it significantly affected its distribution within the soil sample. In the Control, Fng, Bact (in the 0–4% range) and Mix (in the 2–6% range) samples, an increase in wastepaper content was associated with a decrease in  $\text{CaCO}_3$  in the bottom layer of the soil (Figure 3B).

This observation is consistent with the findings of Chen [27], who reported a similar effect in sand biocementation with the addition of wastepaper fibers. They suggested that paper fibers help retain mineralizing bacteria in the top layers of the sample by binding them, preventing their settlement in the bottom layers. In soil, similar to sand, bacterial cells may be washed away from the top layer by gravity, but the presence of paper fibers can prevent their downward migration.

It is important to recognize that, in addition to preventing bacterial leaching, the wastepaper fibers significantly enhance soil strength due to their natural reinforcing properties. Several reports [24,39,40] have shown that the introduction of both synthetic and natural fibers leads to an increase in the compressive strength of soil. In untreated soil, the observed strength enhancement is primarily due to the increased calcium carbonate content precipitated by the microorganisms.

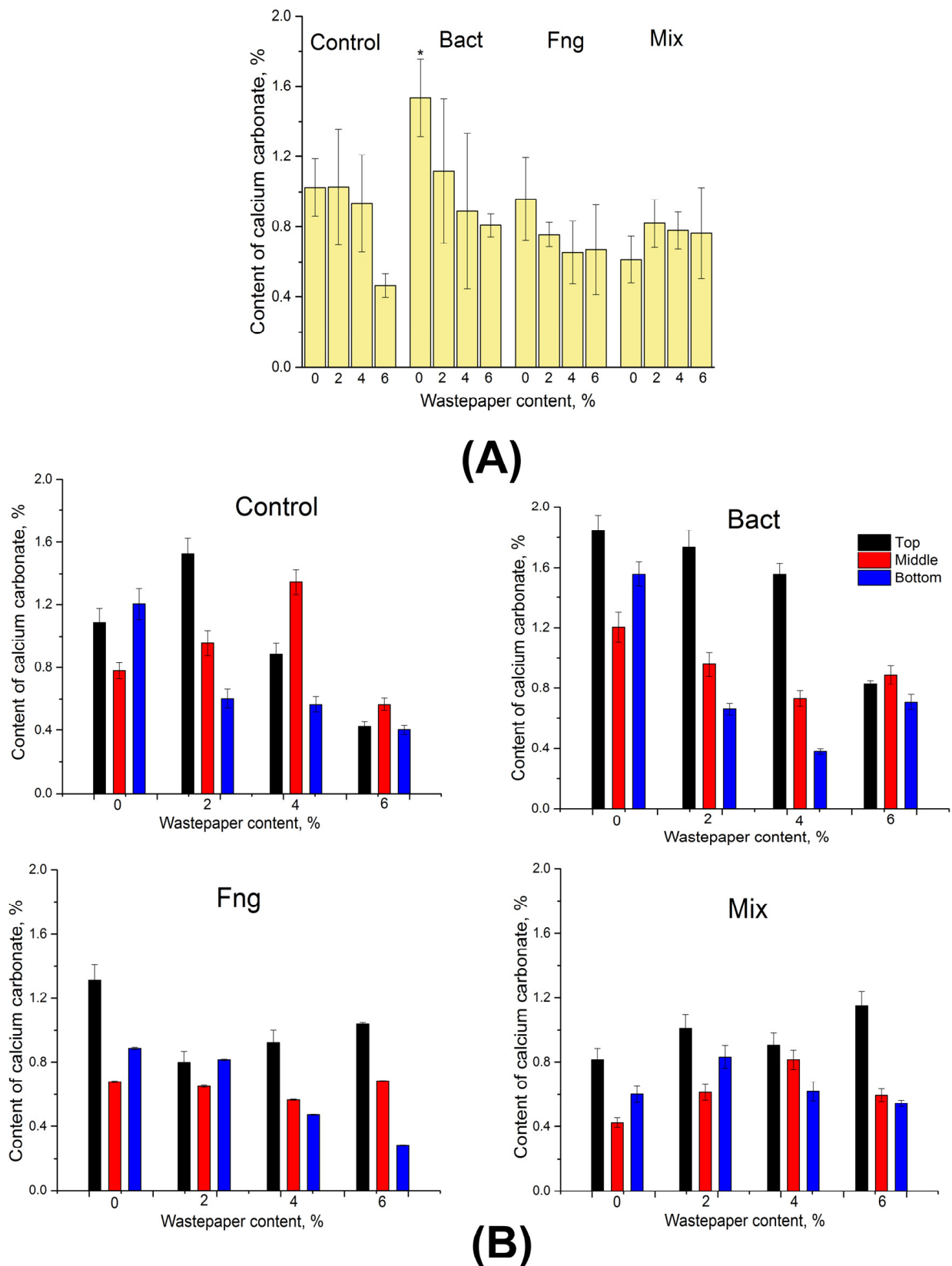


**Figure 3.** Percentages of calcium carbonate formed in MICP-treated soil samples with waste paper additives: (A)—average calcium carbonate content; (B)—CaCO<sub>3</sub> contents derived from top, middle and bottom layers of the soil sample. Soil sample treated with CS (without MICP) was used as control. \* Significantly different ( $p < 0.05$ ).

As can be seen in Figure 3A, the treatment of soil with a *B. licheniformis* suspension, without wastepaper, increased the total  $\text{CaCO}_3$  content by 1.5 times (Bact vs. Control samples) and doubled the strength of the sample (Figure 2). In a previous study [29], treating soil with a mixture of bacteria (without additives) resulted in a similar 1.5-fold increase in precipitated calcium carbonate content. In the present experiment, the strength of Control and Bact samples increased with the addition of wastepaper, while the total  $\text{CaCO}_3$  content decreased (Figures 2 and 3A). In the study by Chen [27], the effect of adding varying amounts (0–8%) of wastepaper fibers to sand was investigated. The results showed that the optimal fiber content was 1%, as further increases in fiber content resulted in a decrease in the strength of the samples. In summary, as the wastepaper content increased, the  $\text{CaCO}_3$  content in most samples decreased. We suggest that 2% wastepaper is the optimal amount, as it does not interfere with microbial biomineralization and enhances strength through reinforcement.

