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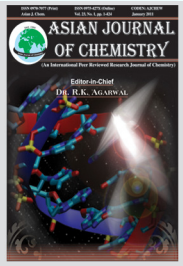

Adsorption Isotherm and Kinetic Modelling of β-Galactosidase Immobilization onto a Basic Resin (Duolite A568)

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This study aimed to determine adsorption characteristics of *Aspergillus oryzae* β-galactosidase onto a basic resin Duolite A568. The experimental data were analyzed by the Langmuir and Freundlich isotherm to describe the adsorption equilibrium. The equilibrium data fitted well with the Langmuir model which confirmed that the Duolite A568 was favourable for adsorption of β-galactosidase enzyme under conditions studied. The maximum adsorption capacity was found to be $(5.1 \pm 0.49) \times 10^{-2}$ mg/g at 35 ºC. The kinetic data were fitted to pseudo-second-order kinetic model of Ho by linear and non-linear regression methods. The enthalpy change (ΔHº), the entropy change (ΔSº) and the Gibb's free energy change (ΔGº) for the sorption processes were calculated to be 15.5 kJ/mol, 30.4 J/mol K and -9.4 kJ/mol, respectively. The positive ΔHº value indicated that the adsorption process was endothermic. ΔGº and ΔSº values showed that adsorption process occurred by physical mechanism and spontaneously.

Key Words: β-Galactosidase, Adsorption kinetic, Duolite A568, Freundlich and Langmuir isotherms, Activation energy.

INTRODUCTION

Biocatalyst immobilization is gaining increased attention for the synthesis of several functional foods such as low lactose milk. Hydrolysis of lactose makes milk consumption easier for the people who suffer from lactose intolerance and also improves product sweetness, increases product quality and process efficiency in dairy industry[1]. Recently, enzymatic method has become an alternative technique to acid hydrolysis due to its many important advantages based on the quality of final product. Soluble and immobilized β-galactosidases are both used for lactose hydrolysis in industrial applications[2,3]. There is a great need for an immobilized enzyme system enhancing stability, imparting reusability and making enzyme-based processes cost-effective and viable in low lactose milk production. The use of immobilized enzyme eliminates the enzyme separation step from the main process thus simplifying and increasing the overall process yield.

There are a lot of immobilization techniques such as adsorption, covalent bonding, entrapping in a polymer matrix and aggregation by cross-linking, *etc*. The first rule of the immobilization is to obtain maximum catalytic activity as well as the binding capacity. The immobilization of enzymes on insoluble solids, especially adsorption of enzymes on solid supports through physical interactions, is proved to be crucial not only to enhance the stability and durability of enzymes under extreme conditions but also to retain the high activity and stereo-selectivity of enzymes in chemical transformations[4]. Moreover, the enzyme immobilization by a physical adsorption method is simple, can be easily operated and induces fewer modifications in the active conformation of the enzyme because the main driving forces for the adsorption are electrostatic, hydrogen-bonding and van der Waals interactions.

Unfortunately, the leaching of the enzymes from the supports is still a drawback for the practical application of a supported enzyme. For this reason, the support is also very important in enzyme immobilization because of its effect on the binding capacity and the catalytic activity of enzyme as much as the immobilization technique. Duolite A568 resin provides significant immobilization yields and enzyme loading. The surface characteristic of Duolite A568 is suitable for adsorption and this resin is also cheap[5]. The Duolite A568 resins differ from most other commercial anion exchange resins in that they remain relatively porous on drying and thus present a much larger active surface than do resins which shrink greatly in drying. Many researchers[6-8] have shown that synthetic carriers such as Duolite A568 are effective adsorbents for some organic and inorganic compounds. However, the studies focusing on β-galactosidase immobilization on a synthetic

carrier *via* simple adsorption have not reported the adsorption isotherm clearly as well as the kinetic and thermodynamic parameters of the adsorption process. In our previous studies, the mechanisms of blood proteins and blood cells on polyurethane and alkylsiloxane plasma treated polyurethane surfaces[9], Cu^{2+} adsorption by *Z. ramigeru* immobilized on Ca-alginate[10], patulin adsorption on activated carbon[11,12] and the adsorption mechanism and the performance of immobilized Pectinex Ultra SP-L on magnetic duolite-polystyrene composite particles[5,13] were well defined. Also, the equilibrium adsorption behaviours of bovine serum albumine[14] and *E. sakazaki*[15] on bare and plasma polymerization modified stainless steel surfaces were clearly explained.

