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Kinetics and Isotherm Studies of the Immobilized Lipase on Chitosan Support

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ABSTRACT

The kinetics and isotherm studies of the immobilized lipase and the mechanism of immobilization on chitosan beads and activated chitosan beads with glutaraldehyde were investigated. The validity of experimental data fitted to Langmuir, Freundlich, Hill, Sips, Temkin, Redlich-Paterson and Dubinin-Radushkevich isotherm models for both immobilization methods were examined. The isotherm models were compatible and confirmed immobilization techniques. In comparing of the isotherm models, the best fit of experimental data was obtained by Langmuir isotherm model for chitosan beads; which is consistent with the heterogeneous behavior of the adsorption sites on the chitosan structure. However, Freundlich isotherm model have corresponded immobilization of lipase on chitosan beads activated by glutaraldehyde so that it can reveal the multilayer adsorption. Also, pseudo-first order, pseudo-second order, Elovich and intra-particle diffusion were studied by experimental results in different concentration of lipase. Pseudo-first order kinetic model were described immobilization of lipase on chitosan beads and corresponds to physical adsorption of enzyme on the carrier. In fact, activated beads have followed pseudo-second order kinetic model which is indicated that chemical adsorption of enzyme occurred in the carrier. In addition, intra-particle diffusion equation for chitosan beads and activated chitosan beads is properly fitted by experimental data with high regression coefficient. In addition, FESEM analysis of chitosan beads and activated chitosan beads demonstrated that glutaraldehyde has significantly enhanced the surface porosity of chitosan beads. Maximum capacity of immobilization was enhanced by 2 folds, when the porosity of chitosan beads were improved by glutaraldehyde. These results were confirmed with adsorption isotherm models and kinetic equations.

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NOMENCLATURE

α	Initial adsorption rate (mg /mg.min)	K_T	Equilibrium binding constant associated (ml/mg)
a_R	R–P isotherm constant (ml/mg)	K_D	Hill isotherm constant
b	Langmuir equilibrium constant (ml/mg)	m	Dry weight of the chitosan (mg)
b_T	Adsorption heat *(kJ/mol)*	n	Model exponent
β	Activity coefficient related to mean adsorption energy	$1/n$	Freundlich constants
β	Desorption constant (mg/mg)	q_{ms}	The Sips maximum adsorption capacity at equilibrium;
C_0	Initial enzyme concentrations	q_t	The amount of adsorption at time t
C_e	Equilibrium concentration of lipase enzyme in (mg/ml)	q_e,	Value of lipase enzyme adsorbed in chitosan beads (mg/mg)
C_t	Enzyme concentration in the solution at time t (mg/ml)	q_m	Langmuir maximum adsorption capacity at equilibrium (mg/mg)
C_i	The intercept at stage i	q_{SH}	Hill maximum adsorption capacity at equilibrium
E	Adsorption energy	R_L	Separation factor

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K_{RP}	R–P isotherm constant (ml/mg)	R	Universal gas constant 8.314×10^{-3} (kJ/mol.K)
K_f	The Freundlich heterogeneous constant ((ml/mg)$^{1/n}$)	R^2	Regression coefficient
K_s	The Sips equilibrium constant	V	The volume of the enzyme solution (ml)
k_1	Rate constant of the pseudo first order adsorption	T	The temperature (*K)*
k_{id}	Intra-particle diffusion rate constant ((mg/mg.min)$^{1/2}$)	t	Time (h)
k_2	Rate constant for the pseudo second order adsorption	X_m	Maximum of adsorption capacity
		ε	Polanyi potential

1. INTRODUCTION

The increasing demands for green and sustainable chemical manufacturing processes promoted the application of enzymes as powerful biocatalysts. The versatility of enzymes attend to several industrial applications. It is extensively applied in food and flavor making, pharmaceuticals and catalysts for biotransformation [1-4]. Obviously, it is desired that enzymes as biocatalysts act and possesses highly specific activities. However, use of free enzyme in industrial operation under severe reaction environments is commonly limited by poor operational stability and low catalytic activity. Destroying the enzyme configuration or falling the conformational flexibility of free enzymes in severe reaction condition may cause inactivation of free enzyme. However, immobilizing the free enzymes on a support is the most effective method to facilitate reusability, improve catalytic efficiency, and adapt to severe reaction conditions. Physical adsorption, and covalent binding are practical methods of enzyme immobilization. However, enzyme leakage from the carrier during use may occur due to easy desorption in physical adsorption that restricts operational stability. Covalent attachment is the suitable method for achieving high operational stabilization and it is also effective and durable [5-8]. It has been recognized that stability and catalytic activity of an immobilized enzyme have greatly depended on the physical characterization of support. Surface functionality, porosity, and surface area of the carriers are of paramount importance for the function of the immobilized enzyme and loading capacity of the carrier [6, 9, 10]. Appropriate linkage of the enzyme to the support enhances enzyme activity by causing proper configurationally changes. Consequently, a proper support is critical for effective enzyme immobilization.

Chitosan is of paramount importance carrier for enzyme immobilization. It is identified as a perfect carrier because of its physical properties such as nontoxicity, biocompatibility, physiological inertness, biodegradability, high affinity to proteins and antibacterial properties. Additionally, the existence of functional amino and hydroxyl groups mark it susceptible for chemical treatments. Furthermore, amino groups create chitosan as a cationic polyelectrolyte (pK_a=˜ 6.5), one of the few found in nature. Subsequently, it is dissolved in aqueous acidic media and amino group got hold of positive charge and so it can be attached to negatively charged surfaces and poly anionic compounds. In addition, it is accessible in various physical forms, like flakes, fibers, beads, gels and membranes [11-14].

The functional groups that take part in the covalent coupling of enzyme to carrier is amino group as a result of chemical reaction. For activation of chitosan beads, chemical modification of the carrier with coupling agents like glutaraldehyde is required. Carrier activation makes high concentration of aldehyde groups on the chitosan surface, which can link with surface amino groups of lipase.

