Examination of urease activity in seeds of tropical fruits

Keywords: plant-derived urease, hydrolysis rate, soil improvement

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Introduction

Application of enzyme as bio-catalysis in calcite precipitation technique has been confirmed as the potential soil improvement method [1)]. In this technique, urease enzyme (EC 3.5.1.5, urea *amidohydrolase*) is used to hydrolysis urea to ammonia and carbonate ions. Hydrolysis of one molecules of urea results in the release of two molecules of ammonia and one molecule of carbon dioxide[2)]. Plant-derived urease has been studied for various possible plants, leaves, and seeds such as jack beans (*Canavalia ensiformis*), soybean (*Glycine max*) leaf and seed, pigweed (*Chenopodium album*) and mulberry leaf (*Morus alba*)[2)] and extract seeds of fruits such as watermelon (*Citrullus vulgaris*), melon (*Cucumis melo*), pumpkin (*cucurbita maxima*) seeds[3)]. In this work the extract seeds of durian (*Durio zibethinus murr*) and watermelon (*Citrullus vulgaris*) were used to examine the urease activity. The purpose of this research to discover a plant species that contains urease activity for Enzyme Mediated Calcite precipitation (EMCP) by catalyzing the hydrolysis of urea and compare the urease activity between factory products (jack beans) and seeds of tropical fruit extract.

Methods

Plant extract having urease activity may enhance the hydrolysis of urea into ammonium and carbonate ion. Therefore, we measured the urease activity of two different solution from two types of fruit-seed extract and factory-product urea. Dried watermelon (*Citrullus vulgaris*) and durian (*Durio zibethinus murr*) seeds were obtained from Indonesia. After removing the seed coat, watermelon and durian seeds were crushed with mortar and soaked in distilled water for 30 min. The amount of crushed seeds is 15 g/L. The crushed-seeds solution was filtered through filter paper (pore size of 11 μm). Volume for each solution to conduct the test is 50 mL.

The concentration of urease solution of the factory product is 15 g/L. The hydrolysis rate was evaluated directly after the mixing. The experimental procedures developed by Whiffin et al.[4)] were adopted. The evolution of the resistance with time was measured using LCR meter KC- 555 KDK from koyo Electronics Industries Co., Ltd, Tokyo, Japan, and the conductivity change was determined. A standard curve was provided by determining the conductivity resulting from the complete hydrolysis of several concentration of urea. The hydrolysis rate is determined by calculating the gradient slope of the conductivity change with time, with equation (1)-(2).

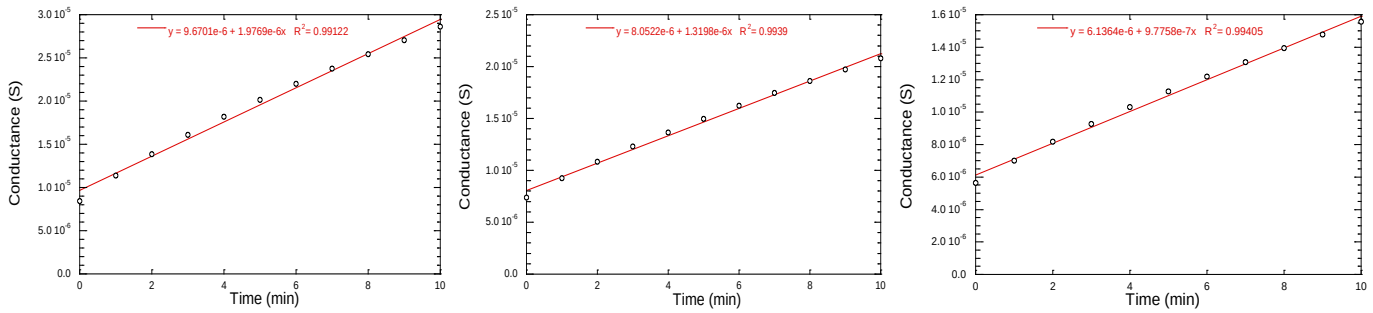
$$Conductance,\ (S) = \frac{1}{R} \qquad (1)$$

$$Hydrolysis\ rate,\ (u/g) = \frac{\theta_{ms}}{\theta_{sc}} \,.\, v.\, N \qquad (2)$$

R is the measured resistance (Ω), θ_{ms} is the gradient slope of measured sample, θ_{sc} is the gradient slope of the standard curve, v is the volume of sample (L), and N is the final concentration of ammonia (mMol/L).

Results

The urease activity tests for factory-product urease and fruit-seed extract were conducted. The evolution of conductivity in urease of factory product is shown in **Fig.1**, and that of conductivity obtained from extracts watermelon and durian seeds with various concentration of urea are shown in, **Fig.2**, and **Fig.3**. A Standard curve for hydrolysis rate analysis are shown in **Fig.4**. The hydrolysis rate analysis are shown in **Table 1**.

