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# Urease Immobilized on Polyethyleneimine Cotton Cloth

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## ABSTRACT

Jack bean urease was immobilized on polyethylenimine-coated cotton cloth by adsorption following by crosslinking with dimethyl suberimidate. Of the various methods used, crosslinking with dimethyl suberimidate was found to stabilize the enzyme with minimal inactivation. Cloth-bound urease showed a shift in pH optimum towards the acidic side without appreciable change in temperature optimum and thermostability. Cloth-bound urease could be used repeatedly for urea hydrolysis without appreciable loss in activity. Alternatively, urease cloth could be stitched in the form of a bag containing ammonia adsorbent and used for urea hydrolysis with simultaneous removal of ammonia.

**Index Entries:** Urease; immobilization; polyethylenimine; cotton cloth; crosslinking; dimethyl suberimidate; urea hydrolysis; ammonia adsorbent; urease bag.

## INTRODUCTION

Biocatalysts have been immobilized on inorganic, synthetic polymer, protein, and polysaccharide supports (*1–3*). These supports in granular or particulate form are often employed in both laboratory and industry either in packed bed or stirred tank reactors. The use of a particulate support has the limitations of high-pressure drop and impeded flow characteristics in packed-bed reactors and also exhibits difficulties in retrieval

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from stirred-tank reactors (*4*). These problems are enhanced when treating viscous, sticky, or particulate substrates as encountered in the food or waste treatment industries. In order to obviate these problems, major emphasis has been directed toward surface immobilization of biocatalysts on glass slides (*5*), or more usefully on woven or knitted fabric material (*6*). Woven materials like cloth not only represent a more compact structure devoid of fines, but could also be easily designed to suit various reactor geometries as well as analytical instruments.

The present paper describes a simple and useful method for immobilization of urease on cotton cloth and delineates its use as an easily retrievable support for the hydrolysis of urea.

## MATERIALS AND METHODS

Dimethyl suberimidate was obtained from Pierce Chem. Co. Rockford, IL, USA. Polyethyleneimine (PEI), Jack bean urease (230 $\mu M$ U/tablet) and glutaraldehyde were obtained from Sigma Chem. Co, St. Louis, MO. Cotton flannel (cloth) with a weight of 21 mg/cm$^2$ was procured from Bombay Dyeing Textile Mills, Bombay, India.

### Preparation of PEI Cloth

Washed and dried 5×5 cm$^2$ cloth pieces were soaked for 2 h in 0.2% PEI, pH 7.0 (adjusted using HCl). The cloth was then rinsed in distilled water and air dried.

### Immobilization of Urease on PEI Cotton Cloth Using Dimethyl Suberimidate

Polyethyleneimine cloth 5×5 cm$^2$ (6 pieces) was incubated with 12 mg enzyme in 30 mL distilled water for 2 h with gentle shaking. Dimethyl suberimidate dissolved in 0.1$M$ sodium phosphate buffer (pH 6.5) was then added to a final concn of 20 m$M$. The crosslinking was allowed to proceed for 4 h at room temperature with gentle shaking. The enzyme-linked cloth was then washed with 0.1$M$ sodium phosphate buffer, pH 6.5, and stored in the same buffer in the cold.

### Immobilization of Urease on PEI Cotton Cloth Using Glutaraldehyde

The enzyme was incubated with PEI cloth as described above. After 2 h incubation, glutaraldehyde (2% final concn) was added in the presence of 100 mg urea and the crosslinking was continued for 5 min.

Alternatively, PEI cloth was first treated with 2% glutaraldehyde for 2 h and washed with excess distilled water. Urease 12 mg/30 mL was then bound to glutaraldehyde-activated cloth by incubating at room temperature for 2 h. The enzyme bound cloth was washed as described earlier.

## Analytical Methods

Urease activity (soluble or bound) was assayed using 0.6% urea in 0.05*M* sodium phosphate buffer (pH 6.5), maintained at 25°C in a constant shaking water bath. The ammonia liberated was estimated using Nessler's reagent, and urea was estimated using dimethyl amino benzaldehyde reagent (*7*).

