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Experimental Study on Influence of Using Urease Enzyme on Stabilized Sandy Soil's Engineering Property by Zeolite and Sawdust

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ABSTRACT

Soil plays an important role in any structure, so soil improvement has become one of the most essential parts of construction projects. This study investigated influence of sawdust admixture on inshore sandy soil stabilized by zeolite and urease enzyme. One of the problems that environmental engineers encounter with is accumulating waste materials ،sawdust is one of these materials. One way for reducing these side products is reusing them, especially for soil improvement. Sandy soil with uniform granulation (SP) due to it's incoherence is one of the most problematic kind of soils. Therefore, sawdust with zeolite has been used to improve soil engineering properties and the results have been compared with the effect of calcium carbonate precipitation ($CaCO_3$) on the soil. Today, by advancement in various sciences and knowledge boundaries elimination in various fields, new and environmentally friendly materials can be used as an alternative method to traditional materials. One of these substances is urease enzymes, which are obtained from natural sources such as Jack Beans or bacteria's activity such as Sporosarcina pasteurii. Samples in this study were made with 4, 8 and 12 percent by weight of zeolite and 4, 8 and 12 percent by weight of sawdust and were cured for 7, 14, 28 and 45 days. According to compaction test results, by increasing zeolite and sawdust's percentage, optimum moisture content has been increased and maximum dry density decreased. The results of unconfined compression strength (UCS) test showed that the samples with 4% sawdust, 8% zeolite and calcite precipitation has about 9% increase in maximum strength compare with the samples without calcite precipitation and with higher zeolite content. Also, the rupture strain of samples with calcite precipitation was higher than the samples without it.

1. Introduction

One of the main characteristics of loose and uniform inshore sandy soils is their low strength and cohesion. To stabilize this type of soil, cement addition is one of the most widely used options by engineers. Producing 1 ton cement will spread about an average of 0.655 tons greenhouse gas and producing one ton of clinker, will spread an average of 0.79 tons of greenhouse gas [1]. Pozzolans, can be used as an alternative to cement. Pozzolans such as zeolite have been able to increase soil's compressive strength and durability. By replacing zaolite with cement we can save energy also reducing pollution [2]. Main reason for using zeolite instead of cement in this research is that zeolite is a natural mineral and there are lots of zeolite mines in Iran, so zeolite can be used as a worthwhile material in Iran. Also sawdust can be used as a waste material in wood industry. Zeolites is crystalline aluminosilicates of alkaline or alkaline earth metals such as sodium, potassium, magnesium, strontium, barium and calcium, which are formed from a combination of $\left[AlO_4\right]^{5-}$ and $\left[SiO_4\right]^{4-}$. These materials were first discovered in 1759 by a Swedish miner named Kronstadt. The word zeolite means a boiling rock, and the reason for this name is large amount of water released in the form of steam when heating zeolites [3].
The structure of zeolite is shown in figure 1.

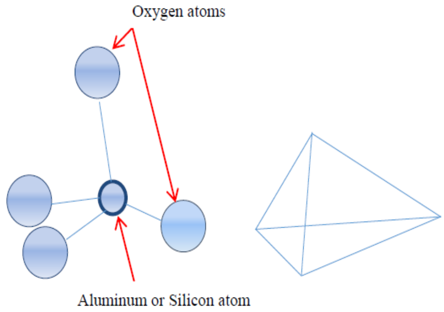

Figure 1. Structure of zeolite [4]

Biological method is an unusual method for soil stabilization and improvement in which calcium carbonate precipitation increases soil strength and hardness by filling soil pores. This method can be a suitable alternative for chemical cement in many improvement projects. Calcite deposition (the most stable form of calcium carbonate) is the result of urea hydrolysis by urease enzyme. The urease enzyme is obtained directly from plant sources such as jack bean or indirectly from urease-positive bacteria. Due to this enzymatic reaction, the pH increases and calcite crystals deposit on the surface of the particles and between the soil pores, eventually leading to the connection between the particles and filling soil pores [5]. Bacterias are the most abundant microorganisms in the soil. There are between 10^8 and 10^{10} bacteria per gram of dry soil. By depth increasing The amount of these bacteria decreases. On average, at a depth of 30 meters (the lowest range of most soil engineering improvement activities), the number of bacteria is about 10^6 cells per gram. Today, bio-cementation has an important role in geotechnical engineering. One of the most common cementation methods that has been researched so far is microbial induced calcite precipitation method (MICP), which has the potential to increase soil strength and hardness [6,7]. This method uses special species of urease-positive bacteria (bacteria that have the ability to hydrolyze urea) that live in the soil and have an alkaline-like property that cause urea deposition in the soil by performing a hydrolysis reaction. This reaction occurs in the soil with two substances, urea and calcium chloride, and raises the soil pH, and finally make calcite precipitation that causes connection between soil particles like a bridge [8,9]. Urease enzymes change urea to carbonate and ammonium ions in presence of water. Urea Hydrolysis without a catalyst is a slow chemical process. Urease enzyme accelerates the hydrolysis reactions of urea by more than 10^{14} times. In fact carries out the process that naturally turns sand into rock sand over millions years in 7 to 10 days. How this process works can be summarized according to reactions (1) and (2) in such a way that urease enzyme hydrolyzes urea and finally leads to calcium carbonate precipitation by reaction with calcium ion [10].

