

Effect of different legume resource and technical parameters on urea hydrolysis

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Abstract

Urea hydrolysis is widely used in agriculture, environment and other engineering fields, among which urease contained in beans can catalyze urea hydrolysis. The urea hydrolysis activity of legume plant leaching solution (LPLS) was investigated, including soybeans, black beans, mung beans, red beans as well as soybean hulls, soybean leaves, soybean stems and soybean pods. For the high urea hydrolysis activity and economic efficiency, soybean is most suitable for agricultural engineering and other fields than other beans and soybean-related parts extract. The urea hydrolysis activity increases with the concentration of LPLS, while decreases gradually with reaction time. When the heating temperature reaches 25, 35, 45, 55 and 65 °C, the urea hydrolysis activity is steady and the enzyme activity is high. Enzyme activity decreases after 65 °C (i.e. 75, 90 °C). Meanwhile, the soaking time of LPLS has a little effect on the urea hydrolysis activity compared with other factors. These results make a positive contribution to domestic production urease experimental basis.

Keywords: Legume Plant Leaching Solution (LPLS); Soybean; Urea Hydrolysis Activity; Enzyme Activity

1. Introduction

Urea from animal's excretion and fertilizer hydrolyze into ammonia and carbonate. Ammonia contributes to plant development, while carbonate anion precipitates to carbonate in the presence of some metal cation, e.g. calcium, vanadium [1]. Urea hydrolyzes at a very slow rate under natural conditions, and it is often desired to accelerate the process. Therefore, understanding the mechanism of urea hydrolysis can reduce environmental pollution, improve nitrogen utilization efficiency, and promote food production [2]. In general, the mostly used bacterial metabolic pathways applied in self-healing concrete are urea hydrolysis [3]. Wang Y et al. [3] has studied in situ MgAl-LDH is synthesized on the surface of η -Al₂O₃, which catalyzes the hydrolysis of urea in wastewater. If wastewater without catalyst is to meet discharge standards, the reaction time must be extended to 210 minutes. The urea catalytic hydrolysis technology meets the demand of denitration reductant supply [5], energy consumption, urea utilization rate and other indicators were optimized. Urea hydrolysis can also prepare engineering cementing materials and free stable heavy metals. When ureolytic bacteria and chemical agents (i.e. urea and CaCl₂) are injected into the soil pores, and calcium carbonate crystals are formed in situ by urea hydrolysis [6-7]. The precipitated crystals cement adjacent soil particles to strengthen and stabilize the soil [8-9]. Metal ions in solidified soil include nickel (Ni), copper (Cu), lead (Pb), cobalt (Co), zinc (Zn), and cadmium (Cd) [10-11].

Beans have many functions. On the basis of traditional products such as edible soybean oil, tofu, soybean milk and curd, they also had soybean protein, margarine, shortening and functional oils produced industrially. Additionally, they have also developed the extraction of small and medium molecular nutrients such as soybean phospholipids, soybean

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isoflavones, soybean urease, etc [12]. Concanavalin and soybean contain urease [13], which catalyzes the hydrolysis process of urea. Rechenmacher et al [14] has reported that there are three urease isoforms in soybean: the embryo-specific encoded by the Eu1 gene, as well as the ubiquitous urease encoded by Eu4 and Eu5. Pure urease extracted from legumes can produce a kit for clinical diagnosis of urea ammonia in serum, regeneration of the dialysate solution in the wearable kidney machine [15]. When urease and urea are present at the same time, it provides N nutrition of high concentration, which is continually beneficial to seed germination, plant development [14] and bioregenerative life support systems [16]. Moreover, ureases play a role in defense against insect and phytopathogenic fungi [17]. It was reported that it can increase the hydrolysis rate of urea up to 10^{14} times [15]. However, in the field of agronomy, the hydrolysis rate of urea is related to the bioavailability of nitrogen [15]. The rapid hydrolysis of urea catalyzed by urease is responsible for that more than 50% of the applied nitrogen (N) lost via ammonia volatilization [15]. The application of seed encapsulation and urease under appropriate conditions can significantly enhance the absorption and utilization of nitrogen nutrients in early stage of plants [18]. However, the price of refined urease is ¥780/100mg, which is too expensive for agricultural and engineering fields.