Surprisingly, the use of fungi in soil treatment led to unexpected results. As mentioned in Section 3.1.1, the introduction of wastepaper reduced the soil strength and was accompanied by a proportional decrease in  $\text{CaCO}_3$  content (Figure 3A). A comparison of the Bact and Fng samples without additives shows that the fungi-treated sample contained 1.6 times less calcium carbonate than the bacteria-treated sample (0.96% in Fng vs. 1.53% in Bact samples). However, the strength of the Fng sample was almost four times higher (236.6 kPa in Fng vs. 60.63 kPa in Bact). This discrepancy may be due to the presence of other microorganisms in the non-sterile soil, which could contribute to mineralization through mechanisms other than ureolysis. For example, phosphate mineralization induced by phytases represents another possible pathway for mineral precipitation [41]. Alternatively, it is possible that fungal biomass itself, through the release of polysaccharides and proteins [42], affects soil aggregate stability, thus enhancing the overall strength. This mechanism remains an area for further investigation.

The strength of the Mix sample without wastepaper was higher than that of the corresponding Bact sample but lower than the strength of the Fng sample. However, the Mix sample containing 2% wastepaper exhibited the highest strength (161.1 kPa) among all samples with the same wastepaper content, which correlated with an increase in calcium carbonate content. The strength of this sample was enhanced compared to untreated soil (61 kPa) and the bacteria-only (66.5 kPa) and fungi-only (140.12 kPa) treatments, by 2.6, 2.4 and 1.15 times, respectively. This suggests that the increased strength of the Mix sample with 2% wastepaper results from the combined effects of wastepaper addition and the mineralizing activity of microorganisms, possibly augmented by an additional, yet unexplained, contribution from the fungal biomass.

The SEM images of the Bact sample (Figure 4A,C) show calcite formation within the soil pores, induced by microbial activity. Seifan [43] demonstrated that bacteria act as nucleation sites for calcium carbonate crystals. In Figure 4A, a trace left by a bacterium on the surface of calcite can be seen, which likely functioned as a crystal nucleation site (indicated by the blue arrows). The light microscope images (Figure 4B,D) show the branched hyphal network formed during the soil treatment with fungal biomass. El Mountassir [21] showed that fungal hyphae of *Pleurotus ostreatus* can lead to the enmeshment and entanglement of sand particles, with hyphae and sclerotia transforming loose sand into a cohesive mass. It can be inferred that the fungi create an organic framework within the soil, providing a surface onto which bacteria can deposit calcium carbonate crystals. This process enhances the strength of the structure and ensures its distribution throughout the sample. However, the calcium content in the Fng samples did not increase after MICP treatment (Figure 3A).