In this research paper, porcine pancreas lipase was immobilized on chitosan beads as an enzyme carrier. The techniques of immobilization of lipase enzyme for chitosan beads and activated chitosan were physical adsorption and covalent bond, respectively. In addition, the kinetics rate model and isotherm studies of the immobilized enzyme and the mechanism of immobilization on the carrier for both methods of immobilization were discussed. The authors investigated that adsorption isotherm models depict the carrier affinity to enzyme, while its parameters reveals the enzyme distribution on the carrier and determining the immobilization capacity. In the present study, the Langmuir, Freundlich, Temkin, Redlich-Paterson, Hill, Sips and Dubinin-Radushkevich isotherm models were deliberated for this purpose. Additionally, the mechanisms of immobilization of lipase onto the carrier for kinetic rate models were investigated. Pseudo first order, pseudo second order, Elovich and intra-particle diffusion model were examined to analyze the experimental data and also determine the rate-controlling step. The aim of the present work was to produce an immobilized form of lipase on chitosan beads and its kinetics and isotherm model were also investigated.

2. MATERIALS AND METHODS

2. 1. Preparation of Chitosan Beads Chitosan (1.5%) was dissolved in 1.5% acetic acid by slightly

heating at 60ºC with continuous stirring unless chitosan is dissolved. This solution was taken in a syringe and allowed to drop in 100 ml solution of 1 M potassium hydroxide. The beads of uniform shape and size were obtained. Beads were then washed with double distilled water and stored in 50 mM phosphate buffer at 4ºC until used [11].

2. 2. Activation of Carrier by Glutaraldehyde Chitosan beads in 50 mM phosphate buffer pH 7 were treated with 3% glutaraldehyde for 24 h at 30°C and 150 rpm. Activated beads were thoroughly washed with distilled water and phosphate buffer to remove excess glutaraldehyde [11].

2. 3. Immobilization of Lipase on Chitosan Beads Chitosan beads or activated chitosan beads by glutaraldehyde were treated with desired protein concentration and left overnight at 30°C and 150 rpm. After 24 h, the solution was decanted and beads were washed with the same buffer solution to remove any unbounded enzyme. Beads were finally stored in 50 mM phosphate buffer, pH 7 at 4°C.

2. 4. Protein Assay Protein concentration was estimated by Bradford protein assay method [15, 16] via bovine serum albumin as a standard of protein solution.

2. 5. FESEM Observation The surface features of chitosan beads were evaluated by a field emission scanning electron microscope (FESEM) (MIRA3, TESCAN). Chitosan beads was dried for FESEM analysis. So, freeze dryer was used for 72 h. In FESEM analysis, the dried beads were placed on a metal stub with double-sided conductive adhesive tape. Then, the samples were coated with a fine coater with a thin gold film under reduced pressure below 5 Pa. After that FESEM analysis was performed.

2. 6. Adsorption Experiments Adsorption experiments were performed in batch mode. For each experiment, 10 chitosan beads (containing 7.55 mg chitosan) were added to 20 ml of the enzyme solution with desired concentration (2-0.25 mg/ml) in a 50 mL Erlenmeyer flask. The flasks were closed with stoppers to avoid any evaporation and then were incubated in an incubator shaker with agitation rate of 150 rpm. In each experiment, sufficient time (24 h) was given to the adsorbate-adsorbent system to reach equilibrium. The enzyme concentration was periodically measured by means of protein assay.

The amount of adsorbed enzyme, q_t (mg/mg) were calculated using the following equation:

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (1)$$

where q_t is the amount of adsorbed enzyme per unit weight of chitosan at time t (mg/mg), C_0 is the initial enzyme concentrations (mg/ml) and C_t is enzyme concentration in the solution at time t (mg/ml), V the volume of the enzyme solution (ml) and m the dry weight of the chitosan (mg) which is used in a chitosan beads.

3. RESULTS AND DISCUSSION

3. 1. Adsorption Equilibrium The adsorption isotherm models were used to find the relationship between the amounts of lipase enzyme adsorbed onto chitosan beads and activated chitosan beads with glutaraldehyde. The equilibrium concentration of enzyme solution were considered after immobilization. In the present study, seven well-known sorption isotherm models such as Langmuir, Freundlich, Temkin, Redlich–Peterson, Hill, Sips and Dubinin-Radushkevich are used to illustrate the adsorption isotherm of lipase enzyme onto chitosan beads and activated chitosan beads with glutaraldehyde. The experimental data were fitted to the above mentioned model isotherms using Matlab curve fitting application (Version R2015a) to find the models parameters and their plot had shown in Appendix Figure A1 to A7. The isotherm models parameters obtained through non-linear fit of experimental data to the isotherm model equations are summarized in Table 1.

The Langmuir isotherms were established based on an assumption of monolayer adsorption onto a surface with a limit number of adsorption sites of uniform energies of adsorption [17, 18]. It is described as follows:

$$q_e = \frac{q_m b\, C_e}{1 + b C_e} \quad (2)$$

where, q_e, is the value of lipase enzyme adsorbed in chitosan beads (mg/mg); C_e is the equilibrium concentration of lipase enzyme in (mg/ml). The q_m is the Langmuir maximum adsorption capacity at equilibrium for a complete monolayer in (mg/mg). The b is the Langmuir equilibrium constant that is mentioned to the bonding energy of sorption in (ml/mg).

The values of q_m and b for chitosan beads and activated chitosan beads with glutaraldehyde are represented in Table 1. Because of higher regression coefficient, Langmuir isotherm model can predicted experimental data of enzyme immobilization on chitosan beads better than chitosan beads activated by glutaraldehyde. It can be concluded that adsorption of lipase on chitosan beads is physical adsorption and monolayer deposition of enzyme on the support surface. Similar results for adsorption of lipase on the various carriers were reported [3-7].

Based on literature, the adsorption of several lipases originated from different sources had followed the Langmuir isotherm adsorption model. In addition, they proved that the deposition of lipase on carrier as adsorption model is monolayer [19]. In addition, separation factor (R_L) is dimensionless constant which is defined based on Langmuir isotherm model. It is define by the following equation:

$$R_L = \frac{1}{1+q_m b\, C_0} \quad (3)$$

where, C_0 (mg/ml) is the initial lipase concentration. The value of R_L reveals that isotherm model to be either unfavorable ($R_L \geq 1$), favorable ($0 \leq R_L < 1$) or irreversible adsorption [19, 20].

The value of R_L, the separation factors are in the range of 0.06 - 0.38 for both chitosan beads and activated chitosan beads with glutaraldehyde, indicating that the immobilization of lipase onto chitosan beads were favorable and irreversible. This confirmed that the immobilization of lipase forms a proper attachment with the chitosan carrier.