The purpose of our present work is modeling of the adsorption of *A. oryzae* β-galactosidase onto a basic resin (Duolite A568). The efficiency of immobilization by adsorption in batch processes has been studied. Furthermore, adsorption was kinetically modeled to explain the mechanism of adsorption and to determine the rate of the adsorption process. Thermodynamic parameters were also evaluated using adsorption data.

EXPERIMENTAL

A. oryzae β-galactosidase obtained from ENZECO® (100,000 lactase unit/gram, Enzyme Development Corporation, USA). Duolite A568, a weak base ion exchange resin was supplied by Rohm and Haas (USA). Bovine serum albumin (BSA) was provided from Acros Organics (Belgium). Buffer solutions such as $NaH_2PO_4{\cdot}2H_2O$ (pH 4.5) and $Na_2HPO_4{\cdot}H_2O$ (pH 9.2) were obtained from J.T. BAKER (Netherlands). All other reagents used were of analytical grade.

Adsorption of β-galactosidase: Adsorption of *A. oryzae* β-galactosidase onto basic resin (Duolite A568) was investigated in the reaction tanks in batch systems. The parameters which affect the rate and capacity of adsorption are temperature and the amount of adsorbent. Adsorption in batch system was investigated in temperature set incubated shaker (SI-300R, Korea).

Adsorption studies were carried out with 5 mL of 4 mg/mL enzyme solution at pH 4.5[16] was mixed with various amounts (25, 50, 75, 100 mg) of support (Duolite A568) and placed in an orbital shaker operating at 150 rpm and different temperature (15, 25, 35 and 45 ºC). Samples (70 µL) were withdrawn periodically during the 24 h of reaction. Shaker was stopped after 24 h and the samples were prepared for the analysis. The enzyme (protein) was determined by the dye binding[17] using bovine serum albumin (BSA) as the standard in protein. Absorbance at 595 nm of the enzyme (protein) solution was measured with a spectrophotometer (Shimadzu 1610, Shimadzu Corporation, Japan). The enzyme (protein) solution was determined by comparing with the standard curve construct using enzyme (protein) solution with known concentrations. The total adsorbed enzyme activity was also compared to free enzyme and active protein amount was reported.

The amount of β-galactosidase adsorption at equilibrium q_e (mg/g) was calculated from the following eqn. 1;

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where C_o and C_e (mg/L) are concentrations of β-galactosidase at initial and equilibrium, respectively, V (L) the volume of the solution and W (g) is the mass of adsorbent used. The procedure of kinetic tests was basically identical to those of equilibrium tests. The aqueous samples were taken to preset time intervals and the concentrations of enzyme were similarly measured. The amount of adsorption at time t, q_t (mg/g), was calculated by eqn. 2;

$$q_t = \frac{(C_0 - C_t)V}{W} \quad (2)$$

RESULTS AND DISCUSSION

Adsorption isotherm: The adsorption isotherm indicates how the adsorption molecules distribute between the liquid phase and the solid phase when the adsorption process reaches an equilibrium state. The analysis of the isotherm data by fitting them to different isotherm models is an important step to find the suitable model that can be used for design purpose[18]. In this study, the adsorption of *A. oryzae* β-galactosidase onto basic resin (Duolite A568) was modelled according to Freundlich and Langmuir isotherms. The Langmuir model as given below is valid for monolayer adsorption onto a surface with a finite number of identical sites, which are homogeneously distributed over the adsorbent surface[19]. The linear expression of the Langmuir model is given by eqn. 3;