Fungal hyphae are known for their high mechanical stability, which is due to the rigid cell wall and the turgor pressure exerted by the protoplast on the inner side of the cell wall. These factors enable hyphae to actively penetrate mineral substrates [17]. It is possible that the addition of wastepaper inhibited the growth and spread of fungal hyphae within the soil, leading to a decrease in strength as the amount of wastepaper increased. Furthermore, the reduction in the reinforcing effect of wastepaper might be due to the

cellulolytic enzyme complex produced by the fungus *Sc. candidum* [44], which could have led to partial decomposition of the wastepaper added to the soil.

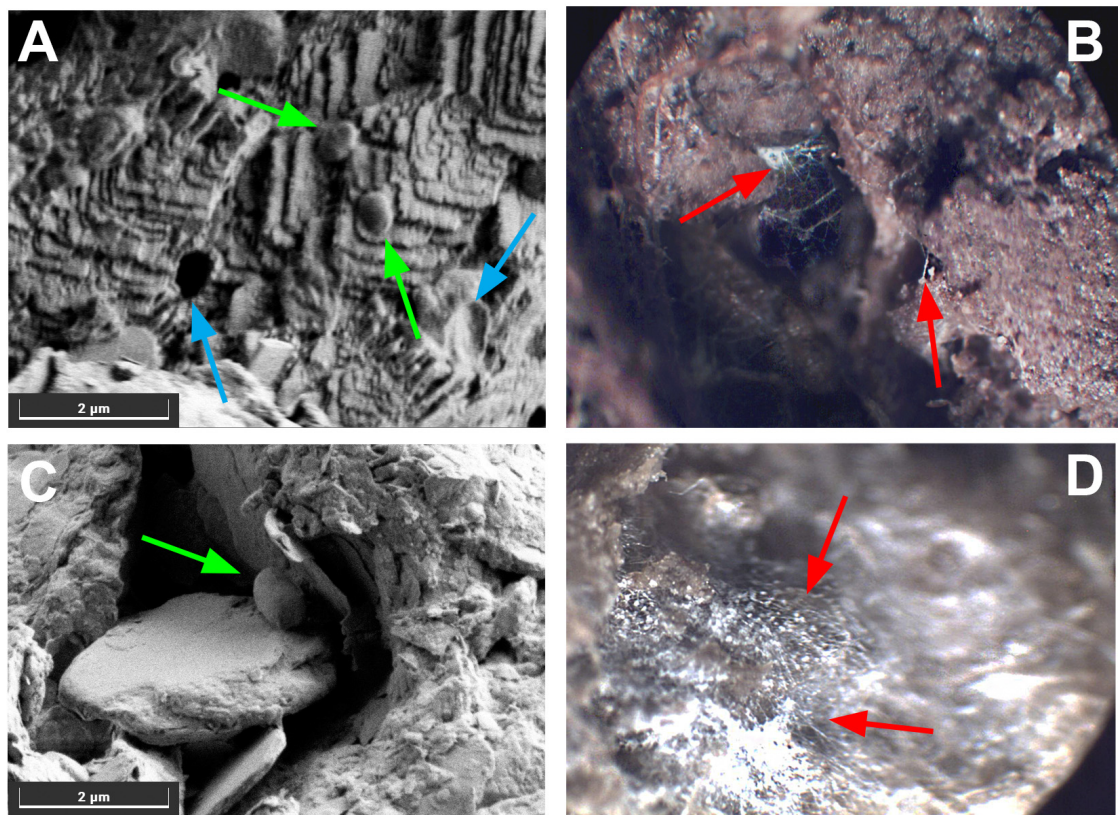


**Figure 4.** Images of soil samples with the addition of wastepaper treated with bacterial and fungal biomass. (A,C) Scanning electron microscopy (SEM) image of Bact sample, where the green arrows show bacteria on the surface of a calcium carbonate crystal deposited in the soil, while the blue arrows show the trace of bacteria around which calcite formed; (B,D) light microscopy image (10×) of Fng sample, where the red arrows mark the hyphae of the fungus inside the soil structure.