The Freundlich isotherm model is an empirical model that can be described the adsorption of an enzyme on a heterogeneous surface of a carrier through multilayer adsorption and that the amount of immobilized enzyme can infinitely increases with an enhanced initial concentration of enzyme which is defined by Equation (4) [10, 19]:

$$q_e = K_f C_e^{\,1/n} \quad (4)$$

where q_e, is the amount of lipase enzyme adsorbed in chitosan beads (mg/mg); C_e is the equilibrium concentration of lipase enzyme (mg/ml). The K_f ($(ml/mg)^{1/n}$) is the Freundlich heterogeneous adsorbent related with the adsorption capacity and "*1/n"* known as the Freundlich constants which is described by the intensity and energy distribution on the adsorbent site. The obtained results are listed in Table 1. Regression coefficient for chitosan beads activated by glutaraldehyde is close to 1 indicating that Freundlich isotherm model can describe enzyme immobilization on activated chitosan beads. In addition, the obtained value of adsorption capacity (K_f) and strength and energy distribution on the adsorbent site (1/n) for activated chitosan beads is higher than chitosan beads. It was found that immobilization on activated chitosan beads are chemical and multi-layers adsorption. Al-Duri and Yong immobilized lipase on a commercial carrier (PE-100) which was heterogeneous adsorption and multilayer deposition of enzyme on support followed Freundlich isotherm model [20].

The Temkin isotherm model has been established based on chemical adsorptions; due to interactions between adsorbate and adsorbent. It is supposed that the heat of adsorption of the sorbate molecules (enzyme) linearly declines with an increase in adsorbent layers [17, 21, 22]. The Temkin isotherm equation is described by the following equation:

$$q_e = \frac{RT}{b_T} \ln(K_T C_e) \quad (5)$$

where b_T, *(kJ/mol)*, is associated to the adsorption heat; K_T, (ml/mg) is the equilibrium binding constant associated with the maximum binding energy; *T* (*K)* is the temperature and *R* is the universal gas constant which is equal to 8.314×10^{-3} (kJ/mol.K). The Temkin isotherm parameters, b_T and K_T, was calculated from the fitted curve and reported in Table 1. It is evident from the obtained data that both parameters for activated chitosan beads are greater than unactivated chitosan beads. It is confirmed that chemical adsorption of enzyme is a valid concept for the activated chitosan beads.

Hill isotherm model, supposed that adsorption process as a cooperative phenomenon, with the ligand binding ability at one site on the macromolecule, may influence the different binding sites on the same macromolecule. It is described by Equation (6):

$$q_e = \frac{q_{SH} C_e^{\,n}}{K_D + C_e^{\,n}} \quad (6)$$

where q_{SH} is the Hill maximum adsorption capacity at equilibrium, "n" and "K_D" are the Hill isotherm constants. One ligand molecule is bound to the enzyme, its affinity for other ligand molecules increases that means positively cooperative binding (n>1). One ligand molecule is bound to enzyme, its affinity for other ligand molecules declines that means negatively cooperative binding (n<1); and if n=1 non-cooperative binding, while the affinity of enzyme for a ligand molecule is not dependent on whether or not other ligand molecules are already bounded. In this case, the Hill equation is equivalent to the Langmuir equation [7, 23]. Based on obtained results immobilization of enzyme on chitosan beads are non-cooperative for both chitosan beads and activated chitosan beads with glutaraldehyde.

Despite of Freundlich equation which amount of adsorbed is continuing growth with an increase in concentration of absorbent, Sips isotherm model has a limit when the concentration of absorbent is sufficiently high. Sips isotherm model expressed as follows:

$$q_e = \frac{q_{ms} K_s C_e^{\,n}}{1+K_s C_e^{\,n}} \quad (7)$$

where q_{ms} is the Sips maximum adsorption capacity at equilibrium; K_s is the Sips equilibrium constant and "n" is the Sips model exponent. Additional parameter "n" in Sips equation is the only difference between this equation and the Langmuir equation. For "n=1", Sips model is converted to Langmuir equation. Therefore, this parameter "n" could be considered as for characterizing the system heterogeneity.

However, maximum adsorption capacity for Freundlich, Sips and Hill isotherm model are

approximately identical. The maximum adsorption capacity for chitosan beads and activated chitosan beads with glutaraldehyde were 0.2 and 0.4 (mg/mg), respectively.

Redlich–Peterson (R–P) isotherm can be described by the following expression:

$$q_e = \frac{K_{RP}\, C_e}{1+a_R C_e^{\,n}} \quad (8)$$

where K_{RP}(ml/mg), a_R(ml/mg) are R–P isotherm constant, and "n" is the exponent which lies between 0 and 1. Redlich–Peterson (R–P) isotherm is well fitted with experimental data and regression coefficient is closed to 1 for both chitosan beads and activated chitosan beads with glutaraldehyde.

Dubinin-Radushkevich (D-R) equation has been widely used to explain energetic heterogeneity of solid at low coverage as monolayer regions in micro-pores.

TABLE 1. Isotherm constants for the immobilization of lipase onto Chitosan beads and Chitosan beads activated with glutaraldehyde

Isotherm Model	Parameters	Chitosan beads	Chitosan beads activated with glutaraldehyde
Langmuir $q_e = \frac{q_m b\, C_e}{1+bC_e}$	q_m	0.158	0.145
	b	797	784.2
	R^2	0.946	0.864
	R_L	0.06-0.36	0.07-0.38
Freundlich $q_e = K_f C_e^{\,1/n}$	K_f	0.212	0.379
	$1/n$	4.361	4.975
	R^2	0.917	0.995
Temkin $q_e = \frac{RT}{b_T}\ln(K_T C_e)$	K_T	50273	58964
	b_T	122.28	137.8
	R^2	0.978	0.983
Redlich-Paterson $q_e = \frac{K_{RP}\, C_e}{1+a_R C_e^{\,n}}$	K_{RP}	591.5	1819
	a_R	2358.5	6944
	n	0.857	0.813
	R^2	0.973	0.994
Hill $q_e = \frac{q_{SH} C_e^{\,n}}{K_D + C_e^{\,n}}$	q_{SH}	0.191	0.41
	K_D	0.041	0.802
	n	0.519	0.271
	R^2	0.989	0.994
Sips $q_e = \frac{q_{ms} K_s C_e^{\,n}}{1+K_s C_e^{\,n}}$	q_{ms}	0.197	0.423
	K_s	24.29	1.23
	n	0.52	0.27
	R^2	0.989	0.994
Dubinin-Radushkevich $\ln(q_e) = \ln(X_m) - \beta\varepsilon^2$	X_m	0.21	0.38
	β	0.009	0.003
	R^2	0.911	0.967
	E	-7.45	-12.9