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(s) \quad (2)$$

Ammonium (NH_4^+) production causes an increase in pH, which results in an increase in hydroxide ions (OH^-). Bacterial cells have a negative electrical charge due to the presence of hydroxide ions on their walls and they stick to the surface of soil grains, which have relatively high concentrations of nutrients. Therefore, calcite joints tend to form soil aggregates at the point of contact with each other [11]. Figure 2 shows the formation of precipitation between soil grains.

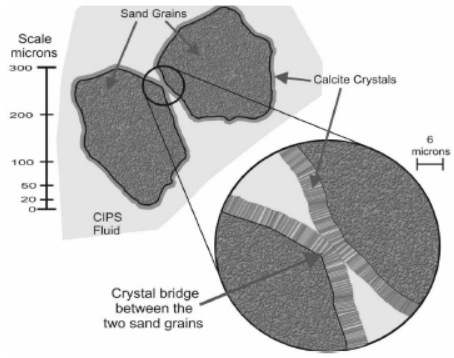

Figure 2. Biological precipitation formed between soil grains [12]

Enzymes are extremely effective catalysts that make a reaction that does not occur easily under normal circumstances or takes a very long time happen, and accelerates the reaction. Most enzymes are highly specific and only catalyze a specific reaction and have no effect on other components of the system. Enzymes are present in all cells of living organisms, wherever a reaction occur in cells, enzymes have main role. They only accelerate very simple reactions and no enzyme can catalyze very complex and multi-step reactions alone. Some of them require special heat and pH for their operation in environment. Enzymes are compounds that can increase the reaction rate by up to 107 times. The enzyme, like an inorganic catalyst, accelerates the reaction by lowering the activation energy. Performing a reaction in a laboratory, requires special conditions such as high temperature and pressure. Therefore, there must be a precise mechanism in the cell in which the environmental conditions are completely constant and such reactions are very slow and This is enzymes duty. Catalysts remain unchanged in reactions, but enzymes, like other proteins, do not remain stable under different conditions. These substances are changeable due to high temperatures and acids and alkalis. Enzymes increase the rate of chemical reactions by reducing the activation energy. Enzymes are protein molecules that have one or more penetration sites to which the substrate or substance is affected by the enzyme binds (Fig. 3). By enzyme action, the substrate changes and becomes one or more products. An important feature of enzymes is that after each reaction and at the end, they remain intact and can catalyze the next reaction [13].

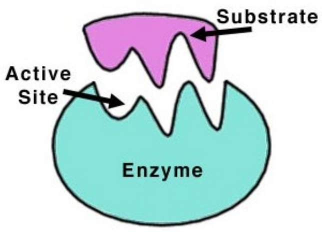

Figure 3. Enzyme action mechanism [13]

In 2016, Abbasi et al. in a study investigated the effect of zeolite replacement by cement on stabilized sandy soil. This research showed that by replacing 50% zeolite with cement, shear strength and rupture strain of the specimens were increased, the amount of increase in shear strength is about 62% compared to the non-zeolite specimens [14]. In 2018, Molla Abbasi and Shoosh Pasha investigated the effect of zeolite replacement with cement in sandy soil samples of Babolsar (IRAN). They concluded that by replacing 30% zeolite with cement compared to non-zeolite samples, the uniaxial compressive strength increased by 20 to 78% [15]. Abbasi et al. in 2018, investigated the effect of zeolite on the tensile strength of stabilized sandy soils by cement. The results showed that by replacing 30% natural zeolite with cement, tensile strength of samples have increased 45% compared to samples without zeolite [16,17]. In another study, the stabilization of expansive soils using zeolite and lime has been investigated. The purpose of this experimental study was to investigate the effects of zeolite and lime and a mixture of these two materials on some soil properties such as atterberg limits, grain size distribution, and soil swelling rate [18]. In one study, the stabilization of BC (Black Cotton soil), which is classified as very poor soil and has very fine particles (about 85 to 100% clay), was investigated. This soil shows volumetric changes of about 200 to 300% and its swelling pressure reaches 10. The results showed that by adding 2% cement with 1% sawdust to the soil, the liquid limit was reduced by about 3.7%. Also, the bearing capacity (CBR) was increased about 4.6 times and the uniaxial compressive strength about 2.1 times [19]. Moravej et al. in 2015, investigated the effect of bacillus sphaericus on stabilization and improvement of loose soil. Pinhole test was used to evaluate the soil dispersive degree. The results showed that the soil dispersive decreased with increasing bacterial solution concentration and also during time [20]. In 2007, Wiffin et al. in a study used bacterial stabilization method for soil stabilization. The microorganism was used in this experiment was sporosarcina pasteurii. results showed an increase in the strength of the stabilized soil. Soil porosity and permeability were also significantly reduced [21].