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b_L} + \left(\frac{1}{Q_0}\right) C_e \quad (3)$$

where q_e = amount of adsorbed β-galactosidase per unit mass of adsorbent (mg/g) and C_e = amount of β-galactosidase at equilibrium (mg/L), Q_0 = maximum amount of β-galactosidase per unit mass of adsorbent to form a complete monolayer on the surface bound at high C_e and b = a constant related to the affinity of the binding sites (L/mg). The plot of C_e/q_e against C_e determines the compatibility of the adsorption with respect to the Langmuir isotherm. The Langmuir constants Q_0 and b were determined from the slope and intercept of the plot and were presented.

Equilibrium adsorption curves of the enzyme on resin (Duolite A568) were obtained by plotting enzyme concentration *versus* time (Fig. 1). As seen from Fig. 1, adsorption process was reached equilibrium more quickly at high resin

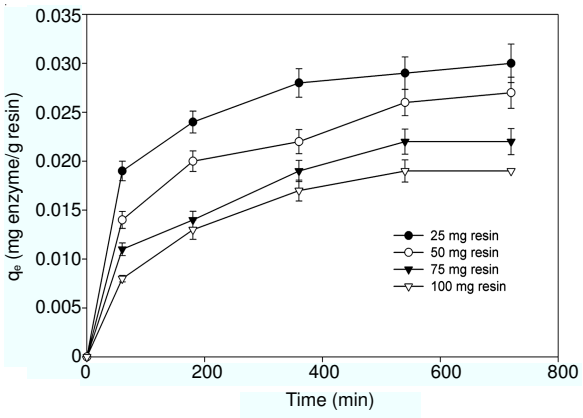

Fig. 1. β-Galactosidase adsorption onto Duolite A568 resin as related to initial resin content at 35 ºC

amount and adsorbed enzyme concentration was determined quite high. However, unit adsorbed enzyme concentration was determined relatively low at higher resin amounts. This condition confirmed that some parts of adsorption capacity of resin were not used at high resin concentration. For different amounts of basic resin in the media, β-galactosidase concentration reached a plateau almost at the same period of 540 min. Also, the changes in the amount of enzyme adsorbed onto resin (q_e) for four different temperatures were shown three dimensionally in Fig. 2. As seen from Fig. 2, a maximum amount of adsorbed enzyme was obtained after 540 min immobilization time and at lower temperatures.

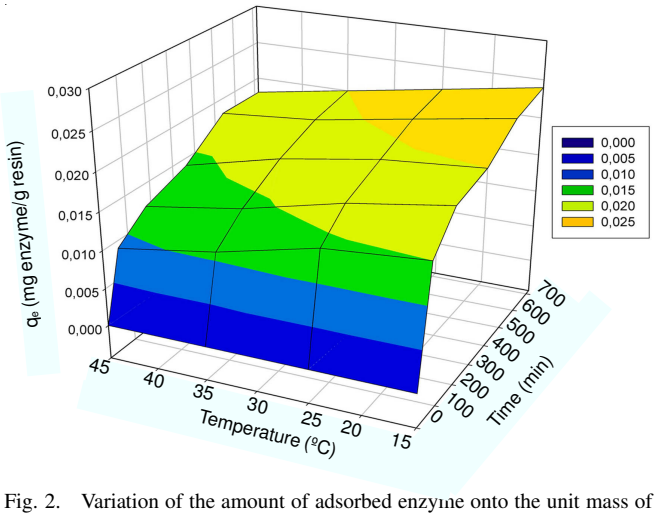

Fig. 2. Variation of the amount of adsorbed enzyme onto the unit mass of resin by time

