

Article

Effect of Temperature on the Efficiency of Enzymatic Soil Stabilization

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Abstract

Enzyme-based stabilizers have been used in soil stabilization in the construction industry for many years as alternatives to traditional stabilizers and have produced successful results. These novel stabilizers gained popularity over standard stabilizers such as cement and lime due to their non-toxic and non-hazardous properties. Although enzymatic stabilizers perform very well for a variety of soil types under different environmental conditions, their effectiveness under varying temperatures has not been sufficiently investigated. This is more important in countries where there are significant fluctuations in temperature that are further exacerbated by the impact of climate change. As enzymatic products are organic and biodegradable, their susceptibility to temperature variations must be well understood. In this study, the efficiency of a commercially available enzymatic stabilizer was investigated. Tests were carried out to assess the influence of temperatures ranging from 4 °C to 80 °C on the geotechnical properties of enzymatically stabilized soil. The investigation was conducted into the compaction characteristics, index properties, compressive strength, and CBR of the stabilized soil. The results indicate that the stabilizing effect of the enzyme remains largely unchanged up to approximately 40 °C. The outcome of the study enables practitioners to use more sustainable stabilizers to treat problematic soils in regions where temperature fluctuations are within the tested range.

Keywords: stabilization; enzymes; compressive strength; CBR

1. Introduction

Problematic expansive soils—soils exhibiting excessive shrink–swell behavior in response to changes in water content [1,2]—are found in many parts of the world and practitioners in the construction industry have used a variety of stabilization techniques to improve the properties of these soils. One of the commonly used techniques is chemical stabilization, where soil is mixed with different additives, such as cement, lime, bitumen, etc. [3–7]. While these traditional additives have worked well, engineers and researchers have been gradually shifting towards less environmentally disturbing materials in the recent past, such as reclaimed waste, geopolymers, natural fibers, etc. [8–16]. Enzyme-based products are a novel and one of the most sustainable additives that have been successfully trialed recently [17–21]. Laboratory testing and field trials from different countries have



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shown that enzymes can be used to effectively improve subgrades and unsealed road pavements [22–29].

Enzyme-based products are organic materials that can be added to expansive soils to improve mechanical and physical properties. They are normally categorized as liquid soil stabilizers, along with other products made of lignosulfonate, petroleum resin, salt, tree resin, polymers, and ionic compounds [11,25]. There are different types of enzyme-based products commercially available in the market. Enzymes were originally used in horticultural applications to increase fertility (by lifting nitrogen, phosphorus, and potassium levels) and to break down adverse chemicals like ammonia. In ground improvement applications, enzyme-based products typically consist of a blend of multiple enzymes combined with surface-active agents and soil nutrients. These stabilizers are organic, non-toxic, non-flammable, and biodegradable, and therefore pose no harm to living organisms. The use of enzyme-based stabilization techniques has gained increasing attention over the past decade, leading to a growing body of research investigating their effectiveness and performance in soil improvement applications across a range of soil types and conditions [8,17,23].

Independent research with encouraging results has advocated the use of enzymatic products for soil stabilization [3,30–33]. Venkatasubramanian and Dhinakaran, in 2011 [33], tested an enzyme-based stabilizer on three types of soils. The results showed an increase of up to about 400% in unconfined compressive strength (UCS) and 600% in California Bearing Ratio (CBR) at the end of a 28-day curing period. Mitikie et al., in 2018 [34], successfully applied an enzyme-based stabilizer to improve the compressive strength and dry density of clay bricks. In both these studies, it was evident that curing time has a great influence on the efficiency of the enzyme.

According to [23], standalone enzyme treatment has demonstrated significant improvements in strength, stiffness, and durability, with increases in the CBR of up to 48–58% (unsoaked/soaked) and UCS gains reaching 42% and 768% under residual/optimum water conditions and saturated conditions, respectively, alongside a six-fold enhancement in permeability resistance and reduced swell and crack propagation during wet–dry cycling. Microstructural and mineralogical analyses (XRD, EDS, and μ CT) confirmed that enzyme stabilization is primarily physicochemical, with no formation of new cementitious phases; instead, strength enhancement can be attributed to densification, pore redistribution, and reduced water affinity, particularly when compacted at the stabilized optimum moisture content (OMC), yielding CBR increments up to 500% [32].