Unconfined compressive strength (UCS) test is one of the most widely used tests in many laboratory applications to evaluate the effect of soil stabilization. Its simplicity and speed compared to similar tests, the availability and low cost of this test are some of the reasons for the widespread use of this test [22].

In this research influence of using urease enzyme for soil improvement has been investigated, how calcite precipitation improves geotechnical properties of loose soils. this method (using urease enzyme for producing calcite precipitation) is abbreviated as EMCP. For more efficacity, zeolite and sawdust has been used. One of the environmental aspects of this research is using zeolite as a substitute for cement, so we can improve soil engineering properties by using less cement. It is a good step for decreasing environmental polluting (as you know cement production process has a lot of bad effects for environment such as air polluting). This study investigated the effect of zeolite and sawdust on uniform sand with poor granulation (SP). In this method (EMCP) urease enzyme can be used directly to the soil, but in MICP method bacteria is used for producing urease enzymes. So using bacteria as a alive organisms in soil has it's difficulties (bacteria need special situation for living. Because these bacteria are alive organisms, they will need laboratory equipment to sterilize the environment to grow their colonies. Laboratory equipment such as autoclave, incubator and spectrophotometer are among these devices). The Most researches are about MICP and using positive urease bacteria such as Sporosarsina (Bacillus) for producing urease enzymes. One of the advantages of this research is to eliminate the complications of using bacteria for producing calcite precipitation. As a result, the direct use of urease enzymes, and limitation laboratory equipments, makes EMCP method more practical that can be used in field, which is vital for research projects in civil engineering.

2. Experimental program

To study the effects of sawdust and urease enzyme on uniaxial compressive strength of poorly granulated sand (SP), the parameters of zeolite content, urease enzyme and curing time have been considered. In order to improve this soil, 4, 8 and 12% by weight of zeolite and sawdust and 5% of fine-grained soil have been used, then the previous values were used with urease enzyme in the amount of 1 gr per 100 ml and 1 mol/L of calcium chloride and urea. curing time for samples with zeolite and sawdust is 7, 14, 28 and 45 days and for samples with urease enzymes is 7 and 28 days. The variables for sample preparation are presented in Table 1.

Table 1. The studied variables

Variables	Details
Soil type	SP
Zeolite type	clinoptilolite
Zeolite content	4, 8 and 12%
Urease enzyme content	1 gr/100 ml
$CaCl_2$ and urea content	1 mol/L
Sawdust content	4, 8 and 12%
Size of sawdust	Residue on sieve No.30
Curing time	7, 14, 28, 45 day

3. Materials

Materials are used in this research include sandy soil, zeolite, clay, sawdust and urease enzyme.

3.1. Sand

The soil is sandy soil with uniform granulation. The granulation of this soil is sand to coarse sand. After transferring this soil to the laboratory by sieving base soil, the grain size passing through sieve No. 8 and residue on sieve No. 10 were considered as the main soil to be tested. By sieve analysis test, it was determined that this soil is poor sand soil (SP). The reason for choosing this soil, is its uniform granulation and non-cohesion, which is one of the worst possible conditions among soils. The gradation curve of this soil according to ASTM C136 standard [23] is shown in Figure 4 and its specifications are shown in Table 2.

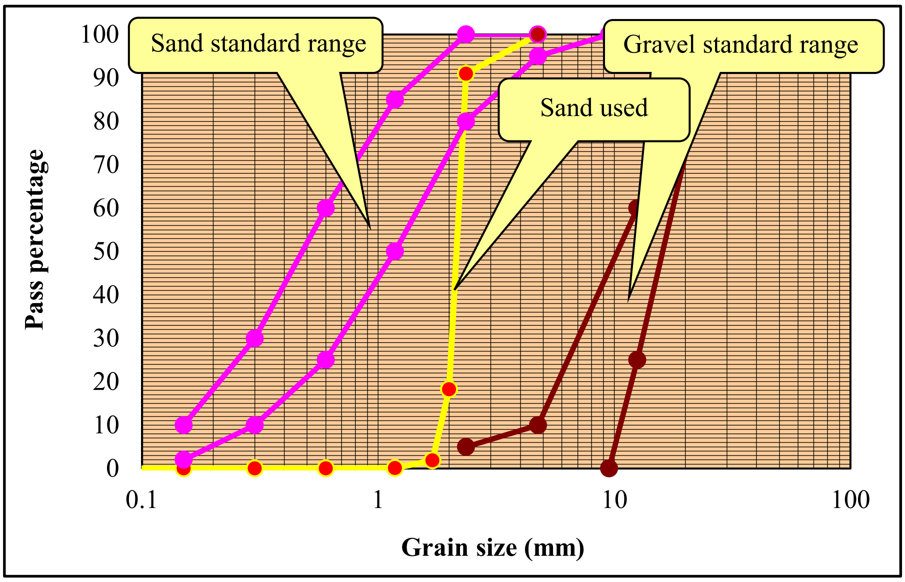

Figure 4. Sand gradation curve

Table 2. Physical characteristic of sandy soil

Characteristic	Value	Standard
Soil type	SP	ASTM 2487 [24]
Water content	8.43 [%]	ASTM D698 [25]
$\gamma_{d,\,max}$	1.59 [gr/cm^3]	ASTM D4253-4 [26,27]
$\gamma_{d,\,min}$	1.47 [gr/cm^3]	
ε_{max}	0.806 [-]	
ε_{min}	0.6 [-]	
G_s	2.65 [-]	ASTM D854 [28]

3.2. Zeolite

The zeolite that is used is clinoptilolite, which is prepared from Kavan mine in Semnan province in Iran. The characteristics of this type of zeolite are shown in Table 3.