Equilibrium study on adsorption has provided information on the capacity of the adsorbent. An adsorption isotherm is characterized by certain constant values, which express the surface properties and affinity of the adsorbent. Adsorption mechanism is relevant to the physical and chemical properties of the adsorbent. Langmuir isotherm which was obtained at 15-45 ºC was shown graphically in Fig. 3. The constant and correlation coefficient Langmuir isotherm were calculated from the graphs and listed in Table-1. As seen from the Table-1, although correlation coefficients ($R^2 > 0.90$) of both equations are considerably well obtained at all temperatures, the Langmuir model exhibited better fit to the adsorption data than the Freundlich model. Although the compatibility of the experimental data to Langmuir isotherm denoted the strong homogeneity of the surface of the adsorbent, it was not acceptable for natural adsorbent such as Duolite A568. A natural adsorbent generally has chemical heterogeneous character including various chemical groups.

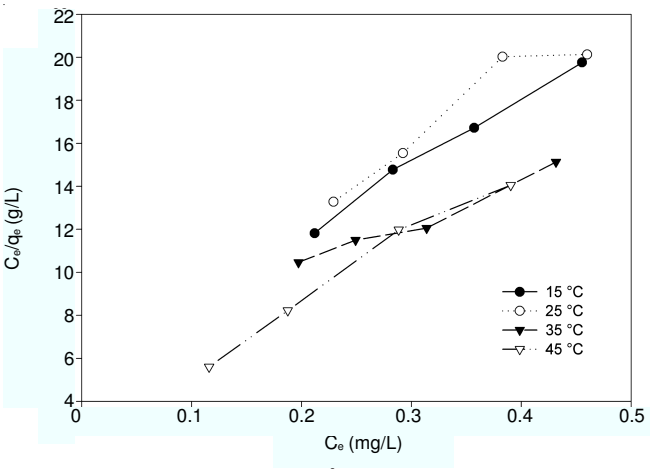

Fig. 3. Langmuir isotherms for *A. oryzae* β-galactosidase onto Duolite A568 resin at different temperatures

The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor R_L that is given by eqn. 4[20];

$$R_L = \frac{1}{1 + bC_0} \quad (4)$$

where C_0 = highest initial concentration of adsorbate (mg/L) and b = Langmuir constant (mg/L). The value of R_L indicates the shape of the isotherm to be either unfavourable ($R_L > 1$), linear ($R_L = 1$), favourable ($0 < R_L < 1$) or irreversible ($R_L = 0$)[21]. Then, R_L constant was calculated to determine the convenience of adsorption process. As seen from Table-1, R_L values showed that the β-galactosidase adsorption onto basic resin was favourable with respect to Langmuir isotherm ($0 < R_L < 1$). Values of R_L were found to be 0.23 ± 0.08, 0.26 ± 0.07, 0.37 ± 0.08 and 0.12 ± 0.03 at 15, 25, 35 and 45 ºC, respectively and confirmed that the Duolite A568 was favourable for adsorption of β-galactosidase enzyme under conditions studied.

Freundlich isotherm which was obtained at 15-45 ºC was shown graphically in Fig. 4. It was seen that experimental data were compatible to Freundlich isotherm nearby Langmuir isotherm because of the high correlation coefficient ($R^2 > 0.90$). The Freundlich isotherm[22] is an empirical equation employed to describe heterogeneous systems. The linear form of Freundlich equation is expressed as;

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (5)$$

where K_F and n are Freundlich constants with K_F = adsorption capacity of the sorbent (L/g) and n = giving an indication of how favourability of the adsorption. The magnitude of the exponent, 1/n, gives an indication of the favourability of adsorption. The value of n > 1 represents favourable adsorption condition[23,24].