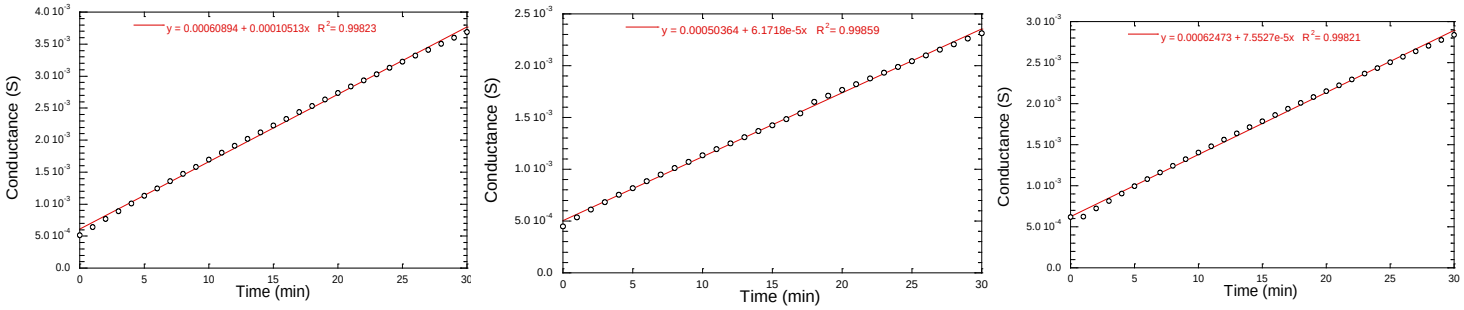


a. urease solution (15g/L) with urea solution (1 mol/L)

b. urease solution (15g/L) on urea solution (1.5 mol/L)

c. urease solution (15g/L) on urea solution (2 mol/L)

Fig 1. The evolution of conductivity after mixing urease and urea solution

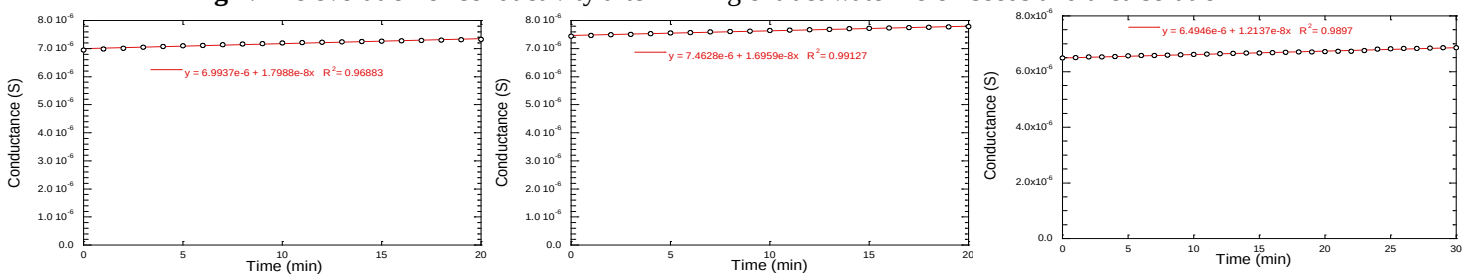


a. Extract watermelon solution (15g/L) on urea solution (0.5 mol/L)

b. Extract watermelon solution (15g/L) on urea solution (1 mol/L)

a. Extract watermelon solution (15g/L) on urea solution (1.5 mol/L)

Fig 2. The evolution of conductivity after mixing extract watermelon seeds and urea solution

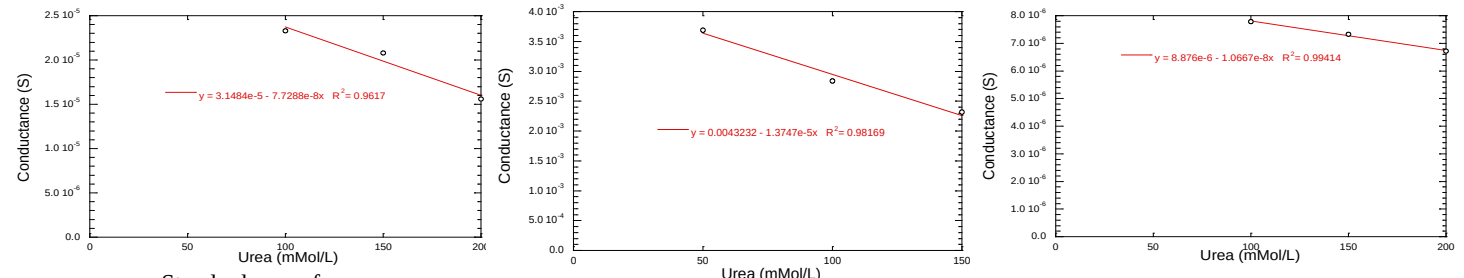


a. Extract durian solution (15g/L) on urea solution (0.5 mol/L)

b. Extract durian solution (15g/L) on urea solution (1 mol/L)

c. Extract durian solution (15g/L) on urea solution (1.5 mol/L)

Fig 3. The evolution of conductivity after mixing extract durian seeds and urea solution



a. Standard curve for urease

b. Standard curve for watermelon seeds

c. Standard curve for durian seeds

Fig 4. Standard curve for hydrolysis rate analysis

Table 1: Hydrolysis rate analysis for various urease solution

Urease solution	Hydrolysis rate (unit)
Jack beans	2386
Watermelon seeds	449
Durian seeds	159

Conclusion

The urease activity was found in extract of watermelon and durian seeds, although the amount of urease activity is smaller than the factory product (jack beans), Further research is needed such as the XRD test and precipitation test to determine that the urease activity in extract seeds can be used as a substitute for factory product (jack beans) on soil improvement using Enzyme Mediated Calcite Precipitation (EMCP) technique.

References

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