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The Enhancement of Sand Properties by the Use of Plant-derived urease-induced calcium carbonate precipitation

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ABSTRACT

There is promise in the application of plant-derived urease enzymes to produce carbonate precipitation (mostly calcium carbonate (CC)). Beneficial mechanical qualities, like bearing capacity and resistance to liquefaction, can be achieved through sand cementation with the help of bio-mineral precipitation, as can the control of soil erosion through surficial stabilization and the reduction of sand's hydraulic conductivity. In this study, plant-derived urease (Chickpea) was selected as the plant source with the highest specific enzymatic activity among them (2.75 U/mg protein) when extracted from ground seeds using 0.1M sodium phosphate buffer pH 7 at a ratio of 1:10 (w/v) for 15 min. Also, the results showed that urease was stable at pH 7 and had the highest activity at 37 ^{o}C for 60 min of reaction time. A notable enhancement in strength, estimated at 1767 KPa, was detected in sand specimens treated with urea-urease in the presence of various concentrations of CaCl2-urea and urease, as well as different curing durations (measured in days). This improvement was not observed in sand specimens that were not treated. The carbonate ions, denoted as CO, that are formed during the hydrolysis of urea have a significant impact on causing the precipitation of CC. The utilization of biominerals that have minimal adverse effects on the ecology and environment is a significant factor in promoting sustainable development.

Keywords: urease enzyme, optimum conditions, compressive strength, sand improvement

1.Introduction

Due to the weak qualities of subsurface soil, terrain, and varying climatic conditions, the amount of land currently available for human activities is limited.

Using a suitable ground improvement method, such as the removal or replacement of unsuitable soils and the modification of existing ground, it is possible to improve the weak qualities of the soil.

The use of ground improvement, which entails changing the underlying soil's engineering properties to increase bearing capacity, can lessen the effects of natural disasters like the liquefaction of saturated loose sand. Cement, chemical compaction, fracture and jet grouting, micropiles, ground anchors, soil nailing, vibro compaction, stone columns, concrete columns, piers, etc. are all widely used ground enhancement techniques [Karol, 2003]. All of the aforementioned methods of soil enhancement involve the use of cement and/or chemical additives. Sodium silicate, acrylate, urethane, lignin, and resin grouts are some of the most common types of chemical grouts used today [US Army Corps of Engineers, 1995]. All of the aforementioned chemical grouts pose risks to groundwater quality. In addition, cement production results in harmful CO2 emissions; hence, the cement-based concrete system isn't eco-friendly either [Gerilla et al., 2007; Mora, 2007]. Therefore, careful preparation is required to enhance the soil's qualities in order to guarantee the least amount of damage to the environment. Traditional methods of enhancing soil quality are typically costly, require significant energy input, and have adverse environmental impacts. In recent times, the concept of biostabilization has gained prominence as a viable and sustainable alternative to address certain constraints associated with conventional soil enhancement techniques. Enzyme-induced carbonate precipitation (EICP) is a very promising technology because of its convenient applicability and compatibility with many soil types. The EICP technique utilizes the urease enzyme as a catalyst to facilitate the hydrolysis of urea inside the pore water. This process, when calcium ions are present, leads to the formation of calcium carbonate precipitation [Ahsan Saif et al., 2022].

Urease enzymes (EC 3.5.1.5) are found in plants, algae, yeasts, bacteria, and filamentous fungi. They are nickel-dependent metalloenzymes responsible for the hydrolysis of urea into ammonia and carbon dioxide. In both fungi and plants, the protein sequences that make up the ureases are highly repetitive. Bacterial ureases, on the other hand, are made up of several copies of only two or three protein subunits [Miyagawa et al., 1999]. Jack bean ureases were the first plant enzymes to be crystallized in the laboratory, and they remain the most thoroughly studied and understood of all ureases. Cajanus cajan [Pandy et al., 1991] and watermelon seeds [Mohammed et al., 1999] both contain urease, albeit for different reasons.

The urease enzyme is a crucial component of this process. Urease plays a crucial role in EICP by catalyzing the first phase of the process, the hydrolysis of urea, by increasing the rate by a factor of 1014 compared to nonenzymatic urea hydrolysis. Under favorable

conditions (e.g., temperature, alkalinity), calcium ions will interact with carbonate ions produced by urea hydrolysis and precipitate as calcium carbonate (CaCO3). Cracks and cavities can be filled, granular particles can be cemented together, and pollutants can co-precipitate with the carbonate [Hamdan et al., 2013]. and experimental conditions will be determined using the central composite design (CCD). ANOVA analysis will be employed to assess the effects of process variables on the responses. The methodology employed seeks to establish a relationship between the desired response or outcome of interest and a set of process variables using statistical techniques. Response Surface Methodology (RSM) offers numerous advantages, including the availability of efficient and straightforward experimental designs [B. Hu et al., 2018]. RSM can reduce the number of required experimental trials and address issues related to linear and nonlinear multivariate regression. The goal of this study was to use crude chickpea seed extract to increase the unconfined compressive strength (UCS) of small samples of sand. This was done through a series of experiments using the Design Expert program.

2.MATERIAL AND METHODS

2.1 Plants

The plants employed in this study were readily available from commercial sources. Namely, tomatoes (Salanum lvcopersicum), soybeans (Glycine max), hummus (Cecil arietinum), watermelon (Citrus lanatus), cotton (Gossypium barbados), pumpkin (Cucurbita pepo), cowpea (Vigna), Liebec (Albizia libback), sesame (Sesame pointer), beans,and peas (Pisum sativum) are used as a source of plant material for measuring the activity of the urease enzyme.

2.2Extraction and recovery of urease enzyme

In a mortar, 1 g of each plant was mixed with 10 mL of phosphate buffer at pH 7.0 for 15 minutes at room temperature to achieve a homogenous mixture. Centrifugation at 10,000 RPM for 15 minutes and filtration through Whatman No. 1 filter paper were used to remove all cellular debris from the slurry [Hussein, S. I., 2018]. The presence of the urease enzyme in the crude extract was determined by testing the clear supernatant that had been collected.

2.3Urease assay determination

The assessment of urease activity was performed via the modified Berthelot reaction [Babazar et al., 2017], which utilises the standard curve of NH4Cl to determine the quantity of ammonia liberated by the enzyme.It is advisable that all glassware intended for sterilisation undergo a washing procedure that involves the use of warm, diluted hydrochloric acid, followed by a comprehensive rinsing with distilled and de-ionized water.The experimental reaction mixture comprised of 1 ml of plant seed extract, 1 ml of a 500 mM urea solution prepared in a phosphate buffer (100 mM, pH 6.8), and 0.8 ml of the identical buffer. Subsequently, the combination was subjected to incubation for a duration of one hour at a temperature of 37°C within a water bath. The experiment was interrupted by the introduction of thermal energy at a temperature of 80°C for a period of 5 minutes.

