Powdered Enzyme from Australian Weed for Bio-stabilisation

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Abstract. The urease enzyme derived from Paddy melon (Australian weed) seeds, is highly effective in catalyzing the hydrolysis of urea in the presence of calcium ions. This reaction results in the precipitation of calcium carbonate, which then binds/cements soil particles. This process is known as the Enzyme-Induced Carbonate Precipitation method (EICP). Recently, EICP has been utilized in geotechnical engineering to enhance various physical properties of soil, such as strength, hydraulic conductivity, and reactivity. However, crude enzyme solution is easily degraded with time, even at low temperatures, making it challenging to produce and store for larger-scale applications. Therefore, this study aims to transform the liquid enzyme solution into a powdered enzyme using the freeze-drying technique (lyophilization) to avoid degradation problems. The catalytic activity of the enzyme powder from Paddy melon was measured at 2.867 KU/g, which is equivalent to commercially available purified enzymes. In addition, cost analysis suggested that the powderisation process can help produce the enzyme powder at a lower cost than commercial products, enhancing the affordability of the EICP application in soil stabilization.

Keywords: EICP · Enzyme powder · Freeze drying · Soil stabilization

1 Introduction

Plant-based urease enzymes catalyse and accelerate the urea ($CO(NH_2)_2$) hydrolysis to produce carbonate ions (CO_3^{2-}), to combine with calcium ions (Ca^{2+}) to precipitate calcium carbonate ($CaCO_3$), which binds soil particles and improves their engineering properties. This is a nature-inspired carbonation process known as the Enzyme-Induced Carbonate Precipitation method (EICP). Enzymes are much smaller (300–500 nm) and percolate through smaller pores of finer soils [1, 16]. Therefore, EICP has the capability of binding/stabilizing finer particles. In engineering applications, calcium chloride ($CaCl_2$) solution can be used as the source of Ca^{2+}. Many researchers suggested that these chemical reactions can be presented by the following equations [3–5, 21]:

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

$$CaCl_2 \xrightarrow{H_2O} Ca^{2+} + 2Cl^- \quad (2)$$

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$$Ca^{2+} + CO_3^{2-} \overset{\text{precipitation}}{\longleftrightarrow} CaCO_3 \quad (3)$$

One of the major challenges for the widespread application of EICP in soil stabilisation is the cost of laboratory-grade pure enzymes, which is about 70–80% of the total stabilisation cost [3]. Recently a few studies suggested that crude enzyme extract from plant sources is effective and economical as an alternative [11, 15]. Despite the success of using crude enzymes in soil stabilisation, most of these studies used food-grade beans or seeds for crude enzyme extraction, which is still not ideal for large-scale engineering applications. Therefore, alternative and cheaper sources are required. In that context, Rahman et al. [17] screened more than 50 native Australian plants and weeds as potential sources for crude enzymes. Using test tube experiments, they showed that crude enzymes from mature Paddy melon seeds, with high enzyme activity, could be much cheaper. However, the crude enzymes were extracted in solution, which is unstable, making it difficult to produce, store and transport. Therefore, this study attempted to transform liquid crude enzyme solutions into powdered enzymes using the freeze-drying (lyophilisation) technique. This study also compares the activity of crude enzymes (solution) and powdered enzymes. A cost analysis is also presented for crude enzyme powder and commercially available purified enzyme powder.

2 Urease Enzyme from Paddy Melon Seeds

Paddy melon (*Cucumis myriocarpus*) is an invasive weed species in Australia (Fig. 1). Control of Paddy melon can be challenging as the plant has a long germination period and produces many seeds that can remain viable in the soil for several years. However, Rahman et al. [17] found Paddy melon as a potential source of crude enzymes, and it has become an economic opportunity as a crop. Although having a lower activity than the enzyme extracted from soybean (in Fig. 2), the enzyme solution extracted from mature Paddy melon seeds had the highest specific activity, which means that it had a higher level of purification than the rest of the samples in the study.

