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Investigating the Possible Bio-stabilization of Rammed Earth Through Microorganisms

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Abstract

In this study, three microorganisms, *Bacillus subtilis*, *Sporosarcina pasteurii*, and *Bacillus subtilis* subsp. *subtilis*, which are capable of inducing calcite precipitation effectively, were used to investigate possible stabilization and improvement in the compressive strength of the rammed earth. The microorganisms are suspended in a solution of yeast extract and ammonium sulfate. The effects of different concentrations of microorganisms and calcium resources were studied by producing a total of eleven mixes. The concentrations of the bacteria were determined by the optical density method, OD_{600}. As calcium resources, low-cost and locally available materials such as air lime and gypsum were used in different amounts. The influence of various microorganisms at different concentrations on earth stabilization was determined by performing uniaxial compression test at different ages, e.g., the 7th and 28th day after production. The cube specimens with a size of 50 x 50 x 50 mm were produced. The obtained results showed that both concentration of the microorganisms and the calcium amount in the medium are the main parameters affecting the mechanical performance of the earth samples. It was found that all microorganisms effectively enhance the compressive strength on and after 28 days. For a definite concentration of the *B. subtilis*, two times higher values, e.g., 8.4 MPa, were achieved on 28 days than reference mixes, which do not contain any microorganisms. Moreover, macro- and micro-scale observations are done to monitor calcite precipitation. It can be deduced that the preliminary test results are promising to be a feasible alternative to cement and or lime-stabilized rammed earth (SRE).

Introduction

In recent years, the construction industry has been meeting challenges in reducing the environmental impacts due to climate change and increased greenhouse gas emissions based on the production of the most used construction materials such as cement and concrete [1, 2]. It is necessary to limit the production and usage of these materials since they cannot be mentioned as environmentally friendly. Thus, the demand for the use and development of alternative materials has been increasing for the last decades. Within this context, earth-based construction materials can be a natural and suitable alternative since their production reduces global carbon dioxide emissions and limits the disposal of raw materials through a circular economy approach.

Bio-based construction materials such as earth, wood, fiber, and plant aggregates have been used for thousands of years. One of the natural materials, earth, is still the most widely used construction material in many countries in the world; one-third of world's population lives in earthen houses in developing countries [3]. Even though the earth has been preferred to use as it has several

advantages such as providing good thermal and acoustic properties, resistance to fire, and low cost, it is known that the earth suffers from having unsatisfying strength and resistance to water [4]. To obtain desired performance, earth can be stabilized with two main methods; one is mechanical stabilization which increases the density and, therefore, the properties of the earth [5] and the second one is chemical stabilization which develops secondary mineralogical phases. Incorporating cement, gypsum, and lime are the most known and effective methods for chemical stabilization [6-11]. However, usage of them, especially cement, limits the recyclability of the materials due to increased carbon dioxide emissions. Instead of stabilizing by hydraulic binders, alternative bio-sourced methods with low environmental impact are increasingly used. These methods can be classified as incorporating bio-additives and bio-stabilizers into the earth. Bio-additives such as plant aggregates and fibers reduce density, improve water sensibility by congesting the open pores, and enhance thermal properties [12]. On the other hand, bio-stabilizers originated from animals, plants, and minerals such as tannins, oils, fats, urine, casein, etc., improve such characteristics as strength and water resistance through induction of calcite precipitation, bio-polymerization, and mineral transformation [13, 14].

Lime stabilization [15, 16] and gypsum stabilization [17] are offered as ideal methods for earth stabilization in previous research. The lime reacts with clay in the earth and forms calcium silicate hydrate (C-S-H) and calcite crystals as it becomes carbonated over time. Gypsum decreases the setting time values and enhances early-age strength. When gypsum and lime are incorporated into the mixture, it is enabled to get stiffening in a short period, about 20 min, giving rigidity to the material and preventing shrinkage [10].

