A Comparative Kinetic Study of Free and Immobilized Urease on Commercial and Glutaraldehyde Activated Cotton

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Abstract: Urease was immobilized on commercial cotton and commercial cotton activated with glutaraldehyde. The kinetic of urea hydrolysis by free and immobilized urease was studied as a function of pH, temperature and time of hydrolysis. High concentration of ammonia was released at pH 10.0 for free enzyme (0.097 mg/mL) and urease immobilized on cotton (0.092 mg/mL) and pH 4.0 for enzyme, immobilized on activated commercial cotton (0.083 mg/mL). High concentration of ammonia was released at 30 °C for free enzyme (0.006 mg/mL), 25 °C for urease immobilized on cotton (0.043 mg/mL) and urease immobilized on cotton activated with glutaraldehyde (0.015 mg/mL). High concentration of ammonia was released after 10 minutes for free enzyme (0.0016 mg/mL), 60 minutes for inactivated (0.043 mg/mL) and for activated cotton (0.015 mg/ml). The result show that immobilized urease is less effective than the free enzyme and is more active in acid medium than the basic medium.

Keywords: Urease, cotton, immobilization, glutaraldehyde, hydrolysis.

1. INTRODUCTION

Urease (EC 3.5.1.5) is a nickel-containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide [1]. It occurs in bacteria, algae, fungi and higher plants. It allows the organism to efficiently use urea as a source of nitrogen. In plants, it is involved in nitrogen transport pathways and also act as a defense protein [2]. In human being, bacterial ureases are considered as important virulence factors in a number of diseases like the urinary tract infection, gastrointestinal region, and cancer [3]. The removal of urea from different environments has aroused biotechnological interest of researcher in this enzyme [4]. Its potential applications range from the treatment of industrial wastes or alcoholic beverages to the design of life-support systems for manned space missions [5-8]. Systems based on immobilized or microencapsulated urease are also being studied for use in hemodialysis [9]. The stability and activity of enzymes can be improved when they are immobilized. The concept of immobilization finds wide application in food industry, brewing, pharmaceutical, medicines, textiles, detergents [10]. Enzymes can be immobilized by several techniques like physical adsorption, cross linking, metal binding ionic binding, covalent binding and entrapment methods. The selection of method entirely depends on the nature of the enzyme and its potential application. A good carrier (matrix) certainly can enhance the operational stability of the immobilized enzyme system.

Barth and Michel [11] analyzed the activity of the 12 S unit of urease in the pH range 4-9 and found a sharp dependence of both *KM* and *vmax* on pH. Krajewska and Zaborska [12] investigated the effects of phosphate buffer on the kinetic behavior of free urease in the pH range 5.8-8.1. These results indicated that buffer can act as competitive enzyme inhibitors. Elcin etal immobilized Urease within polyanionic carboxymethylcellulose/alginate (CMC/Alg) microspheres coated with a cationic polysaccharide, chitosan (C). The optimal pH of urease was not extensively affected by the immobilization procedure.

However, the optimal temperature of urease activity increased up to 60 and 65°C within CMC/Alg and (CMC/Alg) microspheres, respectively, while the optimum temperature for the free enzyme was 50 °C [13].

Neufeld *et al.* encapsulated urease in alginate beads coated with chitosan, poly-L- lysine or poly (methylene-co-gaunidine) membrane to exclude alpha chymotrypsin and other proteases. Urease in uncoated alginate was highly susceptible to 21.6 Kda. Alpha chymotrypsin with 98% of the activity lost within 10 minute [14]. The present study was conducted to compare the catalytic activity of free and immobilized urease on commercial and glutaraldehyde activated cotton.

2. MATERIALS AND METHODS

2.1. Material

Cotton was obtained from local market at Peshawar, washed with distilled water and air dried. All

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ISSN: 1814-8085 / E-ISSN: 1927-5129/17

the chemicals used were of analytical grade and are given below in the Table **1**.