An experimental test sample, commonly known as the "black sample," was generated for the purpose of conducting experiments. The plant seed extract underwent a heating step prior to its introduction into the reaction mixture. The concentration of ammonia was ascertained by the amalgamation of 1 ml of the reaction mixture with 10 ml of Berthelot reagents. The Berthelot reagents were prepared by combining 5 ml of reagent A, which contained 5 g of phenol and 0.02 g of sodium nitroprusside, with 5 ml of reagent B, which had 2.5 g of sodium hydroxide and 8.4 ml of sodium hypochlorite. Both reagents were prepared at a concentration of 0.01 M. Subsequently, the aforementioned combination was diluted with 500 ml of distilled water and subjected to incubation in a water bath maintained at a temperature of 37°C for a duration of 1 hour. The assessment of urease activity involved quantification of the increase in absorbance at a specific wavelength of 625 nm.The term "enzymatic activity unit" is used to describe the quantity of enzyme that releases one μmole of ammonia within a minute under ideal circumstances. The protein concentration was determined by Bradford's method, as described by [Bradford in 1976].

$$Ureas\text{Activity}=\frac{Ab}{Slope}\,(T\times C) \qquad \ldots(1)$$

Where:

$\frac{Ab}{Slop}$: is the concentration of ammonia,

T: is the time of reaction, 60 min.

C: is the constant, (Lyer et al.,2018).

2. 4Urease extraction under optimum condition

2.4.1 Plant sources

Ten different plant species were selected for this study. The seeds of these plants were subjected to crushing and extraction procedures using a phosphate buffer solution with a concentration of 0.02 M. The pH of the buffer solution was adjusted to 7.0. The seeds of each plant weighed one gram and were mixed with ten milliliters of the buffer solution at room temperature for fifteen minutes. Following a centrifugation process at a speed of 10,000 revolutions per minute for a duration of 15 minutes, the assessment of enzyme activity, protein concentration, and specific activity was conducted on the filter [S. I. Hussein et al., 2022].

2.4.2 Extraction buffer

Chickpea seeds were homogenized for 15 minutes at 30°C using a number of different buffers in order to extract urease. For pH 4-6, a sodium acetate buffer of 0.02 M is used; for pH 6.5, 7, and 7.5, a sodium phosphate buffer of 0.02 M is used; and for pH 8–9, a tris-based buffer of 0.02 M is used. [Hussein et al., 2021] In each test, specific activity, enzyme activity, and protein concentration were recorded.

2.4.3 Concentration of extraction buffer

Extraction was carried out in a concentration range of (0.01, 0.02, 0.05, 0.1, 0.15, and 0.2) M of sodium phosphate buffer using a mortar in order to identify the optimal concentration

of extraction buffer. Filtered using filter paper after centrifugation at 10000 rpm for 15 minutes. The protein concentration, activity of the enzymes, and specific activity of the supernatant were both measured [Hussein et al., 2021].

2.4.4 Extraction ratio

Different ratios of 0.1 M sodium phosphate buffer have been used to determine the best urease extraction ratio from chickpea seeds, including 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30 (w/v). After combining 1 g of chickpea seeds with each extraction ratio separately for 15 minutes, the optimal urease extraction ratio was found. After that, it was centrifuged for 15 minutes at 10000 rpm, then filtered by filter paper. The specific activity, enzyme activity, and protein concentration were all measured [Mohanad J. M-Ridha et al., 2023].

2.4.5 Extraction time

To establish the optimum extraction period, the urease enzyme was ground in a mortar for varying amounts of time (5, 15, 30, 60, 90, and 120 minutes), centrifuged at 10000 rpm for 15 minutes, and then filtered through filter paper. The filtrate was analyzed for its enzyme activity, protein concentration, and specific activity [Hussein et al., 2021].

2.5 Characterization of Urease

2.5.1Urease stability affected by pH

At a ratio of 1:1, an equal amount of urease enzyme was combined with buffers at varying pH (4, 5, 6, 7, 8, 9, and 10) and incubated inside a water bath at 37 °C for about 15 minutes. The samples were put in an ice bath right away, and the residual activity percentage was determined [Yi-Ywan et al., 1996].

2.5.2Urease stability affected by temperature

Urease was maintained for 15 minutes at temperatures ranging from 25 to 60 oC, then cooled in an ice bath to determine the enzyme's residual activity percent [Zainab M. D,2020].

2.5.3 Urease activity affected by temperature

Different temperature ranges were used to test urease activity, including 25, 30, 37, 40, 50, 55, and 60 ˚C for 60 min. Following the estimation of urease activity, the connection between enzyme activity and temperature degrees was plotted in order to determine the temperature at which urease activity is most effective.

2.6 Application of (EICP) Bio-mineralization Process

2.6.1Physical and Chemical Properties of the Sand

The locally available sand used in this study was obtained from Tikrit, Salah al-Din. A certain examination was done in the Civil Engineering Laboratories Department of the College of Engineering at the University of Tikrit for specifying the physical properties and oxide compositions of the sand, as shown below in Table 1.

Table (1): Physical and chemical composition of silty-sand used in this study

Sand properties	**Values**
Specific gravity	2.67
Particle size (mm)	4.30
UCS classification	Poorly graded sand
CaO (%)	1.10
pH	8.01
Al2O3 (%)	6.00
Na2O (%)	1.72
Fe2O3 (%)	1.35
SiO2 (%)	79.90

2.6.2 Compressive Strength

The most typical test for sand mortar is its compressive strength. A total of 25 cubes of silty sand were examined to examine the compressive strength in this test, which was conducted on enzyme and control samples [ASTM, 2013]. Using a five-ton machine, compression strength analyses were performed in the Consulting Engineering Bureau Laboratories (CEBL) College of Engineering, University of Baghdad (IKRATRONIX TC-B002, Turkey).

Each sample was separately placed in the compression testing apparatus and exposed to a loading rate of 100 kg/min until the sample's resistance to the cumulative load broke down, at which point no further loading was applied.

3. RESULTS AND DISCUSSION

3.1 Urease extraction under optimum conditions

In order to get the most urease out of the seeds of various plants, a number of bioprocess parameters are improved. Many factors, such as the type of plant used, the length of time it is extracted, the type of buffer used, and the extraction ratio, all play a role in the success of a urease extraction. As a result, improving these factors can lessen the overall cost of the extraction process and maximize the yield of the urease enzyme.