Table 3. Characteristic of zeolite

Characteristic	Value
Water absorption	65 [%]
Average particle size	38 [μ]
Dry density (γ_d)	0.64 [gr/cm^3]
Hardness (E_c)	0.683 [$mmohs/cm$]

3.3. Fine-grained soil

Uniaxial compressive strength test in uniform sandy soils with zeolite will not be possible due to the absence cohesive element, so in this study 5% by weight of fine-grained soil (clay) has been used.

3.4. Sawdust

The sawdust was prepared from wood sawdust waste in carpentry. The sawdust passed through sieve No. 16 and the residue on sieve No. 30 were tested, as shown in Figure 5.

Figure 5. Sawdust used

3.5. Urease enzyme

Enzymes are protein catalysts made by living cells that catalyze biochemical reactions inside or outside the cell. Enzymes increase the rate of reactions (the rate of equilibrium between the substrate and the product) by up to 10^{16} times. Reaction rate in the presence of urease enzyme (urea amidohydrolase EC 3.5.1.4) increases by $3*10^4$/sec and in the absence of enzyme by $3*10^{-10}$/sec and in total the reaction rate increases by 10^{14} times [29]. The enzymes urease, calcium chloride and urea are shown in Figure 6.

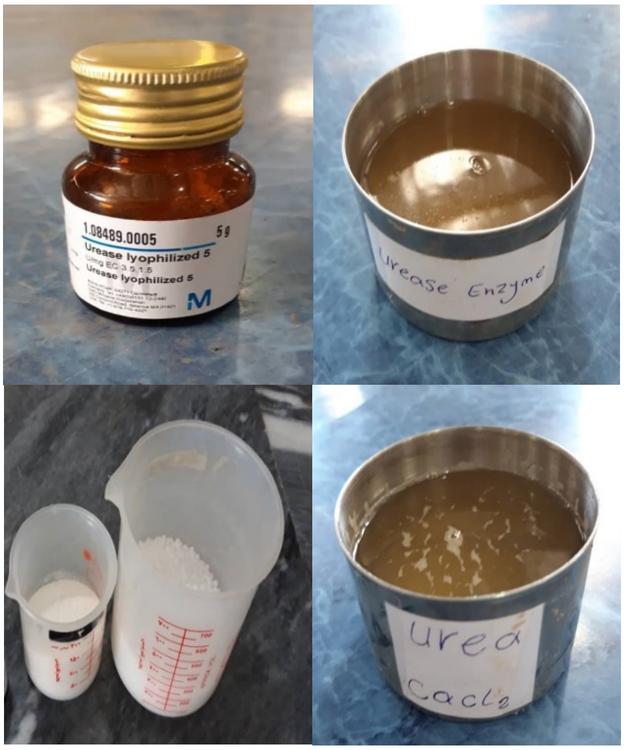

Figure 6. Urease (up), calcium chloride and urea (down)

4. Compaction test

Samples were prepared immediately after mixing and compaction test according to ASTM D698 [25]. Different amounts of sandy soil, zeolite and sawdust based on the stated percentages for each sample were separated by a total of 3 kg and mixed with relatively low humidity and in a standard projector using a 2.5 kg hammer with Free fall was compacted from a distance of 30 cm. Density in 3 layers, each layer is smoothed with 25 compact strokes and the soil surface on the mold without applying pressure. Then the base of the mold was removed and the soil and mold were weighed. Samples were taken from the top, middle and bottom of the soil. This procedure is repeated until the weight of the soil in the mold exceeds the maximum value and begins to decrease (Figure 7).

Figure 7. Compaction test

The compaction test results are shown in Figure 8. by adding 4, 8 and 12% zeolite and sawdust to the sandy soil, the optimum moisture content (OMC) has been increased. The reason is that zeolite absorbs water in the soil and causes more need for water to saturate. On the other hand, by adding zeolite and sawdust to the soil, the maximum dry density (MDD) has been decreased. The reason for the reduction in MDD is that sawdust and zeolite occupy more volume as their low weight.