Although enzyme treatment alone showed limited efficacy in suppressing full heave under saturation, it significantly reduced stresses, strains, and rutting under cyclic traffic loading, as demonstrated through the calibrated 3D finite element modeling and analytical rutting formulations performed by [25]. In addition, the incorporation of stabilization-specific soil–water characteristic curves (SWCCs) improved suction representation and mitigated the overestimation of pavement degradation in numerical simulations [25]. Synergistic enhancements were achieved through hybrid systems by [1] via enzyme–fly ash blends, which accelerated pozzolanic reactions, reducing peak strength attainment from 28 to 4 days and achieving strength gains up to 680% (unsoaked CBR) and 88% (UCS), while additional lime promoted rapid C-S-H/C-A-H formation with CBR improvements approaching 1000%. A comparative assessment of secondary additives by [3] confirmed that fly ash alone was ineffective, whereas fly ash–lime–enzyme systems achieved CBR values exceeding 25% and superior pavement performance through combined pozzolanic, cementitious, and densification mechanisms.

There are several theories about the mechanism of enzymatic stabilization. One of the widely accepted hypotheses further supported by [35] states that the enzyme particles that are positively charged encapsulate the negatively charged organic molecules in the clay

particles. These clay particles contribute to the soil's affinity for water. The double water layer around the clay and the affinity for water are decreased with this process. The stability of the clay particles increases, and the particles bind well [36]. The experiments conducted by [35] showed the expansion of the clay layer and a reduction in pore size and surface area, which is graphically presented in Figure 1. Supporting evidence to this hypothesis at a macro-level was presented by [23], who identified densification as the mechanism of stabilization.

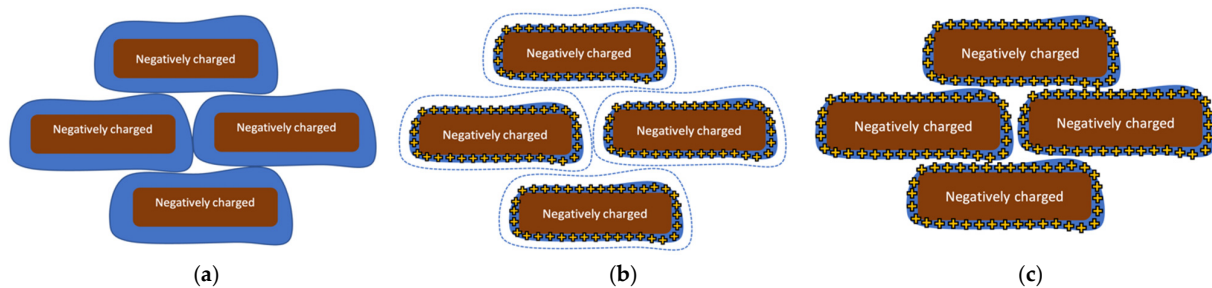


Figure 1. Conceptual illustration of enzyme–clay interaction mechanism (adopted from [32]). (a) Untreated natural clay particles with high affinity to water exhibiting negatively charged surfaces. (b) Adsorption of positively charged enzymes onto clay surfaces, resulting in organic encapsulation. (c) Stable clay sheets bound with other clay particles through reduced repulsive forces and densification.

Previous studies have examined the influence of soil type, fines content, application and dilution ratios, curing time, and the use of secondary additives on the performance of enzymatic soil stabilization. Soils with a high fines content have been shown to respond positively to enzymatic treatment. Optimal enzyme dosages, expressed as the diluted mass ratio (DMR) and application mass ratio (AMR), have been reported as 1:500 and 1%, respectively [32].

Because of their organic origin, enzymatic stabilizers are biodegradable when exposed to elevated temperatures. The thermal effect on enzymatic stabilization was investigated by [32] by drying the raw soil samples at temperatures ranging from 20 °C to 60 °C prior to enzyme treatment. The influence of temperature was assessed using UCS testing, and the results indicated that heating up to 40 °C did not adversely affect stabilization performance. However, in that study, the soil was thermally treated before stabilization, and the impact of thermal exposure on stabilized soils was not examined.