3.1.1Types of plants sources

Using a 0.02 M phosphate buffer and a pH of 7.0 on the seeds of ten different plant species, tests showed that chickpea seeds had the most urease extraction, followed by sesame seeds and cotton seeds, which had specific activities of 2.75, 2.28, and 2.19 U/mg, respectively (see Fig. 1). There may be different levels of urease in plant-based foods because of

differences in genetics, plant type or source, and environmental conditions (like temperature, pH, and soil type) during growth. [Cassone et al., 1987]. Enzyme activity of 33.3 U/ml was achieved by extracting urease from Vicia faba seeds [Bedan, D.S., 2020], whereas enzyme activity of 190 U/g was achieved by extracting urease from germinating Pisum sativum L. seeds by precipitating the enzyme using acetone. [El-Hefnawy et al., 2014].

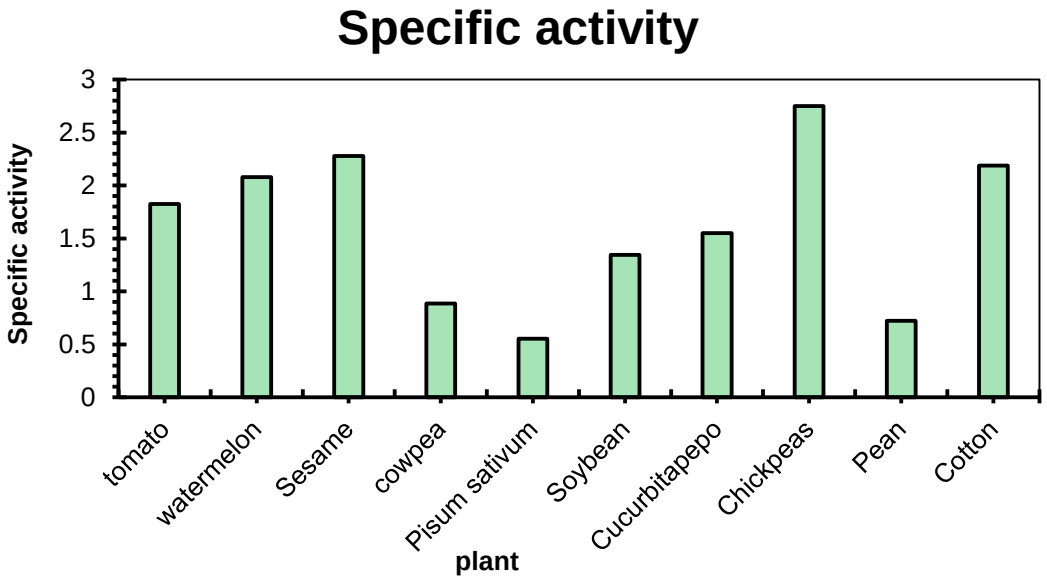


Figure 1. Effect plant sources on urease extraction using chickpeas, 0.02 M phosphate buffer pH 7.0 for 15 min at 30°C.

3.1.2 Type of extraction buffer

Following the extraction of urease using different buffers, the specific activity of the enzyme was determined and the findings are illustrated in Figure 2. The extraction buffer that had the highest efficacy was sodium phosphate (0.02 M, pH 7), exhibiting a specific activity of 3.18 units per milligramme of protein. The inclusion of an appropriate buffer solution in the protein extraction procedure can enhance the stability of protein molecules when exposed to different forces aimed at isolating and analysing them. Buffer solutions are capable of maintaining the structural integrity of proteins by effectively separating them from other biological components. The pH of enzymatic extraction is influenced by the alteration of protein structure in an enzyme molecule due to the modification of ionisation state of certain amino acid residues caused by changes in solution alkalinity and

acidity. This phenomenon occurs due to alterations in the charge state of the solute. According to Mizobutsi et al. [2010], when the pH of a solution matches the net electric charge of a certain molecule, the solute undergoes precipitation and exhibits limited solubility. Several research have utilised different buffers and pH levels to extract urease from diverse sources. In a recent investigation conducted by Hussein et al. [2021], it was shown that the sodium acetate buffer, specifically at a concentration of 0.2M and a pH of 5.0, exhibited the highest efficacy as an extraction buffer for urease derived from chickpea. The urease extracted using this buffer demonstrated a specific activity of 1460 U/mg protein.

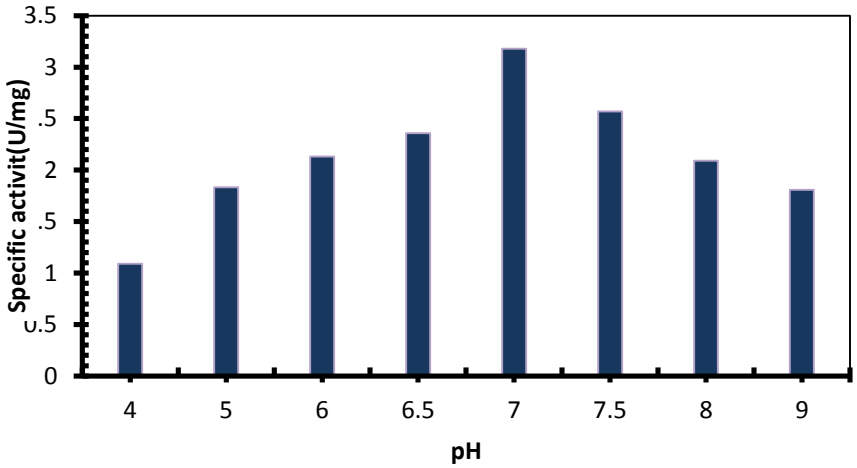


Figure2. Urease extraction affected by types of buffers using chickpeas, phosphate buffer 0.02 M pH 7.0 for 15 min at 30°C

3.1.3 Concentration of sodium phosphate buffer

Testing six different concentrations of sodium phosphate at pH 7 (0.01, 0.02, 0.05, 0.1, 0.15, and 0.2 M) helped find the best buffer concentration for getting urease out of chickpea seeds. Show that the data in Figure 3 supports the hypothesis. For crude extract, the 0.1 M concentration yielded the highest specific activity, at 3.5 U/mg protein, while the 0.2 M concentration yielded the lowest, at 1.66 U/mg protein. Also, the specific activity was low, peaking at 3.25 U/mg of protein at doses of 0.15M, 3.18 U/mg of protein at 0.01M, and 2.25 U/mg of protein at 0.05 M. It has been reported that using a lot of buffers in the extraction can hinder urease activity. This is likely due to the number of ionic groups in the buffer, which can cause activation problems [Yahya et al., 2021]. Urease extraction from Pisum sativum was shown to be most effective at a pH of 7.0 and a sodium phosphate buffer concentration of 0.125 M [Zusfahair et al., 2018].

