

Article

Biostimulation in Desert Soils for Microbial-Induced Calcite Precipitation

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Abstract: Microbial-induced calcite precipitation (MICP) is a soil amelioration technique aiming to mitigate different environmental and engineering concerns, including desertification, soil erosion, and soil liquefaction, among others. The hydrolysis of urea, catalyzed by the microbial enzyme urease, is considered the most efficient microbial pathway for MICP. Biostimulated MICP relies on the enhancement of indigenous urea-hydrolyzing bacteria by providing an appropriate enrichment and precipitation medium, as opposed to bioaugmentation, which requires introducing large volumes of exogenous bacterial cultures into the treated soil along with a growth and precipitation medium. Biostimulated MICP in desert soils is challenging as the total carbon content and the bacterial abundance are considerably low. In this study, we examined the biostimulation potential in soils from the Negev Desert, Israel, for the purpose of mitigation of topsoil erosion in arid environments. Incubating soil samples in urea and enrichment media demonstrated effective urea hydrolysis leading to pH increase, which is necessary for calcite precipitation. Biostimulation rates were found to increase with concentrations of energy (carbon) source in the stimulation media, reaching its maximal levels within 3 to 6 days. Following stimulation, calcium carbonate precipitation was induced by spiking stimulated bacteria in precipitation (CaCl₂ enriched) media. The results of our research demonstrate that biostimulated MICP is feasible in the low-carbon, mineral soils of the northern Negev Desert in Israel.

Keywords: microbial-induced calcite precipitation; desert soil; biostimulation; erosion mitigation

1. Introduction

The ubiquity of bacteria and the diverse roles they play in natural environments have led to growing interest in harnessing bacterial activities for various anthropogenic purposes. From a physical point of view, soil is regarded as an inorganic multiphase system comprising solids, fluids, and gases. However, soil is also a living system, being one of the largest terrestrial carbon pools, constituting about 33% of the total terrestrial carbon [1]. The organic carbon in the top 1 m constitutes more than 50% of the total soil carbon. Prokaryotes comprise up to 17% of the soil organic carbon [2]. These unicellular organisms, mostly bacteria $0.5\text{--}5.0 \times 10^{-6}$ m in size, are about three orders of magnitude smaller than the pore throat size of sand and about the D10 size of kaolinite [3]. Soil bacteria, either motile or fixed to mineral surfaces (grains), may change the chemical and physical properties of their surroundings depending on their metabolism. Microbial biomass and biodiversity often exhibit exponential decreases with depth [4]; nevertheless, there are still active cells in deeper soil horizons [5].

Many bacteria are capable of inducing mineral precipitation through various metabolic paths in both oxic and anoxic environments. Boquet [6] showed that most heterotrophic bacteria can

induce precipitation of calcium carbonate (CaCO_3), a common natural cementing agent, by various metabolic pathways.

Microbial-induced calcite precipitation (MICP) is an emerging technique aiming to mitigate different environmental and engineering challenges, including soil erosion, soil liquefaction, fracture sealing, restoration of stone monuments, and others [7–13]. Hydrolysis of urea, catalyzed by the microbial enzyme urease (urea amidohydrolase, EC 3.5.1.5), is considered the most efficient microbial pathway for MICP [14,15]. The hydrolysis of urea produces ammonium and carbonate, seen in Equation (1), thus increasing the saturation for calcium carbonate, which could lead to its precipitation, usually as calcite, in Equation (2).



Two alternative approaches to facilitate in situ MICP in soils are biostimulation and bioaugmentation. Biostimulation encourages indigenous urea-hydrolyzing bacteria by providing appropriate enrichment and precipitation media; it relies on the natural ubiquity of ureolytic soil bacteria and bacterial spatial distribution [15,16]. Bioaugmentation introduces large volumes of bacterial cultures into the treated soil along with a growth and precipitation medium; therefore, it requires large volumes of pure-cultured specializing ureolytic bacteria, e.g., *Sporosarcina pasteurii*. Producing and transporting large volumes of these cultures is an expensive and delicate procedure; their injection and homogeneous distribution throughout the treated site are difficult to achieve and might encounter regulatory hindrances [17]. Moreover, the introduced bacteria are likely to decline in numbers due to low compatibility with the environment as well as competition and predation by indigenous bacteria [18]. As the two methods involve the introduction of large quantities of urea along with a source of organic carbon, both biostimulation and bioaugmentation are likely to affect and change the indigenous microbial population.

