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Activity and Stability of Urease Enzyme Immobilized on Amberlite Resin

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ABSTRACT

Immobilization of enzymes is a good field of study to expand the life of enzyme in and lowering the cost of the chemical processes such as separation processes. Urease is an important enzyme with medical and industrial applications. The aim of the present study is to prepare an immobilized urease on a strong cation exchange resin (Amberlite IR120 - Na) and study of its activity and stability. We monitored the liberation of Na ions in the collected fractions and searching for protein in the fractions as an indicators of immobilization by ion exchange phenomenon. Sodium is measured using atomic absorption spectroscopy techniques, while protein tested by Bradford's method. Immobilized urease activity was evaluated by salicylate-hypochlorite method. The results indicated a complete immobilization of urease enzyme on the resin surface with reserving 92% of the activity of free enzyme. The immobilized urease enzyme on resin showed good stability and it has a 62% of its activity after 154 days of storage at room temperature. It is concluded that a new immobilized urease enzyme system is prepared with good enzyme activity and stability.

Key words: Amberlite IR120 Na resin, urease, enzyme, immobilization.

INTRODUCTION

Immobilization of enzymes on different surface is an important to increase the life and stability of the enzymes and to decrease the cost of the enzyme reactions[1]. Urease (EC 3.5.1.5) is an enzyme that convert urea to CO_2 and ammonia [2]. Immobilized urease on various materials have many classic applications of immobilized enzymes[3,4,5,6]. Furthermore, many new applications of urease immobilization are introduces including hemodialysis membranes [7], enzymatic bioelectrochemical devices [8], amperometric biosensor for the quantitative determination of urea [9], urea biosensors [10], and study of protein-surface interaction [11]. The enzyme may be immobilized in several ways such as adsorption on a surface where the enzyme is bound to an inactive substance[7] or by ion exchanging [8]. Some immobilization processes reduce the enzyme activity [12]. Different proteins were found to be adsorbed on the adsorbing surfaces with various findings and interactions [13,14,15]. Previously, in our laboratory, we had succeeded in the immobilization of urease enzyme on anionic exchange resin with good stability and activity [16]. However, when immobilization occurred on the nanoceria surface, urease structure lost some of its secondary and tertiary structures [17]. This work aims to identify the ability of urease enzyme to be active when it immobilized on a strong cation exchange resin (Amberlite IR120 Na resin) and study of some properties of immobilized enzyme.

MATERIALS AND METHODS

Preparation of Immobilized Enzyme on cation exchange resin

Twenty five milliliters of Amberlite IR120 Na resin (Rohm and Haas Inc. USA) were put in a glass chromatography column after swelling in 75ml of distilled water and regenerated by 1M hydrochloric acid solution. After incubation for 2 hours, it washed carefully with distilled water until neutralization. The urease enzyme (0.8 mg protein in 0.2 ml of distilled water) was added to the top of the column. The mixture was incubated for 45 minutes at room temperature in order to obtain equilibrium and complete the interaction between the enzyme molecules and the resin. Then, the fractions were collected from the column in 2 ml for each fraction at a flow rate of 1 ml/minute. The concentration of Na ions, H ions and protein were measured in each fraction. Presence if these ions and

lacking the protein in the fractions indicates the immobilization of protein molecules on the surface of resin in exchange with hydrogen or sodium ions, which will appear in the fractions.

Measuring of proteins in fractions

The Bradford method [18] was used for estimation of amount of protein (urease enzyme) in solution and the difference before and after immobilization is equal to the immobilized enzyme on the cationic resin.. We used the albumin as a standard at a concentration ranged from 5 to100 µg in 100µl. The protein solution was added onto five milliliters of Comassie blue dye were added and after incubation for five minutes at room temperature, the absorbance was measured at 595nm [18]. Absence of protein in the collected fractions indicated the immobilization of added enzyme.

