Research Article

Effect of Three Bioenzymes on Compaction, Consistency Limits, and Strength Characteristics of a Sedimentary Residual Soil

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Bioenzymes are organic degradable materials, currently introduced as soil improvement additives. In this experimental study, three types of bioenzymes from three different countries were used to improve Universiti Kebangsaan Malaysia (UKM) soil. UKM soil has properties quite similar to soils recommended as suitable by bioenzyme suppliers. The effect of the three bioenzymes on Atterberg limits, compaction characteristics, and unconfined compressive strength was studied. Controlled untreated and treated samples for two dosages at curing times up to three months were prepared and tested after completion of the curing period. Some results showed little improvement in compaction characteristics, and unconfined compressive strength, but no notable improvement was noticed in Atterberg limits. X-ray diffraction (XRD), X-ray fluorescence (XRF), and field emission scanning electron microscopy (FESEM) tests were conducted for untreated and treated soil samples after two months of curing. XRD and XRF did not show any change in mineralogy and chemical composition between controlled untreated samples and samples treated with the three bioenzymes. However, the FESEM images revealed a denser packing of particles for soils samples treated with two of the bioenzymes.

1. Introduction

In the construction industry, maintaining a balance between performance and cost, while at the same time satisfying environmental regulations, has become a challenge for building material manufacturers, design engineers, and contractors. This challenge has led to identification and use of new construction materials and techniques. Geotechnical engineering projects are closely related to economic and environmental issues; therefore, improving sustainability of materials used in these projects may help in attaining overall sustainable development [1]. Unfortunately, planning and design phases of these projects are dictated by financial interests and are even more affected by lack of knowledge about the effect of the geotechnical process on the environment [2]. Manufacturing of readily used construction materials, such as cement and lime, has a deteriorating effect on the environment; the production of cement and lime is energy demanding, and production of only one ton of cement emits around one ton of CO₂ [3].

Recently, bioenzymes have been introduced for soil stabilization, especially in highway projects. They are organic materials, which are supplied as a concentrated liquid. It is claimed by bioenzyme manufacturers that their products are effective, environmentally friendly (nontoxic), cost-effective, and convenient to use. They are generally extracted by the fermentation of vegetables and sugar canes; thus they are degradable; that is, they easily break down and dissolve with time. They are supplied in liquid form and are easily soluble in water, which is used for soil compaction. This saves time and costs normally consumed by the mixing of traditional solid stabilizers with soil. Kestler [4] suggested that most of the information about enzymes is provided by enzyme suppliers, and, therefore, independent testing information is not readily available. Additionally, enzymes are often reformulated; all of these factors call for product-specific testing before the selection of an enzyme for a project.

Enzymes are biological catalysts present in all living organisms. They are obtained from plants and animals, including microorganisms, by extraction using suitable
solvent. Kestler [4] suggested that enzymes are proprietary of their supplier; unless they provide the composition, it is very difficult to determine the precise composition and stabilization mechanism. He also recommended that some commercial enzymes, for example, Bio Cat 300-1, EMC SQUARED, PermaZyme 11-X, TerraZyme, and UBX No. 0010, should contain protein molecules which react with soil molecules to bind the soil particles together, thus decreasing the affection of soils for moisture.

Scholen [5] proposed that enzymes increase the rate of chemical reaction, which occurs at a much slower speed in the absence of enzymes, without becoming a component of the final product. Enzymes combine with big organic molecules to generate a reactant mediator, which swaps ions with the clay structure and breaks up the clay lattice. As a result, this produces a covering effect, which blocks further absorption of water and loss in density. This reaction regenerates the enzymes again, which sets out and reacts yet again. The enzymes are absorbed by the clay lattice and are afterwards freed upon exchange with metals cations. They have a significant role in the behaviour of the clay lattice, first causing them to get bigger and then to stiffen. Rauch et al. [6], through different chemical and physical tests, endorsed the hypothesis proposed by Scholen [5] that states that enzymes unite with the large organic molecules and adhere to clay surfaces, thus jamming potential cation exchange sites and preventing absorption of moisture and subsequent swelling. Resulting ionized water forms linkages among packed particles to provide a binding effect.