TABLE-1
LANGMUIR AND FREUNDLICH ISOTHERMS AT DIFFERENT TEMPERATURES

T (ºC)	Freundlich constants			Langmuir constants			
	K_f (× 10^{-2})	n	R^2	Q_0 (× 10^{-2})	b	R_L	R^2
15	3.0 ± 0.16	2.96 ± 0.45	0.98	3.1 ± 0.34	5.99 ± 1.38	0.23 ± 0.08	0.99
25	2.9 ± 0.25	2.84 ± 0.56	0.84	3.1 ± 0.28	5.07 ± 1.02	0.26 ± 0.07	0.91
35	4.6 ± 0.65	1.84 ± 0.12	0.96	5.1 ± 0.49	3.02 ± 0.75	0.37 ± 0.08	0.96
45	3.6 ± 0.33	4.44 ± 0.98	0.94	3.2 ± 0.57	13.69 ± 2.46	0.12 ± 0.03	0.98

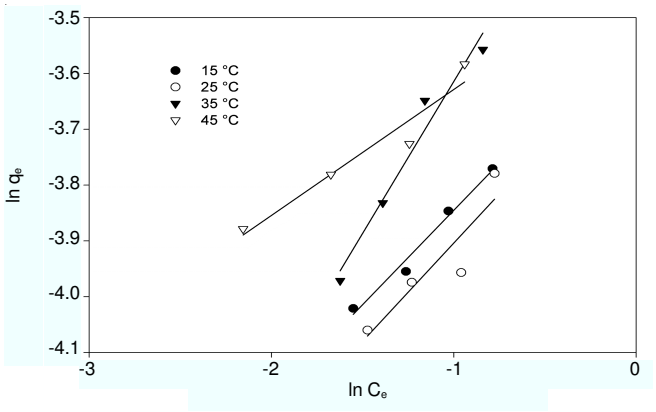

Fig. 4. Freundlich isotherms for *A. oryzae* β-galactosidase onto Duolite A568 resin at different temperatures

The compatibility of the experimental data to Freundlich isotherm denoted the heterogeneity of the surface of the adsorbent. 1/n value informed the compatibility of the system of adsorbent/adsorbate and the capacity[25]. The values between $1 < n < 10$ denoted that adsorption was favourable. As seen from Table-1, n values which were obtained for Freundlich isotherm varied between 1 and 10 at all temperatures. This situation denoted the favourability of the adsorption of the enzyme onto the basic resin. In this study, the greatest K_f value (4.60 ± 0.65) was determined at 35 ºC. It showed that the adsorption capacity was the best at this temperature.

Kinetic studies: In order to investigate the mechanism of adsorption and to determine the rate of the adsorption process, the pseudo first-order and pseudo second-order equations were used to test the experimental data of initial concentration and temperature. In this study, these models are taken into consideration to explain the adsorption mechanism of β-galactosidase enzyme onto the Duolite A568.

Pseudo-first-order kinetic model which was developed by Lagergren is the most current equation in the adsorption of solute from liquid solution. As seen from eqn. 6, the pseudo first-order rate equation is generally expressed as non-linear form;

$$q_t = q_{e,cal}(q - e^{-k_1 t}) \quad (6)$$

and as seen from eqn. 7, Pseudo-first-order Lagergren equation is also expressed as linear form;

$$\ln(q_e - q_t) = \ln q_{e,cal} - k_1 t \quad (7)$$

Generally, the pseudo-second kinetic model developed by Ho and McKay[26] has been applied to heterogeneous systems, where the sorption mechanism is attributed to chemical sorption. As seen from eqn. 8, pseudo-second-order is expressed as non-linear form;

$$q_t = \frac{k_2 q_{e,cal}^2 t}{1 + k_2 q_{e,cal} t} \quad (8)$$

This model can be also represented by the following equation (eqn. 9)[27,28];

$$\frac{t}{q_t} = \left[\frac{1}{k_2 q_{e,cal}^2}\right] + \frac{1}{q_{e,cal}} t \quad (9)$$

where k_1 is the Lagergren adsorption rate constant (min^{-1}), k_2 is the Pseudo-second-order adsorption rate constant (g/mg min), $q_{e,exp}$ is the the amount of substance adsorbed at the equilibrium conditions (mg/g), $q_{e,cal}$ is the calculated amount of substance adsorbed (mg/g); q_t is the amount of enzyme adsorbed at random (any) time (mg/g). The non-linear and linear forms of these equations were both solved by Matlab 7.8 and k_1 and k_2 values were determined. The most suitable adsorption isotherm and order of adsorption rate was found by the evaluation of the data obtained from the experiments with the aid of linear and non-linear graphics.