### 3.1.3. Results of Whole-Genome Sequencing of the Microbiome of Soil Samples Treated with Bacterial and Fungal Biomasses

To understand how treating soil with bacterial and fungal biomass affects its bacterial composition, we analyzed the microbiota of soil samples without wastepaper. As shown in Figure 5, the addition of *Bacillus licheniformis* DSMZ 8782 increased the proportion of bacteria from the genus *Bacillus*, from 0.39% in the Control sample to 1.0% and 1.34% in the Bact and Mix samples, respectively. The number of *Streptomyces* bacteria also increased, from 7.01% in the Control sample to 10.07% in the Bact sample and 8.98% in the Mix sample. Overall, the microbiomes in the Control and Bact samples did not differ significantly.

However, when *Sc. candidum* biomass was added to the soil, the microbial communities in the Fng and Mix samples changed considerably. In these samples, the proportions of most bacteria that were not well-represented in the samples without the fungus increased. Conversely, the population of nitrogen-fixing bacteria from the genus *Bradyrhizobium* decreased almost twofold. The addition of fungal biomass appears to have altered the soil's composition, either promoting or inhibiting the growth of various bacteria. For example, because *Sc. candidum* was pre-cultured on a medium containing filter paper, its biomass may have contained degradation products [44], which could have stimulated the growth of cellulolytic bacteria [45]. One such bacterium is *Cellulosimicrobium*, whose proportion increased from 0.08% in the Control and Bact samples to 0.75% and 2.75% in the Fng and Mix samples, respectively.