Enzyme manufacturers and suppliers claim that enzymes, when used in soil stabilization, can enhance the wetting and bonding properties of the soil particles. The enzymes make the soil more workable, which can be compacted more heavily. Furthermore, the enzymes enhance the chemical bonding of soil particles, which aids in combining them. Thus, a more durable soil structure is built that is more resistant to weathering, traffic, and water infiltration.

Strength tests have shown a considerable increase in strength for soils treated with bioenzymes [7, 8]. Lacuoture and Gonzalez [9] studied the effect of the TerraZyme soil stabilizer product on subbase and subgrade soils. Variation in properties and progressive improvement were observed, but no significant improvement was reported during the early days. Hitam et al. [10] also studied the effect of TerraZyme on plantation roads through field observations. He noticed that the roads, which had serious problems due to monsoons in the past, remained intact after two monsoon seasons.

Soil treated with PermaZyme 11-X showed very modest or no improvement in stiffness, freeze-thaw, leaching, and wet-dry tests [11]. Compaction, Atterberg limits, and strength tests revealed little or no improvement for the soils stabilized with PermaZyme 11-X and EarthZyme [12]. PermaZyme 11-X was also used to treat six single source and three blended soils to conduct Atterberg limits, density, strength, and R-value tests; the overall reduction in the plasticity index and increase in strength were recorded [13]. Rauch et al. [6] conducted experimental studies of three liquid stabilizers including one enzyme. Two natural soils and three reference clays, bentonite, illite, and kaolinite, were treated with this enzyme, and different tests were conducted. Overall, no noticeable improvement was observed.

Soil investigation practices are sometimes criticized for the extensive time needed and high cost involved. However, larger losses can be saved, which may arise if proper soil investigation prior to field application is bypassed. If a stabilizer is not effective in controlled laboratory conditions, then it is likely that it cannot produce the desired results in the field. As discussed previously, the experimental studies conducted to evaluate enzyme’s suitability as a soil stabilizer have revealed dissimilar results. Thus, this experimental study was aimed at evaluating the suitability of three commercial enzymes. The experimental studies conducted thus far used optimum moisture content (OMC) of untreated soil to prepare soil samples treated with enzymes. However, in this study, the effect of enzymes on compaction characteristics was examined. For this purpose, a revised protocol for sample preparation was adopted as recommended by Rauch et al. [6]. Samples were cured for an extended time period of 12 weeks to observe the progressive improvement due to enzyme activity. Instead of using one enzyme to check suitability against different types of soil, in this study, one suitable (presumably) soil was selected to evaluate the improvement by three different enzymes. This study was not restricted to mechanical testing of untreated and treated samples, but XRD and XRF tests were conducted to check any chemical reaction in the treated soil samples. FESEM images were taken to closely investigate the texture of untreated and treated soil samples. Untreated controlled samples were prepared and cured along with treated samples to account for gain in strength due to aging and moisture loss, if any.

2. Test Materials

2.1. Bioenzymes. Three types of bioenzymes, DZ-IX (Boron Innovations Pvt. Ltd., India), EarthZyme (Cypher Environmental Ltd., Canada), and TerraZyme (Nature Plus, Inc., USA), were selected for this study and designated as E-I, E-II, and E-III, respectively. EarthZyme and TerraZyme suppliers provided the material safety data sheet (MSDS), whereas the supplier of DZ-IX did not provide MSDS, though several requests were made. However, some of the properties of the DZ-IX enzyme were determined in the laboratory, and the information contained in MSDS for EarthZyme and TerraZyme is presented in Table 1. The main ingredients of two enzymes (E-II and E-III) are nonionic surfactant and carbohydrates. E-II contained carbohydrates (polysaccharides, oligosaccharides, disaccharides, and monosaccharides) and E-III consisted of fermented vegetable extract. Two dosages were used for all three enzymes: first, single dose (recommended by the supplier) and, second, double dose, that is, two times the recommended dosage, are denoted as D1 and D2, respectively.