The best-fit equations of linear and non-linear forms of these two widely used kinetic models were also compared in this study. The kinetic parameters obtained from the linear (Figs. 5 and 6) and non-linear fittings (Fig. 7) were listed in Tables 2 and 3, respectively. The correlation coefficient of the most compatible model was found by calculation of (R^2) values. As seen from Tables 2 and 3, both linear and non-linear regression analysis showed that pseudo second-order expression as the better expression to predict the kinetics of enzyme/resin sorption system. It was demonstrated that the adsorption

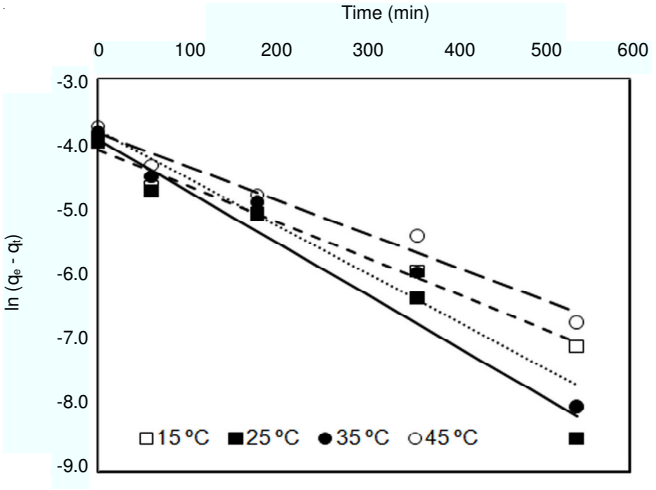

Fig. 5. Pseudo-first-order kinetics for adsorption of *A. oryzae* β-galactosidase onto Duolite A568 resin at different temperatures

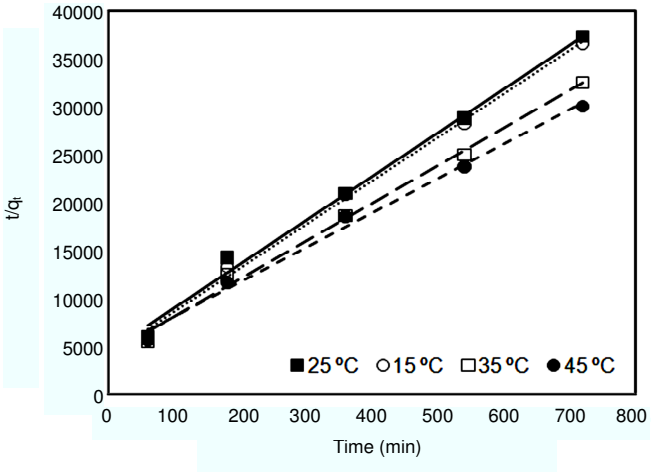

Fig. 6. Pseudo-second-order kinetics for adsorption of *A. oryzae* β-galactosidase onto Duolite A568 resin at different temperatures

TABLE-2
LINEAR FORM OF THE ADSORPTION KINETIC OF THE ENZYME ONTO 75 mg RESIN

T (K)	First-order kinetic model (Lagergren equation)				Second-order kinetic model (Pseudo II. Ho model)		
	$q_{e,exp}$	R^2	k_1 (1/min)	$q_{e,cal}$	R^2	k_2 (g/mg min)	$q_{e,cal}$
288	0.019 ± 0.002	0.98	0.005 ± 0.001	0.017 ± 0.003	1.00	0.52 ± 0.009	0.022 ± 0.001
298	0.019 ± 0.004	0.97	0.007 ± 0.001	0.020 ± 0.005	0.99	0.47 ± 0.113	0.022 ± 0.002
308	0.022 ± 0.006	0.96	0.007 ± 0.002	0.023 ± 0.002	0.99	0.37 ± 0.008	0.025 ± 0.002
318	0.023 ± 0.005	0.97	0.005 ± 0.001	0.022 ± 0.002	0.99	0.29 ± 0.006	0.028 ± 0.004