Table 1:

Chemicals	Company	Purity
Urea	BDH	
Urease (from jack bean)	AVON-CHEM	
H_2SO_4	Riedal-dettaen	98%
Na_2WO_4	SINO-CHEM	
Glutaraldehyde	BDH	25%

2.2. Method of Activation of Support

A known amount of cotton was soaked in known volume of glutaraldehyde for 2 hours. Then cotton was rinsed with known volume of distilled water three times, once with known volume of ethanol and dried in air.

2.3. Method of Immobilization of Urease on Cotton

A known weight of cotton and activated cotton was taken in a test tube. A known volume of urease solution prepared in phosphate buffer of pH 7.0 was added to test tube, the contents were agitated in a centrifuge at 20rpm for 10 minutes. The contents were placed at optimized temperature for optimum time for each carrier .The contents were filtered on filter paper (Whatman 41). The concentration of residual enzyme in the filtrate was determined spectrophotometerically at 500 nm by lowery method [15].

$$\text{Residual protien in filtrate (X)} = \frac{\text{Absorbance offiltrate}}{\text{Absorbance of test solution}} \times 100$$

% Immobilization yield = 100-X

2.4. Preparation of Standard Curve for the Determination of Ammonia

A pure ammonium sulphate solution (47.15mg ammonium sulphate in 250mL water) was prepared. Different aliquots of it were taken and the volume was made up to 3mL with water. To this 1mL of the Nessler's reagent was added and after mixing the color intensity was measured at 500 nm with UV-Visible spectrophotometer [16]. The standard graph was drawn as shown below.

2.5. Kinetics Study of Free and Immobilized Urease

Known amount of immobilized on activated, inactivated and free enzyme were taken in test tubes. 1mL of 150mM of urea solution was added to it and was incubated for various times. 0.3mL of 0.3% sodium tungstate and 0.3mLof 0.68M H_2SO_4 were added to the test tube in the specified time and the volume was diluted to 5mL. 1mL from it was taken, 1mL Nessler's reagent was added and the volume was diluted to 10mL. Absorbance of the solution was measured at 500 nm in a spectrophotometer. The amount of

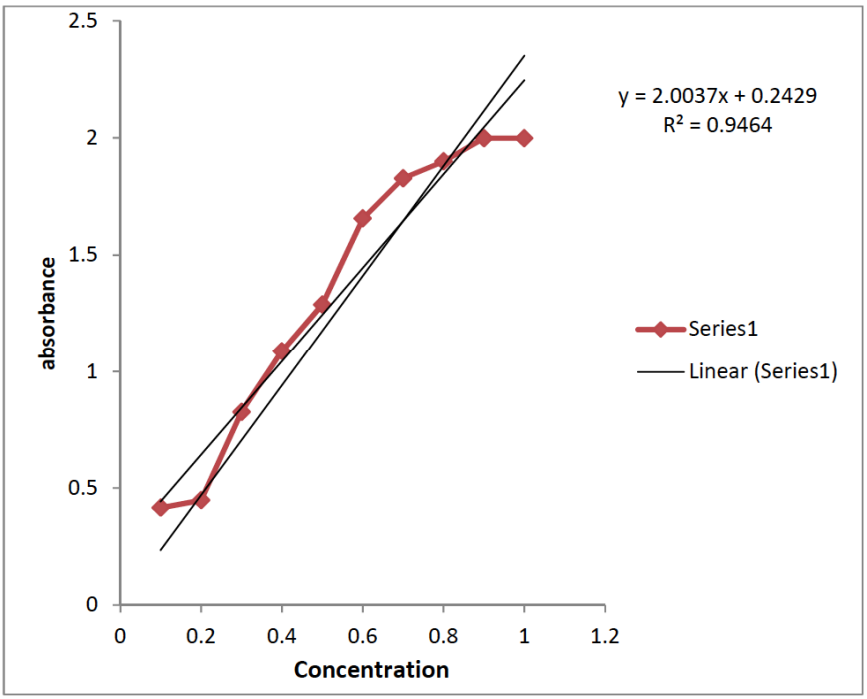


Figure 1: Standard plot of ammonium sulphate.

ammonia released was calculated by comparing the absorbance with the standard curve of ammonium sulfate.