MICP soil improvement was successfully demonstrated at a variety of scales [10,19–22]. However, reliance on bioaugmentation has restricted the technology from becoming a cost-competitive alternative to more traditional ground amelioration techniques. Biostimulation, the use of selective substrates and environmental factors to stimulate the growth of native microorganisms with desirable metabolic capabilities, has been researched extensively in the field of bioremediation [23,24] with success in several notable field-scale applications [25,26]. Despite the frequent use of biostimulation in the field of bioremediation, the use of the biostimulation technique for enabling MICP is more limited [5,27–32].

Biostimulated MICP in desert soils is more challenging than in most soils as the total carbon content is low [33], and the bacterial population is considerably smaller. For example, in coastal sands from southern Israel (31.61°N, 34.50°E), the in situ bacterial population was found to be in the order of 10^4 cells/g [31], three to four orders of magnitude lower than in semiarid soils [34]. For this reason, MICP in low-carbon soils were typically attempted via bioaugmentation [35,36].

In this paper, we present the results of a research aimed to study the biostimulation potential in desert soil from the northern Negev Desert, Israel. The results reported here are part of an ongoing research project aimed to develop a MICP-based technique for the mitigation of topsoil erosion in arid environments and is planned to be followed by laboratory and field experiments. We envision different applications for MICP-based topsoil stabilization, such as the suppression of dust emission from vehicles in construction sites and open-pit mines, improving the efficiency of solar energy facilities and the mitigation of erosion by flash flood, among others.

Biostimulation for ureolytic MICP was confirmed at laboratory conditions by incubating soil samples in urea and enrichment media and monitoring urea depletion and pH evolution within a few days. Precipitation experiments using the indigenous, biostimulated bacteria demonstrated that calcite precipitation could be readily induced. Altogether, we report that urea-hydrolyzing bacteria are

naturally present in the topsoil sampled from the northern Negev Desert and that these bacteria can be effectively stimulated to induce calcite precipitation.

2. Materials and Methods

2.1. Soil Sampling and Chemical–Physical Characterization

Soils were sampled from a depth of 0.4 m from two sites (soil 1 and soil 2 separated by 500 m) of the Rotem Plateau (31.04°N, 35.08°E) at the northern Negev Desert in Israel, an arid region with an average annual rainfall of 70 mm. Samples were stored refrigerated at 4 °C until the biostimulation experiments began. Soils were analyzed for elemental composition by X-ray fluorescence (XRF) using an EX-Calibur spectrometer (Xenometrix, Migdal HaEmek, Israel). Mineralogical phase identification was performed by X-ray diffraction (XRD) using a Bruker D8 Advance system (Bruker, Billerica, MA, USA). Particle size distribution (PSD) was performed by laser diffraction using a Mastersizer 3000 system (Malvern Panalytical, Malvern, UK).

2.2. Biostimulation of Indigenous Ureolytic Microbes

Biostimulation was performed by incubating 10.0 g of soil samples collected from each of the two sites from the Rotem Plateau in 100 mL of different stimulation media at ambient temperature with gentle shaking at 100 rpm for ten days. The media composition is described in Section 2.3. Samples were taken periodically for chemical analysis, as described in Section 2.4.

2.3. Solutions and Stimulation Media

Four different stimulation media used in this study were prepared in artificial groundwater (AGW) consisting of MgCl₂ (1 mM), MgSO₄ (1 mM), NaHCO₃ (2.56 mM), NaCl (14.35 mM), CaCl₂ (2.43 mM), and KCl (0.32 mM); ionic strength was at 31.5 mM and pH at 7.7 [31]. Urea control media consisted of 330 mM urea and served as a control for bacterial stimulation without an energy source. Two stimulation media contained a 330 mM urea supplemented with either a low dose (0.1 g/L) or high dose (1 g/L) of yeast extract. AGW only media was used as a negative control.

2.4. Chemical Analysis of Stimulated Samples

During the bacterial stimulation experiments, samples were taken periodically for pH and urea concentration measurements. pH was measured using a Metrohm pH-meter (Metrohm, Herisau, Switzerland). Urea concentrations were measured according to the Knorst [37] colorimetric method, with minor modifications, on an 8453 Agilent spectrophotometer (Agilent, Santa Clara, CA, USA). During the stimulation experiment, urea concentration values of the urea control media were elevated by a 2.5% increase per day due to media evaporation. Urea concentrations of all urea-containing samples were therefore normalized accordingly.