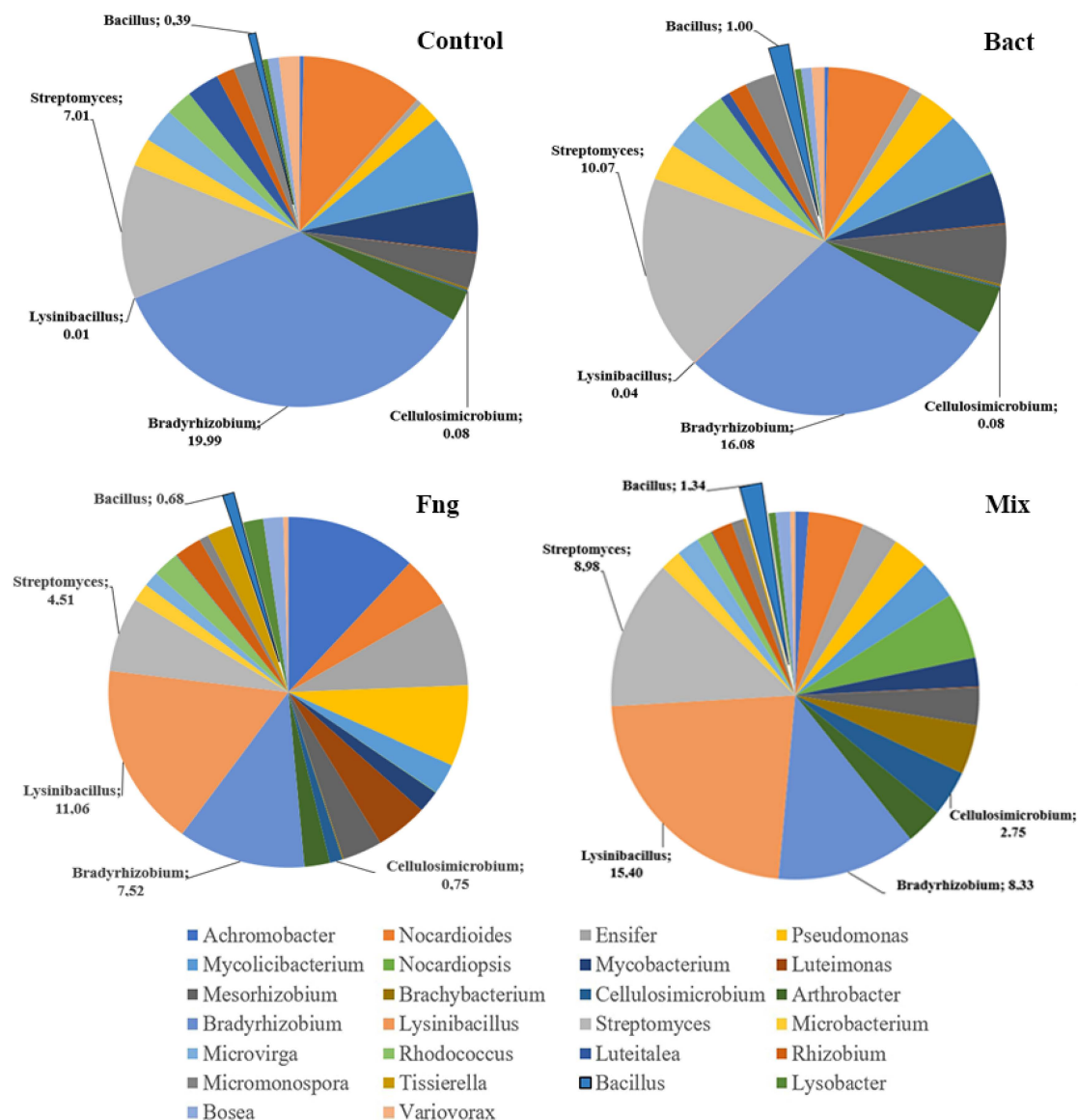


**Figure 5.** Results of whole-genome sequencing analysis of soil samples (with read overlap greater than 0.3%).

Additionally, the content of *Lysinibacillus* bacteria significantly increased in the Fng and Mix samples (11.06% and 15.4% in Fng and Mix samples vs. 0.01% and 0.04% in Control and Bact samples, respectively). Meanwhile, a representative of this genus, *Lysinibacillus xylanilyticus*, a species known for its mineral-forming properties, was used in soil treatment by Chen [27]. This increase in *Lysinibacillus* may explain, in part, the improvement in the strength of the Fng and Mix samples.

#### 4. Conclusions

One of the main objectives of this study was to determine the optimal amount of wastepaper to be introduced into soil treated with microbially induced calcite precipitation (MICP), continuing our previous work [29]. Additionally, we investigated the potential of using the ureolytic bacteria *Bacillus licheniformis* DSMZ 8782 and the yeast-like fungus *Scytalidium candidum* 3C for soil stabilization, both individually and in combination.

The experimental results showed that the optimal amount of wastepaper to be added was 2%, both for soil samples treated with the bacterial suspension of *B. licheniformis* and for soil samples where the natural biomineralizing microorganisms were involved. At

this concentration, the wastepaper did not hinder the biomineralization process, while it enhanced the strength of the samples through reinforcement with the wastepaper fibers.

Treatment with fungal biomass led to a significant increase in strength in the Fng soil sample, which did not contain wastepaper. Its strength increased nearly ninefold compared to the control (236.6 kPa vs. 26.4 kPa) and almost fourfold compared to the bacterial treatment (236.6 kPa vs. 60.63 kPa). However, the introduction of wastepaper into the Fng samples led to a decrease in strength, which correlated with a reduction in the calcium carbonate content.

The high strength of the Mix soil samples (with 0% and 2% wastepaper) treated with a combination of bacterial and fungal biomasses—compared to both the Control and Bact samples with the same wastepaper content—can be attributed not only to the increased calcium carbonate content, which is characteristic of traditional MICP treatment, but also to the synergistic effects of the reinforcing wastepaper fibers and the mineralizing activity of the microorganisms, including the not entirely clear effect of fungal biomass.

Furthermore, we found that the addition of the mycelial fungus *Sc. candidum* to the soil samples led to an increased proportion of bacterial strains capable of biomineralization, particularly from the genus *Lysinibacillus*. Thus, the introduction of *Sc. candidum* into the MICP process yielded promising results in terms of soil strength enhancement, suggesting it could be a valuable addition to the treatment process and merits more detailed study.