In order to provide an economical and convenient method for accelerating urea hydrolysis, it will lead the production engineering of bio-cemented calcium carbonate and soybean-urea mixed fertilizer. This paper introduced the urea hydrolysis ability of LPLS, including different legumes and different parts of soybean, and selected soybean as the best urease source. Urea hydrolysis activity were characterized under different concentration, temperature and time of LPLS.

2. Material and methods

2.1. Selection and processing of materials

The dried soybeans, black beans, mung beans, red beans, soybean hulls, soybean leaves, soybean stems and soybean pods, which obtained from Honggutuan Farmers Market in Nanchang, China, were used. Urea and deionized water was purchased from Nanchang Jingke Organic Fertilizer chemical industry co., Ltd. (Jiangxi, China). Physicochemical properties are shown in Table 1.

Table 1 Physicochemical properties

	Molecular formula	Molecular weight	boiling point	Density (g/cm³)	Melting point(°)	Water solubility
Urea	CH ₄ N ₂ O	60.06	196.6°Cat760mmHg	1.335	132.7	1080 g/L
Deionized water	H ₂ O	18.015	100°C	0.99987	318	

2.2. Preparation of legume plant leaching solution

The different legume plants were weighed with an electronic balance, accurate to 0.01g. The weighed legume plants were shattered in a mechanical grinder, and then sieved to obtain particle smaller than 100 meshes. Put legume plants powder and deionized water into the beaker according to the preset concentrations listed in Table 2. The magnetic stirrer was fully stirred for 30 minutes, and the legume plants powder was soaked at 4 ° C for the corresponding time. Then the leaching solution was centrifuged at 3000 r / min for 15 min to obtain the supernatant.

Table 2 Different concentration of legume plant leaching solution

Concentration(g/L)	40	80	120	160	200
Soaking time	24h				

2.3. Determination of urea hydrolysis activity of legume plant leaching solution

Urea hydrolysis activity is indicative by the μmol of hydrolyzed NH_4^+ from urea per minute [19], as shown in equation (1). Whiffin [20] indicated that the rate of conductivity(mS/min) was converted to urea hydrolysis rate (mM urea hydrolysed/min) by relating conductivity measurement to obtain the urea hydrolysis per minute of the urease extract,

and the value was used to represent the urease activity (equation(2). Equation (3) was derived by combining equation (1) with equation (2).

The electrical conductivity (EC) was determined with a DDS-11A EC meter (Shanghai yidian scientific instrument co., Ltd. Shanghai, China). 1.8 grams of urea was poured into a beaker containing 27 ml of deionized water and fully dissolved. Took 3mL of LPLS and mixed it with the urea solution to stir uniformly. At 20 °C, the EC meter monitored the conductivity change of the above solution within 15 minutes (once every 5 minutes), and the average conductivity change per minute was obtained. Furthermore, equation (3) was utilized to calculate the enzyme activity U.

$$U = \text{hydrolyzed } \text{NH}_4^+ \text{ from urea (mM) / time (min)} \quad (1)$$

$$\text{Urease activity} = \frac{\text{Conductivity value (ms/cm)} \times 11.1 \text{ mM hydrolysed urea/min} \times \text{Dilution multiple}}{\text{Time (min)}} \quad (2)$$

$$U (\mu\text{mol/min}) = \frac{2 \text{ Conductivity value (ms/cm)}}{11.1 \text{ mM hydrolysed urea/min} \times \text{Dilution multiple} \times \text{Time (min)}} \quad (3)$$