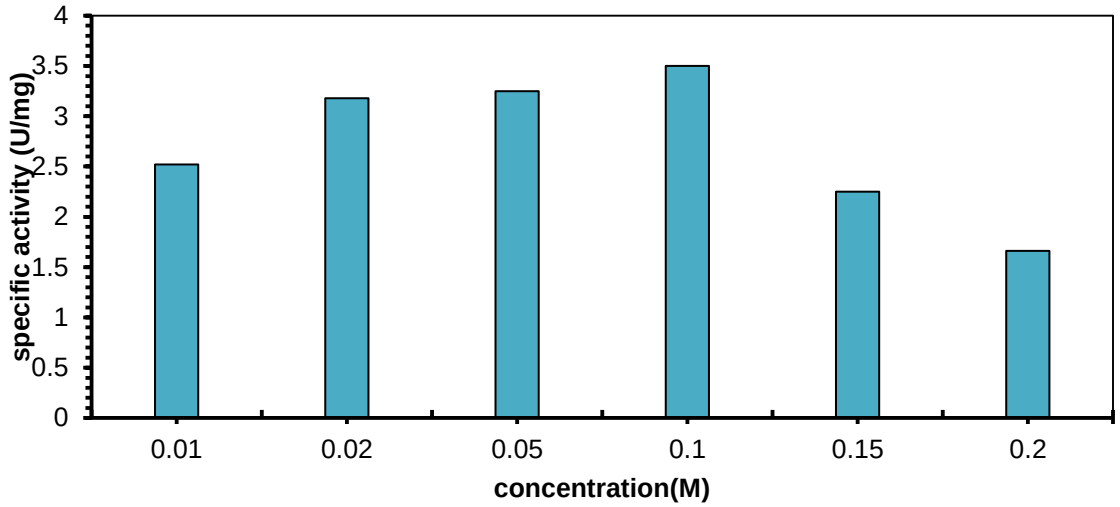


Figure 3. Urease extraction from chickpeas seeds affected by concentration of Sodium phosphate buffer PH7(0.1M) for 15 min, (1:10) at 30C.

3.1.4 Extraction ratio

The extraction ratios of 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30 (w/v) were chosen to find the best urease extraction ratio using sodium phosphate (0.02 M, pH 7). At a ratio of 1:10, crude extract showed the highest specific activity, at 4.04 U/mg protein. Specific activities of 2.31, 3.12, 2.83, 2.54, and 2.12 U/mg protein were obtained from other ratios. The optimal extraction ratio depends on the source and amount of enzyme. If you increase the extraction solution, the enzyme's activity may drop because it takes longer for compounds to form [Cordova et al., 2022]. The optimal ratio for urease extraction from chickpeas using 0.2 M sodium phosphate buffer and the highest specific activity were at a 1:8 ratio, amounting to 1.99 U/mg of protein, according to multiple studies that used buffer solutions with different extraction ratios [Hussein et al., 2021].

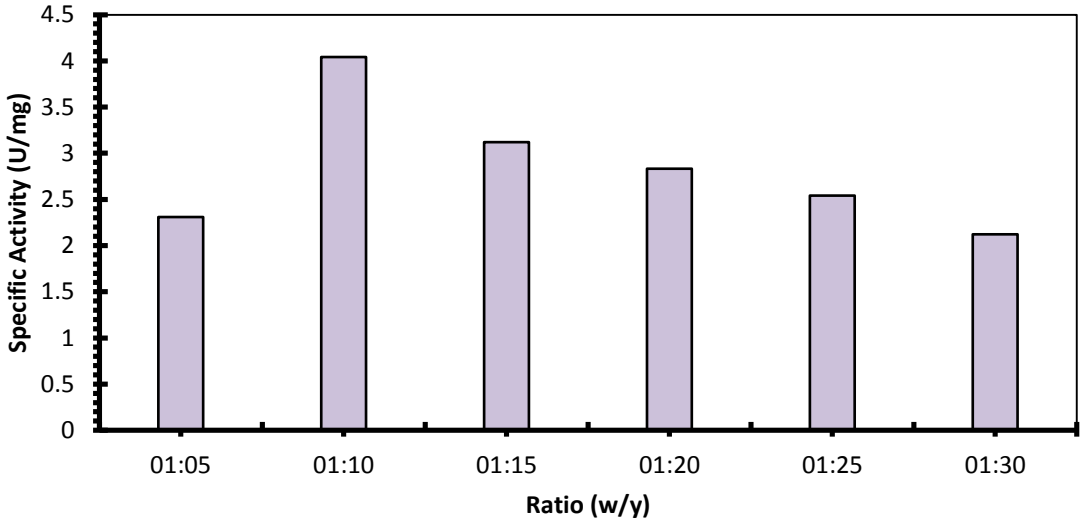


Figure 4. Urease extraction from chickpeas seeds affected by extraction ratio (1:10) using 0.1 M of sodium phosphate buffer Ph7 for 15 min at 30 °C.

3.1.5 Extraction Time

The optimal urease extraction time was determined using sodium phosphate extraction (0.02 M, pH 7) and six extraction periods (5, 15, 30, 60, 90, and 120 minutes). After 15 minutes, crude extract had the highest specific activity of 4.09 U/mg protein, whereas after 5, 30, 60, 90, and 120 minutes, the specific activity decreased to 2.17, 2.82, 2.97, 3.15, and 3.46 U/mg protein, respectively (Figure 5).

The determination of an optimal duration for urease extraction is contingent upon the specific source material and its impact on the enzyme's stability. Consequently, a benchmark was established by Mohanad J. M. Ridha et al. (2023) to obtain a protein extract that exhibits notable resistance to degradation. According to Hussein et al. (2021), the crude urease extract had its highest specific activity of 1.99 U/mg protein after a duration of 15 minutes.