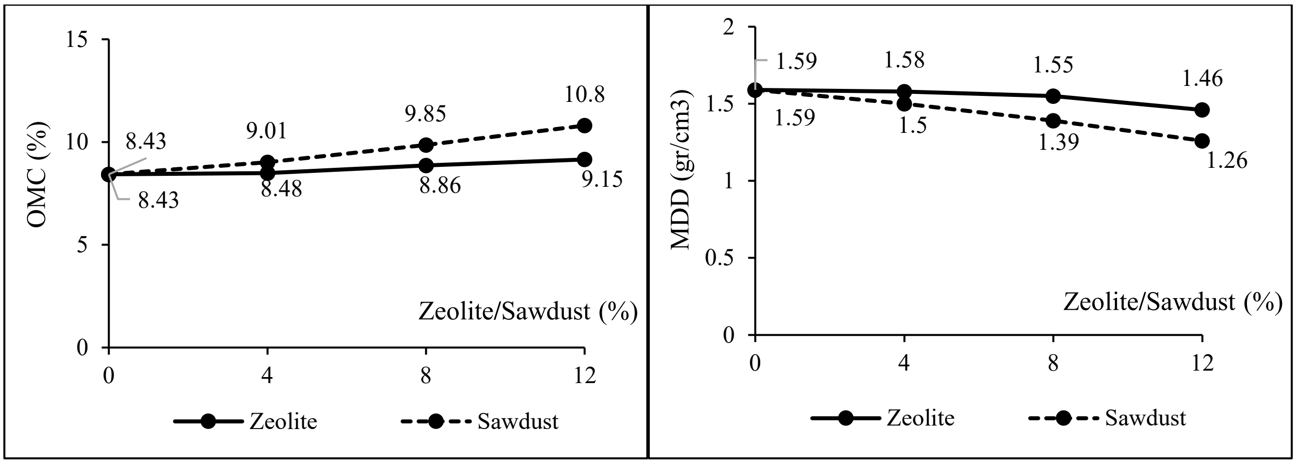

Figure 8. Compaction test results

5. Sample preparation

Samples need time for curing and must be stored inside the mold, so (PVC) pipe is used as the mold. Dimensions of pipe tube molds with outer diameter 40 mm and inner diameter 34 mm, which is cut to a height of 70 mm. In UCS test according to ASTM D2166 [30], the length to diameter ratio of the samples should be between 2-2.5. Also, to facilitate the removal of samples after curing, the molds are divided vertically into two separate parts. PVC molds are shown in Figure 9.

Figure 9. PVC molds

In order to add urease enzyme, calcium chloride and urea, according to the optimal water content of sandy soil, the amount of water required for every sample was divided into two parts. One part was mixed with urea and calcium chloride and the other part with urease enzyme. at first, the sandy soil was mixed with the dry clay, cement and zeolite, then a solution containing urea and calcium chloride was added and mixed, Finally a solution containing urease enzyme was added and mixed, then the mixture was hammered in 3 layers into the mold. In order to properly cure the zeolite, a wet coating with insulation was used to prevent evaporation, which is shown in Figure 10.

Figure 10. Conditions for curing samples with wet coating and insulation

Samples containing calcite precipitation were removed from the mold after 24 hours and the solution containing urea and calcium chloride was sprayed on it, then urease enzyme solution was sprayed on the sample and placed again in the mold with wet cover and insulation to be cured. The reason for spraying is that all specimens must have the same amount of urea, calcium chloride and urease enzyme due to their different optimal water content. In order to ensure the formation of calcium carbonate precipitation, both solution were poured into a container and allowed to form calcite precipitation for one week. Figure 11 shows the calcium carbonate precipitation and an image of a ruptured specimen in a uniaxial device.

Figure 11. Calcium carbonate precipitation (left) and broken sample (right)

6. Results

After curing, samples were taken out of the mold and kept at room temperature for 24 hours and then placed in a single-axis device for testing.

6.1. The effect of urease enzyme addition

The results of unconfined compressive strength test for samples with 4, 8 and 12% of sawdust at 7 and 28 days of curing are presented in Figure 12. As shown in diagrams, the compressive strength and strain rupture of the specimens increased by calcium carbonate precipitation forming between the soil grains.