# RESULTS

## Optimal Conditions for the Immobilization of Urease

Adsorption of urease was optimal when the enzyme and PEI cloth were incubated in distilled water (pH 5.0). The optimum enzyme concentration was 2 mg/5×5 $cm^2$ cloth piece. Incubation of PEI cloth in enzyme solution for 2 h gave maximum binding of urease.

The retention of activity of various immobilized urease preparations is shown in Table 1. The enzyme was found to be adsorbed fairly strongly. However, continuous washing with 0.1*M* KCl was found to elute part of the activity. Similarly activity was found to also decrease during reuse. Crosslinking with glutaraldehyde to prevent desorption resulted in considerable loss of activity. Desorption could be prevented, however, by crosslinking with dimethyl suberimidate without excessive activity loss. Maximum retention of activity with minimum desorption was observed when the enzyme and PEI cloth were crosslinked with 20 m*M* dimethyl suberimidate for 4 h. This preparation was used in all further studies.

Table 1
Activity of Immobilized Urease Systems[a]

| Method of Immobilization | Activity, % |
|---|---|
| Cloth + Enzyme | 10 |
| PEI cloth + Enzyme | 80 |
| PEI cloth + Enzyme + 2% glutaraldehyde in the presence of 100 mg urea | 7 |
| PEI cloth + 2% glutaraldehyde + Enzyme | 23 |
| PEI cloth + Enzyme + dimethyl suberimidate | 43 |

[a] Soluble enzyme activity was taken as 100%. Immobilization of urease on PEI cloth using glutaraldehyde or dimethyl suberimidate was carried out as described in the text. In the case of cloth + enzyme or PEI cloth + enzyme, the cloth containing adsorbed enzyme was washed in 0.1*M* sodium phosphate buffer (pH 6.5) before assay.

## Properties of Immobilized Urease

The pH activity profile of urease was found to be shifted toward the acidic side on immobilization (Fig. 1). The $K_m$ for urea decreased from $40\times10^{-3}M$ to $10\times10^{-3}M$ on immobilization. A slight broadening of temperature optimum was observed on immobilization (Fig. 2). Thermostability was not appreciably changed on immobilization.

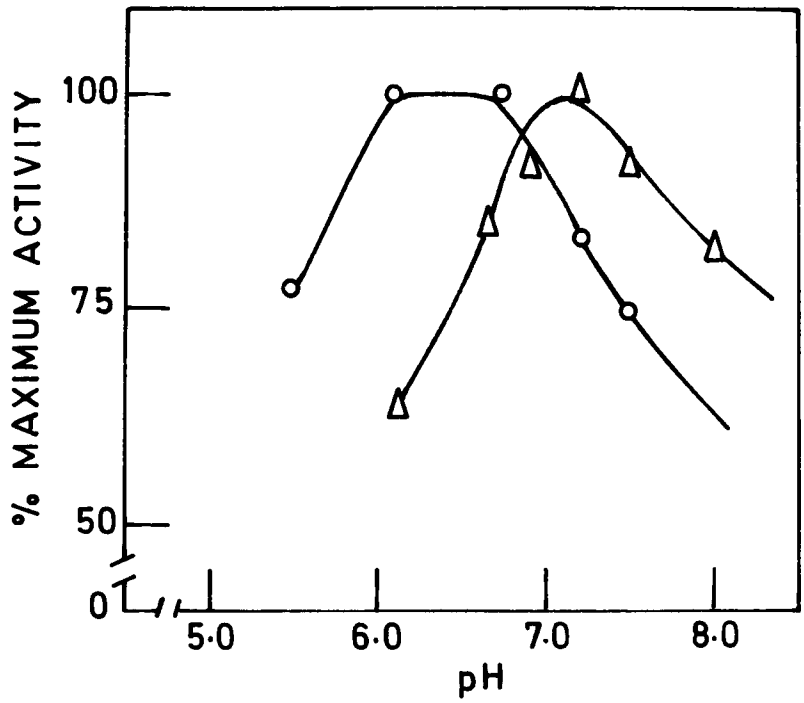


Fig. 1. Effect of pH on urease activity on bound urease. Enzyme assays were carried out using 0.05*M* sodium phosphate buffer. Activity obtained at optimum pH was taken as 100%. Soluble enzyme (△) and immobilized enzyme (○).