3. Results and discussion

3.1. Urea hydrolysis activity of legume plant leaching solution

In order to investigate the enzyme activity of different beans and the most suitable urease materials, the enzyme activity of LPLS was tested. As shown in Fig.1, the enzyme activity (U) of black beans and soybeans was 8.89 μmol/min and 7.54 μmol/min, respectively. The enzyme activity of red beans and mung beans was as low as 0.11 μmol/min, which was 1.4% of that of soybean. Huang et al. [21] studied that 90% of the native soy proteins from soybean dregs are storage proteins that can enhance enzyme activity. In the composting process, the enzyme activity of the test group with 35% soybean dregs was the highest, which was 4.15 mg glucose/g/h [22], the equivalent of 12.45 μmol/min. The results of the current study indicated that urease was abundant in soybean. In soybeans and related parts, the enzyme activity of soybean pods, soybean leaves and soybean stem were 3.3%, 1.9% and 4.8% of soybean, respectively. Soybean hulls showed enzyme activity of 0.92 μmol/min. When the extraction solution was water, the enzyme activity of soybean hulls was 0.608 μmol/min, and the yield of urease was less than 40% of that of raw soybean hulls [12]. The result was consistent with Qi Jia's. Black beans and soybeans were priced at ¥ 17.9/kg and ¥ 12.9/kg, respectively. Therefore, soybean was considered as the best resource of urease.

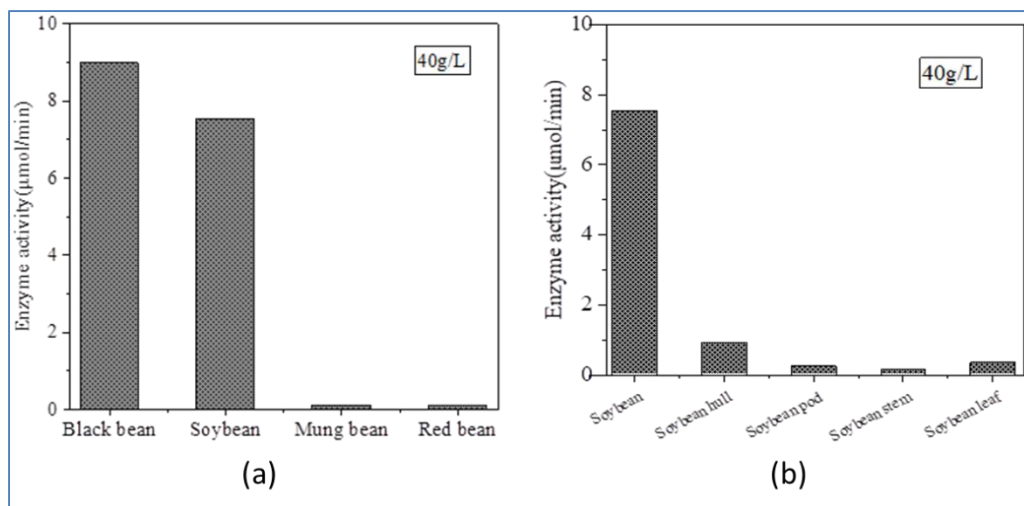


Figure 1 Enzyme activity of legume plant leaching solution.(a) legumes leaching solution and (b) soybean and its related-parts leaching solution

3.2. Urea hydrolysis activity of Soybean leaching solution with concentration

Soybean leaching solution (SLS) was prepared according to the concentrations in Table2, then determined urea hydrolytic activity. The influences of five concentrations on EC values were consistent (Fig.2a). The higher the concentration of SLS caused higher EC values. Pradhan et al. [23] reported that the higher the cell concentration, the

more complete the hydrolysis of urea. When cell density reached the highest value of 108 cells/mL, the urea decomposed increased to 20 g/L, which mean a completely hydrolyzation was achieved [24]. Fig.2b shows the urea hydrolysis activity increased linearly with the rise of the concentration of SLS. Urease concentration affected the process of urea hydrolysis, and urease activity was positively correlated with urea concentration [25]. This also conveyed that the concentration of SLS had a positive correlation to the enzyme activity.

