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Exploring thermodynamics and kinetic parameters of immobilized catalase enzyme via adsorption on krill clay

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ABSTRACT

The thermodynamic parameters and immobilization kinetics of catalase enzyme via adsorption onto krill clay were performed in aqueous solution in batch system. The adsorbent was characterized by X-ray fluorescence, scanning electron microscope and Brunauer–Emmett–Teller N_2 adsorption measurements. The effect of some parameters to the adsorption process such as contact time, stirring speed, initial enzyme concentration, initial ionic strength, temperature and pH were analyzed. Maximum adsorption capacity (q_m) showed a great dependence on pH and initial enzyme concentration. It was found at pH 5.5, and initial enzyme concentration, 0.0075 g L^{-1} values. Pseudo-first-order, pseudo-second-order and intraparticle diffusion were used to fit the kinetics data on kinetic models. Furthermore, thermodynamic activation parameters such as enthalpy, entropy, Gibbs free energy and activation energy ($\Delta H°$, $\Delta S°$, $\Delta G°$ and E_a) which reveal that catalase enzyme adsorption occur spontaneously and in endothermic nature were determined. The obtained results confirmed that the applicability of krill clay is an efficient immobilizing supporter for catalase enzyme and krill clay material can be used as a very effective adsorbent in immobilization of this enzyme from aqueous solutions.

Keywords: Adsorption; Catalase enzyme; Immobilization; Kinetics; Krill clay; Thermodynamics

1. Introduction

Enzymes have been immobilized by three methods: physical adsorption, covalent attachment and involvement [1]. The adsorption consists of the union between the enzyme and the inert support through non-specific physicochemical interactions, van der Waals forces, hydrophobic and ionic interactions, and have shown good cost-effective with regard to efficiency and cost of the immobilization procedure, because it has simple methodology [2]. Enzymes are often immobilized onto solid support or various materials. Immobilized enzymes can be used in the process requiring mechanical strength, microbial resistance, thermostability, chemical durability, chemical functionality, low cost, hydrophilicity, regenerability and high capacity of enzyme [3]. Enzyme immobilization offers advantages over free enzymes in choice of batch or continuous processes, rapid termination of reactions, controlled product formation, ease of enzyme removal from the reaction mixture and adaptability to various engineering designs [4–6]. In the immobilization techniques adsorption has a higher commercial potential than the other methods, because adsorption is simpler and less expensive and a high catalytic activity can be retained. Adsorption method also offers the reusability of expensive supports after inactivation of immobilized enzyme [7–10]. Some materials such as porous glass, silica gels and cellulose are used for the preparation of immobilized enzymes [11,12]. Catalase (EC.1.11.1.6) is an enzyme containing metalloenzyme and is regarded as one of the most common enzymes

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in plant and animal tissues. Immobilization of catalase has useful applications in some industrial fields in the removal of hydrogen peroxide used as oxidizing material [13]. Catalase can be obtained from various organisms and tissues, such as fungal or mammalian sources [14]. The immobilization of catalase is very important for both economical and yielded usage of the enzyme. Many researchers have studied also adsorption systems on various other supports [15]. Samples were characterized by X-ray fluorescence (XRF), scanning electron microscope (SEM) and Brunauer–Emmett–Teller (BET) N_2 adsorption measurements. Adsorption of enzyme was tested in batch and their kinetic parameters were determined.

The aim of this study was to determine the adsorption kinetics of catalase enzyme on krill clay over a range of physicochemical conditions, which is important to indentify various natural environmental systems. A number of experimental parameters in this study are considered, including the effect of stirring speed, initial catalase enzyme concentration, initial solution ionic strength, pH and solution temperatures. The thermodynamic activation parameters of the process, such as activation energy, entropy, enthalpy and the free energy, also determined.

2. Materials and methods

2.1. Materials

Catalase (hydrogen peroxide oxidoreductase; EC.1.11.1.6), from bovine liver (250.000 U mg^{-1}), was obtained from Sigma (St. Louis, MO, USA). All other chemicals and solvents used in this study were purchased from Merck AG (Darmstadt, Germany) and were of analytical grade. Krill clay used in this study was obtained from region of Hoşap (Van, Turkey).

The chemical composition of krill clay is shown in Table 1. The specific surface area of krill clay was measured by BET N_2 adsorption by Micromeritics Flowsorb II-2300 equipment. The results are summarized in Table 2.

The sample of krill clay was subjected to morphological and microstructural analysis using an SCM 5000 Benchtop SEM (Neoscope).