Different suppliers often express the recommended application rates by using different terminology and units. However, it would be advantageous to define the following terms, which were suggested by Rauch et al. [14].
Table 1: Physical and chemical properties of enzymes.

| Item                             | DZ-1X   | EarthZyme | TerraZyme |
|----------------------------------|---------|-----------|-----------|
| Water                            | —       | 21.06%    | >50%      |
| Alcohols, C12–C16, ethoxylated   | —       | —         | <30%      |
| Fermented vegetable extract      | —       | —         | <20%      |
| Nonionic surfactants             | —       | 55%       | —         |
| Polysaccharides                  | —       | 2%        | —         |
| Oligosaccharides                 | —       | 3%        | —         |
| Disaccharides                    | —       | 5%        | —         |
| Monosaccharide                   | —       | 8%        | —         |
| Lactic acid                      | —       | 3.5%      | —         |
| Potassium as the chloride        | —       | 1.2%      | —         |
| Aluminum as the sulphate         | —       | 0.04%     | —         |
| Magnesium as the sulphate        | —       | 1.2%      | —         |
| Total                            | —       | 100%      | —         |
| Specific gravity                 | 1.0     | 1.0 to 1.1| 1.0 to 1.1|
| pH (neat)                        | 4.5     | 3 to 6    | 2.8 to 3.5|
| Boiling point                    | >100°C  | >100°C    | >100°C    |
| Ultimate biodegradability        | —       | DOC² reduction >90% after 28 days | — |
| Composition                      | —       | A blend of fermented carbohydrates, inorganic salts, and surfactants | — |

¹ Concentrated enzyme. ² Dissolved organic content.

Table 2: Recommended dosages, dilution ratios, and diluted application ratios of bioenzymes.

| Stabilizer                  | E-I (DZ-1X)       | E-II (EarthZyme)  | E-III (TerraZyme) |
|-----------------------------|-------------------|--------------------|-------------------|
| Suppliers recommended dosage| 1 liter per 4.2 m³ | 1 liter per 33 m³  | 1 liter per 25 m³ |
| Equivalent dilution mass ratio (DMR) | 5/1000         | 1/1000             | 1/1000            |
| Equivalent application mass ratio (AMR) | 1/7500        | 1/58900            | 1/44600           |
| Diluted application ratios * | 27 mL per kg of soil | 17 mL per kg of soil | 22 mL per kg of soil |

* Maximum dry density of soil was taken for these calculations, that is, 1785 kg/m³.

(i) Dilution mass ratio (DMR) is the mass ratio of a concentrated chemical product to water, used to express the product dilution in water prior to soil application.

(ii) Application mass ratio (AMR) is the mass ratio of a concentrated chemical product to oven-dried material in the treated soil.

In addition, because the recommended doses by the suppliers were very low, the enzymes were diluted in water prior to their application. Suppliers recommended doses, DMR, AMR, and diluted application ratios are given in Table 2.

2.2. Test Soil. The selected soil was taken from within the campus of Universiti Kebangsaan Malaysia in Bangi, Selangor, Malaysia. Therefore, it is abbreviated as UKM soil. It is a residual soil classified as CL, using a plasticity chart. The soil for all the tests was collected at once to reduce the chances of heterogeneity while preparing soil samples.

3. Sample Preparation and Tests

Rauch et al. [6] developed a protocol to prepare soil samples treated with liquid stabilizers. They devised this protocol after consulting a number of industry representatives and the Texas Department of Transportation. After the completion of their studies, this protocol was further modified to determine any change in compaction characteristics brought by liquid stabilizer. The thirteen-step, modified protocol was named “Revised Protocol for Preparing Soil Test Specimens” and is presented here in brief.

AMR was calculated from the dosage recommended by the product supplier, and concentrated stabilizer product was diluted to the recommended DMR. The soil was mixed with initial moisture content $w_o$:

$$w_o = OWC - \frac{AMR}{DMR} + 1\%.$$ (i)

Next, the premoistened soil was allowed to mellow (standing time recommended by ASTM D698-7 to allow the moisture to be absorbed thoroughly by the soil particles) for the minimum of 16 h under sealed conditions. The diluted stabilizer required to attain the recommended AMR in the treated sample was then measured and mixed with the soil samples to achieve a homogeneous mixture. The mixture was allowed to stand for 1 h, again in a sealed condition. The soil was then compacted with the specified compaction method, and the compacted soil was extruded from the mould, sealed, and placed in a moist curing room. The compacted soil sample
was cured for the desired curing period, and the sample was then trimmed to the required size for testing; the moisture content was determined by means of sample trimmings or with the whole sample. Finally, if the moisture content was not within the permitted range, the sample was prepared again.