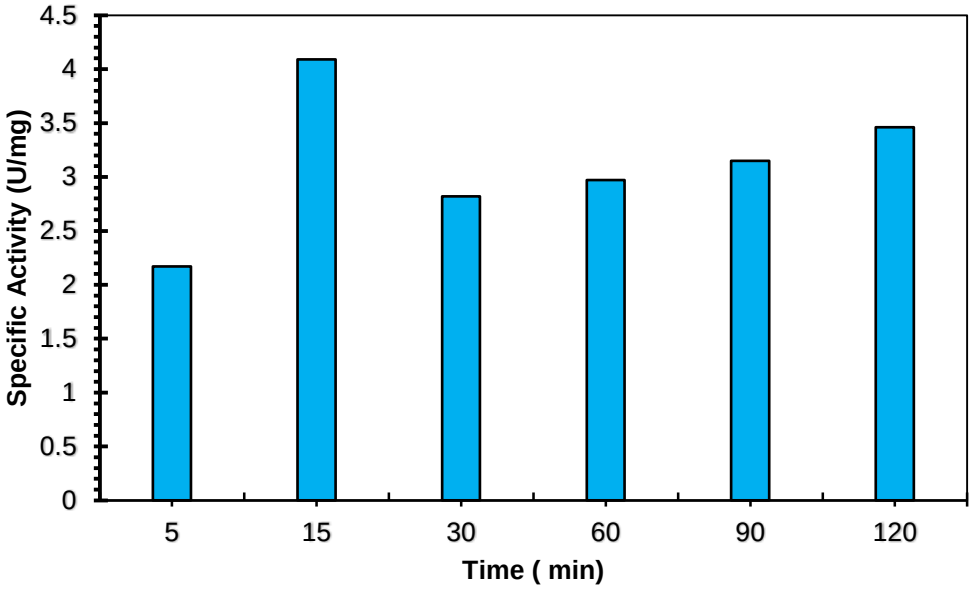


Figure 5. Urease extraction from chickpeas seeds affected by extraction time at 30°C using 0.1 M of sodium phosphate buffer PH7,15min.

3.1.6 Urease stability affected by pH

According to the results shown in Fig. 6, the enzyme preserved a significant portion of its activity at a pH value of 7 and around 66, 71, 87, and 83% at pH 4.0, 5.0, 6.0, and 8.0, respectively. Also, the residual activities at pH 9.0 and 10 were 74.4 and 62%, respectively, as the activity dropped away from the optimal pH values. Enzyme activity was poor at an acidic pH. According to the findings [Mazzei et al., 2019], the urease enzyme may be more stable at pH values near neutral. In general, enzyme activity decreases in buffer solutions

with pH values that are outside of their optimal range because of the effect of pH stability on enzyme structure, which causes denaturing of the enzyme molecule or changes in the ionic state of the enzyme active site [Jasim, 2019]. Furthermore, most enzymes are irreversibly denaturated by solutions that are sufficiently acidic or basic, and the active site's efficacy in forming the enzyme substrate complex is also influenced by the surrounding pH value. Enzyme and substrate activity are affected by changes in pH because of the resulting ionization fluctuations. This improves the efficiency of the product-forming enzyme-substrate interaction during urea breakdown [Whitaker et al., 1972].

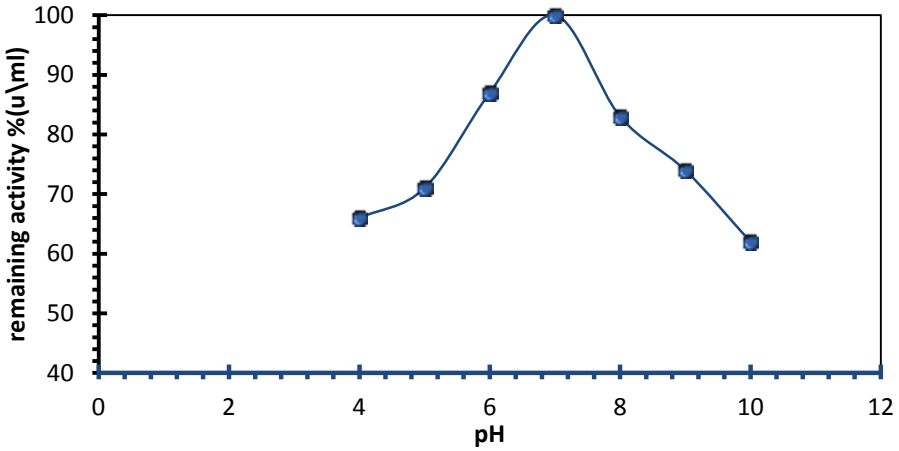


Figure 6. Urease remaining activity changes with different pH values.

3.1.7 Urease activity affected by temp

Urease activity was measured for 60 minutes at various temperatures of 25, 30, 37, 40, 50, and 60°C. Urease activity increased at 30°C, reaching 3.08 U/ml, then decreased depending on the increasing temperature of about 30 °C, reaching a minimum of 1.690 U/ml at 60 °C, as shown in Fig. 7. also, below 30°C, it was reduced too much. The enzymatic reactions have been affected by the temperature in a number of different ways, including enzyme-substrate affinity, pH, and system ionization [Whitaker et al., 1972]. The study indicates that reaction speed increased until it reached 37°C, while above 40°C it began to deteriorate. Even though enzymatic activity decreases at temperatures above 40°C due to changes in the active sites and denatured protein structure, increasing the collision between the enzymatic molecules able to share the reaction with the substrate may be responsible for increasing the molecules' movement energy [Krajewska, 2009]. As the temperature rises, the pace of the reaction accelerates because the molecules' kinetic energy increases. When the heat was turned up even further, the energy barrier between enzyme molecules was overcome. The two hydrophobic hydrogen bonds that maintain the enzyme's three-dimensional structure are broken as a result [Daniel et al., 2008]. It has been determined that the optimal temperature for obtaining optimum urease activity from Pisum sativum L. seeds is 40°C [EL-Hefnawy et al., 2014].

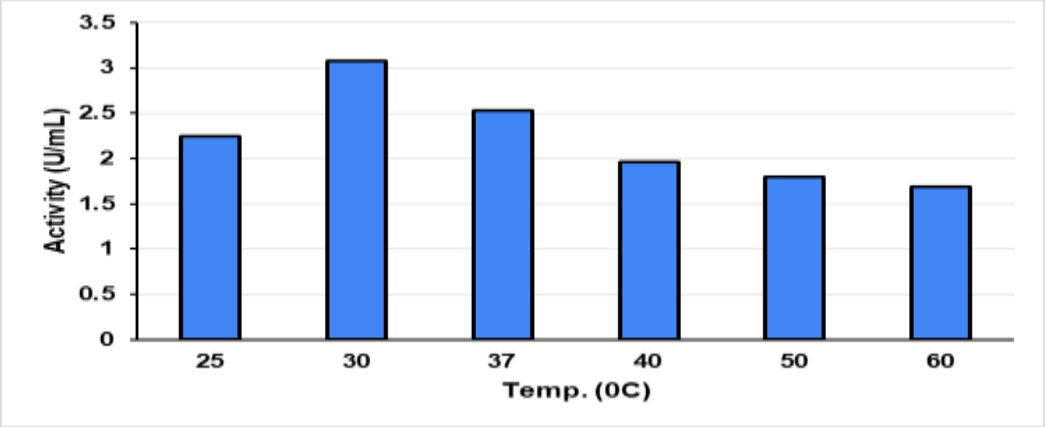


Figure 7. Effect different temperature values on urease activity.