All other chemicals used were of analytical grade. All water used was of Milli-Q quality or doubly distilled.

2.2. Experimental procedure

Adsorption kinetic experiments were carried out using mechanic stirrer. All of the catalase enzyme solution was prepared with ultrapure water. Kinetic experiments were carried out by agitating 1 L of enzyme solution of initial concentration 0.2 g L^{-1} with enzyme of krill clay at a constant agitation speed of 700 rpm, 5 × 10^{-2} mol L^{-1} ionic strength (sodium phosphate buffer), 298 K and pH 7. Agitation was done for 120 min, which is more sufficient than time to reach equilibrium at a constant agitation speed of 700 rpm. Preliminary experiments had shown the effect of the separation time on the adsorbed amount of enzyme. The initial tested concentration of enzyme solution was 0.1, 0.2 and 0.25 g L^{-1}. The effect of pH on krill clay of catalase enzyme was analyzed in the pH range from 5.5 to 9. The pH was adjusted using 0.05 N NaOH and 0.05 N HCl solutions by using an Orion 920A pH meter with a combined pH electrode. The effect of ionic strength was investigated at 0.001–0.0075 mol L^{-1} potassium phosphate solution concentrations. The effect of temperature at experiments was performed at 288, 298, 309.5 and 318 K in a constant temperature bath. Samples of 4 mL were drawn at suitable time intervals.

The samples were then centrifuged for 5 min at 3,000 rpm and the left out concentration in the supernatant solution was analyzed using UV–Vis spectrophotometer (Cary 1E UV–Vis spectrophotometer, Varian) by monitoring the absorbance changes at a wavelength of maximum absorbance. Each experimental run continued until no significant change in the enzyme concentration was measured. The adsorbed amount of enzyme at any time t, q_t, was calculated from the mass balance (Eq. (1)) [16].

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

where C_0 and C_t are the initial and liquid-phase concentrations at any time t of enzyme solution (0.2 g L^{-1}), respectively, q_t is the enzyme concentration on adsorbent at any time t (mg g^{-1}), V is the volume of the enzyme solution (L) and m is the mass of the krill clay sample used (g) [16].

3. Results and discussion

3.1. Effect of contact and equilibrium times and initial enzyme concentration

The adsorption of catalase enzyme on krill clay at different initial concentrations and stirring speed of 700 rpm was studied as a function of contact time in order to determine the equilibrium time. Fig. 1(a) shows the plot of amount of enzyme adsorbed vs. time at different initial enzyme concentrations. From the figure, it was observed that the amount of enzyme adsorbed gets increased from 0.124 to 0.202 m gm^{-1} for an increase in initial enzyme concentration from 0.1 to 0.25 g L^{-1}. As can be seen clearly in Fig. 4, the krill clay

Table 1
Chemical composition of krill clay

Constituent	Percentage present (%)
Mg	20.74
Al	9.20
Si	44.79
Fe	12.48
Ca	10.02
Others	2.77

Table 2
Physicochemical properties of krill clay

Parameters	Value
Particle size (μm)	(325)
Colour	Dark green
pH	9.73
Specific surface areas (m^2 g^{-1})	–
Single point specific surface area	1.484e+01 m^2 g^{-1}
Multipoint specific surface area	1.547e+01 m^2 g^{-1}