To explain the abovementioned procedure, consider the determination of MDD and OMC for E-I-D1 (sample prepared with a single dosage of enzyme DZ-IX). Four samples (2 kg each) of oven-dried soil were taken. Moisture loss during sample preparation was estimated as 1%; therefore, the first trial was made with a moisture content of 2% less than the OMC for untreated (UT) soil, that is, 15%. The volume of the diluted enzyme solution (5 mL in 1 litre of water) for a single dosage (D1) was calculated (54 mL for 2 kg). Thus, 246 mL (0.15 × 2000 mL − 54 mL) was added in 2 kg of soil, and the soil was allowed to mellow for 16 h. After the mellowing period, 54 mL of diluted enzyme solution was added in the soil, and, again, the soil was mellowed for 1 h before compaction. Similarly, the other three samples were prepared with moisture contents of 16, 17, and 18%, and MDD and OMC were calculated for two dosages of all three enzymes.

Three geotechnical tests, that is, Atterberg limits, compaction, and unconfined compression strength (UCS), were conducted in this study. The Standard Test Methods for Laboratory Compaction Characteristics of Soil Using Standard Effort (ASTM D 698) was conducted to evaluate any changes brought by enzymes in compaction characteristics, that is, maximum dry density (MDD) and optimum moisture content. For UCS and Atterberg limits tests, initial curing periods of 7, 28, and 56 days were intended. However, it was later decided to cure the samples for 28, 56, and 84 days because no improvement was observed in UCS after 7 days for all of the enzymes and the two dosages used. For each curing time, one Proctor sample (dia. 101.6 mm, height 116.4 mm) was prepared, cured, and trimmed into three samples (dia. 38 mm, height 76–95 mm) just before the unconfined compressive strength test. Three samples were tested, and an average value of the three was recorded. After the test, the whole sample was used for moisture content determination. In total, three untreated (UT) and 18 treated (E series) samples were prepared. Atterberg limits (plastic limit and liquid limit) of the samples were determined after 56 days of curing.

4. Results and Discussion

4.1. UKM Soil Properties. The indices and other properties of UKM soil are shown in Table 3. Kabir and Taha [15] and M. R. Taha and O. M. E. Taha [16] used the residual soil from Universiti of Kebangsaan Malaysia in their experimental studies. The values of Kabir and Taha [15] were 18.8%, 23%, 15.8%, and 1.781 gm/cm³ and those of M. R. Taha and O. M. E. Taha [16] were 16.96%, 18%, 14.29%, and 1.839 gm/cm³ for the plasticity index, clay fraction, optimum moisture content, and maximum dry density (MDD), respectively. There were minor differences among these values, but, overall, the results are comparable.

| Characteristics                  | Value/description       |
|----------------------------------|-------------------------|
| Plasticity index (PI)            | 19.5%                   |
| Liquid limit (LL)                | 42.3%                   |
| Clay fraction                    | 29.6%                   |
| Soil classification              | CL                      |
| Optimum moisture content (OMC)   | 16%                     |
| Maximum dry density (MDD)        | 1.785 gm/cm³            |
| pH                               | 4.05                    |

Table 3: Characteristics of UKM soil.

1“Standard Test Methods for Liquid Limit, Plastic Limit, and Plasticity Index of Soils,” ASTM D 4318.
2“Standard Test Method for Particle-Size Analysis of Soils,” ASTM D 422.
3“Plasticity chart,” ASTM D 2487.
4“Standard Test Methods for Laboratory Compaction Characteristics of Soil Using Standard Effort,” ASTM D 698.