TABLE-3
NON-LINEAR FORM OF THE ADSORPTION KINETIC OF THE ENZYME ONTO 75 mg RESIN

T (K)	First-order kinetic model (Lagergren equation)				Second-order kinetic model (Pseudo II. Ho model)		
	$q_{e,exp}$	R^2	k_1 (1/min)	$q_{e,cal}$	R^2	k_2 (g/mg min)	$q_{e,cal}$
288	0.019 ± 0.002	0.98	0.0063 ± 0.002	0.020 ± 0.003	0.99	0.33 ± 0.009	0.024 ± 0.001
298	0.019 ± 0.004	0.98	0.0058 ± 0.001	0.020 ± 0.005	0.99	0.45 ± 0.113	0.022 ± 0.002
308	0.022 ± 0.006	0.99	0.0056 ± 0.002	0.023 ± 0.002	0.99	0.23 ± 0.008	0.028 ± 0.002
318	0.023 ± 0.005	0.97	0.0075 ± 0.001	0.023 ± 0.002	0.99	0.34 ± 0.006	0.027 ± 0.004

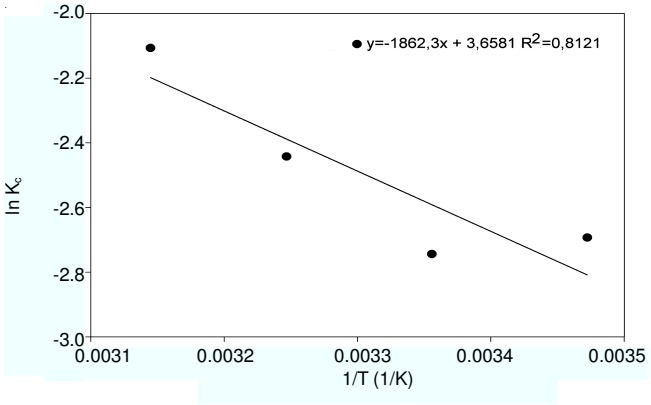

Fig. 7. Experimental data and the fitted non-linear forms of pseudo first order and pseudo second order equations

process of enzyme onto the resin could be explained with the pseudo second-order kinetic model. Non-linear method was found to be a better method than the linear method for predicting the optimum kinetics and the parameters involved in them. By non-linear method, both pseudo first-order and pseudo second-order kinetics well represent the kinetics of enzyme adsorption onto basic resin. Non-linear method is the correct way to obtain the parameters involved in the pseudo first-order kinetics.

The adsorption of the β-galactosidase enzyme onto the Duolite A568 was progressive kinetically. In the first step, enzyme in the solution diffused to the boundary of the film layer covered the adsorbent. This stage was explained by the Lagergren's first-order equation step and generally neglected because it occurred so fast because of the agitation during the adsorption process. Afterwards, enzyme which arrived to the film layer passed the stagnant part, moved into the pores of the adsorbent and went to the surface which adsorption occurred. This was the second step explained by the pseudo-second-order equation. This was the rate determinant step in present study. According to the results that we obtained from the experiments, k_2 values decrease with the increase in temperature. The computed results obtained from the second-order kinetic model were listed in Table-3. The correlation coefficients for the second-order kinetic model were equal to 0.99 for almost all the cases. Also, the calculated $q_{e,cal}$ values also agreed very well with the experimental data.

Thermodynamic analyses: The thermodynamic parameters provided in-depth information regarding the inherent energetic changes associated with adsorption should be properly evaluated. Free energy of adsorption (ΔGº), enthalpy (ΔHº) and entropy (ΔSº) changes were calculated in this study to predict the process of adsorption[29].