Novelty and Significance of Study

Many engineering practitioners and contractors speculate that the enzymes tend to become inactive at lower temperatures and get disintegrated at higher temperatures. Only one study can be found in the literature [37] on the mechanical performance of enzymatic soils subjected to temperature fluctuations. The focus of this study was limited to compressive strength in the temperature range 15 to 60 °C. Enzymatic stabilizers are mostly used in road pavements, where other indicators such as the level of compaction and CBR are also important. The influence of enzymatic stabilizers and temperature on soil plasticity is another area that has not yet been explored. The current study addresses these gaps by investigating the performance of enzymatically treated soil subjected to a wide range of temperatures from 4 °C to 80 °C. The lower boundary, 4 °C, is the refrigerating temperature at which organic matter becomes deactivated. The upper boundary, 80 °C, was selected to obtain a complete understanding of any patterns existing with increasing temperature. A commercially available stabilizer, Eko-Soil, was used as the stabilizing

agent. The performance was evaluated using four indicators, namely index properties, compaction characteristics, UCS, and the CBR.

2. Methodology

This section describes the experimental procedure and materials used.

2.1. Materials

A soil with moderate expansiveness was selected for this study to ensure that the effects of enzyme addition are easily observed. The Atterberg limits, liquid limit (LL), plastic limit (PL), and plasticity index (PI), of the untreated (UT) soil were determined, and the soil was classified as CL. The physical and index properties of the soil are summarized in Table 1. The soil was ground and passed through a 19 mm sieve to ensure that the material was free from large clumps. Figure 2 shows the particle size distribution of the soil used for testing.

Table 1. Properties of UT soil.

Soil Properties	Value
LL (%)	42.9
PL (%)	18.1
PI (%)	24.8
Maximum Dry Density (t/m^3)	1.629
Optimum Moisture Content (%)	22
Unified Soil Classification System classification	CL

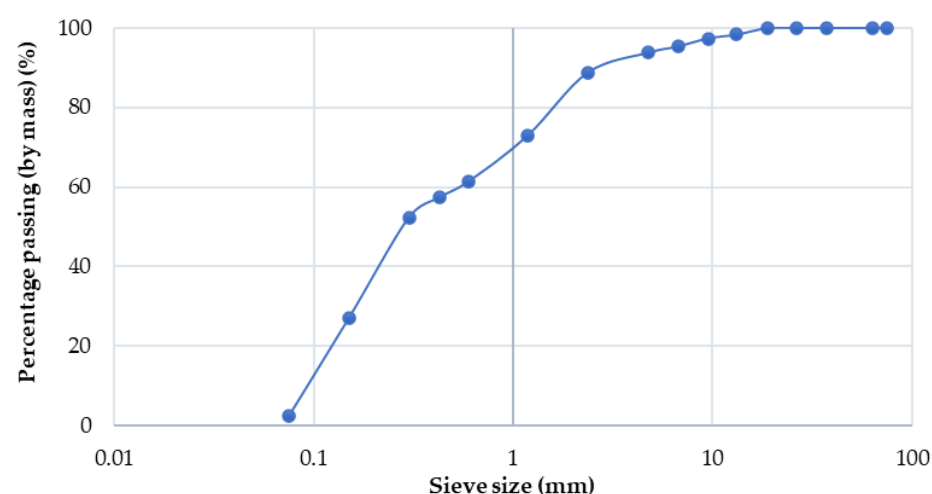


Figure 2. Particle size distribution curve of the untreated soil used in this study.

The enzymatic stabilizer used in this study was Eko-Soil (Norwood Hall (Asia) Pty Ltd., Mount Eliza, VIC, Australia), which is a non-toxic, non-hazardous product. This enzyme is commercially available in several countries, including Australia, India, and the USA, and it imitates other enzymatic products such as TerraZyme and Permazyme [32]. According to the information provided by the manufacturer, Eko-Soil contains enzymes, surface-active agents, soil nutrients, and organic polymers. The three main active enzymes are lipase, amylase, and protease [38]. The manufacturer revealed the general proportion of the enzyme as 20% water, 20% non-ionic surfactant, and 60% ferment base ingredients.

