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Screening and optimization of urase production from various plants

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Abstract

In the present study, we explore plant derived ureases for EICP application. The urease activities of seventeen plant species were assayed, comprising flax seed, lentil, and mung bean. The highest specific activity was found with flaxseed (1.64 U/mg) followed by lentil and mung bean. The optimal conditions of urease extraction were investigated via a series of systematic experiments based on buffer species, extraction ratios and time. The enzyme activity was monitored spectrophotometrically by measuring released ammonia with Nessler's reagent, the latter forms the chromogenic complex. In addition, heavy metals removal potential offered by EICP was investigated as carbonate precipitation has the ability to sequester heavy metals in contaminated environments such as soil and water. This unique bi-function: structure restoration and for environmental treatment highlights that EICP is a green technology. Furthermore, the utilization of urease generated from plants gets beyond the drawbacks of bacterial systems, such as limited enzyme penetration in microcracks and biosafety issues. This study provides a scalable and effective method for maintaining infrastructure by advancing the development of environmentally benign, enzyme-based self-healing systems for concrete. The research emphasizes how indigenous plant materials may be used to produce high-activity urease and optimize extraction techniques to facilitate the broad use of EICP in environmental and civil engineering applications.

KeywordsUrease enzyme, Plant-derived urease, Heavy metals removal, Urea hydrolysis, green technology

Abbreviations

Abbreviation	Meaning
EICP	Enzyme Induced Carbonate Precipitation
MICP	Microbially Induced Carbonate Precipitation
PDUE	Plant-Derived Urease Enzyme
U/mg	Units per milligram (specific enzyme activity)
rpm	Revolutions Per Minute (centrifuge speed)
µM	Micromolar (concentration unit)
pH	Potential of Hydrogen (acidity/alkalinity scale)

1.IntroductionThe longevity and durability of buildings are negatively impacted by weathering, earthquakes, and man-made activities, all of which may cause natural phenomena that result in concrete cracks. Concrete's brittleness and decreased tensile strength were the main causes of the fracture [1]. those related to chemical changes brought about by chemical interactions between the internal components of concrete and external agents, or between internal components themselves with the aid of moisture from the external environment [2]. These fissures may now be repaired using chemicals, grout, or surface treatments, all of which have the potential to be hazardous to both the environment and end users. eco-friendly, sustainable, and creative method an approach to patching the fissures is Enzyme Induce Carbonate Precipitation (EICP), which is a fresh topic of research [3]. Concrete fractures may be sealed using a variety of techniques, including the use of silicone-based polymer materials, epoxy-based polymers, and acrylic resins [4]. In addition to crack sealing, other researchers have thoroughly examined the techniques for mending cracks. Concrete is reported to exhibit some autogenous healing by the hydration of anhydrate cement in a humid environment, but this process is very slow and only works on very small fractures. When water and CO_2 can enter the fracture, the carbonation of leached calcium hydroxide may cause healing. As a result, concrete structure maintenance and inspection have drawn significant attention. At the same time, conventional maintenance and repair techniques are costly and complicated. The potential of concrete fractures to mend themselves in such situations sparked a lot of attention. A microencapsulation technique uses a healing agent in a capsule that is simply shattered when a fracture impinges and polymerizes to close the crack. This is one of the other possible self-healing processes that have been reported by [5]. This has been shown by a number of studies for concretes and other composites. Recently, bio-mediated self-healing of concrete has been extensively investigated. In general, alkaline-resistant spore-forming bacteria and a substrate that are introduced to concrete during the mixing process are the most crucial components of a bacteria-based self-healing system. In the event of a fracture, water and oxygen may awaken the latent spores; active bacteria metabolize the organic substrate, such as urea [6]. Numerous findings claim that microbial species, which are mostly found in natural settings, may produce calcium carbonate precipitate in both controlled and natural settings. Among other abiotic factors, a variety of bacterial species seem to be involved, providing various ways for $CaCO_3$ to precipitate in various settings. Calcium carbonate ($CaCO_3$) and other mineral carbonates are regarded as reactive minerals on Earth. This is due to the fact that they often take part in precipitation and dissolution processes, making them active participants in the planet's carbon cycle. According to reports, the primary cause of concrete's ability to mend itself is calcium carbonate precipitation. thousands of researchers [7]. Showed that some kinds of bacteria could induce or improve the precipitation of CaCO3. This phenomenon was called bio-mineralization, it existed widely in nature. Since the last decade [6] published pioneer papersin1998, biomineralization has been developed to treat concrete cracks. The fundamental principle underlying MICP is that, in the presence of calcium ions, microbial-produced urease enzymatically hydrolyzes urea to cause precipitation of calcium carbonate. Bio-mineralization has been found very effective in the presence of ureaCaCl2 [8]. This process involves the use of plant-derived urease enzymes for catalyzation of the required reaction to induce carbonate precipitation in mortars' cracks [9]. EICP triggers the instantaneous formation of precipitates in calcium acetate and sodium carbonate solution through a mineralization process but also through a cementation process inside the matrix of the crack itself, making the treatment viable to concretes with various uses, from the most structural to the most architectural [10]. Even though MICP is based on the involvement of microbes for the production of the urease enzyme, this research used directly a plant-derived urease enzyme in order to catalyze the reactions required and hence lead to precipitation of carbonates within cracks in mortars, without involving bacteria. Advantages of EICP over MICP include the following: (1) higher efficiency, since it does not consume or compete for urea; (2) easier application in situ for crack control since the enzyme is free and soluble in water; (3) penetration to minor cracks not allowed by the microbiologically generated enzyme due to its much smaller size in the solubilized state; and (4) carbonates produced and enzyme degradation occur rapidly, hence no long-term effects on concrete. The rapid precipitation of carbonate in the EICP makes the process relevant and therefore attractive to management agencies for the crack surface treatment of concrete infrastructures. When developed correctly, encapsulated EICP solutions can be used as self-healing material in concrete. Several techniques were used in removing these heavy metals, of which one of the most conventional techniques is by using chemical precipitation as reported by Gunatilake (2015). The process relies mainly on transforming these heavy metals into the insoluble form of hydroxides, phosphates, carbonates, and sulfides, all reported by Huisman et al. (2006) [11] and Chen et al. (2012) [12]. Although the chemical precipitation method is effective for removing heavy metals at high concentrations, it is quite cost-intensive due to its requirement for large amounts of chemicals. The second disadvantage of this method is its high cost in discarding the produced