## Repeated Use

A 100-cm$^2$ piece of urease bound cloth was suspended in 0.6% urea in 0.05*M* sodium phosphate buffer (pH 6.5) for 30 min. The ammonia formed was estimated. The cloth piece was extensively washed and reused in fresh batches. Intermittently, the cl oth was stored in buffer at 0–5 °C. No significant change in activity was observed when the piece of cloth was used for 18 batches over a period of 3 wk (Fig. 3).

## Removal of Urea Using Urease Cloth Bag

Urea removal using urease bound cloth was studied in a batch reactor system. Urease bound cloth (200 cm$^2$) was suspended in 500 mL of 2% fertilizer urea in 0.05*M* sodium phosphate buffer (pH 6.5). The suspension was mixed by continuous shaking of the bioreactor on a water batch shaker at room temperature. At different time intervals, aliquots were removed for measurement of residual urea and liberated ammonia. The kinetics of urea hydrolysis and ammonia liberation are shown in Fig. 4. Over 90% hydrolysis of urea was achieved in 24 h (results not shown). The cloth suspended in the reactor was washed extensively and reused in

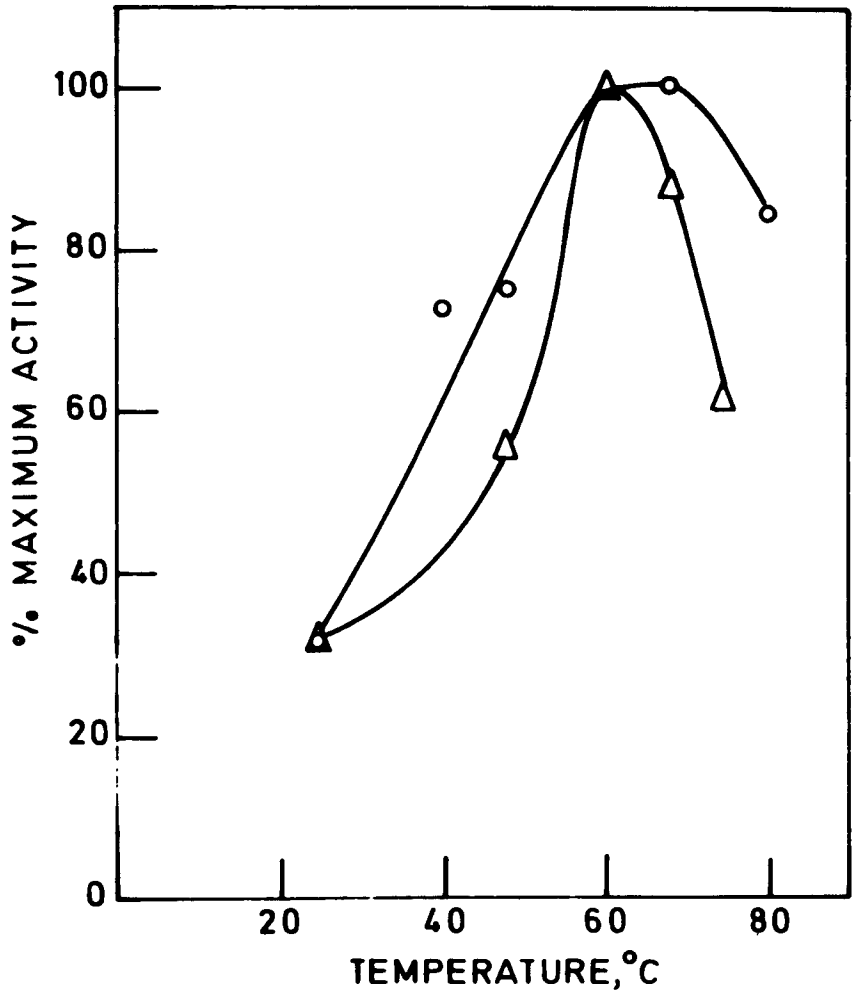


Fig. 2. Effect of temperature on urease activity. The enzyme assays were carried out as indicated in the text. Activity obtained at optimum temperature was taken as 100%. Soluble enzyme (△) and immobilized enzyme (○).

a fresh batch of 2% urea solution. The same cloth was used for 5 batches (each over 24-h period) without appreciable loss in activity.