2.2. Enzyme Dilution

The enzyme was diluted using a DMR of 1:500, in which 1 g of enzyme is mixed with 500 g of water. The diluted enzyme was then mixed with measured masses of soil for each test based on an AMR of 1%. The DMR and AMR were selected based on the optimum dosages reported by [32]. More details and definitions of the DMR and AMR can be found in [32].

2.3. Soil Preparation and Testing

After adding Eko-Soil to the soil, the mixture was left to cure at room temperature for 7 days. The next step was the heat treatment. Five different temperatures—4, 20, 40, 60, and 80 °C—were selected, representing the typical ambient temperatures that can be expected in the field. The highest temperature of 80 °C was selected to determine the maximum temperature under which the enzymatic stabilization will remain uninfluenced. Treated soil samples were placed in the oven to elevate the temperature to 40, 60, and 80 °C. For 4 °C, samples were placed in the refrigerator. Soil to be treated at 20 °C was left under the room temperature, and the actual temperature was monitored, which was recorded in the range 20 ± 2 °C. All the samples were brought back to room temperature before testing.

The Atterberg limits were determined (Australian standards, AS1289.2.1.1 [39] and AS1289.3.2.1 [40]) for all the heat-treated samples. The standard compaction test was conducted to evaluate the compaction characteristics, and the compressive strength was determined using the UCS test. The OMC and maximum dry density (MDD) obtained from the compaction test were used to prepare the UCS test samples. Additionally, a series of CBR (unsoaked) tests was also carried out on treated soil. A constant Eko-Soil dosage was used in all test samples. A summary of the tests conducted in this study is listed in Table 2.

Table 2. Summary of tests conducted in this study.

Test Name	DMR/AMR	Temperature Tested (°C)	No. of Tests Conducted (Including Replicates)
Compaction Test	UT	20 °C	5
	Enzymatic 1:500 (1%)	4 °C, 20 °C, 40 °C, 60 °C, 80 °C	25
UCS	UT	20 °C	3
	Enzymatic 1:500 (1%)	4 °C, 20 °C, 40 °C, 60 °C, 80 °C	15
Atterberg	UT	20 °C	3
	Enzymatic 1:500 (1%)	4 °C, 20 °C, 40 °C, 60 °C, 80 °C	15
CBR	UT	20 °C	3
	Enzymatic 1:500 (1%)	4 °C, 20 °C, 40 °C, 60 °C	12

3. Results and Discussion

3.1. Effect on Consistency Limits

The effect of temperature on the consistency limits of enzyme-treated soil was observed using Atterberg limits. Figure 3 shows the variation in the plastic limit (PL), liquid limit (LL), plasticity index (PI), and linear shrinkage (LS) after the heat treatment. The same results are tabulated in Table 3. All the properties follow a similar pattern. Almost all consistency limits of the enzyme-treated samples at 20 °C are higher than those of the UT sample, which was also prepared at room temperature. This exhibits the impact of the stabilizer on the plasticity of soil. The increase in the PI from 24.8% (UT) to 26.9% (20 °C) suggests that the soil becomes more plastic after stabilization.