Assay of Urease activity

In order to see if the enzyme still active after immobilization, we measure the urease activity after adding urea as substrate and measuring the ammonia liberated from the hydrolysis of urea by the action of urease. Briefly, two milliliters of urea solution (20 mM urea in 2 mM $CaCl_2$) as an enzyme substrate was added on the top of the exchanger and incubated for 30 minutes at room temperature. The immobilized urease will hydrolyze urea into ammonia and CO_2. Then we collect these fractions that contain ammonia (1ml) and incubated with 1ml of Reagent A (62 mM of sodium salicylate, 3.4 mM sodium nitroprusside, and phosphate buffer 20 mM (pH=6.9)) for 10 minutes. Then, the mixture mixed thoroughly with 1ml of Reagent B (sodium hypochlorite 7 mM and sodium hydroxide 150 mM) and incubated for 10 minutes at room temperature. Ammonium ions will react with salicylate in the presence of sodium hypochlorite to produce indophenol, which absorb light at 600 nm. The absorbance of the produced green color that measured spectrophotometrically which is proportional to the amount of concentration of ammonium ions liberated from the hydrolysis of urea by urease [19].

Estimation of sodium by atomic absorption spectroscopy

Sodium concentration in solution was determined by atomic absorption spectrometry [20] after constructing a calibration curve from six known sodium solutions from 0.01 to 1.0 mg/L. The blank was double deionized water to set the digital display to zero.

Stability of the immobilized urease

The activity of immobilized urease was measured weekly to estimate the stability of the complex as cited in the previous paragraph.

RESULTS AND DISCUSSION

The results of the immobilization process, as estimated by Bradford's test method indicated lack of protein in the collected fraction after addition of urease to the resin column. This results revealed a complete immobilization of urease on the resin (negative test). Furthermore the decreased pH on the collected fractions indicated occurrence of ion exchange between urease molecules and the hydrogen ions from the surface of resin. Addition of distilled water to the mixture leads to evacuation of acidic solution until it became neutral. This results refers to a strong interaction between the resin and protein. Furthermore, sodium ions also liberated from the exchange process. Figure 1 showed the liberation of sodium ion after adding the urease solution on the resin as a result of another ion exchange with the urease molecules.

The urease action on the urea was followed by salicylate-hypochlorite method and showed the ability of immobilized urease to hydrolyze urea. Figure 2 showed the concentration of ammonia liberated by the action of immobilized urease on urea substrate. This result indicated an interaction between the resin and urease by sites far away from the active site and the immobilization reserve the enzyme active site intact. The ability of urease to be immobilized with reserving its activity was seen previously with different stationary phases [2,4,5,16]. Furthermore, in a separate experiment on free urease, the activity of immobilized enzyme is about 92% of the activity of the free enzyme. This reduction in activity can be explained by the liberation of hydrogen ions leading to slight increase in acidity that may affecting the activity of urease. Previous work showed 87% activity of the free enzyme after immobilization of urease on the Dowex resin (strong anion exchanger).

[16] This results indicated a different behavior of the urease on different surfaces according to the charges on the surface of resin.

Even though, immobilized enzyme has lower catalytic activity comparing with free enzyme, it has more stability, reusability, and low cost effective [21,22]. This result indicated an interaction between the resin and the sites beyond the active site of the enzyme. Hence, the activity is not affected dramatically due lack of involvement of the active sites in the exchange process. The slight change in activity due to the change in spatial configuration and the orientation of groups beyond the active site. In another study, for comparison, urease covalent immobilization on the template of polysiloxane was reported to be less efficient due to the lack of the enzyme activity. [23] it is found that sloping immobilized urease presents higher temperature resistance, higher similarity to the substrate, and higher constancy of operation [24]. In some previous works[25], the immobilization of urease enzyme needed a chemical cross-linking using formaldehyde, glutaraldehyde and chromium (III) acetate while in the present work, there is no need for this treatment and the urease still binds to the resin tightly. Our study is safer as chromium ion is toxic and some important application of immobilized urease is for reducing urea levels in uremia patients.[26] Many factors affecting the activity of protein adsorbed on any surface. It is found previously that high ionic strength produce huge interaction between protein and the adsorbing surface [27]. The activity of immobilized urease on strong cation exchange resin in hydrogen from showed good stability but liberation of Na during exchange increased the acidity.