2.5. Calcite Precipitation

Biostimulated bacteria (100 µL aliquots taken from stimulation supernatants) were spiked into 100 mL of yeast extract and CaCl₂-containing enrichment media at ambient temperature with gentle shaking to allow for calcite precipitation. Urea concentrations were systematically measured, as described in Section 2.4. At the end of the experiment (12–14 days), filtered and dried precipitates were analyzed by XRD.

3. Results

3.1. Chemical and Physical Characterization of Soils

Soils were collected from two sites of the Rotem Plateau at the northern Negev Desert in Israel, a broad plateau covered with clastic sediments of the Miocene Hazeva Fm. Soils are mainly composed

of quartz and calcite, as shown by XRD analyses (Figure 1). XRF analyses showed that the two soils are similar in their elemental composition; however, significantly higher levels of Si and lower levels of Ca were identified in soil 1 than soil 2 (Table 1). Particle size distribution analysis showed that the soils are classified as sand (Figure 2 and Table 2), with soil 2 showing a higher coarse sand fraction 94%, compared to 64% in soil 1, and a lower fine sand fraction, 6% and 36% in soil 2 and soil 1, respectively.

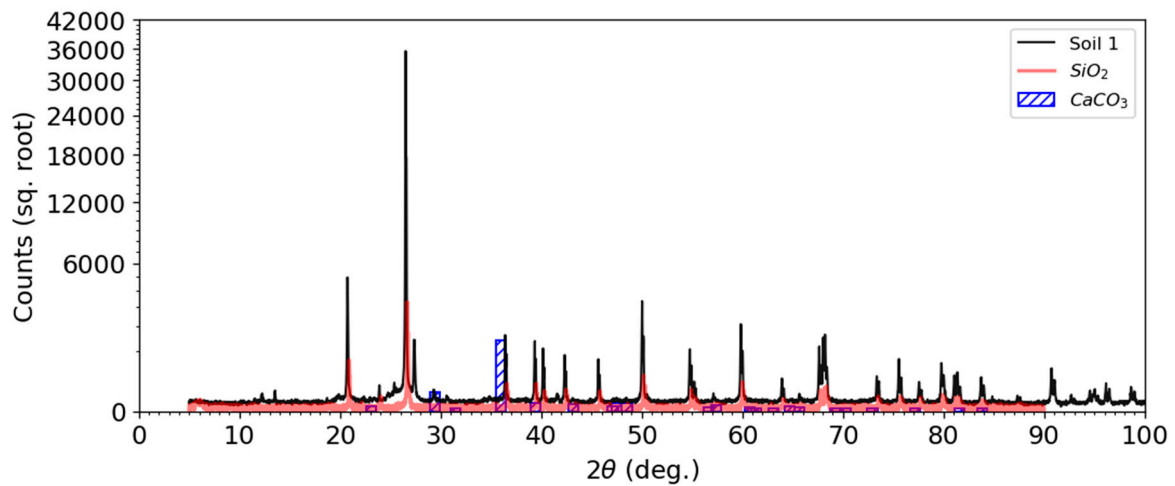


Figure 1. X-ray diffraction patterns of soil 1 sampled from the Rotem Plateau. Counts in arbitrary units.

Table 1. Elemental composition X-ray fluorescence (XRF) of soils from the Rotem Plateau.

Element	Soil 1 (%wt)	Soil 2 (%wt)
Si	76	47
Ca	14	40
Fe	10	13

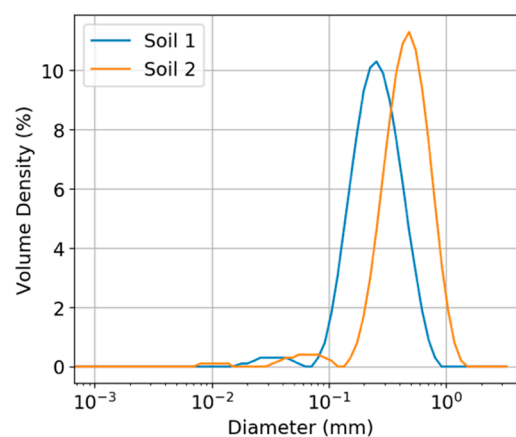


Figure 2. Particle size distribution of soils sampled from the Rotem Plateau in Israel.

Table 2. Particle size distribution (by volume) properties of soils from the Rotem Plateau.