The feasibility of hydrolyzing urea with the simultaneous removal of ammonia was also investigated. Urease-bound cloth (200 $cm^2$) was stitched in the form of a bag and filled with 5 g of cation exchange resin (Dowex 50w-x12). The bag was suspended in 500 mL of 2% fertilizer urea in 0.05*M* sodium phosphate buffer (pH 6.5). The urease cloth bag containing ammonia adsorbent could hydrolyze urea to the same extent as urease cloth. However, no significant amount of free ammonia was detected in the aqueous solution (Fig. 4).

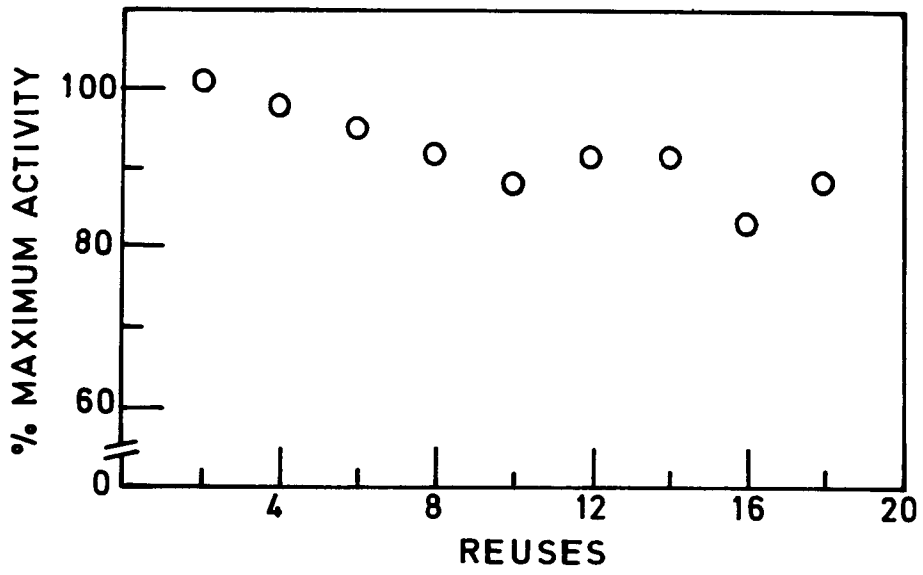


Fig. 3. Reuse of immobilized urease cloth.