According to Kestler [4], enzymes may work suitably for soils containing 12–24% clay fraction with a plasticity index between 8 and 35. UKM soil almost falls into this category. Thus, it was assumed that the UKM soil is quite fitting for enzyme functioning.

4.2. Compaction Test. Compaction characteristics of UKM soil (untreated) were determined using the standard compaction effort (ASTM D698), and the same procedure was used to identify any change in compaction characteristics due to enzymes. During the preparation of untreated and treated soil samples, an increment of 1% moisture content was chosen so that precise compaction characteristics could be determined. Three important factors that affect the compaction of soil are moisture content, soil type, and compaction effort. For a given soil, as the compaction effort is improved, the MDD is increased and OMC is decreased. The bell-shaped curves with single peak, which were achieved in this study, are typical of clayey soils with liquid limits between 30 and 70 as observed by Lee and Suedkamp [17] in their studies of compaction curves of different soils. The compaction curves for untreated and treated samples for the two dosages are shown in Figures 1 and 2. The OMC and MDD for untreated soil were 16% and 1.785 gm/cm³, whereas those for samples treated with a single dose of three enzymes were 16.1% and 1.788 gm/cm³, 16% and 1.787 gm/cm³, and 16.2% and 1.786 gm/cm³ for E-I, E-II, and E-III, respectively. Similarly, for double dosage, no reduction in OMC was observed, but modest improvement was noted in the increase of the maximum dry density.

The two enzymes E-II and E-III (chemical composition of DZ-IX) are not provided by the supplier, but during the dilution of DZ-IX, foaming was formed, showing its surfactant-like behavior) contained nonionic surfactants, yet improvement in compaction was not observed. The reason for this performance could be a very low quantity dose of the enzymes. Therefore, all the treated samples were prepared with optimum moisture content of the control untreated soil samples. The average dry densities of all untreated and treated samples are given in Figure 3. The maximum increase in dry density was observed in samples treated with a double.
dose of enzyme E-I, and even this increase was only 3%. This increase can be attributed to the high application rate of enzyme E-I. In their study, Rauch et al. [14] examined the effect of the enzyme on compaction characteristics of three reference clays (kaolinite, illite, and montmorillonite) and two native Texas clays (from Bryan and Mesquite, Texas) but found no improvement in the dry density. They used the OMC of untreated soils to prepare soil samples treated with the enzyme. They then suggested that the compaction characteristics (OMC and MDD) for treated soils should be determined separately. Milburn and Parsons [11] conducted compaction tests on ML and SM soils. The moisture content for the treated soil samples was kept at 1% less than the optimum moisture content, but only 4% and 1% increases in dry density were found for ML and SM soils, respectively.

The moisture content determined at the time of compaction and moisture content at the time of testing (UCS) for all the prepared samples are given in Table 4. In moisture contents at the time of sample preparation and after, the curing was compared, and, in all samples, the difference remained within 1%. There could be two reasons for this moisture conservation: the samples were cured in a bigger sized and were trimmed to the required size at the time of testing and no chemical reaction took place that possibly could have changed the moisture content.

4.3. Atterberg Limits Test. Liquid limit and plastic limit were determined after 56 days of curing. Marasteanu et al. [8] recommended an extended period of curing up to four months. Additionally, considerable improvement in treated soil is generally required to be reflected consistently through Atterberg limit tests. Thus, curing periods of 56 days and 84 days were chosen. No noteworthy improvement was found after 56 days. Furthermore, the UCS results for the 84-day cured sample did not show substantial improvement; therefore, Atterberg limits for 84 days were not determined. The plasticity indices for untreated and treated samples for two doses are given in Figure 4. It showed marginal change from the untreated samples. It is indicated in the ASTM (1998) that determinations of plastic and liquid limits of a certain soil, even by a single operator, can vary by 2.6 and 2.4, respectively. Therefore, the difference in plasticity indices may be attributed to the routine inconsistency which is encountered in the laboratory.
Table 4: Moisture content of prepared samples.