The equation is given by:

$$\ln(q_e) = \ln(X_m) - \beta\varepsilon^2 \quad (9)$$

where, β is the activity coefficient related to mean adsorption energy, X_m the maximum of adsorption capacity and ε is the Polanyi potential, which is equal to:

$$\varepsilon = RT\ln(\frac{1}{C_e}) \quad (10)$$

where, R and T are the gas constant (kJ/mol.K) and temperature (K), respectively. The adsorption energy (E) defined as:

$$E = -\frac{1}{\sqrt{(-2\beta)}} \quad (11)$$

where, it is corresponding the nature of adsorption. The value of E reveals that adsorption process to be either physical adsorption ($-1 \le E \le -8$ (kJ/mol)) or chemical adsorption ($-9 \le E \le -16$ (kJ/mol)). The model parameters of the D-R equation based on experimental data are given in Table 1. Adsorption energy for chitosan beads and activated chitosan beads with glutaraldehyde were determined -7.45 and -12.6, respectively. Subsequently, these results confirmed the other isotherm model findings.

3. 2. Adsorption Kinetic Studies The effect of contact time on the adsorption of lipase enzyme on chitosan beads was studied and the results showed that adsorption increased with an increase in contact time. The experimental data were examined by pseudo-first-order, pseudo-second-order, Elovich, and intra-particle diffusion kinetic equations to understand the mechanism of the adsorption process and the rate limiting step in overall transport process [17-19, 21, 23, 24]. It also provides some information about how fast the species are adsorbed on the adsorbent surfaces; such characteristic is important in designing and modeling of the adsorption process.

3. 3. Pseudo-first-order Kinetic Model The pseudo-first-order equation is usually associated with the physical adsorption in which the adsorption process is controlled by weak interactions between the adsorbate and the adsorbent surface. A simple pseudo-first order equation was used; that is given by Equation (12):

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (12)$$

where, q_e and q_t are the amount of lipase enzyme adsorbed at equilibrium and at time t (min), respectively. The k_1 is the rate constant of the pseudo first order adsorption process. The linear form of the equation is given by Equation (13):

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}\mathrm{t} \quad (13)$$

The values of k_1 and q_e were calculated from the slope and intercept of the linear plot of $\log(q_e - q_t)$ versus t (see Figure 1). Tables 2 and 3 summarized kinetic model parameters for chitosan beads and chitosan beads activated by glutaraldehyde, respectively.

3. 4. Pseudo Second Order Kinetic Model The pseudo second order kinetic model is based on the assumption that the adsorption is controlled by chemisorption. The corresponding pseudo second order rate equation is given as Equation (14):

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{1}{q_e}\, t \tag{14}$$

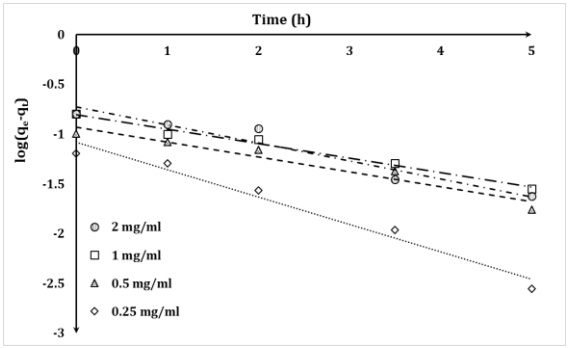
where, k_2 is the rate constant for the pseudo second order adsorption process (mg/mg.min). The slope and intercept of the plot of $\frac{t}{q_t}$ versus t (see Figure 2) were used to calculate the values of q_e and k_2. All rate constants are presented in Tables 2 and 3 for chitosan beads and chitosan beads activated by glutaraldehyde, respectively. The value of the regression coefficient calculated from the plot of the pseudo second order kinetic plot shows that it best fitted with the experimental data and can be used to describe the adsorption of lipase onto chitosan beads [25, 26].

3. 5. Elovich Kinetic Model The Elovich equation was first developed to describe the kinetics of chemisorptions of gas onto solids. The linear form of the Elovich model is presented by the following Equation (15):

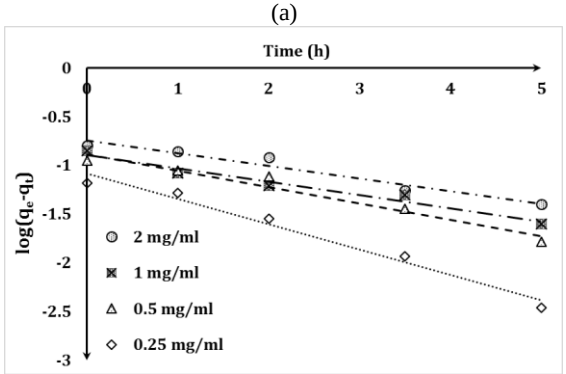
$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \tag{15}$$

where α is the initial adsorption rate (mg/mg.min) and β is the desorption constant (mg/mg). The slope and intercept of the plot of q_t versus *ln(t)* (see Figure 3) were used to calculate the values of the constants α and β. Also, these constants are shown in Tables 2 and 3 for chitosan beads and chitosan beads activated by glutaraldehyde, respectively.

3. 6. Intra-particle Diffusion Study The adsorption mechanism of adsorbate on to adsorbent follows three steps: (1) transport of adsorbate from the boundary film to the external surface of the adsorbent; (2) adsorption at a site on the surface; (3) intra-particle diffusion of the adsorbate molecules to an adsorption site by a pore diffusion process. The slowest of the three steps controls the overall rate of the process. The possibility of intra-particle diffusion was explored by using an intra-particle diffusion model.