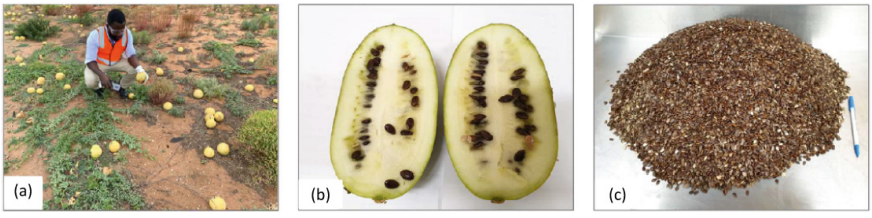

Fig. 1. Paddy melons **a** grown plant species; **b** cross-section of the fruits; and **c** extracted seeds [1]

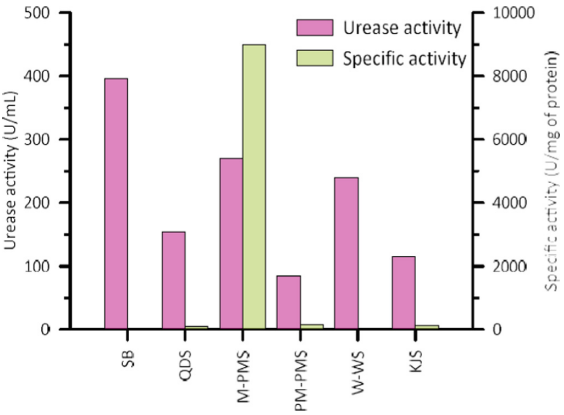

Fig. 2. Urease activity and specific activity of crude extracts [2, 17]

3 Material and Methods

3.1 Crude Enzyme Extraction

Crude urease enzyme extracted from seeds of Paddy melon was used. The detail of the crude extraction technique is described below:

- Seeds were air dried at 25 ± 1 °C for 48 h for better storage [8, 18].
- 300g of seeds were then soaked overnight in 1 L of DI water at 4 °C [11, 18]. DI water was used as an extraction solution to reduce the costs and complexity of the extraction process [11, 18].
- The solution was homogenised in a blender for 5 min to disrupt the cells and tissues [11, 18].
- The mixture was filtered by a cloth fabric to separate the coarse solids from the enzyme solution [11].

The enzyme-containing solution was centrifuged at 14000 rpm for 1 h at 4 °C then filtered to eliminate the organic content. After the centrifuge process, 900 ml of enzyme solution was obtained as a crude extract.

3.2 Powderisation

Javadi et al. [10] attempted to powderise crude enzyme solution from Jackbean by lyophilization method. This technique is also known as freeze-drying, a process of removing water from a substance, typically a liquid or a solution, by freezing it and then subjecting it to a vacuum under low temperatures. During lyophilization, the substance is first frozen, and then the water is removed from it by sublimation, which is a process where the water goes directly from a solid (ice) to a gas (water vapour) without passing through the liquid state. This is done by placing the frozen substance in a vacuum chamber and applying low heat, which causes the ice to evaporate directly into a vapour without melting, as shown in Fig. 3.

In past research, Javadi et al. [10] powderised the enzyme solution without centrifugation. This might help shorten the production time and increase the powder yield; however, it raises concerns about impurities, which may affect the treatment process. Therefore, to remove the impurities, the enzyme solution was centrifuged at 14,000 rpm with a high-speed centrifuge machine for 1 h at 4 °C. Then, 900 ml of crude enzyme solution was filled into 50 ml tubes and got frozen at −85 °C for 24 h. Then the tubes were put into the vacuum machine (Fig. 3) to eliminate water content at −48 °C. It took three days for the liquid solution to dry completely. About 7 g of powder was collected at the end of the process.