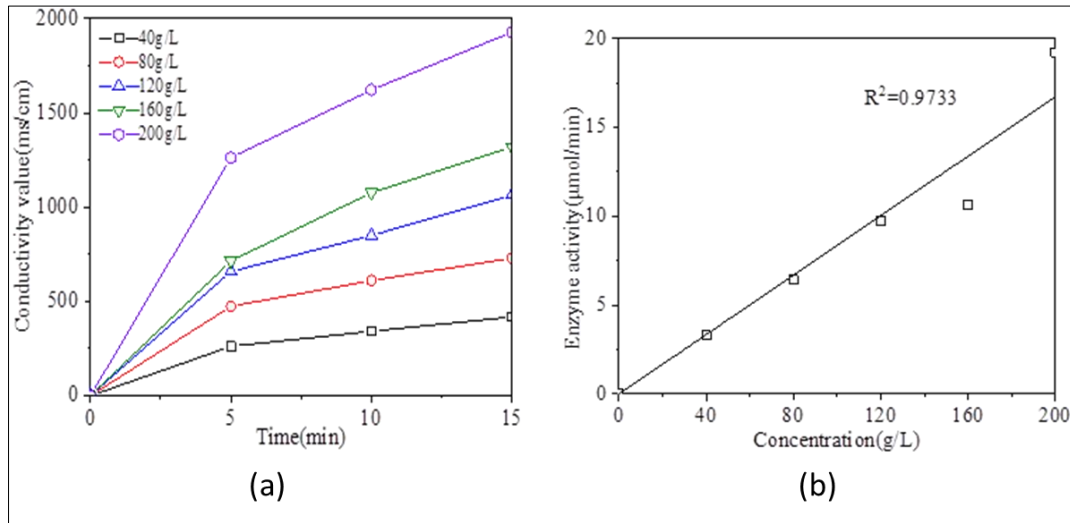


Figure 2 Hydrolytic urea activity of Soybean leaching solution.(a) Changes in electrical conductivity of Soybean leaching solution and (b) Enzyme activity of Soybean leaching solution with different concentration

3.3. Evolution of urea hydrolysis activity of Soybean leaching solution with reaction time

Fig. 3a shows the average conductivity of different concentration of SLS in different time periods. It could be seen that the conductivity values of different concentration of SLS show: the first 5 minutes > the middle 5 minutes > the last 5 minutes. With increasing in reaction time, the urea degradation rate rapidly increased [4]. On the contrary, with the increase of reaction time between SLS and urea, urease activity gradually decreased (Fig.3b). Because of the early mixing of SLS and urea, SLS was abundant in urease, which reacted fully with urea and had good hydrolysis activity. Thereafter, urease was gradually consumed and the enzyme activity of the solution decreased.

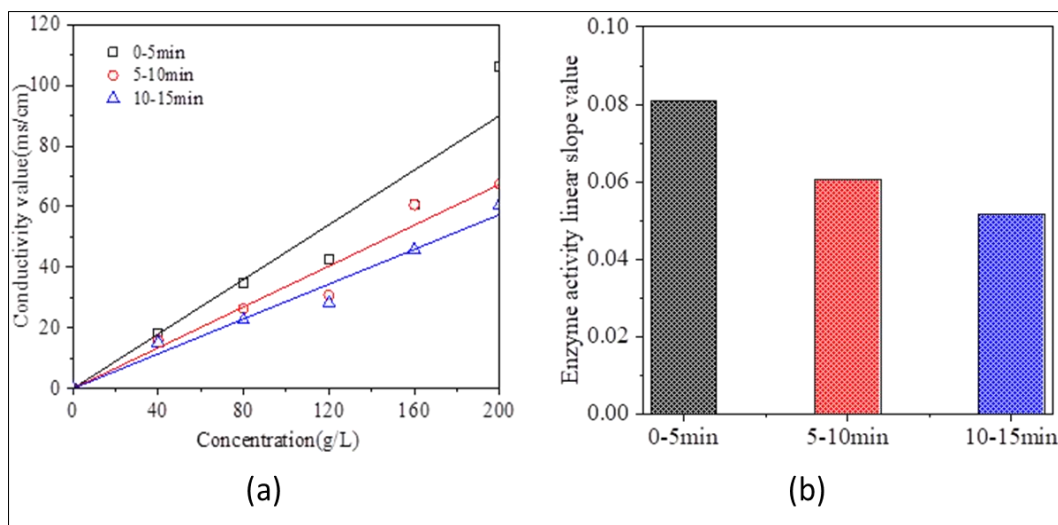


Figure 3 Effect of reaction time on urea hydrolysis activity of Soybean leaching solution. (a) Enzyme activity of Soybean leaching solution with different concentration in different periods and (b) Linear slope of enzyme activity in different periods

