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Preparation and Properties of Urease Immobilized onto Glutaraldehyde Cross-linked Chitosan Beads

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Abstract: Urease was immobilized onto the glutaraldehyde cross-linked chitosan beads that were prepared under microwave irradiation. The activity and the yield of activity of immobilized urease was 10.83 U/g B and 47.7%, respectively. The conditions of urease immobilization were optimized. The properties of the immobilized urease were investigated and compared with that of the free enzyme.

Keywords: Urease, immobilization, chitosan, bead, microwave.

Urease (urea amydohydrolyse, EC 3.5.1.5) is a highly efficient enzyme for converting urea to ammonium and carbon dioxide[1]. The enzyme plays an important role in the determination of urea in blood, urine and wastewater, in the process of dialysis for removal of urea from blood in the treatment of uremia, *etc*.[2].

The use of enzymes is often limited due to their high cost, unavailability, instability and difficulty of recovery from a reaction mixture. Although the immobilized enzyme usually shows lower catalytic activity than the free one, it is more stable, reusable, and in consequence less costly. Hence, up to now enzyme immobilization has been of great interest for many researchers[3-5].

The present paper reports the study of urease immobilization onto glutaraldehyde cross-linked chitosan beads prepared under microwave irradiation. In immobilization runs, urease was immobilized covalently onto activated chitosan *via* free amino groups of enzyme protein coupling with aldehyde groups of glutaraldehyde cross-linked chitosan beads. In order to design an effective immobilized enzyme matrix, the influence of several immobilization parameters in the immobilization were investigated, such as glutaraldehyde volume fraction [φ(GA)], urease/bead weight ratio (E/B ratio), processing time of immobilization, and pH of the reaction medium during immobilization. The kinetic parameters, properties and stabilities of immobilized enzyme produced under optimum conditions were determined.

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Experimental

Preparation of activated chitosan beads

0.1 g of chitosan was dissolved in 10 mL acetic acid solution of 1% volume fraction with stirring for 1 h. This solution was introduced in a 20 mL syringe and extruded through a 5 mm-diameter needle by compressed air. The droplets were pulled off in tripolyphosphate solution of 20 g/L, and the formed chitosan beads were then allowed to harden for 2 h. The beads were rinsed with deionized water till the solution became neutral. The chitosan beads were added in the flask containing 20 mL glutaraldehyde solution of 1% φ(GA). The flask containing mixture of reactant was placed in the reformed microwave oven. The mixture of reactant were irradiated under irradiation power 450 W for 2 min. The reaction mixture was allowed to cool to room temperature, washed several times with deionnized water to remove excess glutaraldehyde. The absorbance of the solution was continuously monitored with UV at 280 nm till the absorbance was less than 0.01 indicating glutaraldehyde was washed away.

Urease immobilization

The glutaraldehyde cross-linked chitosan beads were immersed in 5 mL urease solution with a given E/B ratio. The mixture was gently stirred for 10 min and then placed in refrigerator at 4℃ for 24 h. The supernatant was removed, and the beads were washed three times with deionized water. The immobilized urease was recovered from the solution and stored at 4℃ for next use.

Urease activity assay

The activities of both free and immobilized urease were determined by measuring the amount of ammonia liberated from the urease-catalyzed hydrolysis of urea per unit of time and was expressed in μmol NH_3 g^{-1} min^{-1} of urease(U/g E) and μmol NH_3 g^{-1} min^{-1} of urease-containing chitosan beads(U/g B) for free and immobilized urease, respectively. The reaction was carried out in the phosphate buffer of pH 7.0 at 37℃ for 30 min. The enzyme activity of the free and the immobilized urease was determined by NF Ⅷ method[6].

Urease immobilization were carried out in phosphate buffer under described conditions. Runs of immobilization were repeated twice and the average values are presented in the **Tables**. In the **Tables**, the yield of enzyme activity denoted the amount of enzyme activity immobilized to that added initially during the immobilization, and maximum activity%(MA%) was defined as the percent maximum activity achieved in a particular set of experiments.

Table 1 shows the effect of φ(GA) of glutaraldehyde solution used during the activation step on enzyme immobilization. Maximum activity of immobilized urease occurred at 1% φ(GA). This indicates that as more aldehyde groups are available on the activated chitosan beads, multiple-point attachments of urease molecules to the beads will likely occur and this leads to inactivation of urease.

Table 1 Effect of φ(GA) on activity of urease immobilization at E/B 10 mg/g, reaction time 24 h and pH 6.5

φ(GA) (%)	Activity(U/g B)	MA%
0.50	6.53	60.3
0.75	8.25	76.2
1.00	10.83	100
1.25	6.18	57.1
1.50	3.41	31.5

Table 2 Effect of E/B ratio on urease immobilization at φ(GA) 1%, reaction time 24 h and pH 6.5

E/B ratio (mg/g)	Activity (U/g B)	MA%	Activity yield (%)
5	5.89	52.2	49.3
10	10.83	95.9	47.7
15	11.14	98.7	39.8
20	11.29	100	28.0

Table 3 Effect of reaction time on activity of urease immobilization at φ(GA) 1%, E/B 10 mg/g and pH 6.5

Time (h)	Activity(U/g B)	MA%
12	8.27	76.4
16	9.48	87.5
20	10.17	93.9
24	10.83	100
28	10.61	98.0

The effect of E/B ratio with a constant beads weight of 1 g on urease immobilization was studied, and the results are shown in **Table 2**. After E/B ratio of 10 mg/g, the activity of immobilized urease did not significantly increased, while the yield of enzyme activity of immobilized urease decreased gradually. In this set of experiments the activity and the yield of enzyme activity for the immobilized urease were 10.83 U/g beads and 47.7% at E/B ratio of 10 mg/g, respectively.

The effect of processing time on the activity of immobilized urease is shown in **Table 3**. A processing time of 24 h was found sufficient to reach the maximum activity (100%). Extended processing time is not appropriate because enzyme denaturation or conformation changes in tertiary structure may occur during the immobilization reaction.

As seen in **Table 4**, the highest enzyme activity was obtained at pH 6.5. While it is decreased below or above that, especially in alkaline conditions. This may be due to urease denaturation at high or low pH.

The properties of the immobilized urease were investigated and compared with that of the free enzyme. The optimum pH value was 7.0 and 6.5 for the free and the immobilized urease, respectively. The optimum temperature was 60 ℃ for the free urease, and 65℃ for the immobilized enzyme. The Michaelis constant K_m was 12.5 mmol/L and 9.1 mmol/L for the free and the immobilized urease, respectively. The

immobilized urease retained 40% of its initial enzyme activity even after 10 repeated uses. The immobilized urease stored at 4℃ retained 46% of its initial activity even after 35 days.

Table 4 Effect of pH on urease immobilization at φ(GA) 1%, E/B ratio and reaction time 24 h

pH	Activity(U/g B)	MA%
5.3	10.35	95.6
5.9	10.66	98.4
6.5	10.83	100
7.0	9.94	91.8
7.4	8.35	77.1

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