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## References

1. United Nations Environment Programme. *Emissions Gap Report 2023: Broken Record—Temperatures Hit New Highs, Yet World Fails to Cut Emissions (Again)*; United Nations Environment Programme: Nairobi, Kenya, 2023; ISBN 9789280740981.
2. Chavan, S.; Yadav, B.; Atmakuri, A.; Tyagi, R.D.; Wong, J.W.C.; Drogui, P. Bioconversion of Organic Wastes into Value-Added Products: A Review. *Bioresour. Technol.* **2022**, *344*, 126398. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Zhang, K.; Tang, C.-S.; Jiang, N.-J.; Pan, X.-H.; Liu, B.; Wang, Y.-J.; Shi, B. Microbial-induced Carbonate Precipitation (MICP) Technology: A Review on the Fundamentals and Engineering Applications. *Environ. Earth Sci.* **2023**, *82*, 229. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Bibi, S.; Oualha, M.; Ashfaq, M.Y.; Suleiman, M.T.; Zouari, N. Isolation, Differentiation and Biodiversity of Ureolytic Bacteria of Qatari Soil and Their Potential in Microbially Induced Calcite Precipitation (MICP) for Soil Stabilization. *RSC Adv.* **2018**, *8*, 5854–5863. [\[CrossRef\]](#)
5. Han, J.; Lian, B.; Ling, H. Induction of Calcium Carbonate by *Bacillus Cereus*. *Geomicrobiol. J.* **2013**, *30*, 682–689. [\[CrossRef\]](#)
6. Krajewska, B. Urease-Aided Calcium Carbonate Mineralization for Engineering Applications: A Review. *J. Adv. Res.* **2018**, *13*, 59–67. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Liu, S.; Du, K.; Wen, K.; Huang, W.; Amini, F.; Li, L. Sandy Soil Improvement through Microbially Induced Calcite Precipitation (MICP) by Immersion. *J. Vis. Exp.* **2019**, *151*, e60059. [\[CrossRef\]](#)
8. Zheng, X.; Lu, X.; Zhou, M.; Huang, W.; Zhong, Z.; Wu, X.; Zhao, B. Experimental Study on Mechanical Properties of Root–Soil Composite Reinforced by MICP. *Materials* **2022**, *15*, 3586. [\[CrossRef\]](#)