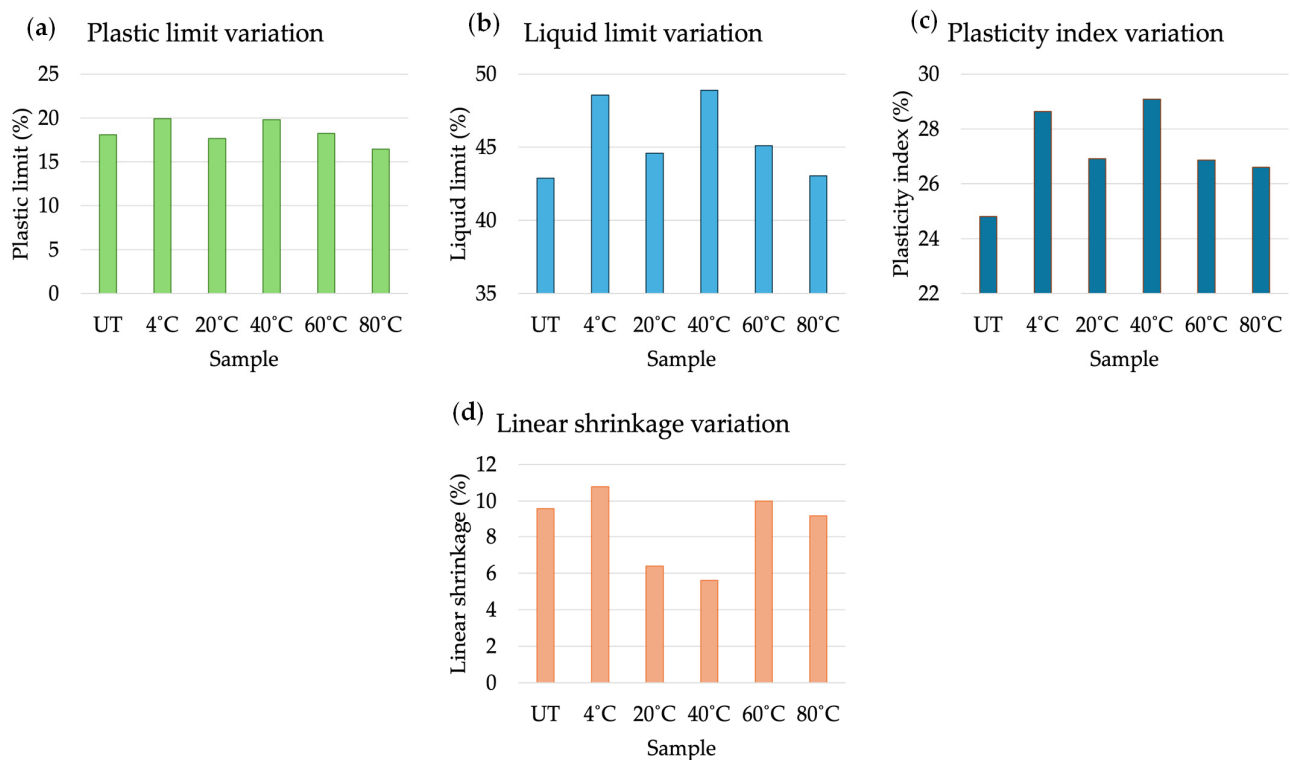


Figure 3. Atterberg limits variation between UT and enzymatic samples treated at different temperature conditions: (a) plastic limit, (b) liquid limit, (c) plasticity index, (d) linear shrinkage.

Table 3. Numerical results of the Atterberg limits tests.

Sample Type	Subjected Temperature	PL (%)	LL (%)	LS (%)	PI (%)
Enzymatic	UT	18.07	42.89	90.4	24.81
	4 °C	19.93	48.57	89.2	28.64
	20 °C ¹	17.66	44.58	93.6	26.92
	40 °C	19.81	48.89	94.4	29.08
	60 °C	18.24	45.10	90.0	26.86
	80 °C	16.45	43.05	90.8	26.599

¹ 20 °C—room temperature.

Though soil stabilization usually results in reduced plasticity, an increase in the PI after enzymatic stabilization is not uncommon. Previous studies [41,42] have reported several cases where plasticity increased after the application of enzymes. The impact of enzymes on plasticity, therefore, is not universal and depends on fine content, original PI, enzyme dosage, and curing time.