3.1.8 Stability of urease at various temperatures

The stability of urease at various temperatures has been assessed by incubating the enzyme at several temperatures ranging from 25 to 60°C and then measuring residual activity after 15 minutes at 37 °C. According to the results in Fig. 8, the enzyme maintained its activity at temperatures ranging from 25 to 37°C, after which the activity began to decrease with temperature increase. A significant drop in stability resulted in lower temperatures. In addition, the enzyme preserved 74% of its initial activity at temperatures of 60°C, while the remaining urease activity was 73.6% at 25°C. Temperatures up to or below the optimal temperature for any enzymatic activity will limit the reaction rate dramatically. Enzymes derive their catalytic activity from a precise and highly ordered tertiary structure. The enzyme's tertiary structure is primarily maintained by a significant number of weak noncovalent bonds. That can be affected by the temperature. A high temperature will enhance the collision between the enzyme and substrate, but this will be offset by denaturation [Segal, 1977]. The stability of the urease enzyme isolated from pisum sativum seeds was shown to be highest at 60°C [Lyer et al., 2018].

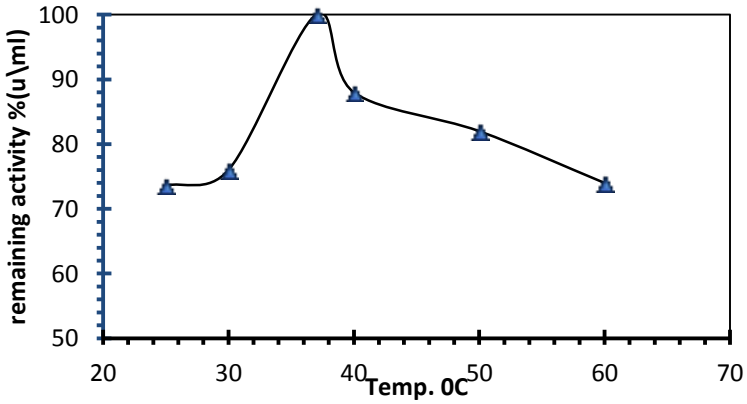


Figure 8. Effect different temperature values on urease chickpeas, sodium phosphate PH7, (0.1M),(1:10), (15 min)

3.2Application of (EICP)

3.2.1 Sand Preparation

Locally available sand with a particle size less than 4.75 mm and a specific gravity of 2.69 was used in the present work and was sieved with respect to the USCS grain size distribution method. The soil test results were used to generate the curve shown in Fig. 9. The particle size distribution of the sand utilized in the subsequent experiment was examined, revealing a D50 value of 0.58mm. The size of the sand grains utilized in the subsequent experimental experiments was less than 1 mm.

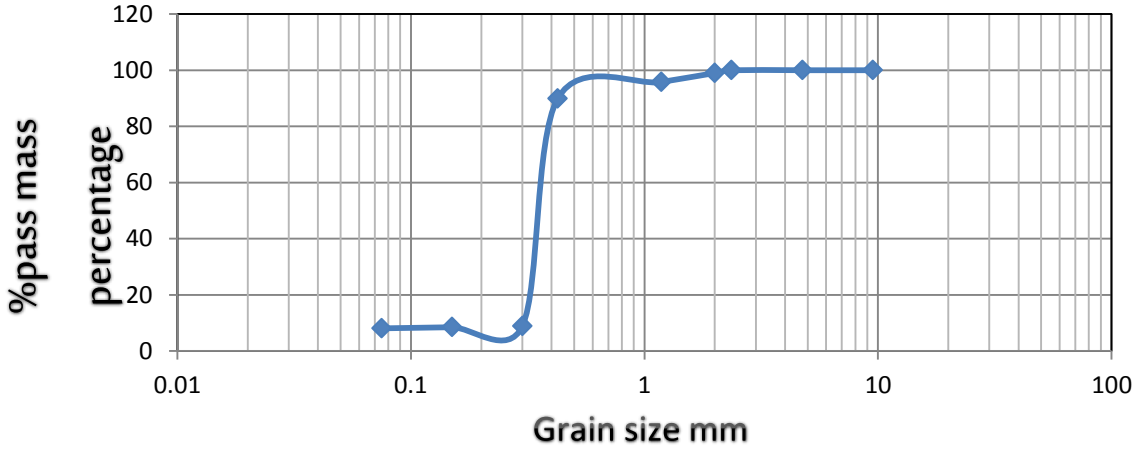


Figure (9): Grain size distribution of the sand used in experiment.

3.2.2 Design expert modeling –RSM-CCD

The experimental conditions are summarized along with the results obtained from 25 experiments according to the design of theresponse surface methodology (RSM) in Table2. As shown in Table 2.

Table (2): Design Experiment Program to obtain maximum compression strength for silty sand

			Factor 1	Factor 1	Factor 1	Response 1
Std	**Group**	**Run**	**A: CaCl2-Urea**	**B: Enzyme**	**C: Time**	**UCS**
Unit			**M**	**ml**	**day**	**KPa**
21	1	1	0.75	30	10.5	581
20	1	2	0.75	30	10.5	667

22	1	3	0.75	30	10.5	712
10	2	4	0.75	30	10.5	639
9	2	5	0.75	30	10.5	683
11	2	6	0.75	30	10.5	592
24	3	7	0.75	30	10.5	648
23	3	8	0.75	30	10.5	702
25	3	9	0.75	30	10.5	613
2	4	10	0.46	35.77	8.47	359
1	4	11	0.46	24.22	8.47	347
4	4	12	0.46	35.77	12.52	487
3	4	13	0.46	24.22	12.52	434
14	5	14	1.25	30	10.5	1633
15	5	15	1.25	30	10.5	1767
5	6	16	1.03	24.22	8.47	989
8	6	17	1.03	35.77	12.52	1328
6	6	18	1.03	35.77	8.47	1192
7	6	19	1.03	24.22	12.52	1086
16	7	20	0.75	20	10.5	743
18	7	21	0.75	30	7	671
17	7	22	0.75	40	10.5	817
19	7	23	0.75	30	14	896
12	8	24	0.25	30	10.5	207