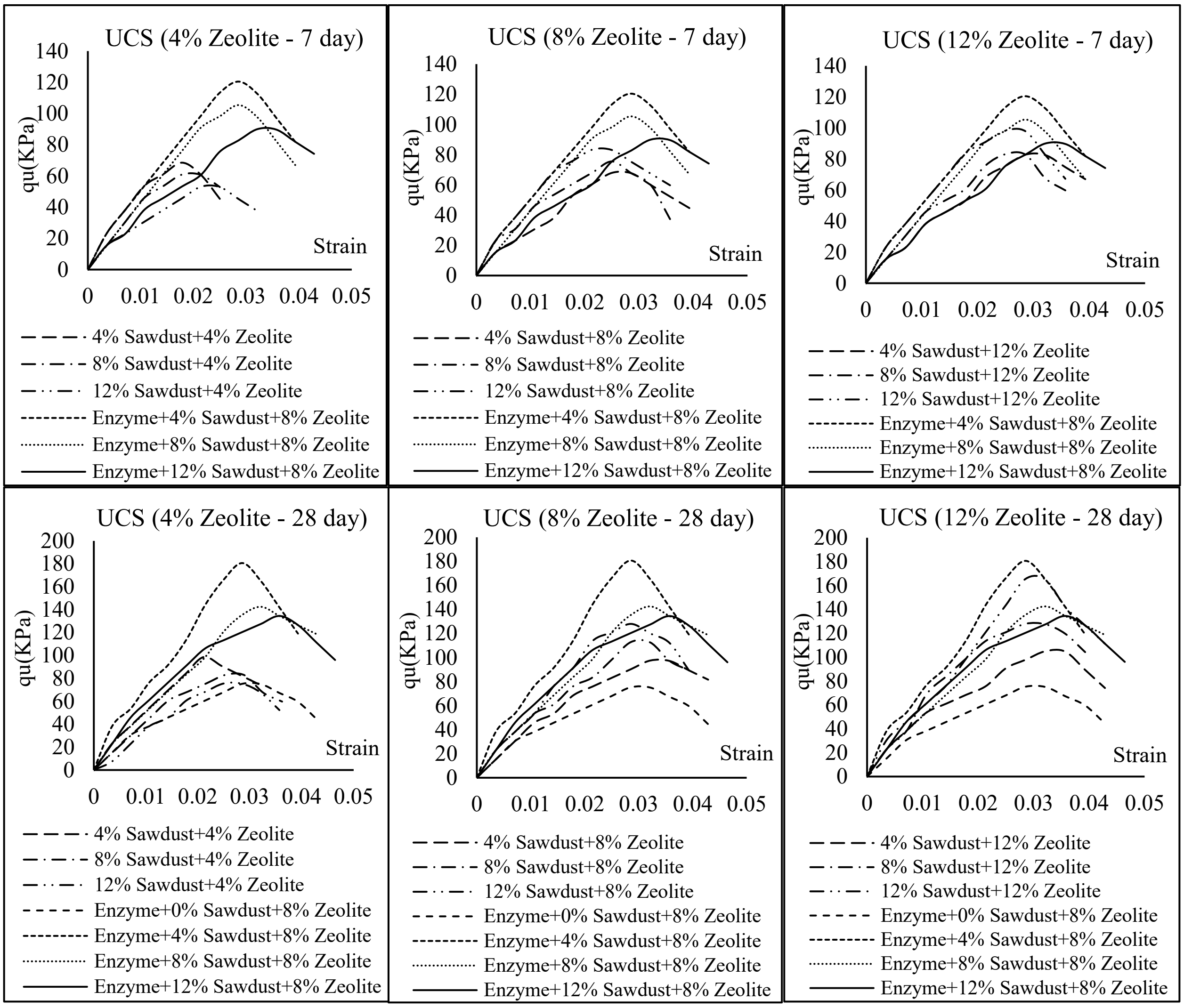

Figure 12. Unconfined compressive strength test results in 7 and 28 days curing

6.2. The effect of sawdust addition

The results of unconfined compressive strength test are shown in Figure 13. According to the diagrams, the maximum increasing in the strength of samples is in samples with 4% sawdust, by increasing the percentage of sawdust, the compressive strength has decreased. This may be due to the fact that by increasing the percentage of sawdust, the cohesion between soil particles has decreased and because of sawdust's high absorbency, large amount of water was absorbed by sawdust and soil particles did not have sufficient amount of adhesion solution. The diagrams also show that by increasing the percentage of sawdust, the rupture strain of the samples has increased.