Heat treatment at higher temperatures tends to increase the plasticity of soil further. The highest PI of 29.1% was observed at 40 °C. However, this trend is not continuous, and the plasticity of the treated soil starts to diminish when subjected to temperatures above 40 °C. The increased plasticity is expected to lead to greater shrinkage upon drying. Established empirical correlations between PI and LS suggest a linear increase in LS with PI. For example, BS 1377:1967 [43] suggests $PI \approx 2 \times LS$. These correlations are, however, applicable to natural, untreated soils and the results for enzymatically treated soil in the current study show an inverse behavior. The sample treated at room temperature shrank by 6.4% in comparison to 9.6% for the untreated sample. The sample at 40 °C, which had the highest PI of 29.1%, shrank the least with an LS of 5.6%. This proves the early findings

regarding the ability of enzymatic stabilizers to improve water retention in clayey soils. It is evident that the stabilizing agent remains active up to about 40 °C.

The fact that the effect of a lower temperature (4 °C) on treated soil is at the same level as soil heated to 40 °C is an interesting phenomenon. The PI of 4 °C samples is 28.6%, which is not far from the 29.1% of the 40 °C sample. The LS of the 4 °C (10.8%) is, however, nearly double the LS of the 40 °C sample (5.6%). It is possible that the active ingredients of the stabilizer become permanently dormant or destroyed under very low temperatures.

3.2. Effect on Compaction Characteristics

A standard compaction test was performed on all the soil samples after heat treatment. One compaction curve was generated for each temperature tested. The OMC and MDD were determined from the peak of each compaction curve. As the compaction test yields a curve-based characteristic response rather than independent replicate peak values, statistical analysis such as standard deviation was not applicable for OMC and MDD.

The measured OMC and MDD values are given in Table 4. There is an initial reduction in OMC coupled with an increase in MDD in treated soil at room temperature, a phenomenon observed by other researchers and has been attributed to the densification of soil upon enzymatic stabilization, as proposed by [32].

Table 4. Change in OMC and MDD compared to the control sample.

Sample	OMC (%)	MDD (t/m ³)	Change in OMC Compared to UT Soil	Change in MDD Compared to UT Soil
UT soil at room temperature (20 °C)	22	1.629	-	-
Enzymatic soil—4 °C	21.8	1.654	−0.2	+0.025
Enzymatic soil—20 °C	20.9	1.666	−1.1	+0.037
Enzymatic soil—40 °C	20.69	1.664	−1.31	+0.035
Enzymatic soil—60 °C	21	1.65	−1	+0.021
Enzymatic soil—80 °C	23.64	1.69	+1.64	+0.061

The changes in OMC and MDD compared to the UT sample are presented in Figure 4. The OMC exhibits a parabolic trend, with temperature attaining its minimum value at 40 °C. Eko-Soil reduces the interparticle friction and alters the soil fabric, allowing particles to pack more efficiently. Concurrently, the affinity of water for the clay surface is reduced, thereby allowing higher densities to be achieved at lower moisture contents.

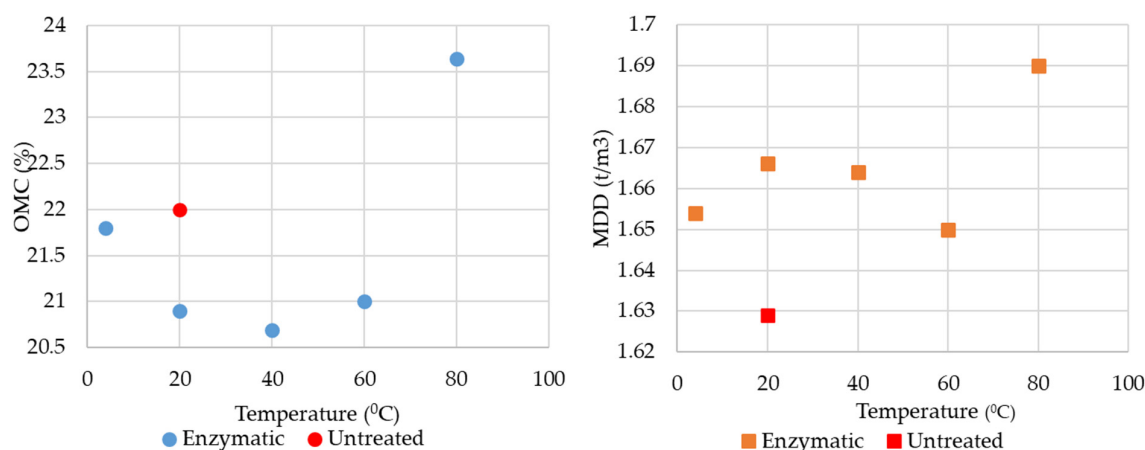


Figure 4. Effect of temperature on compaction characteristics OMC (left) and MDD (right) of UT and enzymatic soils.