| Sr. number | Enzyme & dosage | One month $w_o^1$ | One month $w_f^1$ | Two months $w_o^2$ | Two months $w_f^2$ | Three months $w_o^3$ | Three months $w_f^3$ |
|------------|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 1          | UT             | 16.0              | 15.7              | 15.9              | 15.8              | 15.7              | 15.1              |
| 2          | E-I-D1         | 15.3              | 15.2              | 16.2              | 16.0              | 15.9              | 15.9              |
| 3          | E-I-D2         | 16.0              | 15.8              | 16.2              | 16.1              | 15.9              | 15.5              |
| 4          | E-II-D1        | 16.5              | 16.1              | 16.1              | 15.9              | 16.3              | 16.2              |
| 5          | E-II-D2        | 16.4              | 16.2              | 16.1              | 15.7              | 16.3              | 16.2              |
| 6          | E-III-D1       | 15.7              | 15.6              | 16.1              | 15.9              | 16.4              | 16.0              |
| 7          | E-III-D2       | 16.7              | 16.6              | 16.1              | 16.1              | 16.1              | 15.8              |

$^1$Moisture content in % at the time of compaction.

$^2$Moisture content in % at the time of UCS test.

Mgangira [12] performed Atterberg limits tests on two soils treated with two enzymes (PermaZyme II-X and Earth-Zyme) after 28 days of curing, and no significant improvement was observed. Similarly, Rauch et al. [14] also found no decrease in plasticity indices for the five soils they tested, as mentioned previously.

4.4. Unconfined Compressive Strength Test. The Standard Test Method for Unconfined Compressive Strength of Cohesive Soil (ASTM D 2166) was used to evaluate the ultimate compressive strength of untreated and treated soil samples. Untreated control samples were also prepared and cured for three curing periods. After the curing period, the sample was unsealed and trimmed into three samples of the required size, and all three samples were weighed before testing. The three samples were then tested on a strain controlled machine, and the average of the three ultimate compressive stresses was taken as the final value. The tested samples were then placed in an oven for moisture content determination.

The results are shown in Figures 5 and 6. The results of E-I for two doses showed no improvement, whereas, for enzymes E-II and E-III, there was little improvement. The maximum increase in strength was recorded for E-III-D2, which was 17%. It is evident that the results were generally moderate, or the improvement was, in individual cases, without any persistent pattern.

Controlled untreated samples were prepared to account for any strength gain due to thixotropy or aging. Some of the clayey soils were reported to reduce their unconfined compression strength when tested after remoulding without any change in moisture content. The main reason behind this loss was the destruction of clay particle structure, which developed from the original sedimentation process. However, when remoulded samples are held for some time, keeping the moisture content unchanged, they may gradually gain strength [18].

Unconfined compression tests on different soils treated with PermaZyme II-X were also carried out by Peng et al. [19]. The treated samples were cured in two different conditions, that is, sealed and air-dry. No improvement was recorded for soil samples cured in a sealed condition, whereas a maximum of 10% gain in strength was observed for soil samples cured in an air-dry condition. However, Venkatasubramanian and
Table 5: Comparison of XRF results for untreated (UT) soil samples and soils treated with three enzymes.

| Formula | UT Concentration (%) | E-I-D2 Concentration (%) | E-II-D2 Concentration (%) | E-III-D2 Concentration (%) |
|---------|----------------------|--------------------------|---------------------------|---------------------------|
| SiO₂    | 49.07                | 51.42                    | 46.17                     | 48.74                     |
| Al₂O₃   | 28.89                | 29.84                    | 30.35                     | 29.80                     |
| Fe₂O₃   | 9.07                 | 9.20                     | 9.78                      | 9.70                      |
| TiO₂    | 1.59                 | 1.55                     | 1.60                      | 1.61                      |
| K₂O     | 0.48                 | 0.55                     | 0.57                      | 0.57                      |
| MgO     | 0.40                 | 0.44                     | 0.45                      | 0.44                      |
| ZrO₂    | 0.14                 | 0.16                     | 0.12                      | 0.14                      |
| SO₃     | 0.08                 | 0.12                     | 0.08                      | 0.09                      |
| V₂O₅    | 0.04                 | 0.04                     | 0.04                      | 0.04                      |
| CaO     | 0.03                 | 0.04                     | 0.08                      | 0.07                      |
| Cr₂O₃   | 0.01                 | 0.01                     | 0.02                      | 0.01                      |