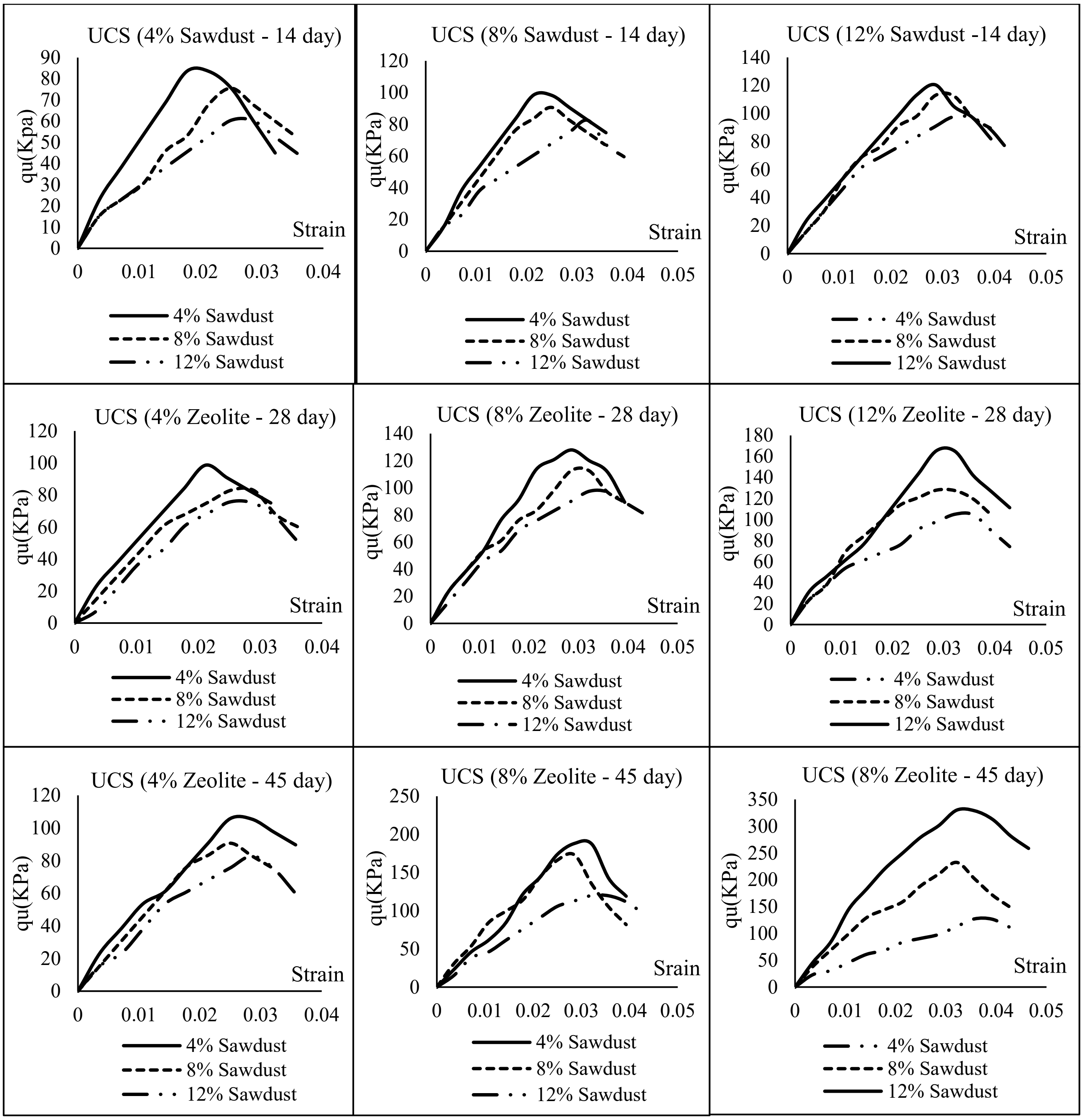

Figure 13. Results of UCS test at 14, 28 and 45 days curing time

The general results of unconfined compressive strength test for samples with 4, 8 and 12% of sawdust and in 7, 14, 28 and 45 days curing are presented in Figure 12. As Figure 14 shows, the strength of the specimens decreased by increasing the percentage of sawdust more than 4%. Also, the highest effect of sawdust was 4%. By increasing the percentage of zeolite, the slope of increasing the compressive strength at 28 to 45 days curing, has increased significantly. The strength of samples with 4% zeolite, 4% sawdust and 5% fine-grained soil at 45-days was 2.31 times higher than samples without sawdust. This ratio of increasing, in samples with 8% and 12% zeolite and 4% sawdust was 3.01 and 4.81, respectively. Another point is that the lack of unconfined compressive strength of 7-day samples without sawdust, is due to the time-consuming process of zeolite hydration process.

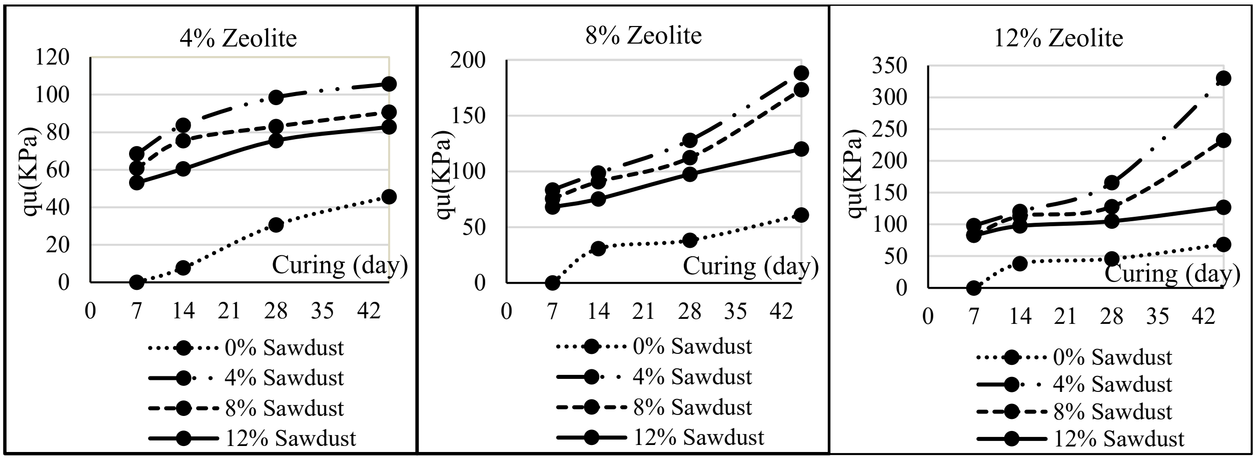

Figure 14. Uniaxial compressive strength of samples with sawdust and 4, 8 and 12% zeolite at different curing times

6.3. Result comparison between samples with and without urease enzyme

According to Figure 15, the highest compressive strength of samples with fine grained soil, sawdust, urease enzyme and 8% zeolite during 28 days of curing, compared with samples without enzyme, in 4%, 8% and 12% zeolite were 1.83, 1.41 and 1.09, respectively, Therefore, samples with 4% sawdust and enzyme, then samples with 4% sawdust showed the highest compressive strength.