The MDD follows a relatively inverse parabolic trend to the OMC, apart from the value at 80 °C. A peak MDD appears to occur within the temperature range of 20 °C to 40 °C. Beyond this range, MDD decreases while OMC increases, indicating a reduction in the performance of the stabilizing agent at both lower and higher temperatures. This behavior suggests that the stabilizing activity remains intact within the 20–40 °C temperature range.

3.3. Effect on Compressive Strength

The results obtained from the UCS tests are shown in Figure 5. The stabilizing treatment provides an initial improvement in UCS, as seen from the sample at room temperature (20 °C) in comparison to the UT sample. This observation is consistent with the underlying mechanism of enzymatic stabilization, whereby improvement in soil properties is achieved through particle rearrangement and densification.

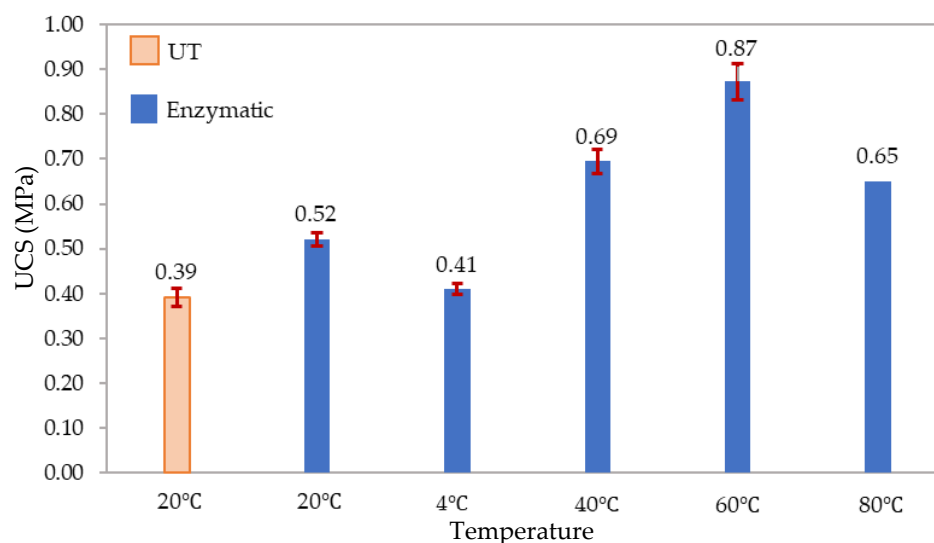


Figure 5. Effect of temperature on UCS of UT and enzymatic soils, with each value representing a mean of three replicates.

The variation in UCS across the temperature range tested is parabolic. Notably, the peak value that was apparent in MDD shifted to 60 °C from about 20 to 40 °C. The improvement in UCS needs to be discussed in conjunction with the changes to the plasticity of the soil, as reflected by the PI. In untreated soils, high-plasticity soils tend to have lower UCS. The high compressibility and high-water affinity of these soils make them more prone to swelling, resulting in reduced UCS. However, in enzymatically stabilized and compacted soil, increased plasticity contributes to greater apparent cohesion, improving UCS. Additionally, flocculation and bonding mechanisms can be induced by enzyme activity favoring further improvement in UCS. It should also be noted that changes in the PI are inversely related to LS, which can be attributed to high water affinity. Reduced LS also suggests a higher shrinkage limit and a more flocculated structure. It is well-known that lowered LS results in higher UCS and MDD. Thus, the parabolic trends in compaction and strength correspond to the parabolic trends in PI and LS. Overall, the UCS results indicate that temperature fluctuations in the tested range do not hinder the stabilizing action of the enzyme.