Parameter	Soil 1 (μm)	Soil 2 (μm)
D ₁₀	136 ± 0.7	233 ± 12.8
D ₅₀	251 ± 2.8	442 ± 11.4
D ₉₀	467 ± 15.0	751 ± 29.1

3.2. Biostimulation of Indigenous Urea-Hydrolyzing Bacteria

Biostimulation experiments were conducted on soils sampled from the Rotem Plateau to test whether indigenous urea-hydrolyzing bacteria are naturally present in this region and can be effectively stimulated. Soils were incubated in different media for ten days, and urea hydrolysis was tested by pH and urea concentration measurements (Figure 3). Based on our previous experience [31], it was expected that if indigenous urea-hydrolyzing bacteria would be present in the soil, then their stimulation in the presence of urea would result in a pH rise due to the production and accumulation of ammonium ions. Stimulation of urea-hydrolyzing bacteria was evident in soils incubated in the presence of urea and a carbon source (yeast extract), as shown by pH elevation and urea degradation (low yeast extract concentrations (YE_L) and high yeast extract concentrations (YE_H)). The addition of a carbon source was required to achieve the stimulation, as no significant ureolysis was observed in the absence of yeast extract (Urea). Low yeast extract concentrations (YE_L) resulted in inefficient stimulation when compared to high yeast extract concentrations (YE_H) in the two soils studied. As expected, the pH of the negative control samples from the two sites remained unchanged, and urea concentrations were negligible throughout the biostimulation experiment (Figure 3, AGW).