sludge. As a result, the total operating cost for this technology is found to be very high. Another advantage of the application of EICP is its selectivity for the separation of heavy metals in different environmental matrices, including soil and water[13]–[15]. One major issue that has been raised is the removal of heavy metals from contaminated sites due to their negative effects on human health, plant life, and the environment [16]. For this purpose, PDUE was employed to remedy of the biocarbonation mechanisms enhanced, including urea hydrolysis and heavy metal precipitation. The time necessary for PDUE facilitated the removal of heavy metals and further discussed by comparing the time requirements of bacterial bioremoval processes reported in the literature data as follows: [17], [18]

2. MATERIAL AND METHODSPlants: The plants used throughout this study were locally available in the market. Namely, Lentil (Lens culinaris), Eggplant (Solanum melongena), Cowpea (Vigna unguiculata), Rapeseed (Brassica napus), Onion (Allium cepa), Acacia (Acacia nilotica), Lima bean (Phaseolus lunatus), Flaxseed (Linum usitatissimum), Jujube (Male) (Ziziphus jujuba), Jujube (Female) (Ziziphus jujuba), Indian gooseberry (Phyllanthus emblica), Sweet potato (Ipomoea batatas), Turnip (Brassica rapa), Broad bean (Vicia faba), Mung bean (Vigna radiata), Cantaloupe (Cucumis melo), and Kidney bean (Phaseolus vulgaris (var.)) were used as a source of material to screen for urease enzyme activity**.**

2.1 Extraction and recovery of urease enzymeA mortar was used to combine 1.1 grams of each plant individually with 11 milliliters of 0.02 M phosphate buffer pH 7.1 for 15 minutes at 25 degrees Celsius. To get rid of any remaining cell debris in the setup, the slurry was centrifuged for 14 minutes at 10500 rpm and then filtered through filter paper. The basic extract was indicated by the clear supernatant that was collected and tested for the urease enzyme.