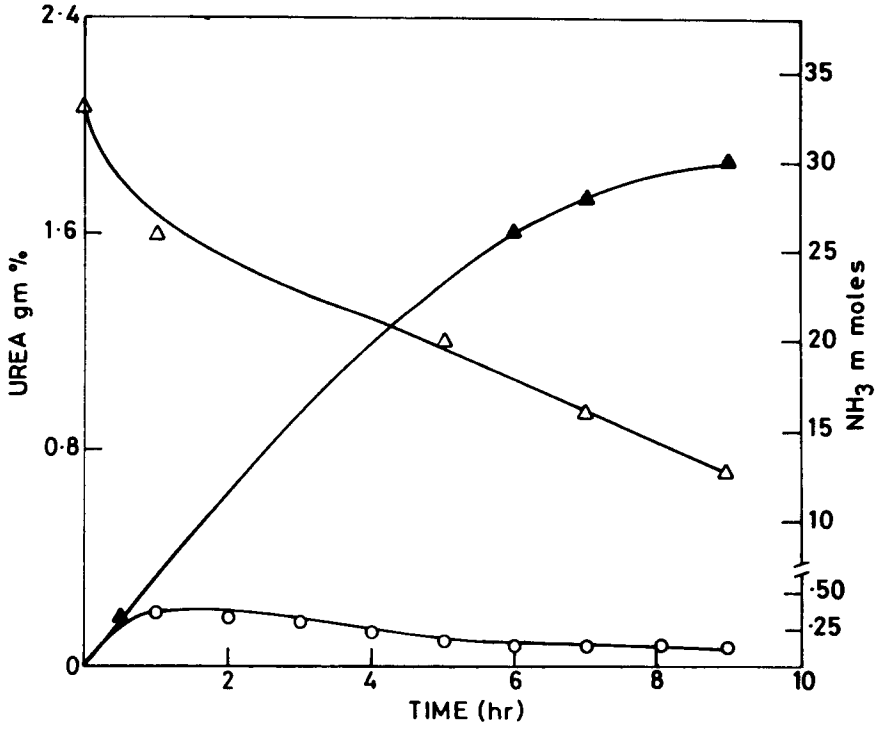


Fig. 4. Kinetics of urea hydrolysis by urease-bound cloth: urea concentration (△), ammonia concentration in absence of resin (▲), and in presence of resin (○).

## DISCUSSION

Cellulose has been widely used as a support for immobilization of biocatalysts, because of its ready availability and ease of derivitization (*1*). Cellulose in fiber form, however, has the limitations of attrition and compression when used in stirred or packed-bed reactors. In order to obviate these problems, enzymes have been immobilized on cloth treated to possess hydrophobic or anion exchanger characteristics (*8,9*). Polyethylenimine has been used for coating cellulose powder, cotton cloth, glass beads, as well as microbial cells, in order to convert them into anion exchangers (*5,8,10*). Enzymes of low p*I*, such as invertase and glucoamylase, are known to bind to the PEI-coated surfaces (*8,9*). In the present studies, jack bean urease, which as a p*I* of 5.0–5.1, was found to be effectively adsorbed on PEI cloth.

Enzymes adsorbed on PEI-coated surfaces have been normally stabilized by crosslinking with glutaraldehyde. Urease, however, is extremely susceptible to glutaraldehyde crosslinking, possessing essential -SH groups that are known to react with glutaraldehyde (*11*). The present studies also showed a considerable amount of inactivation of urease even when crosslinked in the presence of urea. Riesel and Katchalski have suggested the modification of -SH groups of urease with *p*-chloromercuribenzoate prior to glutaraldehyde crosslinking, followed by reactivation of the crosslinked enzyme by cysteine (*12*). However, in the present studies, dimethyl suberimidate was found to serve as useful for crosslinking. Dimethyl suberimidate reacts primarily with amino groups and has been extensively used in the *in situ* crosslinking of protein in active form (*13,14*).

The pH optimum of cloth-bound urease was found to be shifted toward the acidic side. The immobilization of an enzyme on any charged support generally causes a shift in the apparent pH optimum (*15*). The shift in pH optimum, observed in the present study, may perhaps be owing to the positive charge of the PEI-coated cloth. A similar shift toward the acidic side has been observed for an enzyme bound on polycationic carriers (*16*).

Urease has been immobilized on different supports with varying degrees of success (*11,12,15,17–19*). Immobilized urease has been examined for its use in analytical and biomedical applications and possible future potential for the treatment of urea effluents (*15,17,18*). The limitations of bound urease, for the treatment of urea effluent, is the need for bioreactors that have good flow properties, in view of the large volumes of waste streams to be treated, that at times may contain suspended particulate materials. Moreover, the immobilized urease system, as well as the bioreactor, should be able to release carbon dioxide and volatile ammonia freely without any gas build-up. Unlike the frequently used particulate supports, cloth-bound urease may be more suitable for designing such bioreactors for specific end use. In addition, because of the ease of incorporating ammonia adsorbents and simplicity of retrieval from suspended solids, it may find applications in the removal of urea in some industrial

processes, especially in the food industry. The principle of urea removal using urease and ammonia adsorbent resin has been successfully applied in biomedical applications (*19*).

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