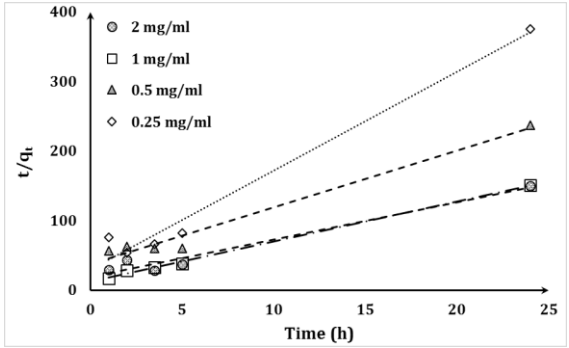


(a)

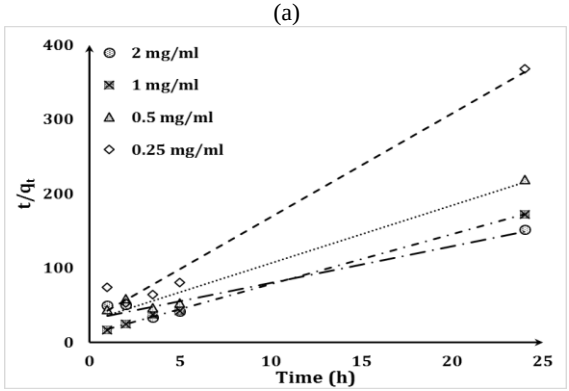


(b)

Figure 1. Plot of the pseudo-first order kinetic model for chitosan beads (a) chitosan beads activated by glutaraldehyde (b)

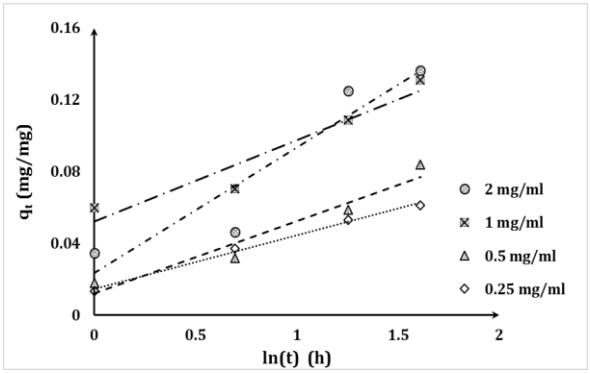


(a)

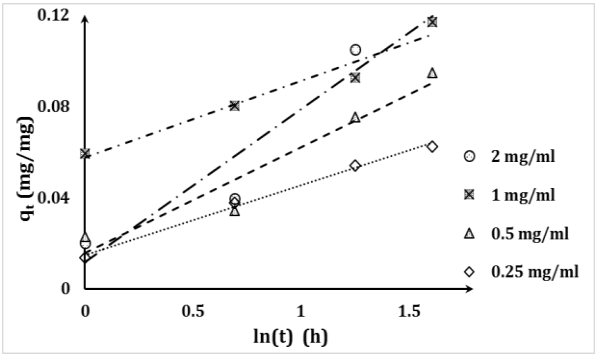


(b)

Figure 2. Plot of the pseudo-second order kinetic model for chitosan beads (a) chitosan beads activated by glutaraldehyde (b)



(a)



(b)

Figure 3. Plot of the Elovich kinetic model for chitosan beads (a) chitosan beads activated by glutaraldehyde (b)

The intra-particle diffusion varies with square root of time and is given in Equation (16):

$$q_t = k_{id} t^{0.5} + C_i \quad (16)$$

where, q_t is the amount of adsorption at time t; t is the square root of the time, k_{id} is the intra-particle diffusion rate constant ((mg/mg.min)$^{1/2}$), and C is the intercept at stage i and is related to the thickness of the boundary layer. Large C_i represents the greater effect of the boundary layer on molecule diffusion. The intra-particle diffusion rate constant was determined from the slope of the linear gradients of the plot q_t versus $t^{0.5}$ as shown in Figure 4 and their values are presented in Tables 2 and 3. The intra-particle diffusion process is controlled by the diffusion of ions within the adsorbent. The intra-particle diffusion models proved the significant role of intra-particle diffusion as one of the probable rate controlling mechanisms during enzyme adsorption on chitosan beads.

The obtained result for chitosan beads showed that pseudo first order kinetic can predicted experimental data with higher regression coefficient than pseudo second order kinetic. In addition, most of the calculated values of q_e from the first order kinetics model are higher than those from the experimental value of q_e. The insufficiency of the pseudo first order model to fit the kinetics data could possibly be due to limitations of the boundary layer that controls the sorption process.

TABLE 2. Parameters of the Pseudo first order, Pseudo second order, Elovich, and Intra-particle kinetic models together with their regression coefficients chitosan beads

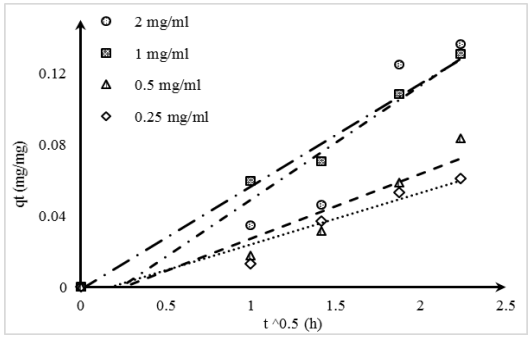
Kinetic model	Enzyme Concentration	q_e*	q_e	K_1	R^2
Pseudo -first order	2	0.160	0.1766	0.415	0.966
	1	0.151	0. 1633	0.334	0.981
	0.5	0.143	0.1317	0.344	0.988
	0.25	0.062	0.0743	0.633	0.970
		q_e*	q_e	K_2	R^2
Pseudo-second order	2	0.160	0.146	1.494	0.964
	1	0.151	0.124	2.649	0.937
	0.5	0.143	0.102	1.808	0.956
	0.25	0.062	0.050	6.773	0.929
		α	β	R^2	
Elovich	2	0.097	14.245	0.889	
	1	0.143	22.026	0.927	
	0.5	0.054	24.813	0.936	
	0.25	0.048	33.445	0.931	
		k_{id}	C_i	R^2	
Intra-particle Diffusion	2	0.0642	0.0153	0.875	
	1	0.0578	0.0015	0.988	
	0.5	0.0365	0.0093	0.985	
	0.25	0.0291	0.005	0.971	

q_e*: Experimental q_e

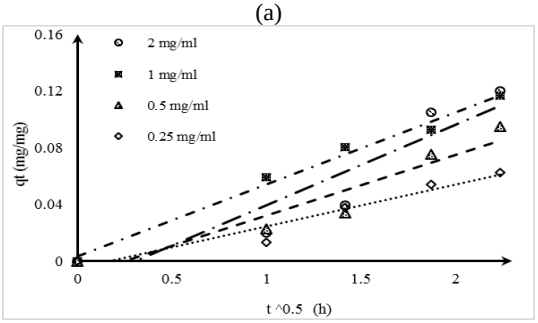
TABLE 3. Parameters of the pseudo first order, pseudo second order, Elovich, and intra-particle kinetic models together with their regression coefficients chitosan beads activated by glutaraldehyde