The results of immobilized enzyme activity expressed as ammonia concentrations with time are presented in Figure 3. The immobilization of urease on the Amberlite IR120 Na resin is easy, and the enzyme still active with good stability even after 154 days of incubation in distilled water. The enzyme has 62.07% of its activity after 154 of incubation in the sterile condition to keep it free from contamination with foreign organisms. In previous study, The immobilized urease on Dowex resin showed good stability and slight change noticed in its activity after 15 days of storage at room temperature. However, this study did not monitor the activity of immobilized enzyme for a longer time as done in our study. The thermal stability of the immobilized urease was higher than free enzymes in terms of the pH and the stop action of some metal ions or organic substances. The stability

over time of the immobilized urease is high, its enzymatic activity was stable at 85% of the first value three months after synthesis [28].

CONCLUSIONS

A new immobilized urease enzyme on the strong cation exchange resin (Amberlite IR120 Na resin) in sodium form is prepared with good enzyme activity (92% of free enzyme activity) and stability up to 154 days.

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Conflict of interest

The authors declared no conflict of interest.

Author's contributions

All authors have participated in the work and preparation of the manuscript.

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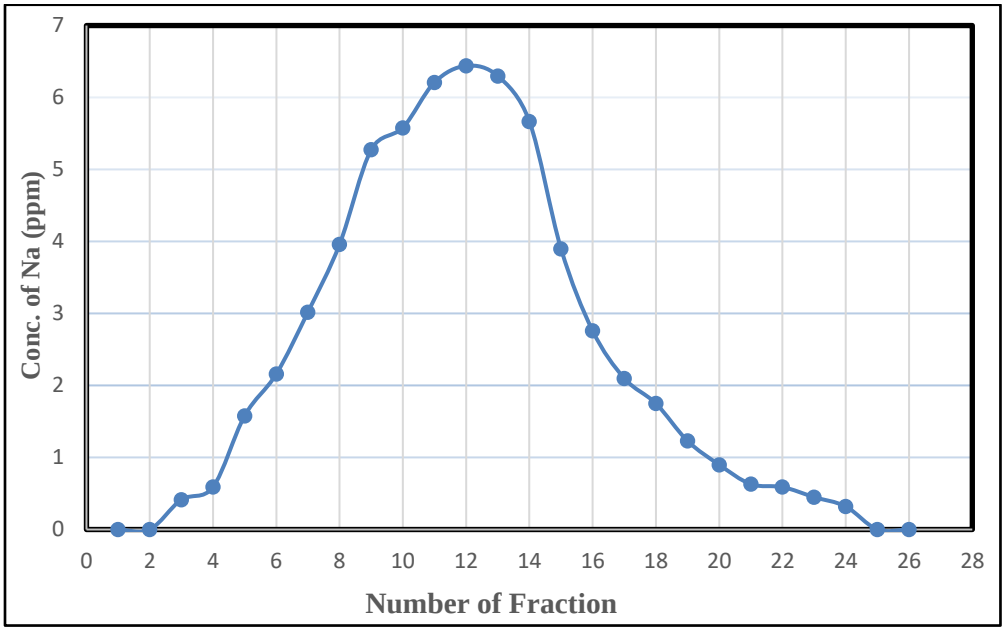

Figure 1: The liberated sodium concentration in the collected fractions after exchange with the urease enzyme on the surface of Amberlite.