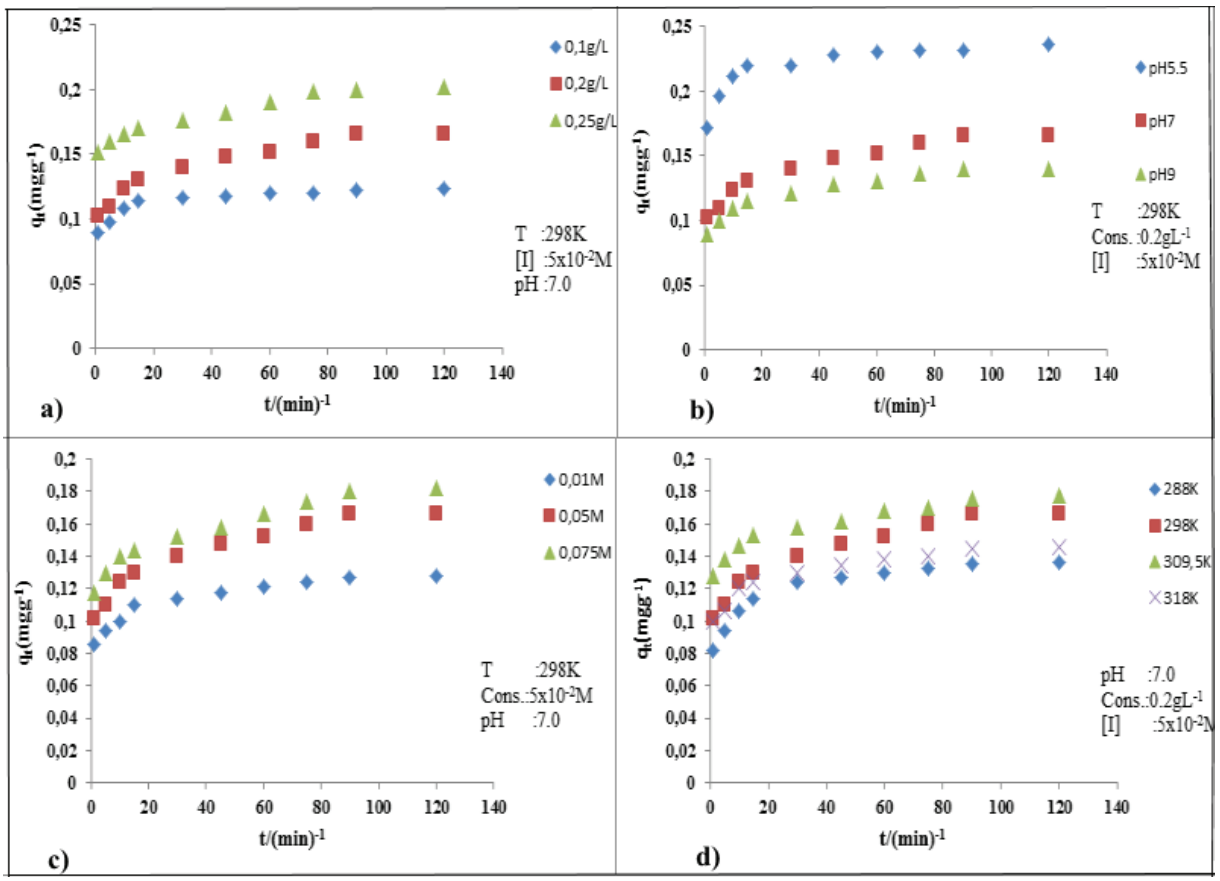

Fig. 1. The effect of parameters to the adsorption rate of catalase enzyme on krill clay: (a) the effect of initial enzymes concentration, (b) the effect of solution of pH, (c) the effect of ionic strength, (d) the effect of temperature.

and the enzyme adsorbed on the surface of krill clay show time-dependent morphological changes. Krill clay surface morphology completely changed after 120 min. It can be concluded that the images are consistent with experimental data.

3.2. *Effect of solution pH*

The variation in the adsorption rate of catalase enzyme with respect to pH can be elucidated by considering the surface charge of the adsorbent materials. From Fig. 1(b), it was observed that the solution pH affected the amount of enzyme adsorbed. Fig. 1(b) demonstrates that the adsorption decreases with the increasing of pH. Zero charge point, in which hydroxyl and proton ions are zero, is considered to be the pH where the adsorption occurs. Zero charge point expresses the same thing with the isoelectric point [17]. Because the net charge is zero at the isoelectric point in which enzymes or proteins has a very stable structure. But these structures will start to decompose stable values below or above the isoelectric point. In this case, the amount of adsorption is affected. It was stated that the amount of positive charge in the molecule at pH values above the isoelectric point of the biomolecule is less than the amount of negative charge [18].

Therefore, biomolecules such as catalase enzyme are positively charged at pH values below the isoelectric point and the negatively charged at pH values above the isoelectric point. The pH values of the catalase enzyme which is a biomolecule are below the isoelectric point due to the reduction in the negative charge increases the number of positive charges on the surface of krill clay. The pH of the catalase enzyme which is a biomolecule is below the isoelectric point of causing reduction in negative charge in the surface of krill clay. This situation increases the number of positive charges. In this case, biomolecules of the excess positive charge cause much more difficult to approach in terms of electrostatics. The same is the catalase enzyme which biomolecules have negative charges increases at pH values above the isoelectric point leads to an increase in negative charge in the surface of krill clay as it is. Demirbaş [19] stated this situation in a nice way (Fig. 2).