Incorporating microorganisms in concrete has been investigated by researchers to heal the microcracks in concrete structures for longer than a decade [18-20]. Improved compressive strength through the bacterial activation of sand columns has been established by other researchers [21]. However, investigation on incorporating bacteria in earth/soil mixes and their possible favorable effects in rammed earth mixes has remained unexplored. Normally, microorganisms, especially bacteria, already exist in soils at very high concentrations (around 10^{14} bacteria/kg). However, all types of bacteria may not be efficient, or the environmental conditions may not be appropriate to produce calcite in the earth mixes. In previous studies, it was declared when nutrient-rich additives such as eggs, animal blood, and molasses are mixed with soil, the bacteria are activated [22]. The bacterial cells act as nucleation sites and create micro niche conditions favoring the precipitation of deposits within soil particles.

In this study, the possible stabilization of earth by microorganism was investigated and within this regard, three microorganisms, *B. subtilis*, *S. pasteurii*, and *B. subtilis* subsp. *subtilis*, which are capable of inducing calcite precipitation effectively, were used. The effects of different concentrations of microorganisms and calcium resources were studied by producing a total of eleven mixes. The concentrations of the bacteria were determined by the optical density method, OD_{600}. As calcium resources, low-cost and locally available materials such as air lime and gypsum were used in different amounts. The influence of various microorganisms on earth stabilization was determined by performing a uniaxial compression test at different ages, e.g., the 7th and 28th day after production. Moreover, macro- and micro-scale observations are done to monitor possible calcite formation.

Materials and Method

The earth sample was collected from a depth between 0.5 and 1.5 m from Kemerburgaz, Istanbul. CL 80S-type air lime and gypsum were used as physicochemical stabilizers. Table 1 presents the chemical composition of stabilizers determined using an X-ray fluorescence spectrometer. The specific gravities of slaked lime and gypsum were 2.2 and earth was 2.6. Sieve analysis test results of earth are shown in figure 1 and engineering properties are given in table 2. Rammed earth through microorganisms is nanoscale material.

Bacterium types and cultivation

Three types of bacteria, *B. subtilis, S. pasteurii,* and *B. subtilis* subsp. *subtilis*, are incorporated in rammed earth and gypsum + lime SRE mixes, and the compressive strength performance was compared.

All batch experiments were initiated by cultivating cultures of bacteria, which are *B. subtilis, S. pasteurii,* and *B. subtilis* subsp. *subtilis* obtained from American Type Cell Culture. The

Table 1: Chemical composition of slaked-lime and gypsum.

	Chemical composition (% mass)						
	SiO_2	Al_2O_3	CaO	MgO	Fe_2O_3	Na_2O	K_2O
Slaked lime	0.80	0.40	69.00	1.30	1.20	-	0.10
Gypsum	1.74	0.51	32.46	0.54	0.27	0.05	0.07

Table 2: The engineering properties of the earth.

Property	Parameters	Details
Atterberg limits	Liquid limit, WL	36%
	Plastic limit, PL	18%
	Plasticity index, IP	18%
Proctor test	Optimum moisture content, OMC	18%
	Maximum dry density, MDD	17.3 kN/m³

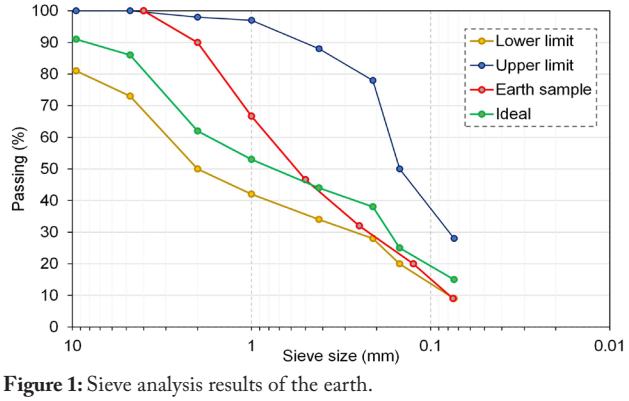

Figure 1: Sieve analysis results of the earth.