Kinetic model	Enzyme Concentration	q_e*	q_e	K_1	R^2
Pseudo first order	2	0.157	0.128	0.302	0.931
	1	0.139	0.049	0.316	0.962
	0.5	0.109	0.053	0.387	0.956
	0.25	0.070	0.034	0.603	0.946
		q_e*	q_e	K_2	R^2
Pseudo second order	2	0.157	0.203	0.794	0.976
	1	0.139	0.149	3.965	0.999
	0.5	0.109	0.128	2.119	0.974
	0.25	0.070	0.072	6.636	0.989
		α		β	R^2
Elovich	2	0.079		14.947	0.929
	1	0.185		29.858	0.953
	0.5	0.065		21.551	0.925
	0.25	0.049		32.784	0.914
		k_{id}		C_i	R^2
Intra-particle Diffusion	2	0.0566		0.0169	0.858
	1	0.0508		0.0035	0.987
	0.5	0.0426		0.0102	0.975
	0.25	0.0296		0.0051	0.991

q_e*: Experimental q_e



(a)



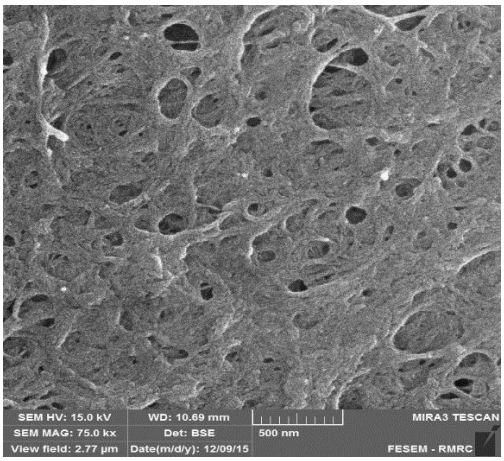
(b)

Figure 4. Plot of the Intra-particle Diffusion for chitosan beads (a) chitosan beads activated by glutaraldehyde (b)

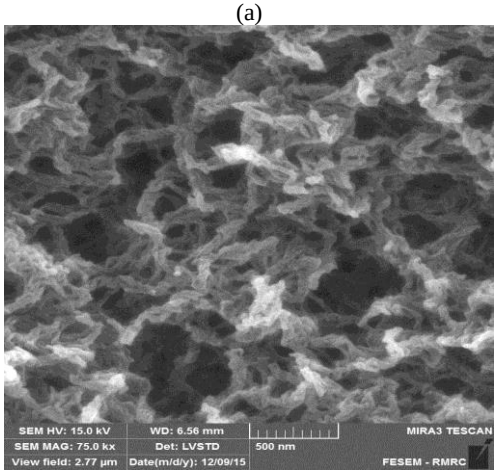
Consequently, immobilization of lipase on chitosan beads is physical adsorption and follow pseudo first order kinetic model. However, chitosan beads activated by glutaraldehyde was fitted by pseudo second order kinetic model with good regression coefficient ($R^2 \geq 0.97$). Also the q_e of predicted by pseudo second order kinetic model is very close to experimental value of q_e. Subsequently, immobilization of lipase on activated chitosan beads with glutaraldehyde is chemical adsorption with strong covalent bond. The value of the determination coefficient obtained from the linear plot of Elovich models are not high ($R^2 < 0.95$), suggesting that the applicability of this model to describe the immobilization of lipase on chitosan beads and activated chitosan beads with glutaraldehyde is not feasible.

The intra-particle diffusion models proved the significant role of intra-particle diffusion as one of the probable rate controlling mechanisms during immobilization of lipase on chitosan carrier. The value of the regression coefficient calculated from the plot of the liner form of Equation (16) that it best fitted with the experimental data ($R^2 \geq 0.97$).

Figures 5a and 5b show FESEM with 75 kx magnification of exterior surface of chitosan bead and activated chitosan bead with glutaraldehyde, respectively. As it was observed, glutaraldehyde has greatly increased the surface porosity of chitosan bead. Due to increasing in porosity of chitosan beads activated by glutaraldehyde, maximum capacity of immobilization was enhanced by 2 folds. These results were confirmed with adsorption isotherm models and kinetic equations.



(a)



(b)

Figure 5. FESEM image of (a) chitosan bead; (b) activated chitosan bead with glutaraldehyde; image magnification of 75 kx

4. CONCLUSIONS

The lipase enzyme was immobilized on chitosan beads and activated chitosan beads by glutaraldehyde. The isotherm adsorption models and kinetic equations were considered for both of immobilization method to evaluate the mechanism of adsorption on chitosan carrier. Immobilization of lipase on chitosan beads is physical adsorption and this results confirmed by Langmuir adsorption model and Dubinin-Radushkevich isotherms. In addition, immobilization on lipase on activated chitosan beads by glutaraldehyde is chemical adsorption and it is followed by Freundlich isotherm model and Dubinin-Radushkevich isotherms. Maximum adsorption capacity for chitosan beads and activated chitosan beads were predicted approximately 0.2 and 0.4 (mg/mg), respectively. Also it is corresponds to Hill, Sips, and Dubinin-Radushkevich isotherm model. Pseudo-first order, pseudo-second order, Elovich and intra-particle diffusion kinetic model were evaluated for both chitosan beads and activated chitosan beads with glutaraldehyde. It can be concluded that chitosan beads have fitted with pseudo-first order kinetic model which corresponding its physical adsorption. While, the activated chitosan beads have resembled by pseudo-second order kinetic model indicating chemical adsorption. However, Elovich may not proper choose for predicting immobilization of lipase on chitosan carrier because of low regression coefficient.