2.2 Estimation of the standard curve of NH_4Cl

Serial doses (100–500 µM) were made in triplicate from a stock solution of NH4Cl (0.5 mM) to create the NH4Cl standard curve for the urease test. The NH4Cl standard curve was drawn between the ammonium chloride concentrations (µM) and the standard ammonium chloride absorption at 625 nm, as seen in Figure 1.

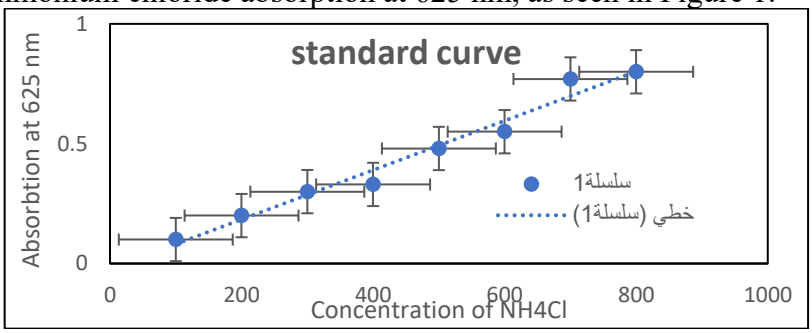

Figure 1 Ammonium chloride standard curve

2.3 Determination urease Assay Ammonia and carbon dioxide are produced when urea is hydrolyzed by urease. When the released ammonia combines with Nessler's reagent, a yellow to reddish-brown complex is created. All sterilized glassware should be thoroughly rinsed with distilled and de-ionized water after being cleaned with warm, diluted hydrochloric acid. 1 milliliter of plant seed extract, 1.1 milliliter of 100 mM urea made in 100 mM, pH 7 phosphate buffer, and 0.55 milliliters of the same buffer made up the test reaction combination. The combination of was incubated for an hour at 37°C in a water bath for 30 minutes. To render the enzyme inactive, immediately bring the process to a boil for three to five minutes. Allow the sample to cool down. To the reaction combination, add 1.1 milliliter of Nessler's reagent.$Urease\ Activity\ (U/ml) = \frac{\mu mol\ NH_3\ liberated}{Time(min.)}$ … (1)

2.4 Type of plant material Lentil, Eggplant, Cowpea, Rapeseed, Onion, Acacia, Lima bean, Flaxseed, Jujube (Male), Jujube (Female), Indian gooseberry, sweet potato, Turnip, Broad bean, Mung bean, Cantaloupe, and Kidney bean. Each plant material (1 gram) was homogenized with 10 ml of 0.02 M phosphate buffer (pH 7.1) using a mortar and pestle for 15 minutes at room temperature. The homogenate was centrifuged at 10,100 rpm for 15 minutes, and the supernatant was filtered using Whatman No. 1 filter paper. The resulting filtrate (crude extract) was used to determine the urease enzyme activity, protein concentration, and specific activity.**2.5 Type of extraction buffer**Mung bean seeds homogenized with different types of buffers for 15 min at 30oC for urease extraction. These buffers are 0.02 M sodium acetate buffer (pH 4, 5 and 6), 0.02 M sodium phosphate buffer (pH 6.5, 7.1 and 7.5) and 0.02 M Tris-based buffer (pH 8, 8.5 and 9). The enzyme activity, protein concentration, and specific activity were assayed in each experiment (13). After homogenization, the mixtures were centrifuged at 10,100 rpm for 14 minutes and filtered through Whatman No. 1 filter paper. The supernatant was analyzed to determine the enzyme activity, protein concentration, and specific activity [19].

2.6 Extraction ratio Different plant matter to buffer (w:v) ratios were examined in order to identify the ideal extract ratio for urease enzyme separation from flaxseed (Linum usitatissimum). Various amounts of 0.02 M phosphate buffer (pH 7.1) were combined with precisely 1 gram of flaxseed seeds to produce the following extraction ratios: 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30 (w:v) After fifteen minutes at room temperature, each mixture was homogenized using a mortar and pestle. Next, it was centrifuged for 14 minutes at 10,100 rpm. We used Whatman No. 1 filter paper to filter the clear supernatant.

2.7 Extraction time To find the ideal extraction duration for the urease enzyme, the purification process was carried out by mortar for 5, 10, 30, 60, 90, and 120 minutes, followed by centrifugation at 10100 rpm for 14 minutes and filtering through filter paper. Protein content, specific activity, and enzyme activity were measured in the filtration.