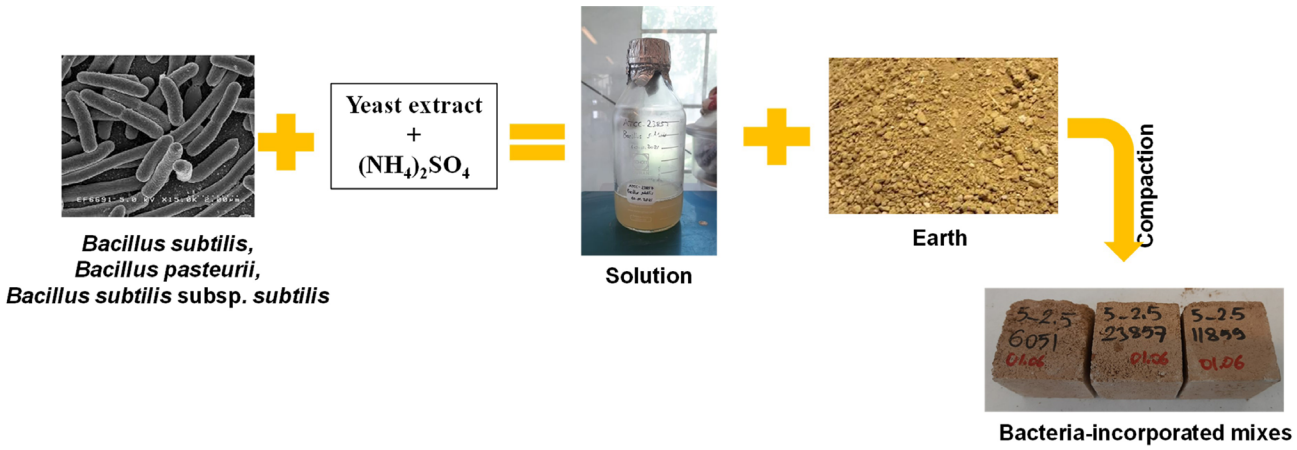

Figure 2: Flow chart of production of bacteria-incorporated mixes.

plate cultures were transferred to 1 L sterilized stock liquid growth solution containing 20 g yeast extract, 10 g $(NH_4)_2SO_4$ and 0.13 M of tris buffer. A single culture was introduced into the growth solution. The bacteria were incubated at 30 °C and mixed at 180 rpm until growth (approximately 24 hours). At this point, 50 ml of the microbial solution was transferred to fresh media bottles containing growth solution to run an optical density analysis at 600 nm to produce growth curves of the bacteria.

Mix proportions

Eleven different mixes were produced: a plain rammed earth mixture shown as RE, a 10% gypsum + 5% of slaked lime-containing rammed earth mixtures (SRE) which can be defined as reference mixes, and bacterium-incorporated RE and SRE mixes having different OD_{600} values. Bacteria-incorporated mixes are designated with their commercial numbers, which 6051, 11859 and 23857 define *B. subtilis, S. pasteurii,* and *B. subtilis* subsp. *subtilis,* respectively (Table 3). Two batches of bacteria incorporated SRE mixes were produced to evaluate the density of bacteria in the SRE mixes.

Table 3: Mix proportions of rammed earth mixes.