3.3. *Effect of ionic strength*

The presence of ionic solution had significantly influenced the adsorption rate of catalase enzyme. As seen in Fig. 1(c) the adsorption was found to increase with increasing ionic strength. Catalase enzyme in the surface of krill clay with increasing ionic strength was determined to increase the adsorption capacity. Adsorption medium added presence of sodium phosphate salts causes two opposite effects in the first case while improving the separation of the catalase enzyme molecule with krill clay added to the sodium phosphate salt solution medium. On the other hand sodium

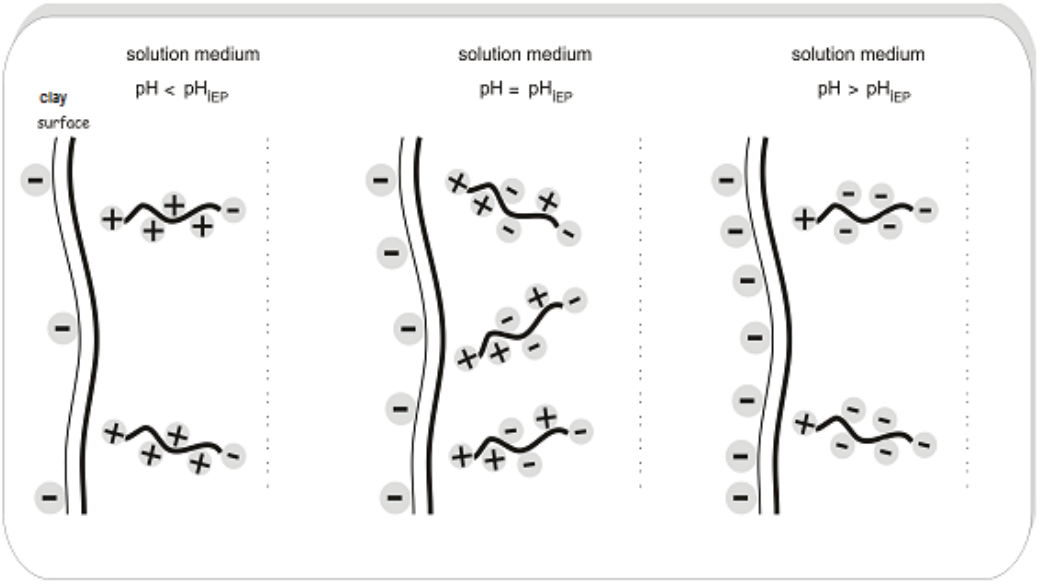

Fig. 2. Effect on the krill clay surface at different pH medium of the catalase enzyme.

phosphate salt, entering between catalase enzyme with krill clay leads to decrease electrostatic interaction. The increase in the adsorption capacity of these two processes can be said that higher dominant effect. The same situation is observed in the interaction of dyes with kaolinite clay surface [20–23].

3.4. Effect of temperature

Fig. 1(d) shows the adsorption kinetics of catalase enzyme at 15°C, 25°C, 36.5°C and 45°C by plotting its uptake capacity, q_t, etc. time at the initial enzyme concentration of 0.2 g L^{-1}.

The speed of any reaction catalysed by enzymes heat generally increases until the optimum value. The temperature increase also increases the molecular kinetic energy and movement. This increases interference in the active site of catalase enzyme and the surface of krill clay. And the maximum adsorption between the krill clay with the catalase enzyme was determined at 36.5°C. Three-dimensional structure of the enzyme at points above this temperature begins to deteriorate and lose their activity. In this case, it affects the adsorption between the krill clay with the enzyme negatively. In a study, authors found that immobilized from different sources have been found to be the optimum temperature 37°C [22], in parallel with this work, Alkan et al. [24] maximum adsorption between montmorillonite clay with catalase were obtained at 35°C. In another study, Çetinus and Öztop [25] catalase enzyme showed maximum activity at 35°C, and it decreased activity at temperatures above this temperature.

As can be seen clearly in Fig. 3, the krill clay and the enzyme adsorbed on the surface of krill clay show time-dependent morphological changes. Krill clay and catalase enzyme adsorbed by krill clay of thermal gravimetric analysis (TGA) was measured as shown in Fig. 4. A big mass loss in this figure is seen among 100°C–500°C after the departure of water which physically adsorption to adsorbent in the heat treatment analysis. It is seen that catalase enzyme which adsorb by krill clay shows a higher thermal stability than natural krill clay.