3.Optimum conditions for urease extractionFor optimal enzyme synthesis, several bioprocess parameters that impact urease extraction from certain plants were adjusted. Urease extraction is influenced by several variables, including the kinds of plant material that Seventeen regularly utilized plant varieties were employed to investigate the impact of plant type on the enzyme extraction process. Using 0.02 M of phosphate buffer pH 7.0, the following were tested: Lentil, Eggplant, Cowpea, Rapeseed, Onion, Acacia, Lima bean, Flaxseed, Jujube (Male), Jujube (Female), Indian gooseberry, sweet potato, Turnip, Broad bean, Mung bean, Cantaloupe, and Kidney bean. The results showed that the highest urease extraction was in Flaxseed, followed by Lentil and Mung bean, with specific activities of 1.63, 0.893, and 0.74 U/mg, respectively (Fig. 2). According to ElHefnawy [20], urease isolated from Pisum Sativum L. seeds has a specific activity of 0.19 U/mg protein (Fig.2). Our results show that flaxseed produced the highest specific urease activity among the 17 plant sources tested, at 1.63 U/mg, followed by lentils (0.893 U/mg) and mung beans (0.74 U/mg). This value is moderately higher than previously reported crude plant extracts, such as peas (Pisum sativum), which produced approximately 0.19 U/mg of urease under similar testing conditions. Practical studies, including a 2020 American Society of Civil Engineers (ASCE) technical paper and another 2024 paper on soybean-derived crude urease, confirm the effectiveness of crude plant extracts in promoting carbonate precipitation and structural self-healing, due in part to cofactor proteins and biomolecules that may enhance crystallization or enzyme stability [21]. Therefore, our finding—the specific activity of flaxseed of 1.63 units/mg—represents strong performance within the range of environmentally friendly, plant-derived crude urease sources. While it does not match the highest values from highly purified preparations, it significantly outperforms low-activity extracts and represents a practical and scalable option for real-world EICP-assisted crack repair strategies.

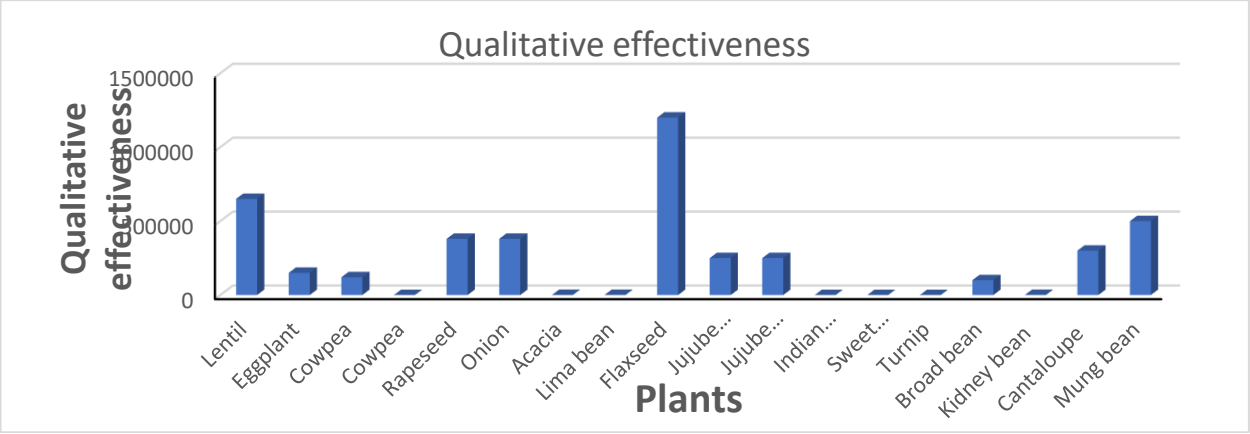

Figure 2 Effect the types of plant material on urease extraction.