Dhinakaran [20] used three soils with clay content of 20, 12.5, and 8% with PIs of 6, 5 and 6, respectively. Increases in unconfined compression strength went to 200% and 400% after 2 weeks and 4 weeks, respectively, and were recorded for the enzyme treated soils. A maximum increase of 450% in unconfined compression strength was recorded by Shankar et al. [21] when a lateritic soil (LL = 45 and PI = 10) was treated with 4 times the recommended dosage by the enzyme supplier TerraZyme. Part of this substantial improvement could have been due to moisture loss because the moisture content at the time of the sample preparation and testing was not mentioned in these two studies.

4.5. XRD, XRF, and FESEM. Willie and Norman [22] suggested that the XRD technique for identification of soil mineralogy is used a great deal and is considered one of the most dominant techniques used for mineral identification in soils and rocks. XRD, XRF, and FESEM tests were conducted for untreated (UT) and treated (E series) soil samples after 56 days of curing. The XRD results for the untreated (UT) soil and soil samples treated with three enzymes are given in Figure 7. The peaks were matched with different possible compounds present in the soil. The matching revealed SiO₂, Al₂O₃, and Fe₂O₃ as the three main ingredients (compounds) of UKM soil as verified by the chemical composition obtained from XRF test results for the untreated and the three treated soils as given in Table 5. The XRD results of the untreated and the three treated soil samples are stacked for comparison. It was evident that no chemical change took place to alter the chemical composition of the soil treated by any of the three enzymes. This conclusion is further verified by the XRF results, which showed no variation in chemical composition in any of the three treated soil samples. However, by looking at FESEM images in Figure 8, it can be observed that the particles for E-II-D2 and E-III-D2 samples were more closely packed and agglomerated than the untreated (UT) and E-I-D2 samples. The voids or pores can be seen as shadows in Figure 8 due to looseness of particles in untreated (UT) and treated samples (E-I-D1), with boundaries marked in lines. These results are consistent with the hypothesis suggested by Scholen [17] and Rauch et al. [16], which states that the enzymes combine with organic molecules, which then surround the clay minerals, nullifying the negative charge on the clay surface and lessening the clay’s affinity for water.

5. Conclusions

In this experimental study, the effects of three enzymes on Atterberg limits, compaction characteristics, and unconfined compressive strength were evaluated. A Standard Proctor test was carried out to examine any change in optimum moisture content and maximum dry density with two doses of all three enzymes. The same test was conducted to prepare control untreated soil samples and soil samples treated with two doses of three enzymes for three curing periods (28, 56, and 84 days). The Atterberg limits test was carried out on untreated and treated soil samples after 56 days of curing. XRD, XRF, and FESEM were conducted to identify if any chemical change had occurred.
It was found that the three enzymes did not produce any comprehensible improvement in the three tests conducted, that is, Atterberg limits, compaction, and unconfined compression tests. Little improvement, in some cases, could be related to the hypothesis that the enzymes did not produce any chemical change, and they only prevented moisture absorption to bring the particles closer. Therefore, when selecting an invalidated stabilizer, it is imperative to check its suitability before using it on larger scale. It is hoped that this study will be beneficial for designers, contractors, and constructors when choosing bioenzymes as a soil stabilizer.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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**References**

[1] S. A. Jefferis, “Moving towards sustainability in geotechnical engineering,” in Proceedings of the Geoenvironment Annual Congress of the Geo-Institute of ASCE (GeoCongress ’08), pp. 178–844, March 2008.

[2] D. G. Abreu, I. Jefferson, P. A. Braithwaite, and D. N. Chapman, “Why is sustainability important in geotechnical engineering,” in Proceedings of the GeoCongress, Geotechnical Special Publication no. 178 on Geosustainability and Geohazard Mitigation, pp. 821–828, 2008.

[3] J. Khedari, P. Watsanasathaporn, and J. Hirunlabh, “Development of fibre-based soil-cement block with low thermal conductivity,” Cement and Concrete Composites, vol. 27, no. 1, pp. III–116, 2005.