Mixes	Binders (%)		Bacteria (OD_{600})			Water (%)
	Lime	Gypsum	6051	11859	23857	
RE	-	-	-	-	-	25
SRE	5	10	-	-	-	
RE-6051	-	-	3.25	-	-	
RE-11859	-	-	-	4.01	-	
RE-23857	-	-	-	-	1.80	
SRE-6051*	5	10	2.48	-	-	
SRE-11859*	5	10	-	3.70	-	
SRE-23857*	5	10	-		2.26	
SRE-6051**	5	10	1.80	-	-	
SRE-11859**	5	10	-	1.00	-	
SRE-23857**	5	10	-	-	3.00	

Note : 6051: *B. subtilis*, **11859:** *S. pasteurii*, **and 23857:** *B. subtilis* **subsp. s***ubtilis*. *First batch, **Second batch

Production and curing

The production steps of bacteria-incorporated mixes are shown in figure 2. The water-to-dry material (earth + gypsum + lime) ratio was kept at 0.25, which is a close value to the optimum moisture content. After adding water/bacteria solution to the dry mixture, it was rapidly cast into the molds within a few minutes by compacting it with a wooden hammer. After 24 hours, the samples are demolded and placed into the moisture cabinet having 65 ± 5% relative humidity and 30 °C temperature.

Compressive strength test

The compressive strength of the rammed earth specimens was tested on triplicate 5 cm-cubic specimens that took place on the 7th and 28th day, where the average was considered for the evaluation.

X-ray diffraction (XRD)

Crystal structures were examined in the hardened RE and SRE samples by XRD tests. The XRD analysis was performed by using a Cu tube (45 kV, 40 mA) XPERT-PRO diffractometer at 28 days. The patterns were collected in the range of 5° to 65° (2θ) with a step size of 0.02° (2θ) and a measuring time of 1 s/step.

Macro- and micro-scale observations

Bacterium-stabilized mixes are monitored 28 days after the production to ascertain the possible calcite formation on the samples. The micro-scale observation was done by scanning electron microscopy (SEM) analysis, which was performed on small, broken samples of selected samples.

Results and Discussion

Compressive strength

To ascertain the compressive strength of rammed earth mixes, three replicates are produced for each mix and the average value was considered. The compressive strength development of RE, SRE, and two batches of SRE-bacteria incorporated mixes are presented in figure 3, figure 4, and figure 5, respectively. It can be seen that plain rammed earth mixes reached 3.1 MPa at 7 days and with a slight increase, designated 3.6 MPa at 28 days. On the other hand, gypsum + slaked lime-stabilized mixes (SRE) demonstrated much higher values than RE at 7 and 28 days (4.4 MPa and 4.7 MPa). Accord-

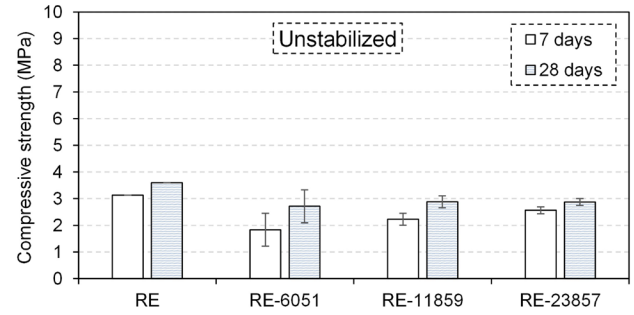

Figure 3: Compressive strength test results of RE and RE-bacteria incorporated mixes.

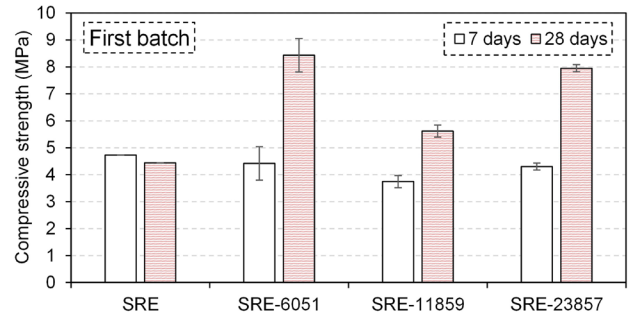

Figure 4: Compressive strength test results of SRE and the first batch of SRE-bacteria incorporated mixes.