The thermodynamic parameters such as change in standard free energy (ΔGº), enthalpy (ΔHº) and entropy (ΔSº) were determined by using the following equations:

$$\ln K_c = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{R} \times \frac{1}{T} \quad (10)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (11)$$

where R (8.314 J/mol K) is the gas constant, T (K) the absolute temperature and K_c (L/g) is the standard thermodynamic equilibrium constant defined by q_e/C_e. ΔHº and ΔSº can be calculated from the slope and intercept, respectively, by plotting a graph of ln K_c *versus* 1/T (Fig. 8)[30].

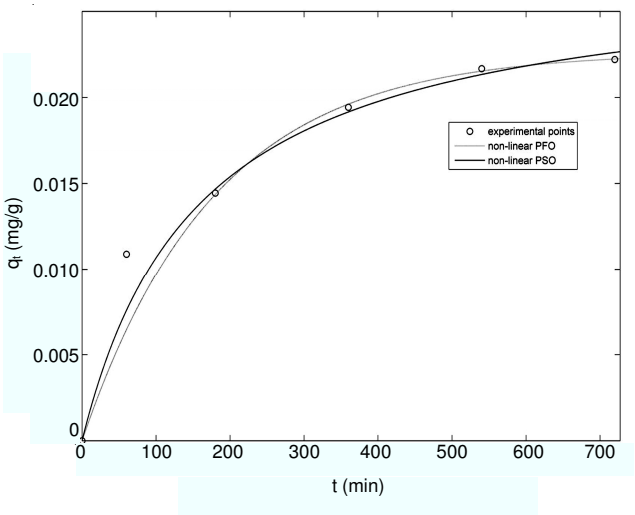

Fig. 8. Van't Hoff plot belongs to the adsorption of *A. oryzae* β-galactosidase onto Duolite A568 resin

The enthalpy change (ΔHº) and the entropy change (ΔSº), the free energy change (ΔGº) for the sorption processes were calculated to be 15.48 kJ/mol and 30.4 J/mol K, -9.367 kJ/

mol, respectively. The negative values of ΔGº confirmed the feasibility of the process and the spontaneous nature of sorption with a high preference of enzyme on resin. The positive value of ΔHº, indicating that the sorption reaction was endothermic. The positive value of ΔSº reflected the affinity of the resin for enzyme and suggested some structural changes in the enzyme. In addition to this, positive value of ΔSº also showed the increasing randomness at the solid/liquid interface during the sorption of the enzyme on the resin.

When the temperature increased from 288-318 K, ΔGº became a high negative value, suggesting that adsorption was more spontaneous at high temperature. Generally, ΔGº for physisorption is between -20 and 0 kJ/mol and it is between -80 and -400 kJ/mol for chemisorption[31]. Therefore, the ΔGº results implied that physisorption might dominate the adsorption of the enzyme onto the resin.

Conclusion

In food industry, there is great need to produce milk with low lactose content by a immobilized enzyme system due to its several expected economical and health benefits. In this study, *A. oryzae* β-galactosidase was immobilized onto Duolite A568 resin by simple adsorption mechanism. Duolite A568, an inexpensive and easily available support, was found very effective to immobilize β-galactosidase enzyme. The temperature dependence of the adsorption kinetic was successfully described by the Langmuir model in Arrhenius form. The adsorption of the enzyme onto Duolite A568 resin was determined as an endothermic process. The maximum adsorption capacity was found to be 5.1×10^{-2} mg/g from the Langmuir isotherm model at 35 ºC. The kinetic parameters were also calculated and it was shown that they could be fitted well to the pseudo-second-order kinetic model in non-linear form. The thermodynamic parameters confirmed that adsorption process was endothermic and occurred by physical mechanism and spontaneously. Duolite A568 resin proved to be an attractive and efficient support for β-galactosidase immobilization due to the simplicity of immobilization procedure. Lactose hydrolysis by immobilized enzyme in packed bed column reactor is still under investigation.

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