3.5. Adsorption kinetics

In order to examine the controlling mechanism of sorption process, several kinetic models were used to test the experimental data. From a system design viewpoint, a lumped analysis of sorption rates is thus sufficient for practical operation.

3.5.1. Pseudo-first-order equation

The pseudo-first-order equation is generally expressed as follows [26]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{2}$$

where q_e and q_t are the amount of enzyme ions adsorbed at equilibrium at time t (mol g^{-1}), respectively, and k_1 is the rate constant of pseudo-first-order adsorption (min^{-1}). The fitting results are given in Table 3.

3.5.2. Pseudo-second-order equation

If the rate of adsorption is a second-order mechanism, the pseudo-second-equation is expressed by Eq. (3) [25]:

$$\frac{t}{q_e} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{3}$$

where q_e is the amount of enzyme ions adsorbed at equilibrium rate constant of pseudo-second-order sorption (g mol^{-1} min^{-1}).

The half-adsorption time of the enzyme ions, $t_{1/2}$, is expressed by Eq. (4):

$$t_{1/2} = \frac{1}{k_2 q_e} \tag{4}$$

The initial adsorption rate, h (mol g^{-1} min^{-1}), is expressed by Eq. (5):

$$h = k_2 q_e \tag{5}$$

The values k_2, q_e, $t_{1/2}$ and h are given in Table 3. As shown in Table 3, experimental data can be explained by pseudo-second-order kinetic equations.

3.5.3. Intraparticle diffusion equation and mass transfer

The initial rate of the intraparticle diffusion is calculated by the following Eq. (6) [18]:

$$q_t = k_{int} t_{1/2} + C \tag{6}$$

where k_{int} is the intraparticle diffusion rate constant (mg g^{-1} min$^{-1/2}$) and is given in Table 4.

The intraparticle diffusion coefficient for the sorption of catalase enzyme was calculated from the slope of the plot of square root of time (min$^{1/2}$) vs. amount of enzyme adsorbed (mg g^{-1}) previous studies by various researchers showed that the plot between q_t and $t^{1/2}$ represent multilinearity, which characterizes the two or more steps involved in sorption process [27,28].

Fig. 5 shows the plot between q_t and $t^{1/2}$ for catalase enzyme onto krill clay particles. From Fig. 5, it can be seen that the sorption process tends to be followed by two phases. It was found that the initial linear portion ended with a smooth curve followed by second linear portion. The two phases in the intraparticle diffusion plot suggest that the

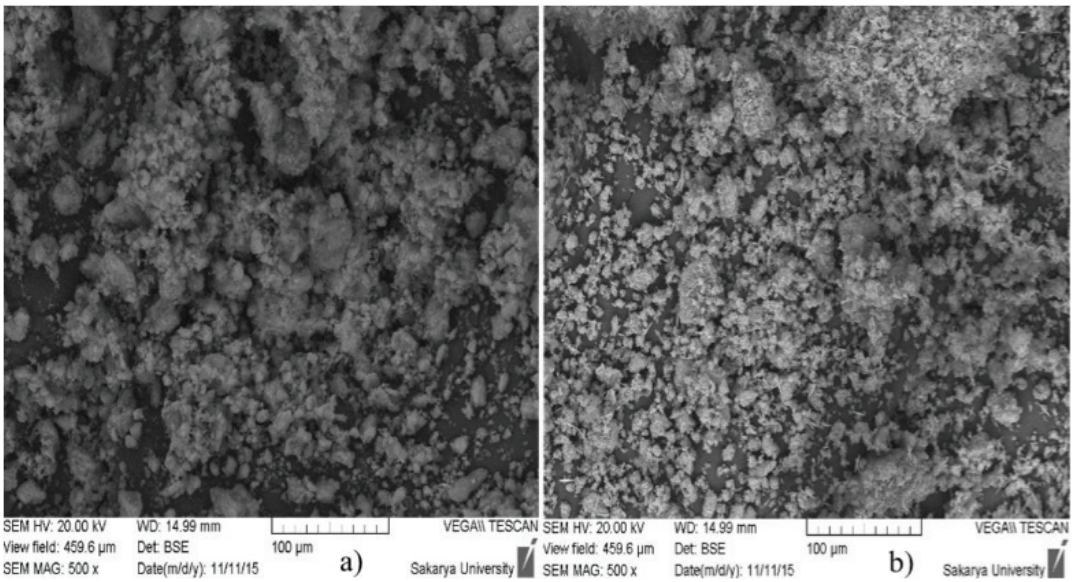

Fig. 3. SEM microphotographs of: (a) krill clay and (b) catalase enzyme adsorbed by krill clay after 120 min.