[4] M. A. Kestler, Stabilization Selection Guide for Aggregateand Native-Surfaced Low Volume Roads, Forest Service, San Dimas.
[5] D. Scholen, “Non-standard stabilizers,” Report FHWA-FLP-92-011, Department of Transportation, 1992.
[6] A. F. Rauch, L. E. Katz, and H. M. Liljestrand, An Analysis of the Mechanisms and Efficacy of Three Liquid Chemical Soil Stabilizers, Center for Transportation Research, The University of Texas at Austin, 2003.
[7] M. Shukla, S. Bose, and P. Sikdar, “Bio-enzyme for stabilization of soil in road construction a cost effective approach,” in Proceedings of the IRC Seminar Integrated Development of Rural and Arterial Road Networks for Socio-Economic Development, New Delhi, India, 2003.
[8] M. O. Marasteanu, R. Hozalski, T. R. Clyne, and R. Velasquez, “Preliminary laboratory investigation of enzyme solutions as a soil stabilizer,” Tech. Rep., Department of Civil Engineering, University of Minnesota, Minneapolis, Minn, USA, 2005.
[9] A. Lacouture and H. Gonzalez, Usage of Organic Enzymes for the Stabilization of Natural Base Soils and Sub-Bases in Bagota, Faculty of Engineering, Pontificia Universidad Javeriana, 1995.
[10] A. Hitam, A. Z. Yusof, and O. Samad, “Soil stabilizer for plantation road,” in Proceedings of the National Seminar on Mechanization in Oil Palm Plantation, Palm Oil Research Institute of Malaysia (PORIM 99), Bangi, Malaysia, 1999.
[11] J. P. Milburn and R. Parsons, Performance of Soil Stabilization Agents, Kansas Department of Transportation, 2004.
[12] M. Mgangira, “Evaluation of the effects of enzyme-based liquid chemical stabilizers on subgrade soils,” in Proceedings of the 28th Southern African Transport Conference, Pretoria, South Africa, July 2009.
[13] F. Bron, C. Ding, H. Gary, and R. Charles, Permazyme Testing Volume I: Final Testing Summary Report, California Pavement Preservation Center, 2010.
[14] A. F. Rauch, J. S. Harmon, L. E. Katz, and H. M. Liljestrand, “Measured effects of liquid soil stabilizers on engineering properties of clay,” Transportation Research Record, vol. 1787, no. 1, pp. 33–41, 2002.
[15] M. H. Kabira and M. R. Taha, “Sedimentary residual soil as a waste containment barrier material,” Soil & Sediment Contamination, vol. 13, no. 5, pp. 407–420, 2004.
[16] M. R. Taha and O. M. E. Taha, “Influence of nano-material on the expansive and shrinkage soil behavior,” Journal of Nanoparticle Research, vol. 14, article 190, 2012.
[17] P. Y. Lee and R. Suedkamp, “Characteristics of irregularly shaped compaction curves of soils,” Highway Research Record 381, 1972.
[18] B. M. Das, Principles of Geotechnical Engineering, Cengage Learning India Private Limited, 5th edition, 2001.
[19] H. T. Peng, H. T. Su, X. P. Zhang, and J. Wang, “An experimental comparison of compressive strengths of soils stabilized with enzyme and ground quicklime,” Advanced Materials Research, vol. 280, pp. 9–12, 2011.
[20] C. Venkatasubramanian and G. Dhinakaran, “Effect of bio-enzymatic soil stabilisation on unconfined compressive strength and California Bearing Ratio,” Journal of Engineering and Applied Sciences, vol. 6, no. 5, pp. 295–298, 2011.
[21] A. Shankar, H. K. Rai, and R. Mithanthaya, “Bio-enzyme stabilized lateritic soil as a highway material,” Indian Roads Congress Journal, vol. 70, no. 2, 2009.
[22] H. Willie and W. Norman, Methods of Soil Analysis. Part 5. Mineralogical Methods, SSSA Book Series, No. 5, Soil Science Society of America, Madison, Wis, USA, 2007, edited by L. R. Drees, A. L. Ulery.
