Investigation for the Alteration in Some Kinetics Parameters of Free and Immobilized Cellulase from Local Fungus *Trichoderma viride*

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Abstract

Investigations were carried out to study the immobilization behavior of cellulase *Trichoderma viride* on polystyrene an-ion nature of the AM-21-A, and cat-ion exchange fiber VION KN-1.Immobilization of cellulase change the values for K_m which were $2.5x10^{-5}$ ، $4.7x10^{-5}$, $6.8x10^{-5}$ for free and immobilized enzyme on VION KN-1, AM-21-A respectively, and V_{max} was 75,59,33 $g/lmin^{-1}mg^{-}$ for free and immobilized enzyme on VION KN-1, AM-21-A respectively. The optimum temperature of the activity of the cellulase was affected by immobilization where the enzyme showed maximum activity at 50 ,60 ,70 C˚ for free and immobilized enzyme on VION KN-1, AM-21-A respectively, however, the immobilized cellulase showed a higher thermal stability, with respect to temperature, compared to the free cellulase.

Key words: kinetics parameters, Immobilized cellulase, *Trichoderma viride*.

البحث في التغيرات التي تحدث في بعض الصفات الحركية لانزيم السيليليز الحر والمقيد من الفطر المحلي Trichoderma viride

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الخلاصة

اجريت دراسة حول التغيرات التي تحدث على انزيم السيليليز بعد تقييده باستخدام مادتين ايونيتين سالبة وموجبة . وقد وجد ان هنا ك اختلافا في قيم بعض المؤشرات الحركية بين الانزيم الحر والمقيد فيما يخص قيمة ثابت ميكاليس حيث كانت

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$6.8x10^{-5}$, $4.7x10^{-5}$ ، $2.5x10^{-5}$ والسرعة القصوى للتفاعل $g/lmin^{-1}mg^{-}$ 75,59,33 للانزيم الحر والمقيد على التوالي.اما درجة الحرارة المثالية لفعالية الانزيم ايضا تأثرت بعملية التقييد وكانت 50,60,70 م ه للانزيم الحر والمرتبط على التوالي حيث تبين الثبات والاستقرار الحراري للانزيم المقيد مقارنة بالانزيم الحر .

الكلمات المفتاحية : المؤشرات الحركية ، السليليز المقيد ، *Trichoderma viride*.

Introduction

Cellulase is a generic name for the group of enzymes which catalyze the hydrolysis of cellulose and related cellu-oligosaccharide derivatives .Enzyme, cellulase finds wide application to a variety of fields such as textile, paper and pulp, food and animal feed, fuel and chemical industry. Additionally, they can be used in waste management, pharmaceutical industry, protoplast production, genetic engineering and pollution treatment [1]. The main problems of using the enzymes industrially are the difficulty of their separation from the solution and their inactivation by organic solvents and extreme pH or temperatures. Novel designs with immobilized enzymes and without need of separation are of major concern. This also reduces the loss of enzymes and offers the opportunity to use a continuous reactor with a reuse of the enzyme for many reaction cycles, thus lowering the total production cost of enzyme mediated reactions [2]. Many immobilization methods have been tried, ranging from covalent attachment to adsorption or physical entrapment. There are many methods for immobilization enzymes, one of them is adsorption method which is considered as a simple method for immobilization as well as the immobilized enzyme maintain a large percentage of its ability for analysis, cheaper method compared with other methods and it can be used many times [3].So the objective of this study is to use for the first time solid carriers AM-21-A , VION KN-1 for immobilizing cellulase using adsorption method and evaluate its kinetic parameters.

Material and Method

Enzyme cellulase was procured from ***Trichoderma viride*** in the previous study [4]**.** Cellulase immobilized on polystyrene an ion nature of the AM-21-A,and cation exchange fiber VION KN-1, preparation of ion exchange resins for immobilization was carried out by conditioning of ion

exchangers and transferring them to the desired shape of the ion exchange [5,6]. For immobilization of sorption carrier 5g allowed to overnight at room temperature in 50 ml of acetate buffer (pH 5). To a suspension of ion exchanger was added 5 ml of enzyme in a flask and stirred with a power mixer for 1.5 hours at 25 ° C, more centrifuged for 5 min at 250 g. For covalent immobilization of the method glutaraldehyde 2.5g of resin was left overnight at room temperature in 20% glutaraldehyde solution with ratio 1:1 with a stoppered. Thereafter, the carrier was washed with distilled water, was added 2.5 ml of an enzyme solution (pH 5) and incubated overnight in the closed vessel. The immobilized preparation obtained during the sorption and covalent immobilization, washed with acetate buffer until no protein in the washings. Monitoring was carried out in a spectrophotometer SF-46 at $\lambda = 280$ nm. In order to enhance the catalytic activity of cellulase we used a modified glutaraldehyde method. The experiment was conducted according to the following procedure: To 2 g of anion exchanger was added 45 ml of a 4% solution of succinic anhydride in chloroform and refluxed for 4.5 hours, the mixture was further incubated for another 16 hours at room temperature. The resin was washed chloroform and air dried, and then 10 ml of thionyl chloride and refluxed for 30 minutes. To the washed with toluene and dried carrier was added drop wise 20 ml of ethylenediamine, maintaining the temperature at 20 ° C, and the mixture stood 20 hours, it was washed 10 times with distilled water, 5 times - ammonia solution (3%) and then - water. To 10 ml of ion exchanger solution of glutaraldehyde (2%) and stirred with a magnetic stirrer for 3 hours at 50 ° C. The resin was separated from the solution and washed. . Determination of enzyme activity.Enzyme activity was determined using spectrophotometer by the method of Mandels*et al*, [7].The relative activity of immobilized cellulase is the ratio of the specific activity of the immobilized enzyme to that of the free enzyme under the same conditions. The activities measured and plotted in the figures are the result of the average of the triple measurement. By using the Lineweaver–Burk plot the Km and Vmax values for cellulase in both situation (free and immobilized) were studied.