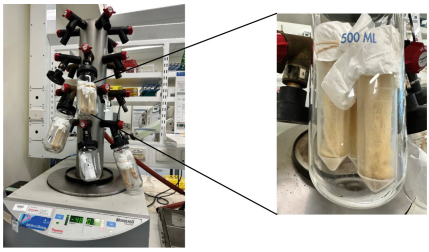

Fig. 3. ModulyoD Freeze Dryer

3.3 Enzyme Activity Measurement

The research used the electrical conductivity (EC) method to determine the urease activity of the enzyme solution [20]. This method involves measuring the rate of change in EC due to the conversion of urea into ammonium and carbonate ions catalysed by the urease enzyme. 3 mL of crude extract or enzyme powder solution (0.5 g of powder) was added to 7 mL of 1.0 M urea solution in test tubes and immediately sealed them using stoppers with aluminium seals to minimize the loss of ammonia gas generated during the reaction. The tubes were then gently shaken, and EC readings were taken at different time intervals over 5 min. The rate of change in EC over time was found to be consistent, indicating a minimum loss of ammonia gas.

The enzyme activity of the solution was calculated by dividing the slope of the EC-time curve by enzyme concentration. The rate of mS/cm/min was found to correspond to 11.11 mM urea/min, taking into account a dilution factor of 10. This approach was previously used to determine the urease activity of Soybeans and Jack beans [7, 9, 19].

4 Results and Discussion

Electrical conductivity was measured in 5 min intervals from 3 to 7 min after mixing the enzyme and cement solution. The enzyme solution produced from 0.5 g of powder had a steeper slope of the EC-time curve with a coefficient at 0.52, about two times higher than the figure obtained for crude enzyme solution (Fig. 4a). This result indicated that there was a much faster rate of NH_4^+ ion created in the solution, which means that the enzyme powder facilitated the urea hydrolysis process at a much higher reaction speed.

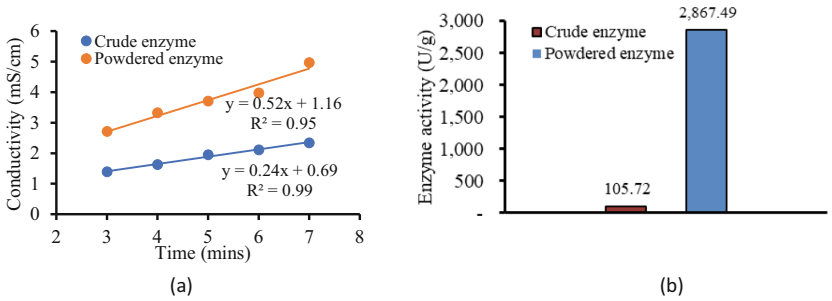

Fig. 4. Comparison between crude enzyme and powdered enzyme: **a** EC-times curves, **b** Activity (U/g)

Enzyme powder has a much higher activity than the crude extracted one, about 27 times better in terms of the number of units per gram of enzyme source. In detail, 1 g of Paddy melon seeds contains 105.72 U, whereas 1 g of enzyme powder consists of 2,867 U (Fig. 4b). This is an expected result because enzyme powder was concentrated from the crude extracted liquid solution while a gram equivalent of Paddy melon seed contains other impurities. Compared to a previous study conducted by Javadi et al. [10], 1 g of powdered enzyme extracted from Jackbean had about 3700 U, nearly 900 U higher than powder from Paddy Melon. The higher activity value of the Jackbean is understandable because Jackbean is known as one of the richest urease-contained plants [13].

Test tubes were used to investigate the $CaCO_3$ precipitation. 25 ml of enzyme solution was mixed with an equivalent amount of cement solution. Then, test tubes were cured in an oven with a constant temperature of 30 °C. After 48 h, test tubes were then taken for SEM analysis. SEM images showed that the powdered enzyme created mainly calcite and vaterite crystals, whereas the crude enzyme generated aragonite and vaterite (Fig. 5). In terms of thermal stability, calcite is well-known as the most stable form of $CaCO_3$ crystal. In contrast, vaterite is the least stable one and can be easily turned into the other crystal types. Aragonite is slightly more stable than vaterite. However, it is well known as a filler that can improve the material's strength when added [12].