3.4. Evolution of urea hydrolysis activity of Soybean leaching solution with soaking time

The relationship between the soaking time and the urea hydrolysis activity of the SLS was shown in Fig.4a, in which soaking time was 3, 6, 9, 12, 18 and 24h, respectively. With the increase of the concentration of SLS, the enzyme activity showed a linear increasing trend. The formula for the relationship between the concentration of SLS c_b and the enzyme activity U of soybean urease solution can be expressed as follows: $U=kcb$. The difference in slope K values was not large, very close to 0.07, as shown in Fig. 4b. Moreover, R^2 of the six fitted line segments were 0.9984, 0.9861, 0.9975, 0.9959, 0.9988 and 0.9924, respectively; this finding showed a satisfactory fitting accuracy. The reason for the above results was that the solubility of protein in water is faint [26]. Therefore, the soaking time had a negligible effect on urea hydrolysis activity. This means that the predecessors did not need to soak for as long as 24 hours for the urease hydrolysis activity test [27]. Experiments can be carried out to find a shorter optimal soaking time in the future.

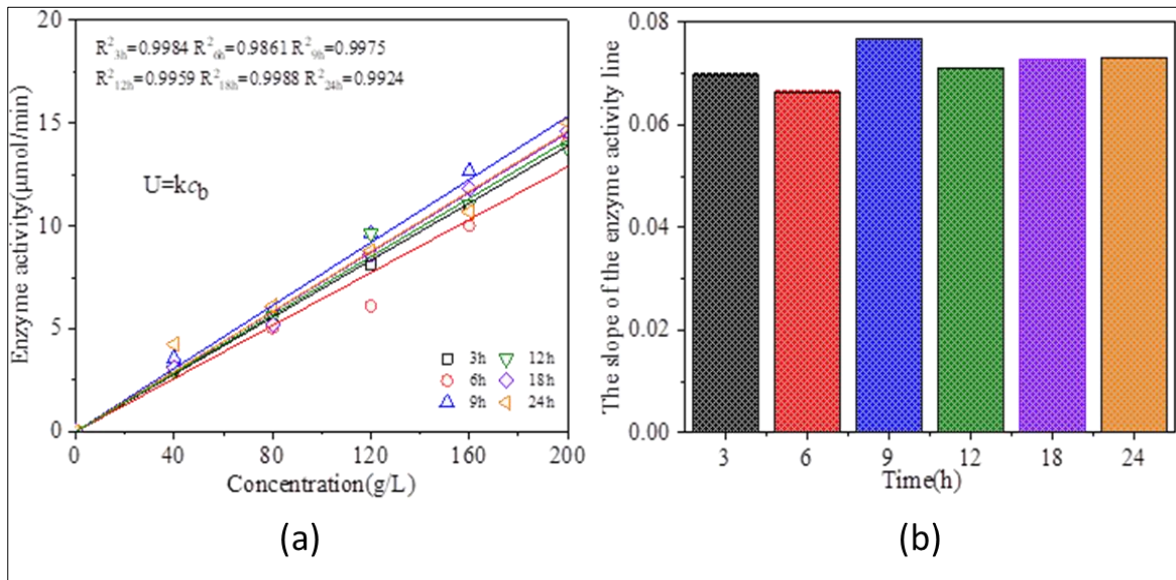


Figure 4 Effect of the soaking time on urea hydrolysis activity of Soybean leaching solution. (a) Enzyme activity of Soybean leaching solution with different concentration and (b) Linear slope of enzyme activity

3.5. Effect of heating treatment on urea hydrolysis activity of Soybean leaching solution

The change of temperature affects the urease activity and the degree of urea ionization. When the temperature increased from 10 °C to 20°C, the ionization constant of urea in the solution would increase about 10 times, and the faster the urea decomposition rate [28].