4.Extraction Time

From (5-120 min) of extraction used to determine the optimum extraction time of urease. The time chosen was (10, 15, 35, 90, 120, 160) min. The highest crude extract was obtained at 90 minutes, reaching 1.11 U/mg protein, compared to the rest of the results obtained at 10, 15, 35, 120 and 160 minutes, 0.5, 0.6, 1, 1.1 and 0.8 units/mg protein, respectively (Figure 6). It was found necessary to determine the optimal time period due to the variation of urease enzyme extract from one source to another, due to the difference in the materials present in that source that interfere with the enzyme as shown in figure 3. Our investigation into flaxseed urease extraction determined that a 90-minute extraction period yields optimal results, specifically achieving the highest specific activity at 1.11 U/mg protein. This outcome corresponds closely with findings reported in the broader scientific literature. For example, comparative studies examining urease extraction across various plant matrices have noted optimal extraction times ranging between 60 and 90 minutes, depending on both the plant source and buffer conditions employed [22].Furthermore, research into microbial urease extraction highlights the interplay between temperature and extraction duration, with peak enzyme activity recorded after 90 minutes under controlled thermal conditions [23]. Conversely, a study focused on crude soybean urease for EICP applications selected a shorter extraction time of approximately 60 minutes yet still reported high enzymatic efficiency in downstream processes such as bio cementation [24].Taken together, these studies underscore that the optimal extraction period is highly contingent upon the specific biological source and extraction protocol. The fact that flaxseed achieved maximal urease activity at the 90-minute mark is well-supported by international literature and represents a practical balance between extraction efficiency and procedural simplicity, making it suitable for scalable EICP applications.

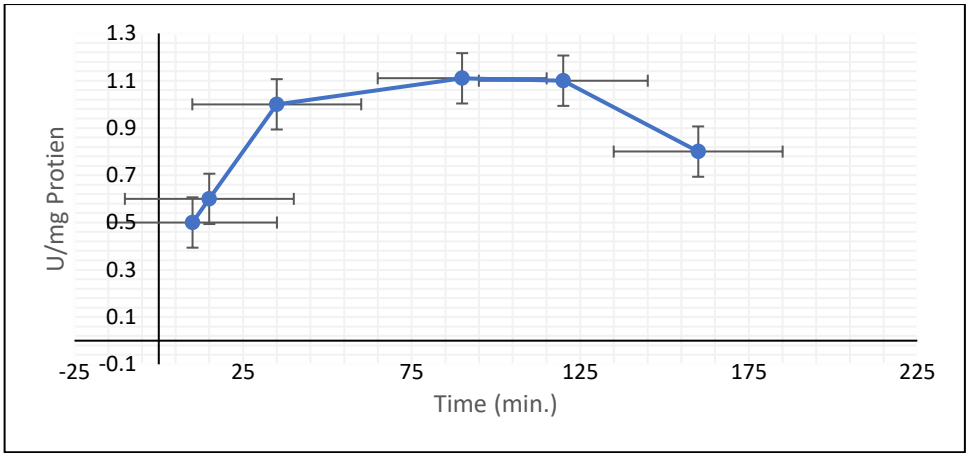

Figure 3. Effect of extraction time on urease extraction.

5.Effect pH on Urease stability The data in figure 4 show that pH ranges between 6.5 and 7.5 were optimal for urease stability; the enzyme preserved 90% of its activity at pH 6.5, 50% at pH 4.5, and about 60% and 40% at pH 7.5 and 8, respectively. At both acidic and alkaline pH values, the enzyme activity was very low. The findings could support the idea that the urease enzyme is more stable at pH values that are almost neutral. Generally speaking, the effect of pH stability on enzyme structure, which can denaturize the enzyme molecule or alter the ionic state of the enzyme active site, as well as its effect on the secondary and tertiary structure of the enzyme, which can result in the loss of activity in buffer solutions that are too far from the ideal pH, may be the cause of this decreasing activity at pH values that deviate from the ideal condition. At pH 6.5, the enzyme retains nearly 90% of its activity, indicating considerable stability under these conditions. When the pH drops to 4.5, however, activity declines sharply to about 50%. Alkaline environments are similarly detrimental; at pH 7.5, activity falls to 60%, and by pH 8.0, only 40% remains. These observations suggest that urease is highly sensitive to both acidic and basic extremes, with optimal performance near neutral pH. This likely results from pH-induced alterations in enzyme structure—such as disruptions in folding or changes to the active site—which impair catalytic function. Inappropriate buffer systems can exacerbate this by modifying the ionization state of critical residues, destabilizing secondary and tertiary structures, and thereby reducing overall activity. These findings are consistent with previous research on microbial and plant ureases, which also identified near-neutral pH as optimal for catalytic stability and efficiency [25], [26]