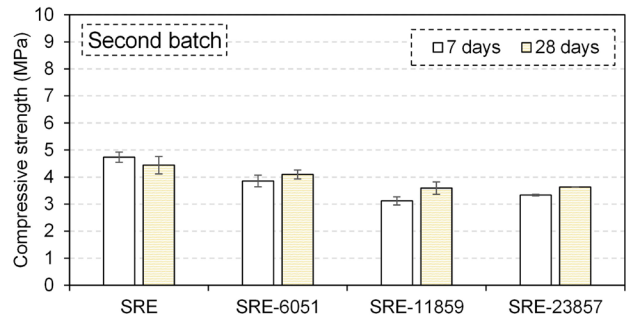

Figure 5: Compressive strength test results of SRE and the second batch of SRE-bacteria incorporated mixes.

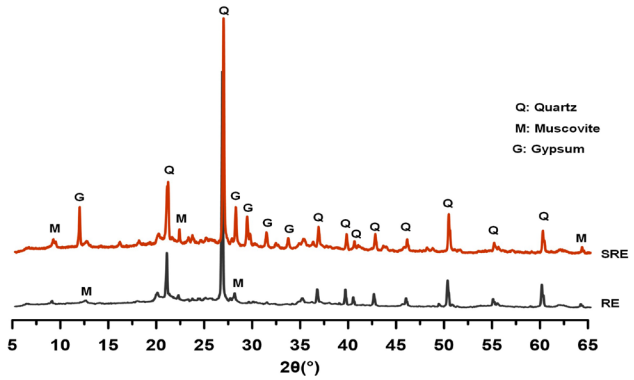

Figure 6: XRD patterns of RE and SRE.

ing to the New Zealand standard, the dry density of rammed earth ranges between 1800 and 2200 kg/m^3 and should have a minimum characteristic unconfined compressive strength of 1.3 MPa [23]. Accordingly, all the produced mixes can be regarded as appropriate earthen construction material.

The bacteria incorporation did not improve the compressive strength in plain RE mixes, which was attributed to the lack of calcium resources or any nutrients for bacteria. On the contrary, in some of the gypsum and slaked lime-stabilized mixes, a significant increase was observed. For the first batch, similar or slightly lower strength values are observed for 7 days than SRE. However, at 28 days all first batch mixes presented higher strength values than SRE (Figure 4). The maximum strength values obtained in the *B. subtilis*-incorporated mixes reached higher than 8 MPa at 28 days, representing two times higher values than the reference mix, SRE. Moreover, *B. subtilis* subsp. *subtilis* displayed 8.0 MPa of compressive strength at 28 days. This increase accounted for the possible calcite formation which occurs over time [24, 25].

On the other hand, for the second batch of SRE-bacteria incorporated mixes, lower compressive strength values than the SRE were obtained. This trend was attributed to the lower concentrations of bacteria compared to the first batch and thus, inadequate formation of calcite precipitation (Figure 5).

The obtained results showed that both concentrations of the microorganisms and calcium in the medium are the main parameters affecting the mechanical performance of the earth samples. It was found that microorganisms can effectively enhance compressive strength in 28 days. For a definite concentration of *B. subtilis*, two times higher values, e.g., 8.4 MPa, were achieved on 28 days than the reference mixes SRE, which do not contain any microorganisms. It can be deduced that the preliminary test results promise to be a feasible alternative to cement and or lime-stabilized rammed earth.

XRD analysis

CL 80S-type air lime and gypsum were used as physicochemical stabilizers and XRD patterns of RE and SRE are given in figure 6. The XRD patterns of the plain rammed earth sample (RE) display high amounts of quartz (SiO_2) and muscovite ($KAl_2(Si_3Al)O_{10}(OH,F)_2$) which can be present on a large scale in sand and earth. Gypsum and slaked lime stabilized mixes (SRE) presented the same peaks. The only difference between RE and SRE was found as gypsum ($CaSO_4.2H_2O$) crystals, as expected.