9. Sharaky, A.M.; Mohamed, N.S.; Elmashad, M.E.; Shredah, N.M. Application of Microbial Biocementation to Improve the Physico-Mechanical Properties of Sandy Soil. *Constr. Build. Mater.* **2018**, *190*, 861–869. [\[CrossRef\]](#)
10. Li, J.; Achal, V. Self-assembled Silk Fibroin Cross-linked with Genipin Supplements Microbial Carbonate Precipitation in Building Material. *Environ. Microbiol. Rep.* **2023**, *15*, 797–808. [\[CrossRef\]](#)
11. Zhu, T.; Dittrich, M. Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review. *Front. Bioeng. Biotechnol.* **2016**, *4*, 4. [\[CrossRef\]](#)
12. Chuo, S.C.; Mohamed, S.F.; Mohd Setapar, S.H.; Ahmad, A.; Jawaid, M.; Wani, W.A.; Yaqoob, A.A.; Mohamad Ibrahim, M.N. Insights into the Current Trends in the Utilization of Bacteria for Microbially Induced Calcium Carbonate Precipitation. *Materials* **2020**, *13*, 4993. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Wang, Y.; Sun, X.; Miao, L.; Wang, H.; Wu, L.; Shi, W.; Kawasaki, S. State-of-the-Art Review of Soil Erosion Control by MICP and EICP Techniques: Problems, Applications, and Prospects. *Sci. Total Environ.* **2024**, *912*, 169016. [\[CrossRef\]](#)
14. Ezzat, S.M. A Critical Review of Microbially Induced Carbonate Precipitation for Soil Stabilization: The Global Experiences and Future Prospective. *Pedosphere* **2023**, *33*, 717–730. [\[CrossRef\]](#)
15. De Muynck, W.; De Belie, N.; Verstraete, W. Microbial Carbonate Precipitation in Construction Materials: A Review. *Ecol. Eng.* **2010**, *36*, 118–136. [\[CrossRef\]](#)
16. Oualha, M.; Bibi, S.; Sulaiman, M.; Zouari, N. Microbially Induced Calcite Precipitation in Calcareous Soils by Endogenous *Bacillus Cereus*, at High PH and Harsh Weather. *J. Environ. Manag.* **2020**, *257*, 109965. [\[CrossRef\]](#)
17. Bindschedler, S.; Cailleau, G.; Verrecchia, E. Role of Fungi in the Biomineralization of Calcite. *Minerals* **2016**, *6*, 41. [\[CrossRef\]](#)
18. Ahmad, A.; Rautaray, D.; Sastry, M. Biogenic Calcium Carbonate: Calcite Crystals of Variable Morphology by the Reaction of Aqueous  $\text{Ca}^{2+}$  Ions with Fungi. *Adv. Funct. Mater.* **2004**, *14*, 1075–1080. [\[CrossRef\]](#)
19. Menon, R.R.; Luo, J.; Chen, X.; Zhou, H.; Liu, Z.; Zhou, G.; Zhang, N.; Jin, C. Screening of Fungi for Potential Application of Self-Healing Concrete. *Sci. Rep.* **2019**, *9*, 2075. [\[CrossRef\]](#)
20. Takey, M.; Shaikh, T.; Mane, N.; Majumder, D.R. Bioremediation of Xenobiotics: Use of Dead Fungal Biomass as Biosorbent. *Int. J. Res. Eng. Technol.* **2014**, *3*, 565–570. [\[CrossRef\]](#)
21. El Mountassir, G.; Minto, J.M.; van Paassen, L.A.; Salifu, E.; Lunn, R.J. Applications of Microbial Processes in Geotechnical Engineering. *Adv. Appl. Microbiol.* **2018**, *104*, 39–91. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Amobonye, A.; Bhagwat, P.; Singh, S.; Pillai, S. Plastic Biodegradation: Frontline Microbes and Their Enzymes. *Sci. Total Environ.* **2021**, *759*, 143536. [\[CrossRef\]](#)
23. Sukumaran, R.K.; Christopher, M.; Kooloth-Valappil, P.; Sreeja-Raju, A.; Mathew, R.M.; Sankar, M.; Puthiyamadham, A.; Adarsh, V.-P.; Aswathi, A.; Rebinro, V.; et al. Addressing Challenges in Production of Cellulases for Biomass Hydrolysis: Targeted Interventions into the Genetics of Cellulase Producing Fungi. *Bioresour. Technol.* **2021**, *329*, 124746. [\[CrossRef\]](#)
24. Imran, M.A.; Gowthaman, S.; Nakashima, K.; Kawasaki, S. The Influence of the Addition of Plant-Based Natural Fibers (Jute) on Biocemented Sand Using MICP Method. *Materials* **2020**, *13*, 4198. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Yao, D.; Wu, J.; Wang, G.; Wang, P.; Zheng, J.-J.; Yan, J.; Xu, L.; Yan, Y. Effect of Wool Fiber Addition on the Reinforcement of Loose Sands by Microbially Induced Carbonate Precipitation (MICP): Mechanical Property and Underlying Mechanism. *Acta Geotech.* **2021**, *16*, 1401–1416. [\[CrossRef\]](#)
26. Wen, K.; Bu, C.; Liu, S.; Li, Y.; Li, L. Experimental Investigation of Flexure Resistance Performance of Bio-Beams Reinforced with Discrete Randomly Distributed Fiber and Bamboo. *Constr. Build. Mater.* **2018**, *176*, 241–249. [\[CrossRef\]](#)
27. Chen, M.; Gowthaman, S.; Nakashima, K.; Komatsu, S.; Kawasaki, S. Experimental Study on Sand Stabilization Using Bio-Cementation with Wastepaper Fiber Integration. *Materials* **2021**, *14*, 5164. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Avramenko, M.; Nakashima, K.; Takano, C.; Kawasaki, S. Eco-Friendly Soil Stabilization Method Using Fish Bone as Cement Material. *Sci. Total Environ.* **2023**, *900*, 165823. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Golovkina, D.A.; Zhurishkina, E.V.; Filippova, A.D.; Baranchikov, A.E.; Lapina, I.M.; Kulminskaya, A.A. Integration of Organic Waste for Soil Stabilization through MICP. *Appl. Sci.* **2023**, *14*, 62. [\[CrossRef\]](#)
30. Pavlov, I.Y.; Bobrov, K.S.; Sumacheva, A.D.; Masharsky, A.E.; Polev, D.E.; Zhurishkina, E.V.; Kulminskaya, A.A. *Scytalidium candidum* 3C Is a New Name for the *Geotrichum candidum* Link 3C Strain. *J. Basic Microbiol.* **2018**, *58*, 883–891. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Golovkina, D.A.; Zhurishkina, E.V.; Ivanova, L.A.; Baranchikov, A.E.; Sokolov, A.Y.; Bobrov, K.S.; Masharsky, A.E.; Tsvigun, N.V.; Kopitsa, G.P.; Kulminskaya, A.A. Calcifying Bacteria Flexibility in Induction of  $\text{CaCO}_3$  Mineralization. *Life* **2020**, *10*, 317. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Shoba, S.A.; Rozhkov, V.A.; Alyabina, I.O.; Kolesnikova, V.M.; Urusevskaya, I.S.; Molchanov, E.N.; Stolbovoy, V.S.; Sheremet, B.V.; Konyushkov, D.E. *Soil Geographic Database of Russia*, 2nd ed.; Huang, P.M., Li, Y., Sumner, M.E., Eds.; CRC Press: Boca Raton, FL, USA, 2011.
33. ASTM D2487-17; Standard Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System). ASTM International: West Conshohocken, PA, USA, 2000.
34. ASTM D7012-14e1; Standard Test Methods for Compressive Strength and Elastic Moduli of Intact Rock Core Specimens Under Varying States of Stress and Temperatures. ASTM International: West Conshohocken, PA, USA, 2014.
35. ASTM D 4373; Standard Test 5 Method for Calcium Carbonate Content in Soil. ASTM International: West Conshohocken, PA, USA, 2021.