3. RESULTS AND DISCUSSION

3.1. Immobilization of Urease

Urease was immobilized on cotton. The carrier was characterized by FTIR to know the functional moieties on the carrier for binding the enzyme. The spectra of cotton and cotton activated with glutaraldehyde are shown in the Figures **2** & **3** respectively. The various specification bands assigned and various groups are presented in the Table **2**.

3.2. Comparative Kinetics Studies of the Immobilized and Free Enzyme

The kinetics of urea hydrolysis by urease was studied as a function of time, pH, and temperature keeping the enzyme and substrate concentrations constant.

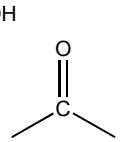
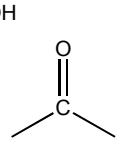
3.2.1. Effect of Time

Effect of time on urea hydrolysis by urease is shown in Table **3**. The high concentration of ammonia is released at 10 minutes for free enzyme (0.00158), 60 minutes for inactivated (0.0433) and activated cotton (0.0150).

3.2.2. Effect of pH on the Activity of Free and Immobilized Urease

A change in optimum pH occurs when an enzyme is immobilized on a matrix. This change in pH is helpful in understanding the structure–function relationship of enzyme .Thus; it is necessary to compare the activity of the free and immobilized urease as a function of pH. We studied the activity of all the three forms of urease in the pH range 4-10. The results are shown in Table **4**. The high concentration of ammonia is released at pH 10.0 for free enzyme (0.09712), pH 10.0 for inactivated

Table 2: FTIR Bands of Cotton and Activated Cotton

S. No	Carrier	Frequency (cm^{-1})	Functional group
1	Cotton	3342.64 1712.79	
2	Activated Cotton	3338.78 1716.65	

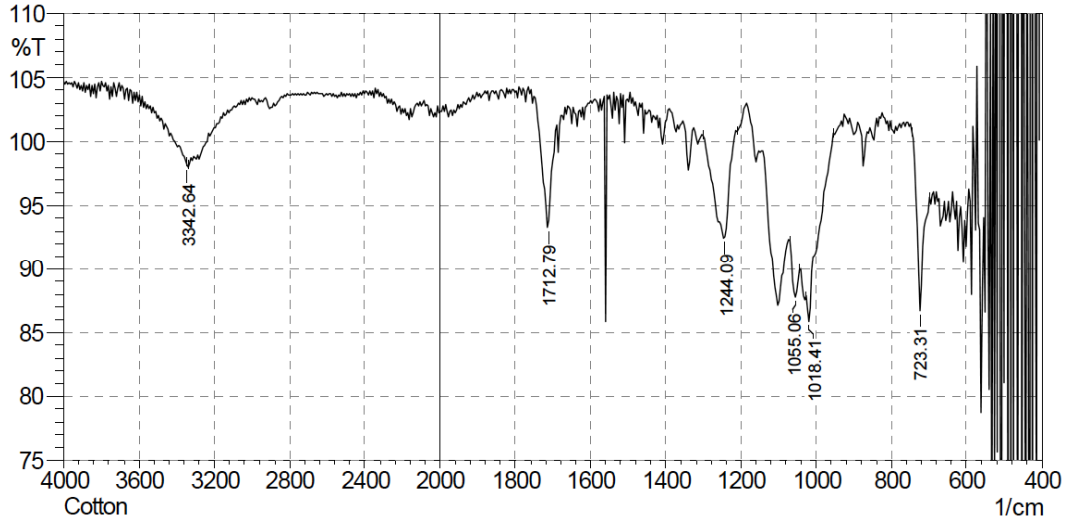


Figure 2: FTIR Spectra of Pure Cotton.