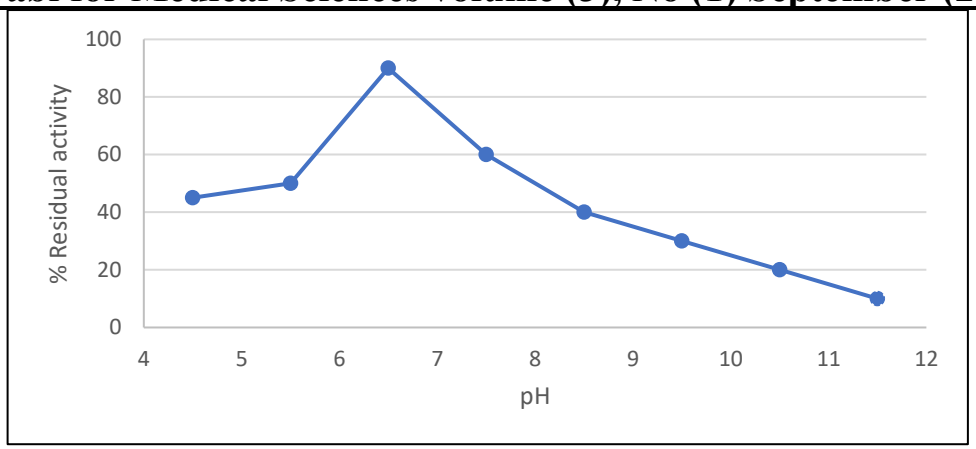

Figure 4. Effect of pH in urease stability.

6.Effect substrate concentration In this experiment, a change in the concentration of urea significantly affected the activity of the urease enzyme. The analysis was prepared by adjusting the volumes of a 5% urea solution, bromothymol blue, distilled water, keeping the volume of the crude enzyme constant at 4 ml (see Figure 5). The highest observed activity was recorded at 0.9 U/mL, using a mixture containing 1 ml of urea, 2 ml of bromothymol blue, and 1.5 ml of distilled water. An increase in the volume of urea to 2 ml led to a decrease in activity to 0.7 U/mL, which indicates inhibition of the substrate at elevated urea concentrations. This behavior corresponds to Michaelis-Menten dynamics, in which the reaction rate increases with the concentration of the substrate up to a specific point, after which inhibitory effects appear, reducing enzymatic activity [25].These results are supported by globally published studies. For example, Yahya et al. (2021) reported that in a membrane-based system, urease activity was sharply reduced at high urea concentrations, which corresponds to substrate inhibition or the phenomenon of enzyme degradation under excessive substrate conditions . Similarly, Mutassim et al. (2024), in the soil context, observed a decrease in urease activity with high urea application rates - attributing this decreases to substrate inhibition with pH changes - confirming the importance of optimal substrate dosage [27]–[29].Together, these data confirm the urgent need to improve the concentration of urea in enzymatic systems. Specifically in applications such as enzyme-catalyzed carbonate precipitation (EICP), control of substrate levels is essential to improve the performance of urease-mediated precipitation and self-healing in concrete.

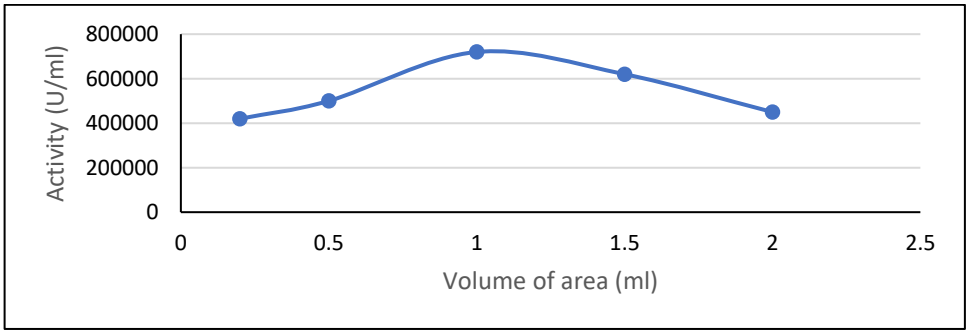

Figure 5. Effect substrate concentration on urease activity

7. Acknowledgment

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