3.4. Effect on California Bearing Ratio

CBR values of treated soils are affected by temperature variations, according to the results shown in Figure 6. The results suggest a continuously increasing trend in the CBR with temperature. This is a crucial finding for practical applications such as unsealed

road surfaces and subgrades. The construction of roads can span over multiple seasons, exposing the stabilized soil to varying temperatures. Since the CBR value is an important material quality indicator, it is crucial that the CBR remains unaffected by the variations in temperature.

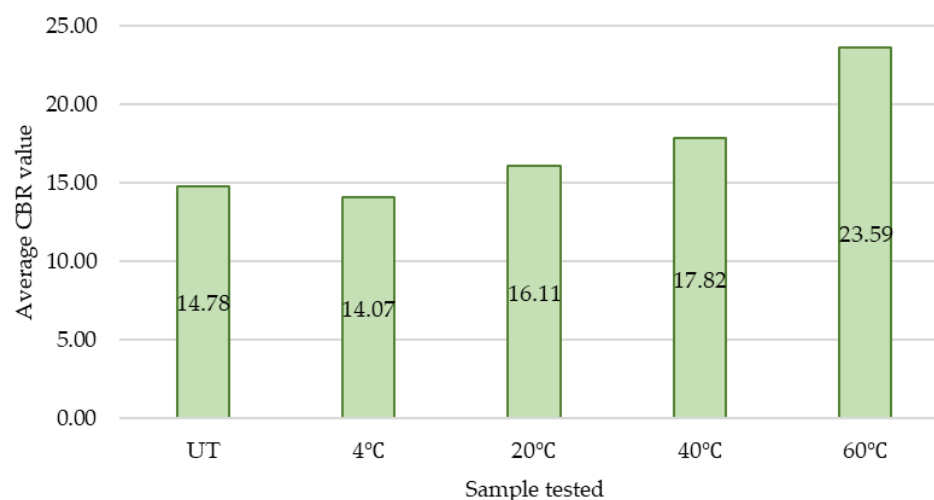


Figure 6. Effect of temperature on average unsoaked CBR values of UT and enzymatic soils.

Apart from the absence of a peak CBR value, the increase in the CBR agrees well with increased density (MDD) and UCS. Unlike chemical stabilizers such as cement and lime, which develop cementitious bonds with soil particles, enzyme stabilizers rely on particle re-arrangement and apparent cohesion generation. The peak values in MDD and UCS indicate that the effect of stabilizers on cohesion withers at very low or very high temperatures. Nevertheless, the denser matrix achieved through the re-arrangement of particles is not affected when soil undergoes temperature variations. Micro-structural observations by [30,34] support this explanation. Their observations show that enzymatic soil has a significantly lower number of pores, resulting in a denser structure compared to untreated soil. This structure tends to get denser at high temperatures [34]. Hence, the CBR continues to increase across the tested temperature range.

4. Conclusions

The impact of temperature variations on the efficacy of enzymatic soil stabilization was investigated. Stabilized soil samples were subjected to different temperatures and were tested for Atterberg limits, compaction, UCS, and CBR. Based on the experimental results, it can be concluded that the properties of enzymatically stabilized soil are largely unaffected by moderate temperature changes. Enzymatically stabilized soil can be safely exposed to temperatures up to 40 °C without compromising the engineering properties of soil tested in this study. According to the results presented, exposure to varying temperatures can

- Increase the PI and make enzyme-treated soil more plastic;
- Lower the OMC while MDD is increased;
- Enhance the UCS;
- Increase the CBR value.

The performance of enzymatic stabilization depends on soil type as well. Hence, the above findings are applicable to soils similar to the one used in this study. Overall, it can be concluded that enzyme-based soil stabilizers can be safely used to improve problematic soils under a variety of climatic conditions.

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

Abbreviations

The following abbreviations are used in this manuscript:

DMR	Dilution mass ratio
AMR	Application mass ratio
UCS	Unconfined compressive strength
OMC	Optimum moisture content
MDD	Maximum dry density
PL	Plastic limit
LL	Liquid limit
PI	Plasticity index
LS	Linear shrinkage
UT	Untreated

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