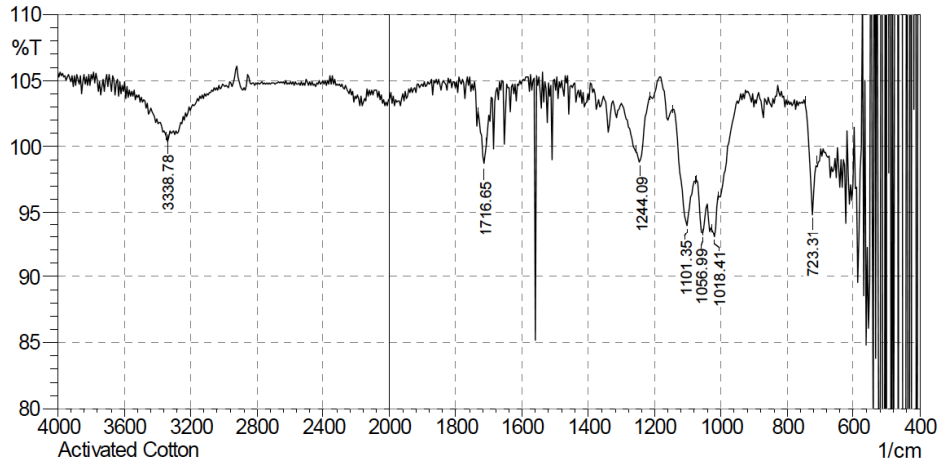


Figure 3: FTIR Spectra of Cotton activated with Glutaraldehyde.

Table 3: Effect of Time on Urea Hydrolysis by Urease at Cotton: 0.1g, pH: 7.0, Temp: 25°C, Urea Conc: 150mM

S. No	Time (minutes)	Free Enzyme (0.25mg/mL)	Immobilized Enzyme (Pure Cotton) (0.4mg/mL)	Immobilized Enzyme (Activated Cotton) (0.4mg/mL)
1	10	0.00158	0.00537	0.003877
2	20	0.000793	0.00387	0.001498
3	30	0.000705	0.01004	0.00202
4	40	0.000176	0.0191	0.00590
5	50	0.000440	0.0143	0.0105
6	60	0.000793	0.0433	0.0150

(0.0921) and pH 4.0 for activated (0.0828). It is clear from the data that activated immobilized urease show high activity toward the acidic side, around pH 4. This shift toward acidic pH upon immobilization could be because of the urease-catalyzed degradation of urea into ammonia and carbon dioxide. The liberated ammonia increase the local pH, and due to the diffusional constraint of the support retaining a higher concentration of enzyme product, in the vicinity of the support that adsorbed enzyme favors the shift in optimum pH toward acidic side. Several such reports are available with similar observations upon immobilization of urease and other enzymes [17]. Thus, the microenvironment around the enzyme was more alkaline than that of the bulk solution [18].

3.2.3. Effect of Temperature on the Activity of Free and Immobilized Urease

The rate of the enzymatic reaction increases usually with the increase in temperature.

At higher temperatures, beyond a certain limit, the counteracting force of protein denaturation becomes prevalent, which leads to severe decrease in the activity. In order to examine the effect of temperature the catalytic activity of the free and immobilized urease was studied over a temperature range of 25–35°C. The results are shown in Table **5**. The high concentration of ammonia was released at 30 °C for free enzyme (0.00616), 25 °C for inactivated (0.0433) and 25 °C for activated (0.0150). No significant differences were

Table 4: Effect of pH on Urea Hydrolysis by Urease at Cotton: 0.1g, Time: Optimized Time, Temp: 25°C, Urea Conc: 150mM

S. No	pH	Free Enzyme(0.25mg/mL)	Inactivated Immobilized Enzyme (0.4mg/mL)	Activated Immobilized Enzyme (0.4mg/mL)
1	4.0	0.0423	0.00185	0.0828
2	7.0	0.00158	0.0433	0.0150
3	10.0	0.09712	0.0921	0.07535

Table 5: Effect of Temperature on Urea Hydrolysis by Urease at Cotton: 0.1g, Time: Optimized Time, pH: 7.0, Urea Conc: 150mM

S. No	Temperature(°C)	Free Enzyme (0.25mg/mL)	Inactivated immobilized enzyme (0.4mg/mL)	Activated immobilized (0.4mg/Ml
1.	25	0.00158	0.0433	0.0150
2.	30	0.00616	0.003877	0.00440
3.	35	0.00185	0.0110	0.01128

found in the activity of free and immobilized enzyme. It has been found that free urease loses its activity above 70°C [17].

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Received on 11-02-2017 Accepted on 20-03-2017 Published on 19-06-2017

https://doi.org/10.6000/1927-5129.2017.13.53