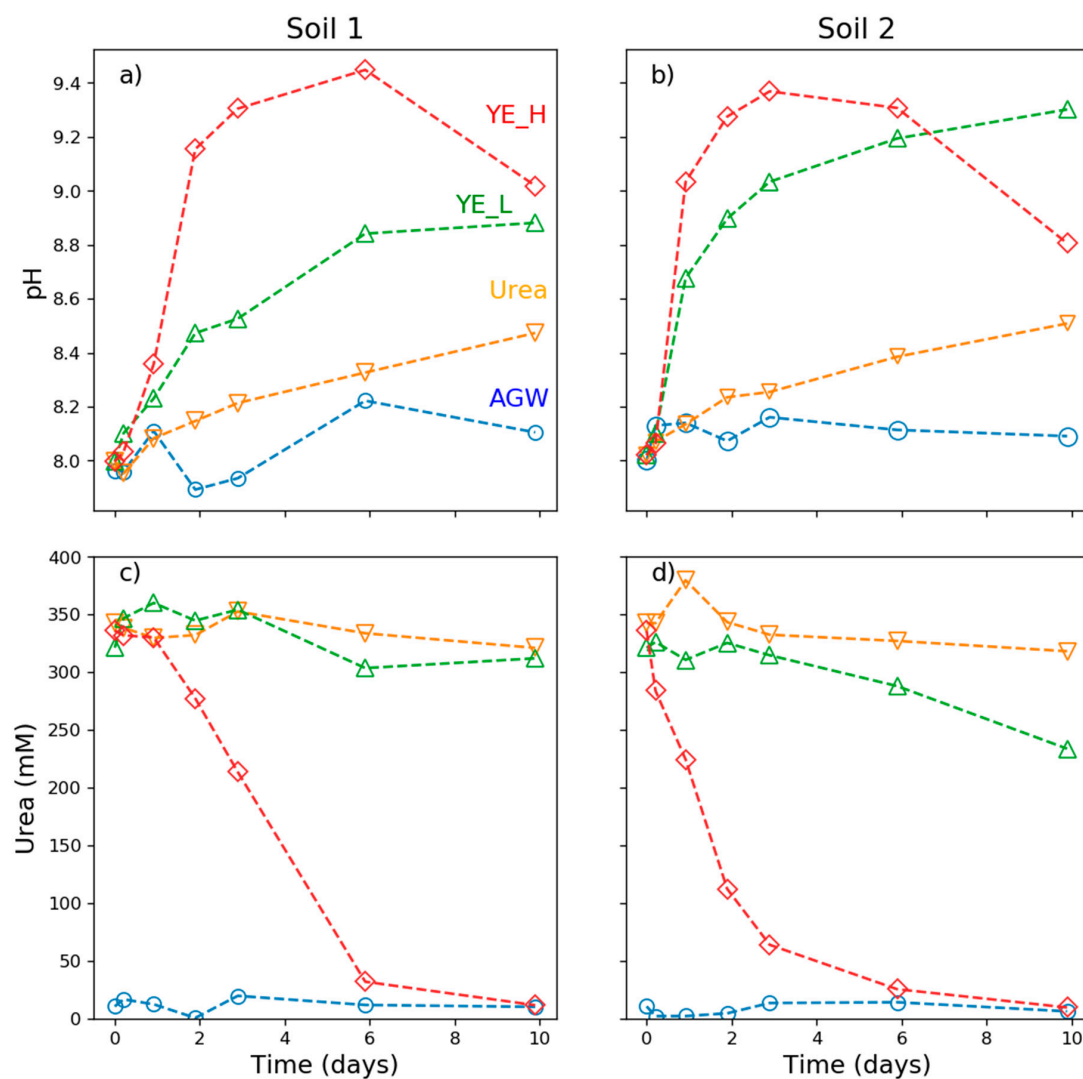


Figure 3. Biostimulation of soils from the stimulated soils from the Rotem Plateau. (a) pH of soil 1; (b) pH of soil 2; (c) urea concentration of soil 1 and (d) urea concentration of soil 2. AGW is artificial ground water (o); urea is urea control (V); YE_L is low (0.1 g/L) yeast extract enriched medium (Δ); YE_H is high (1.0 g/L) yeast extract enriched medium ($\diamond$).

To provide direct evidence that MICP can be induced in these soils, precipitation experiments were conducted using bacteria stimulated with urea and 1.0 g/L yeast extract concentrations. Aliquots of bacteria stimulated from soils for seven days were spiked into fresh urea, and yeast extract-containing media were supplemented with CaCl_2 . Within two days, the media became turbid, and white precipitates appeared at the bottom of the Erlenmeyer flasks (data not shown). Urea hydrolysis was confirmed in samples provided with stimulated bacteria from either soil, albeit higher rates were observed in soil 2 than in soil 1 (Figure 4). This effect probably resulted from differences in biostimulation rates between the two soils (Figure 3). After two weeks of incubation, precipitates were collected, dried, and identified by XRD as calcite (Figure 5). Neither precipitation nor urea hydrolysis took place in negative control samples (AGW) incubated in the same conditions.

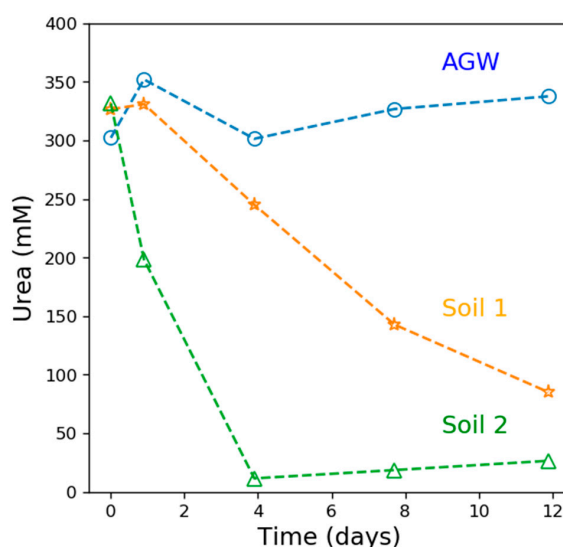


Figure 4. Urea concentrations measured in precipitation experiments following bacterial stimulation in soils from the Rotem Plateau.

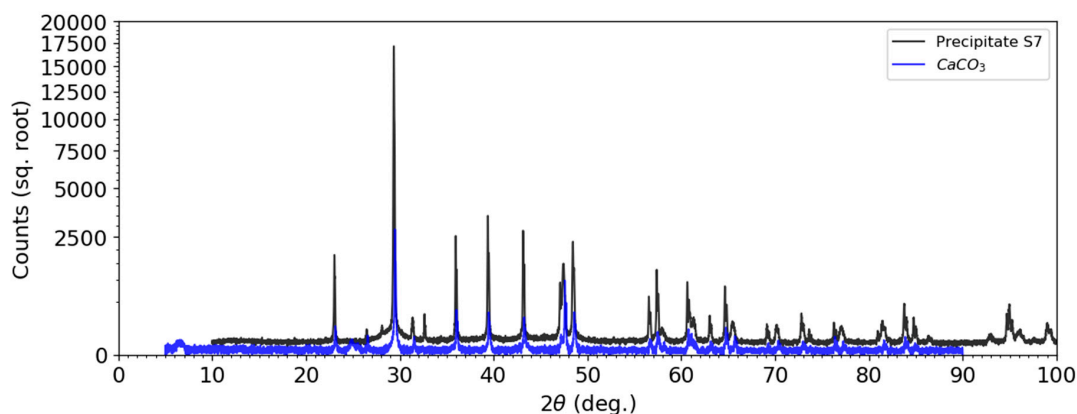


Figure 5. XRD patterns of precipitates produced by biostimulated soil (1) bacteria grown in CaCl_2 precipitation media. Counts in arbitrary units. CaCO_3 is calcite reference RRUFF R040070.