Macro- and micro-scale observations

To observe possible calcite precipitation on bacteria-incorporated mixes, macro- and micro-scale observations are done (Figure 7, figure 8, and figure 9). It was observed that different from RE or SRE mixes, in all bacteria-incorporated mixes, *SRE-6051, SRE-11859,* and *SRE-23857*, white-colored calcite precipitation was observed (Figure 7) and it was more pronounced in the first batch. This product was also

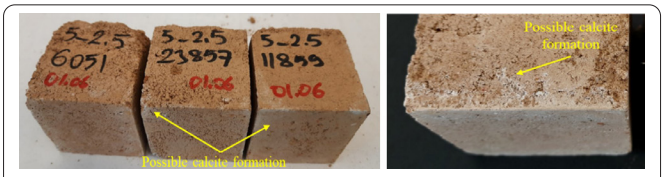

Figure 7: Calcite precipitation on first SRE-bacteria incorporated batch samples.

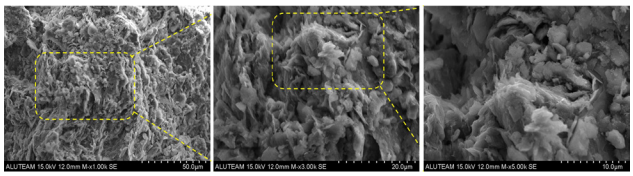

Figure 8: SEM images of SRE-6051.

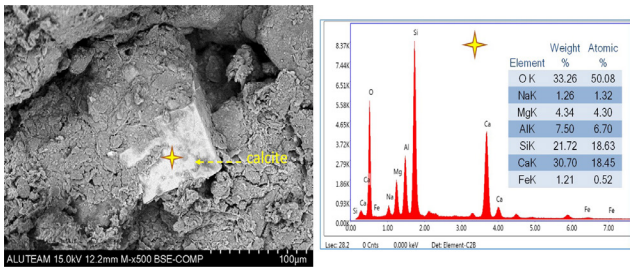

Figure 9: SEM image and point EDS spectrum of SRE-11859.

examined with a SEM under high magnifications and SEM images and energy dispersive spectroscopy analysis results also demonstrated calcite precipitation (Figure 9).

Conclusions

This study aimed to investigate the possible stabilization of the earth with the aid of gypsum, lime, and bacteria. Different types of bacteria are incorporated into both plain and chemically SRE mixes and the effects of different concentrations of the bacteria are investigated. The compressive strength of the mixes is examined at 7th and 28th days after production. The conclusions drawn from the results of this paper are stated below.

- The plain rammed earth mixes achieved only 3.0 MPa in 7 days and did not gain considerable strength over time.
- The addition of gypsum and lime as chemical stabilizers increases the compressive strength considerably both for early and final ages.
- Incorporating bacteria does not improve the compressive strength of the plain rammed earth mixes since there are no nutrient or calcium resources for bacteria in the medium. However, in gypsum and slaked lime-stabilized mixes, bacteria incorporation at a definite concentration increased the compressive strength significantly, which reached up to 2 times higher values (>8 MPa) after 28 days.
- The effect of bacteria incorporation on compressive strength depends on the bacteria type and concentration.
- Calcite formation can be monitored by macro- and micro-scale investigation.

In a nutshell, gypsum and lime can be used as chemical stabilizers and bacteria incorporation can enhance the compressive strength of rammed earth significantly when used at definite ratios. Based on the visual inspection of the hardened rammed earth samples, calcite formation can be seen, and this might give strength to the material. However, more robust investigations should be done for different concentrations of bacteria and different nutrients. This bio-stabilization process through bacteria, enhancing mentioned properties of rammed earth mixes will be further explored within an architectural design and production process. Biologically improved rammed-earth blocks are designed to form various wall patterns and will be fabricated in the factory as compressed earth blocks within the scope of BIRE-PAN research project. The results will be shared in further studies.

Acknowledgments

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Conflict of Interest

None.

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