13	8	25	0.25	30	10.5	138

3.2.3analysis of (ANOVA)

The analysis of variance (ANOVA) results for the three variables are presented in Table 3. The data were well-fitted using the selected regression models, as indicated by the high R2 value of 0.9815, to get maximum comprehension strength for silty sand. Moreover, the adjusted R2 (R2ad) value of 0.9704 and the expected R2 value of 0.9209 demonstrate a close alignment, with a deviation of less than 0.2. The AP value of 32.99 demonstrates a strong signal-to-noise ratio, implying that the used models possess statistical significance and effectively capture the experimental results [Mohammed-Ridha et al., 2021]. Additionally, the F-values for lack-of-fit were found to be (2.88, 0.0727) in Table 3, implying a lack of significance, further supporting the significance and precision of the quadratic model. The low coefficient of variation (CV%) of 9.05% indicates that the model is both reliable and accurate in predicting experimental outcomes [Ridha et al., 2020]. It also highlights the significance of selecting appropriate factor levels and closely approaching the average efficiency of the theoretical system [Ridha et al., 2020]. Figure 10a depicts the interactive effects of enzyme and CaCl2-Urea, with values of 30–35 ml and 1.05–1.25 M, respectively, to get maximum compression strength for silty sand; this reached 1750 KPa. The interactions between experimental times of 12–14 days and enzymes of 30–35 ml seen in Figure 10b show that the maximum compression strength for silty sand is 1000 KPa. The results showed that the UCS for sand by the urease enzyme is 500–4000 KPa after 14 days [Dilrukshi et al., 2016].

Figure 10c shows the 3D response surface and contours of how CaCl2-Urea and time affect the compression strength of silty sand. These are the two most important factors. Figure 10c shows that the compression strength increased with increasing CaCl2-Urea. During the biocementation of sand and silty sand by the urease enzyme, one of the most important things that affects the growth of calcium carbonate crystals is the amount of cementation reagent. Muhammed et al. [2021] found the UCS for sand is 161–552 KPa by urease enzyme after 21 days, and the range of UCS for soil by urease enzyme reached 1200–2500 KPa after 14 days [Al-Imran et al., 2021]. Figure 11a clearly demonstrates the observable link between the forecast obtained from the RSM model and the empirical data gathered, thereby indicating the model's precision. The optimization tool yielded the optimal compression strength (UCS) of silty sand in Figure 11b as 1837.52 KPa. This result was produced using 1.24 M CaCl2-Urea, a 31.42 ml enzyme dosage, and a 13.74-day extraction period.

Table 3. ANOVA results for response surface of quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.73	9	4.15	88.38	< 0.0001	significant
A-CaCl2-Urea	3.41	1	3.41	725.87	< 0.0001	
B-Enzyme	29089.98	1	29089.98	6.19	0.0251	

C-Time	50126.41	1	50126.41	10.67	0.0052	
AB	18050.12	1	18050.12	3.84	0.0689	
AC	40.51	1	40.51	0.008	0.9273	
BC	799.98	1	799.98	0.17	0.6857	
A^2	2.18	1	2.18	46.40	< 0.0001	
B^2	18366.87	1	18366.87	3.91	0.0667	
C^2	19725.02	1	19725.02	4.20	0.0584	
Residual	70484.98	15	4699.00			
Lack of Fit	41600.26	5	8320.05	2.88	0.0727	not significant
Pure Error	28884.72	10	2888.47			
Cor Total	3.80	24				

R^2 = 0.9815, $R^2_{adjusted}$ = 0.9707, $R_{predicted}$ =0.9209 ,AP = 32.99, CV = 9.05%

3.2.4 Final Equation in Terms of Actual Factors for Sand

UCS (KPa) = + 2343.85 -1085.93X1 -80.31X2 -170.23X3

+28.49X1X2+3.85X1X3 +0.85 X2 X3 +1080.66 $X1^2$ +0.96 $X2^2$ +8.15 $X3^2$ … … … (2)

Where:

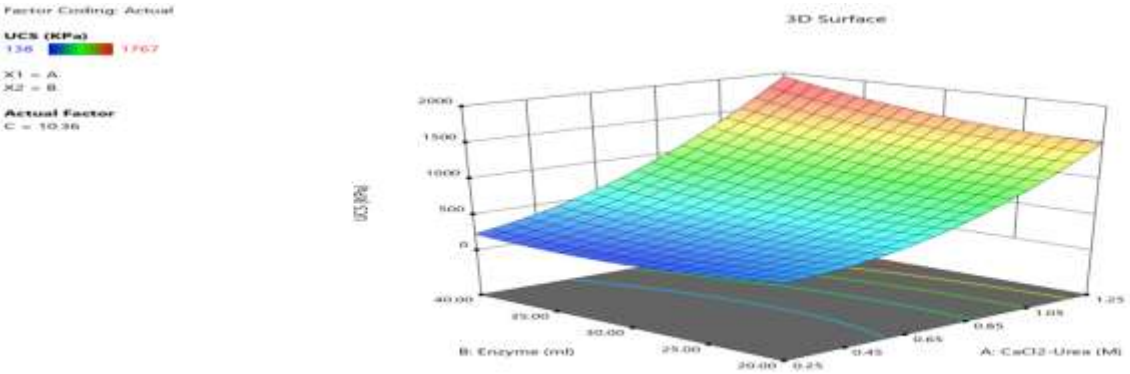
X1: is the concentration of (CaCl2-Urea) M

X2: is the volume of enzyme (ml)

X3: is the time of period (day)

By utilizing the equation expressed in terms of the real components, it is possible to forecast the corresponding outcome for certain quantities of each constituent. In this context, it is necessary to represent the levels of each component in their respective original units.