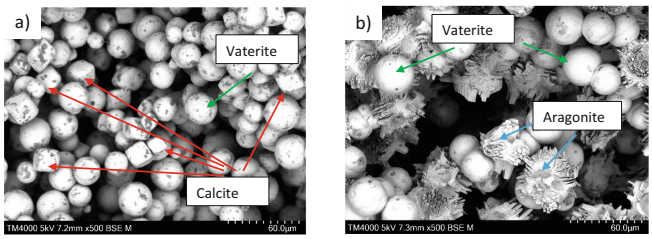

Fig. 5. SEM analysis $CaCO_3$ precipitated by: **a** enzyme powder, **b** crude enzyme solution

5 Cost Analysis

The process of powderisation of urease enzyme from Paddy melon seeds includes three stages. Firstly, Paddy melons were harvested from Port Pirie, South Australia. The seeds were collected and dried to store in the fridge at 4 °C for the next stage. Secondly, the crude extracted process was used to produce the crude enzyme solution. Finally, the enzyme solution was frozen at −85 °C before putting into the freeze dryer to get the powder.

According to the Australian Bureau of Statistics [6], the average hourly rate of a worker in the construction sector was 40.9 AUD/hour. Based on this number, it costs 163.6 AUD for the labour to harvest and dry 10 kg of the paddy melon seeds in 4 h. Meaning, it took about 0.01936 AUD to harvest 1g of seeds (Table 1).

Table 1. Seed harvesting cost.

Seeds (kg)	Transportation cost ($AUD)	Labour cost ($AUD)	Cost of seeds ($AUD/g)
10	30	163.6	0.01936

In the second and third stages of powderisation, the major expense came from the rent of the freeze dryer and the labour cost (Table 2). After the process, the total cost of producing 7.13 g of powder was 81.21 AUD. Since the activity of the enzyme powder was measured at 2867 U/g by the conductivity method, the overall cost for 1 U of enzyme powder was 0.004 AUD/U. This is cheaper than commercial purified products packed at 20,000 and 100,000 U from Sigma-Aldrich but higher than the big packages at 500KU and 1MU (Fig. 6).

Table 2. Cost for powdered urease enzymes

Cost objects		Unit	Cost ($AUD)
Seed	300	g	$ 5.81
Water added	1.2	l	$ –
Grind, filter, Centrifuge	900	ml	$ –
Freeze drying	3	days	$ 34.50
Labour cost	1	hour	$ 40.90
Powder yield	**7.1328**	**g**	**$ 81.21**

It is noticeable that the average cost of weeds for agricultural industries in Australia is about $AUD 3.9 billion annually [14]. So, the benefit of using Paddy melon seeds as a urease enzyme source is not limited to the revenue of selling the urease enzyme but also from the cost saving in controlling this invasive plant.

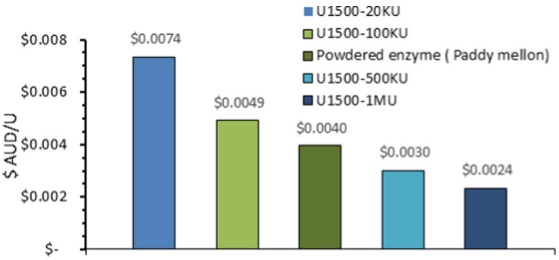

Fig. 6. Unit cost comparison with commercial products from Sigma-Aldrich

6 Conclusions

This study has proposed a production process on a laboratory scale to produce urease enzyme powder from Paddy melon seeds (an Australian weed). The main conclusions are as follows:

- Urease enzyme powder produced by the freeze-drying process can create a high-activity enzyme solution, allowing the control of the enzyme concentration in the EICP process at a low cost.
- Different urease activity can lead to varying formations of calcium carbonate crystals.
- The cost analysis suggested that the powderisation process for Paddy melon was economical and could be effective if it is adopted as a source of urease enzyme.

The cost analysis in the research was conducted on a laboratory scale, which is supported by available machines at the university. However, commercial production on a large scale may be able to decrease the cost and need to be investigated in future research.

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