36. Ignatov, K.B.; Blagodatskikh, K.A.; Shcherbo, D.S.; Kramarova, T.V.; Monakhova, Y.A.; Kramarov, V.M. Fragmentation Through Polymerization (FTP): A New Method to Fragment DNA for next-Generation Sequencing. *PLoS ONE* **2019**, *14*, e0210374. [[CrossRef](#)]
37. Lu, J.; Rincon, N.; Wood, D.E.; Breitwieser, F.P.; Pockrandt, C.; Langmead, B.; Salzberg, S.L.; Steinegger, M. Metagenome Analysis Using the Kraken Software Suite. *Nat. Protoc.* **2022**, *17*, 2815–2839. [[CrossRef](#)]
38. Golovkina, D.A.; Zhurishkina, E.V.; Xu, J. Field Trials of Soil Improvement Technology with a Bacterial Mixture. In *Biogenic—Abiogenic Interactions in Natural and Anthropogenic Systems 2022*; Springer Nature: Berlin/Heidelberg, Germany, 2023.
39. Qiu, R.; Tong, H.; Fang, X.; Liao, Y.; Li, Y. Analysis of Strength Characteristics of Carbon Fiber-Reinforced Microbial Solidified Sand. *Adv. Mech. Eng.* **2019**, *11*, 168781401988442. [[CrossRef](#)]
40. Imran, M.A.; Nakashima, K.; Evelpidou, N.; Kawasaki, S. Durability Improvement of Biocemented Sand by Fiber-Reinforced MICP for Coastal Erosion Protection. *Materials* **2022**, *15*, 2389. [[CrossRef](#)]
41. Roeselers, G.; Van Loosdrecht, M.C.M. Microbial Phytase-Induced Calcium-Phosphate Precipitation—A Potential Soil Stabilization Method. *Folia Microbiol.* **2010**, *55*, 621–624. [[CrossRef](#)] [[PubMed](#)]
42. Rillig, M.C.; Mummey, D.L. Mycorrhizas and Soil Structure. *New Phytol.* **2006**, *171*, 41–53. [[CrossRef](#)] [[PubMed](#)]
43. Seifan, M.; Samani, A.K.; Berenjian, A. Bioconcrete: Next Generation of Self-Healing Concrete. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 2591–2602. [[CrossRef](#)]
44. Pavlov, I.Y.; Eneyskaya, E.V.; Bobrov, K.S.; Polev, D.E.; Ivanen, D.R.; Kopylov, A.T.; Naryzhny, S.N.; Kulinskaya, A.A. Comprehensive Analysis of Carbohydrate-Active Enzymes from the Filamentous Fungus *Scytalidium candidum* 3C. *Biochemistry* **2018**, *83*, 1399–1410. [[CrossRef](#)]
45. Kimeklis, A.K.; Gladkov, G.V.; Orlova, O.V.; Afonin, A.M.; Gribchenko, E.S.; Aksenova, T.S.; Kichko, A.A.; Pinaev, A.G.; Andronov, E.E. The Succession of the Cellulolytic Microbial Community from the Soil during Oat Straw Decomposition. *Int. J. Mol. Sci.* **2023**, *24*, 6342. [[CrossRef](#)] [[PubMed](#)]

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