5. ACKNOWLEDGMENTS

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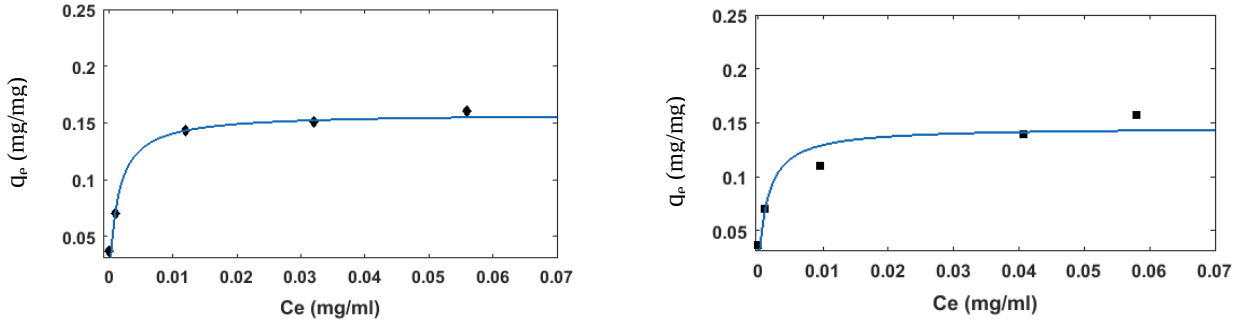
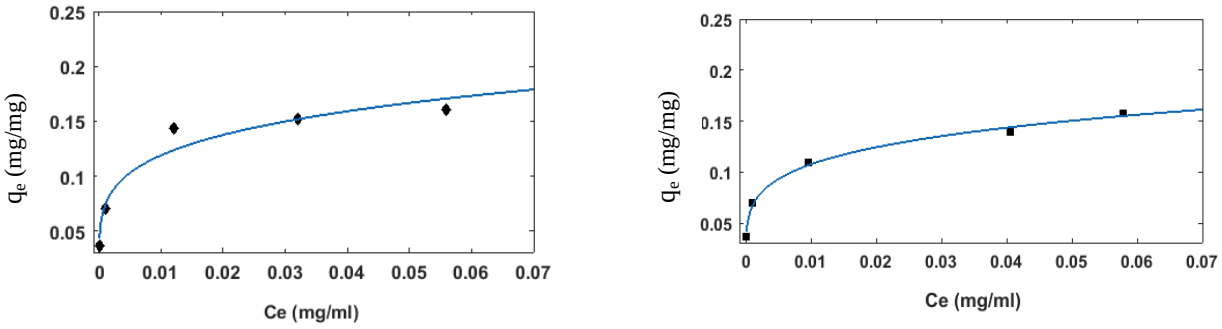
5. APPENDIX

The experimental data were fitted to the mentioned isotherm model using Matlab curve fitting application (Version R2015a) to find the models parameters and their plots are shown in Appendix Figures A1 to A7.



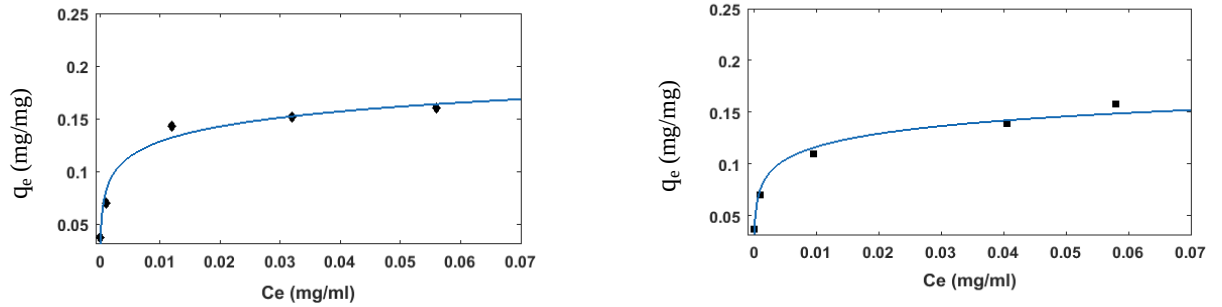
(a) (b)

Figure A1. Langmuir isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)



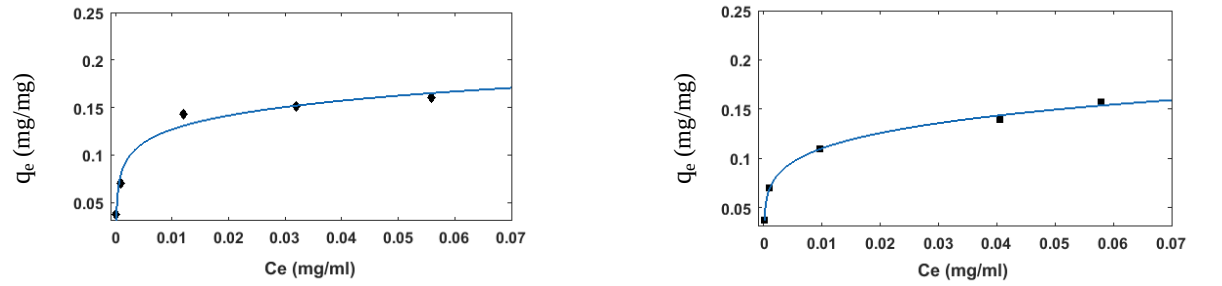
(a) (b)

Figure A2. Freundlich isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)