(a)



(b)

(c)

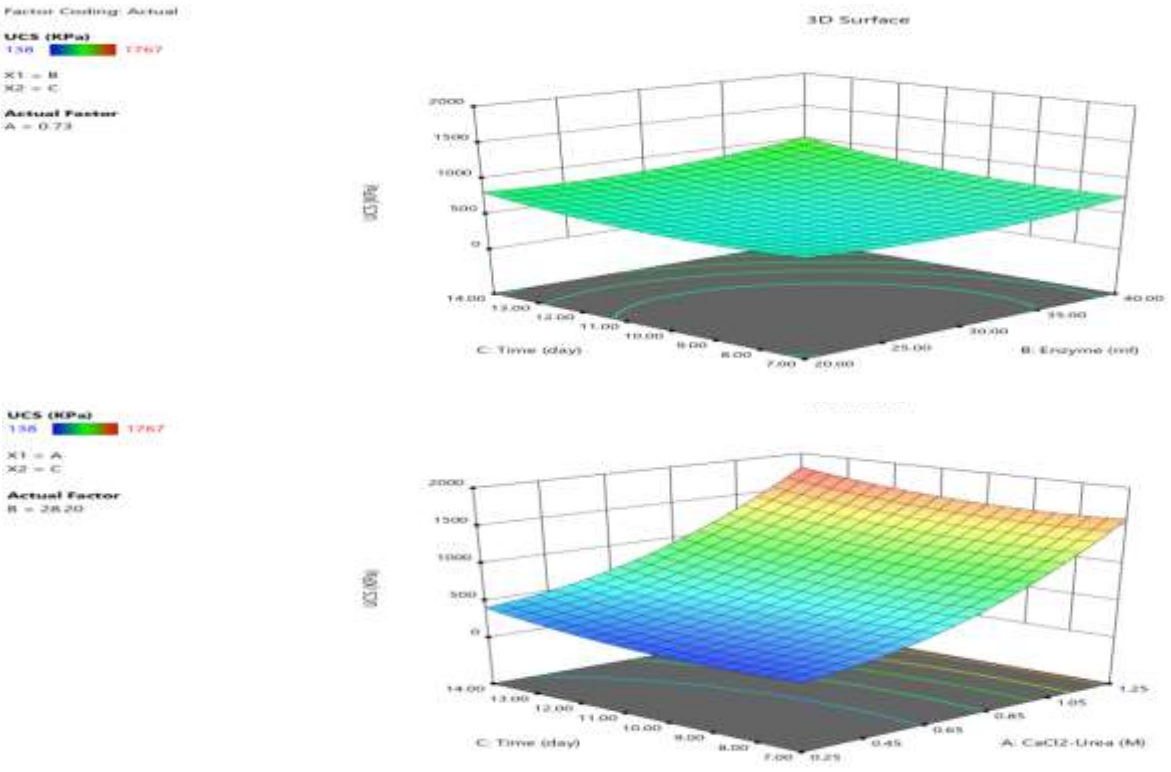
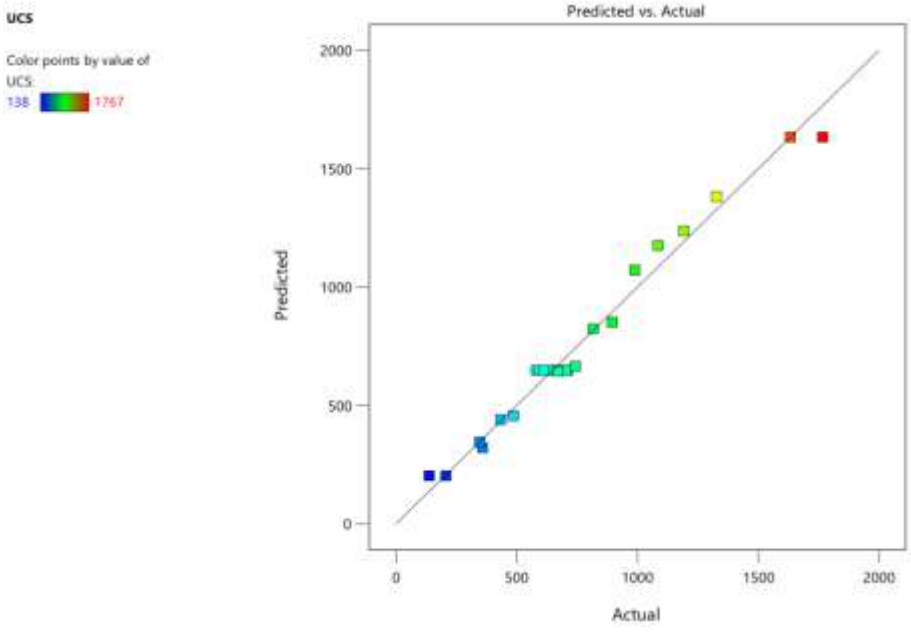
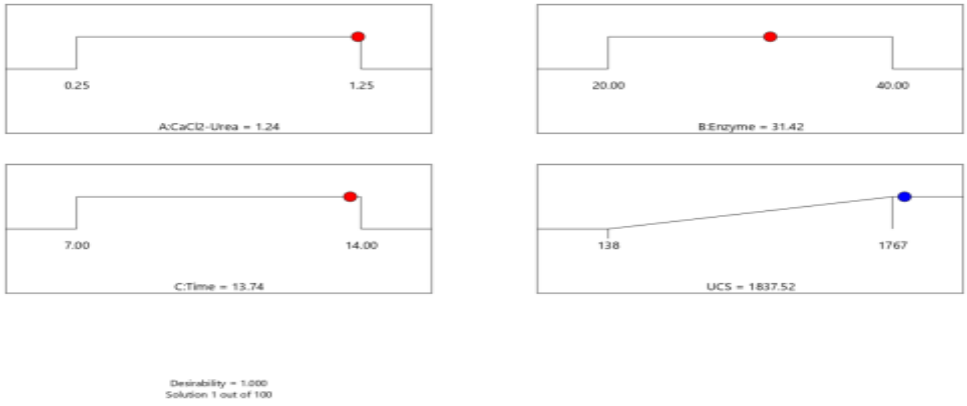


Fig. 10. 3D surface plots for multiple interactive effects on compression Strength for Silty Sand.



(a)



(b)

Fig. 11. (a) Actual experimental data versus predicted data; (b) optimization results judged by the desirability function.

4.Conclusion

The objective of this study was to determine the optimal conditions for the extraction of urease from indigenous plant species. Furthermore, it will be employed to evaluate the participation of the enzyme in certain applications. The improvement of sand properties. The present work utilized a crude extract derived from crushed chikpea seeds. Small-scale test specimens were created using commercially available silty sand and treated with the extract. The extension of the curing duration led to an increase in the estimated unconfined compressive strength (UCS) measurement. It was found that a rise in the CaCl2-urea concentration was directly linked to a rise in the calculated value of unconfined compressive strength (UCS). The possible uses of this technique encompass a range of areas, including the improvement of stability in weak, unconsolidated soil. One specific application involves addressing the issue of liquefaction in saturated, loose sand with the aim of reducing its potential impact.

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