Seven groups of SLS with the same concentration were heated to 25, 35, 45, 55, 65, 75 and 90 °C in water bath for 10 minutes. Took it out and cooled it at room temperature, and its urea hydrolysis activity was measured. Under different heating temperature, the conductivity increased with reaction time (Fig.5a). In Fig.5b, the enzyme activity showed a slight upward trend and the U values did not differ much when the heating temperature was 25-65°C. Bei Wang et al. [29] reported that hydrothermal pre-treatment of bean dreg was conducive to promote the absorption for methylene blue. At 25°C, the enzyme activity was 44.88 $\mu\text{mol}/\text{min}$, about 13 times higher than that of microbial urease activity [28]. The enzyme activity decreased rapidly at 75 and 90 °C, which was caused by excessive temperature. Enzyme activity was shown at high temperature due to the high concentration of SLS [120 g/L] used in the experiment. It further illustrated that the concentration of SLS could affect urea hydrolysis more than heating temperature. Meanwhile, the response intensity of the urease activity in temperature may differ under different concentrations [24]. Yang Wang et al [30] showed that following with the increase in temperature, the urease activity of ureolytic bacteria is gradually increased. In addition, some scholars indicated that the optimum extraction temperature of soybean dreg is 50°C [31], and the optimum extraction temperature of soybean hulls is 25-55°C [12]. The results showed that the optimum heating temperature range of SLS catalyzed urea hydrolysis was 25-65°C.

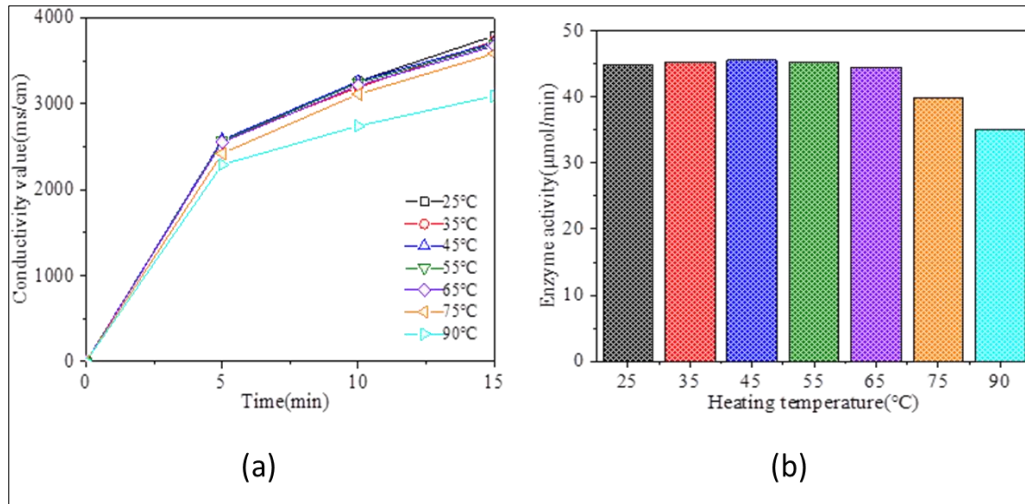


Figure 5 Effect of heating temperature on urea hydrolysis activity of Soybean leaching solution. (a) The conductivity changes with different heating temperature and (b) Enzyme activity at different heating temperature

4. Conclusion

This paper has demonstrated that soybean and black bean leaching solution showed high enzyme activity in different legumes and soybean-related parts, which are 3.78 and 4.49 $\mu\text{mol}/\text{min}$, respectively. Considering its economic efficiency, soybean was considered as the best source of urease. The urea hydrolysis activity has affected by the concentration of SLS, the reaction time of SLS and urea, and the heating temperature. The soaking time of the SLS does not impact the urea hydrolysis activity much. The results have shown that the higher the concentration, the shorter the reaction time, and the heating temperature range of 25–65 °C, which expresses better enzyme activity. SLS has a fine catalytic effect on urea hydrolysis, which provides a possibility to lead the production of soybean urease from bio-cemented calcium carbonate for production engineering and soybean-urea mixed fertilizer.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There are no conflicts of interest to declare.

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