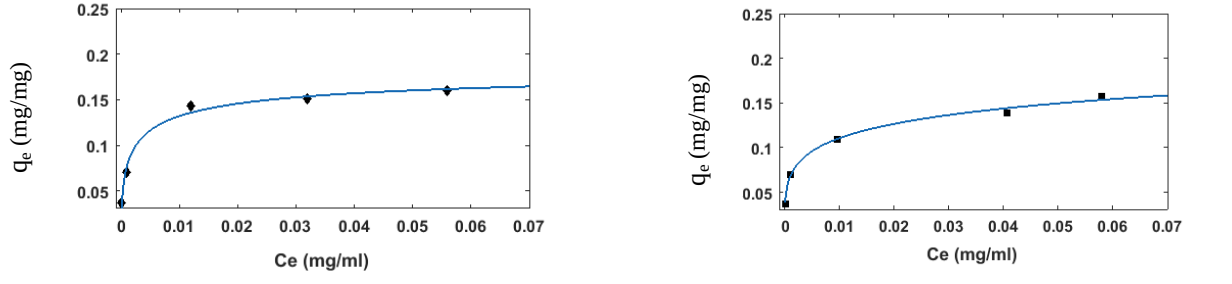
(a) (b)

Figure A3. Temkin isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)



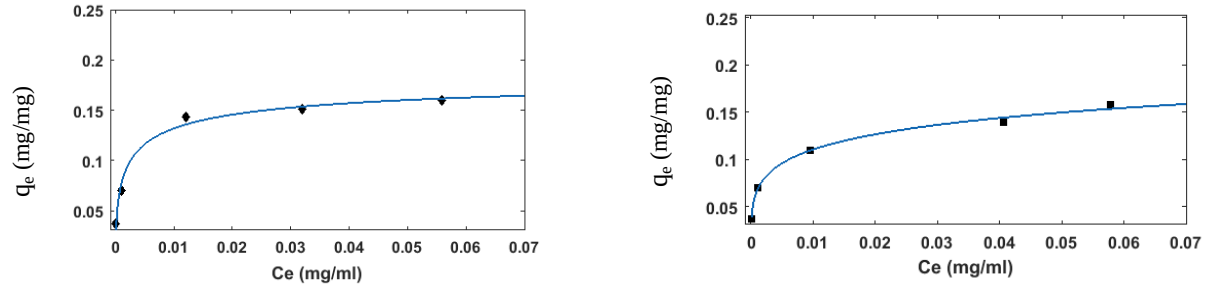
(a) (b)

Figure A4. Redlich-Paterson isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)



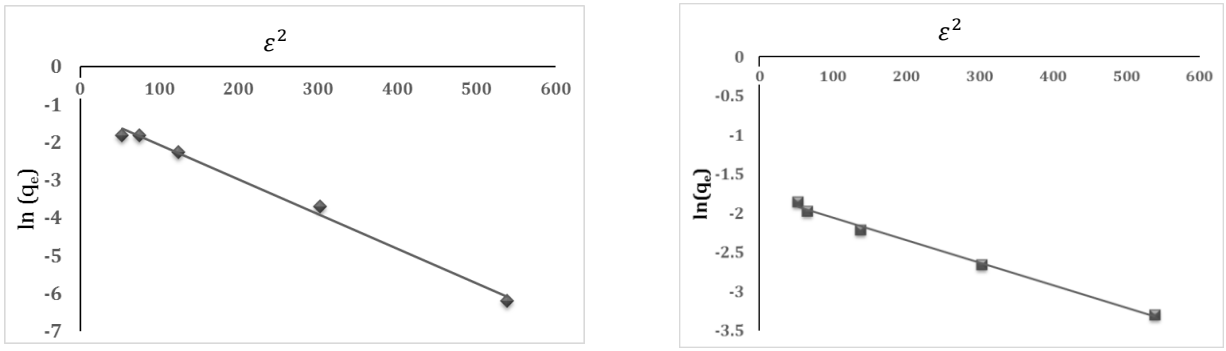
(a) (b)

Figure A5. Hill isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)



(a) (b)

Figure A6. Sips isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)



(a) (b)

Figure A7. Dubinin-Radushkevich isotherm plot for the immobilization of lipase on chitosan beads (a), activated chitosan beads by glutaraldehyde (b)

Kinetics and Isotherm Studies of the Immobilized Lipase on Chitosan Support

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چکیده

در این مقاله، مطالعات ایزوترم و سینتیکی آنزیم تثبیت شده و مکانیزم تثبیت بر روی دانه های کیتوزان و دانه های فعال شده با گلوتارآلدئید مورد بررسی قرار گرفت. داده های تجربی برای هر دو روش تثبیت با مدل های ایزوترم لانگمایر، فرندلیچ، هیل، سیبز، تمکین، ردلیچ پترسون و دوبینین رادوشکویچ انتطباق داده شده است. مدل های ایزوترم مورد بررسی با روش های تثبیت مورد استفاده، سازگاری مناسبی نشان داده است. مدل لانگمایر در مقایسه با مدل های بررسی شده دیگر، بهترین انطباق را برای دانه کیتوزان نشان داد که بیانگر رفتار هتروژن جایگاه های جذب کیتوزان می باشد. در حالیکه مدل فرندلیچ برای جذب توسط دانه های کیتوزان فعال شده با گلوتارآلدئید انطباق بهتری نشان داده است که نشان دهنده جذب چند لایه می باشد. همچنین به منظور بررسی سینتیکی جذب، معادلات سینتیکی شبه درجه اول، شبه درجه دوم، الوویچ و نفوذ درون ذره ای در غلظت های مختلف آنزیم لیپاز برای داده های تجربی مورد بررسی قرار گرفت. معادله سینتیکی شبه درجه اول با داده های تجربی فرایند جذب لیپاز بر روی دانه های کیتوزان انطباق نشان داده که به علت جذب فیزیکی آنزیم بر حامل می باشد. در حالیکه دانه های فعال شده از معادله سینتیکی شبه درجه دوم پیروی کرده که نشان دهنده جذب شیمیایی آنزیم بر روی حامل می باشد. همچنین معادله نفوذ درون ذره ای برای هر دو روش تثبیت مورد استفاده، تطابق مناسب با داده های تجربی و ضریب همبستگی مطلوبی نشان داده است. در ادامه آنالیز FESEM برای دانه های کیتوزان و دانه های فعال شده با گلوتارآلدئید انجام شد که نشان داد گلوتارآلدئید تا حد چشمگیری تخلخل سطحی دانه های کیتوزان را افزایش داده است. بیشترین ظرفیت تثبیت با فعال سازی دانه های کیتوزان توسط گلوتارآلدئید به دو برابر افزایش پیدا کرد. نتایج تجربی به دست امده از ازمایشات با معادلات ایزوترم و سینتیکی تطابق خوبی نشان داده است.

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