Results and Discussions

The *K*m values signify the extent to which the enzymes have access to the substrates [8]. Km, *V*max values for the free and immobilized cellulase shown in table1.

Table 1: The values of Km and Vmax for the hydrolysis reaction of Carboxymethyl cellulose.

Enzyme	Km, g/l	V_{max} g/lmin^{-1}mg^{-}
Free Cellulase	2.5x10^{-5}	75
Immo.En.VION.KN.1	4.7x10^{-5}	59
Immo.En.Am-21-A	6.8x10^{-5}	33

These values showed That immobilized cellulase had a higher Km and lower Vmax, while the free cellulase had lower Km and higher Vmax ,the importance of Km represent higher affinity between enzymes and substrates while Vmax indicates the difficulty of enzyme substrate interaction.Increasing Km after immobilization may be due to diffusional resistance of the carrier against substrate and/or product.The low value of Vmax for both the immobilized enzyme indicates the difficulty of enzyme substrate interaction. This suggests that the immobilized cellulase on AM-21-A has more affinity towards the substrate than VION KN-1 and the enzyme is less active in catalyzing the degradation of methylcellulose [9].Thus, a comparative analysis of the basic kinetic parameters enable us to suggest that the greatest affinity for the substrate exhibits cellulase, adsorption associated with the ion-exchange fiber VION KN-1. In this case, optimal and constitute 4.7x10^{-5}g/l and 59 g/lmin^{-1}mg^{-}, respectively. Clearly, ion-exchange fiber VION KN-1, first, quite firmly associated with cellulase, providing better thermal stability of the protein, and secondly, breach of the native conformation of the subunits of cellulase in comparison with the previous carrier are expressed to a lesser extent, as the immobilized enzyme exhibits a sufficiently high catalytic ability after interaction with the matrix fibers.

Thermal Stability of the Immobilized Enzyme:

Temperature increase the affinity of cellulase to cellulose and also increase the relative adsorption of endoglucanases and cellobiohydrolases [10].So Basic anion exchange polystyrene nature of the AM-21-A, and cation exchange fiber VION KN-1 shown that cellulase immobilized on VION KN-1, retained 57% of the activity of free enzyme, while the adsorption immobilization exchanger AM-21-A cellulase retained 42% of the catalytic activity compared with soluble enzyme (Figure1**).**

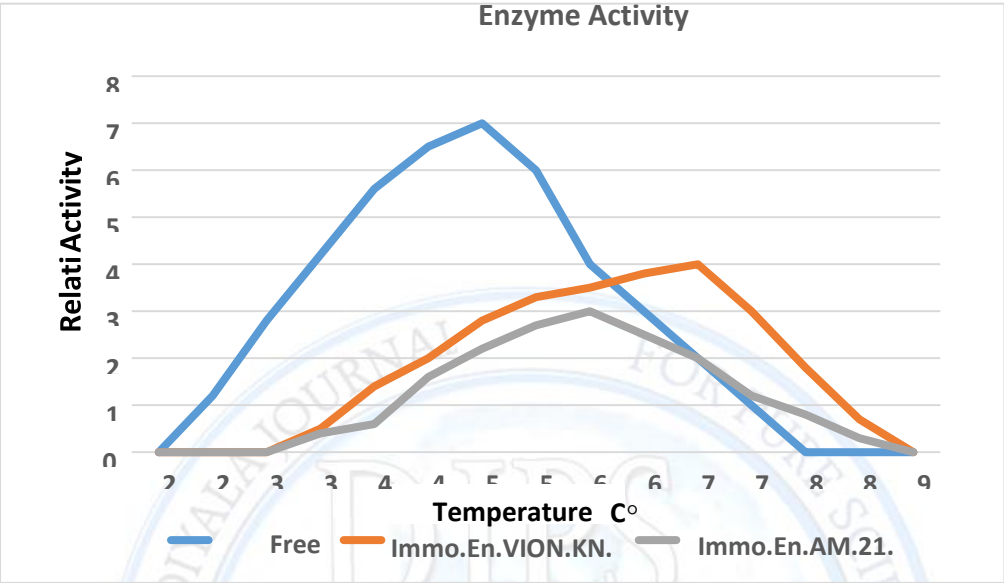

Figure 1: Effect of temperature on free and immobilized cellulose

Also in order to understand the effects of different temperatures on the conformation of macromolecules *Trichoderma viride* cellulase experiments were conducted to study the thermal stability of the enzyme.From figre1 we can observed that the increasing in the rate of inactivation of the enzyme with increasing temperature from 60 C˚ to 80C ˚ C for *Trichoderma viride* cellulose which can be explained by the fact that the excess thermal energy causes the destruction of the hydrophobic interactions that are making an important contribution to protein stability, resulting in a better deployment of the polypeptide chain.Thus, the process of inactivation of cellulase is complex, and mechanisms for the enzymes from different sources are not identical. Immobilization imparts greater rigidity of the polypeptide chain of protein and thus increases its stability.

Conclusion

Cellulase immobilized on granular and fibrous ion exchangers AM-21-A, VION KN-1 respectively .Adsorption method for cellulase is simple and Suitable for immobilization. Immobilized enzyme showed thermal stability higher than free enzyme, however the ion

exchange fiber VION KN-1 allows us to recommend it for reuse in the laboratory and industrial conditions.

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