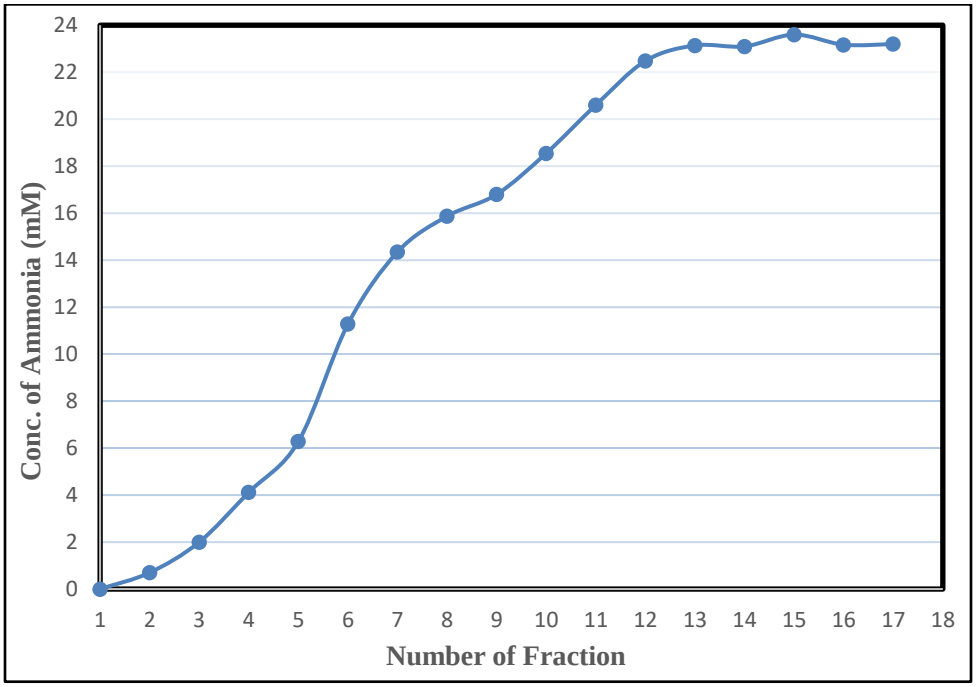

Figure 2: Ammonia concentrations in the fractions collected after adding urea solution to the immobilized urease enzyme on the Amberlite resin.

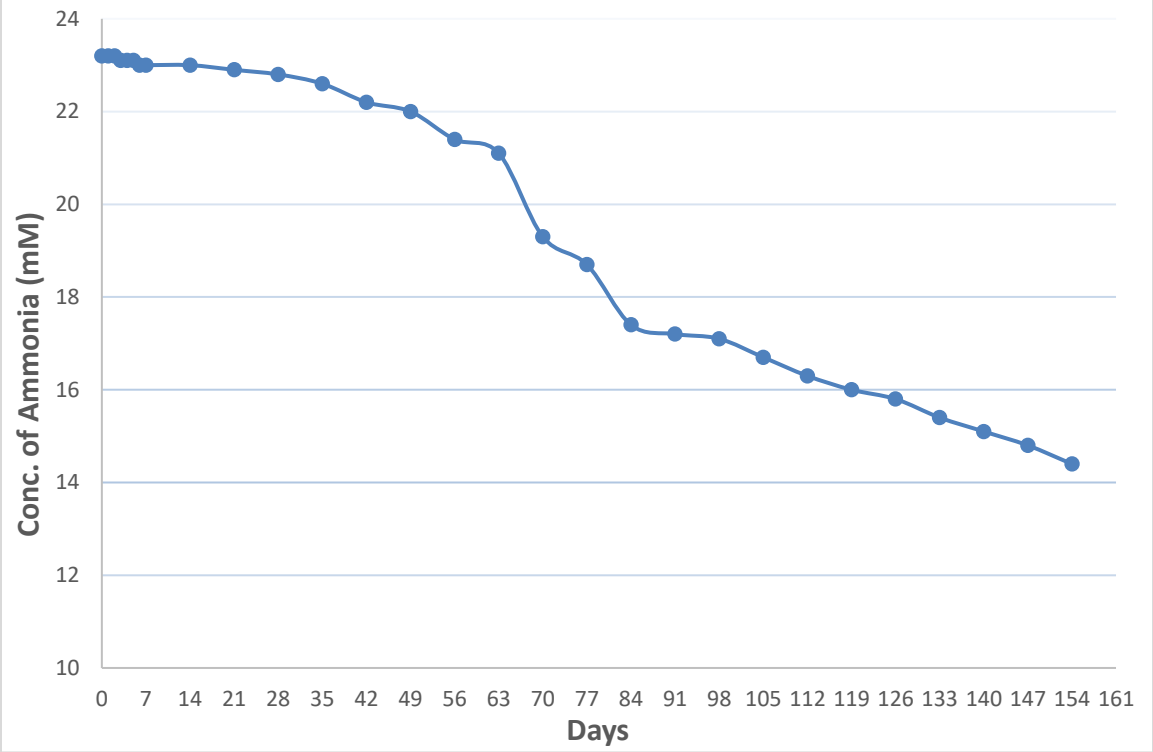

Figure 3. Ammonia concentration as function of immobilized urease activity with time of storage.