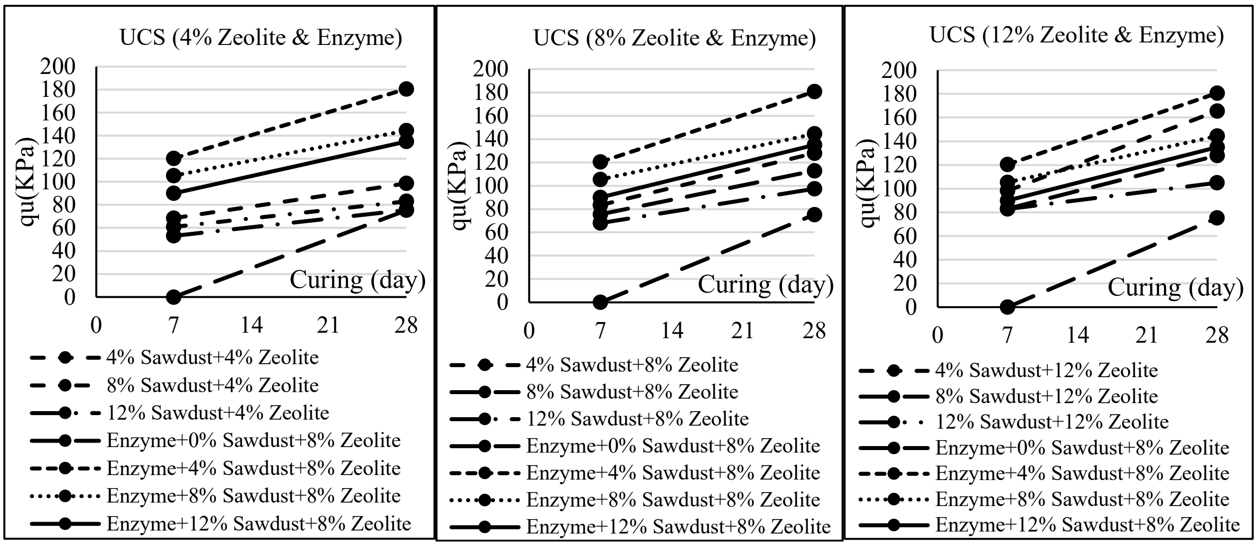

Figure 15. Comparison between the results of UCS by urease enzyme and samples with 4, 8 and 12% zeolite

7. Conclusion

This research is an initial step in biological soil improvement, to become fully implementable as a reliable, functional and economic technique, it still needs much exploration involving the resolution of the limitations. This technique has been remained under laboratory experimentation, so it needs up-scaling and life-size field experiments. by effort of microbiologists, chemists and biochemists and civil engineers this technique will finally bring this environmentally safe, energy-saving and convenient technology from laboratory to field applications.

The use of sawdust has increased the strain rupture of the samples. On the other hand, increasing the percentage of sawdust (more than 4%) has reduced the unconfined compressive strength of the soil, which may be due to the high water absorption of sawdust and absorbing large amount of adherence solution and make sawdust aggregate between soil particles that make separate particles in soil, it causes lack of adherence between soil particles. Therefore, 4% sawdust has the most compressive strength. The reason is that low amount of sawdust absorb low amount of water and cohesive solution can be distributed between soil particles and sawdust, sawdust particles like a bridge can connect soil particles to each other.

The compaction test results are shown in Figure 8 by adding 4, 8 and 12% zeolite and sawdust to the sandy soil. As can be seen, by addition of zeolite and sawdust, the optimum moisture content (OMC) has increased. The reason for this increase is that sawdust and zeolite absorbs some moisture in the soil and causes the soil to need more water to reach optimal moisture content. On the other hand, with the addition of zeolite and sawdust, the maximum dry density (MDD) has been reduced. The decrease in dry density of soil can be due to low specific gravity of zeolite and sawdust

Samples with 7-days curing time and without sawdust did not show resistance due to the time-consuming zeolite pozzolanic reactions. Urease enzyme showed the highest increase in resistance by 4% of sawdust. It also increased the unconfined compressive strength and strain rupture of the samples compared to the enzyme-free state.

In this study, zeolite has been selected as a pozzolanic mineral to increase the strength of stabilized sandy soil. Zeolite is more stable in corrosive environment than chemical materials such as cement. Although using completely natural, cheap and environmentally friendly materials can be initial step for providing new and completely environmentally friendly methods in soil improvement and stabilization. Also this research can be the first step to further reduction of chemical materials such as cement.

As a suggestion for future researches, it can be a good choice to use this method in the field that has been tested in only a few limited research projects, which indicates more studies on a real scale. Also, using of materials such as zeolite, rice husk ash or mulch ash instead of cement can be considered. On the other hand, three-axis tests, direct cutting, CBR, Brazilian tension and permeability can be investigated in future researches. It is also possible to study the possibility of plant growth on soil improved by urease enzyme or software modeling of this method and comparing with laboratory results. One thing that is important for future researches is that does solid sawdust (sawdust with cement and zeolite and calcite precipitation) have less decomposition ability like fossils.

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