4. Discussion

Soil erosion, carbon sequestration, infrastructure rehabilitation, hazardous waste disposal, and water resources protection are all 21st-century global challenges. Many of these challenges occur within or are supported by soil. The conventional perspective views soil as an infinite resource, composed of discrete functions, e.g., hydraulic, mechanical, and ecological, among others. However, soil is also a diverse ecosystem. The biotic potential in soil offers prospects for innovative and sustainable

solutions for some of these challenges. Harnessing natural biogeochemical processes to improve the environmental conditions and/or engineering properties of geological deposits has received significant attention in the scientific community [5,22,36,38,39].

Microbial-induced calcite precipitation (MICP) is one of the most promising biogeochemical treatments that effectively changes the hydromechanical and environmental properties of materials. Potential applications range from groundwater remediation and sequestration of radionuclides [40] to self-healing concrete [41]. For engineered materials, such as concrete, MICP can be achieved via bioaugmentation only, i.e., the introduction of monoclonal cultivated bacteria. In natural environments, such as soils, effective bioaugmentation by exogenous bacteria is not warranted, as the introduced bacteria are likely to decline in numbers due to low compatibility to the environment, as well as competition and predation by indigenous bacteria [18]. Moreover, producing and transporting large volumes of these cultures is an expensive and delicate procedure; their injection and homogeneous distribution throughout the treated site are difficult to achieve and might encounter regulatory hindrances.

The ability to hydrolyze urea is widely distributed among indigenous bacteria in soils [15]. Hence, the biostimulation approach of MICP in soils is tangible. The main challenge with biostimulated MICP is inducing effective urea hydrolysis, which requires a sufficiently large ureolytic population. This challenge is even greater in nutrient-poor soils, where the initial bacterial abundance is low.

Gat [31] showed that for MICP, effective biostimulation of an indigenous, ureolytic population in soil from a semiarid region (BSh (hot semiarid climate) in Köppen climate classification) requires an energy (carbon) source, in addition to urea. No ureolysis was observed in the absence of a carbon source. The authors showed significant changes to the indigenous bacterial population following biostimulation, where ureolytic bacteria population increased from 5% in the native sand up to 99% in carbon high dosage treatments.

The primary goal of our research was to evaluate the potential of biostimulating urea-hydrolyzing bacteria in desert soils of the Rotem Plateau of the northern Negev Desert, Israel (BWh (hot desert climate) in Köppen climate classification), for soil erosion mitigation via MICP biocementation of the topsoil. We found that indigenous urea-hydrolyzing bacteria are naturally present in the desert soils and can be readily stimulated to achieve effective urea hydrolysis and calcite precipitation within days. These results are consistent with the results of [31]. It was also found that bacteria stimulation was not affected by differences in soil mineralogy observed between the two soils. Gomez [5] showed that urea hydrolysis could be effectively stimulated even at greater depths (10 m) using low doses of yeast extract and alkaline pH adjustment of the treatment media. Biostimulation rates in the desert soils were accelerated by providing a yeast extract dose of 1.0 g/L, whereas a lower dose of 0.1 g/L was found to be ineffective. Other more cost-effective energy sources can be utilized to promote biostimulation, such as off-the-shelf molasses [31] with similar concentrations of 1.0 g/L.

To conclude, the results presented above demonstrate that desert soils from the Rotem Plateau of the northern Negev Desert (Israel) are susceptible to the biostimulation of ureolytic native bacteria. Effective urea degradation is a necessary requirement for in situ MICP, which is designed to achieve effective biocementation to mitigate topsoil erosion.

Author Contributions: H.R.-A. and M.T. designed the research methodology; H.R.-A. performed the physiochemical analyses; H.R.-A. and M.T. analyzed the data and wrote the original draft; H.R.-A. and M.T. secured the research funding. The authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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