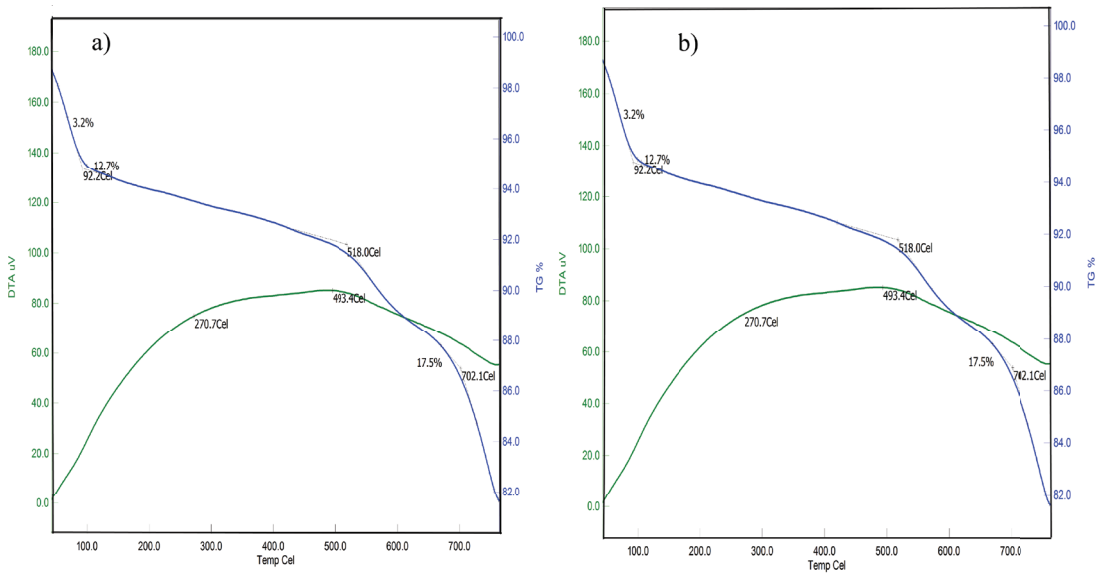

Fig. 4. Thermal gravimetric analysis of: (a) krill clay and (b) catalase enzyme adsorbed by krill clay between 0°C and 500°C.

Table 3
Kinetic data calculated for adsorption of catalase enzyme on krill clay

Kinetic models											
Parameters					Pseudo-second-order						
T (K)	Concentration (mol L^{-1}) 10^1	pH	Stirring speed (rpm)	[I] (mol L^{-1}) 10^3	First-order R^2	q_e (cal.) (mg g^{-1})	q_e (exp.) (mg g^{-1})	k_2 (g mg^{-1} min^{-1})	R^2	h (mol min^{-1})	$t_{½}$ (min)
288	2	7	700	5	0.96	0.136	0.138	2.6614	0.99	0.3673	2.7228
298	2	7	700	5	0.97	0.166	0.169	1.4807	0.99	0.2502	3.9968
310	2	7	700	5	0.92	0.178	0.178	2.2145	0.99	0.3941	2.5374
318	2	7	700	5	0.9	0.146	0.147	2.5590	0.99	0.3761	2.6588
298	1	7	700	5	0.91	0.124	0.124	5.1987	0.99	0.6446	1.5513
298	2	7	700	5	0.97	0.166	0.169	1.4873	0.99	0.2514	3.9785
298	3	7	700	5	0.93	0.202	0.204	1.8026	0.99	0.3677	2.7194
298	2	5.5	700	5	0.9	0.236	0.236	3.3126	0.99	0.7818	1.2791
298	2	7	700	5	0.97	0.166	0.169	1.4873	0.99	0.2514	3.9785
298	2	9	700	5	0.96	0.14	0.142	2.2021	0.99	0.3127	3.198
298	2	7	700	1	0.94	0.128	0.129	2.8738	0.99	0.3707	2.6975
298	2	7	700	5	0.97	0.166	0.169	1.4873	0.99	0.2514	3.9785
298	2	7	700	7.5	0.9	0.182	0.1847	1.3985		0.2583	3.8714

Table 4
Kinetic data calculated for adsorption of catalase enzyme on krill clay

Mass transfer					Intraparticle diffusion				
Temperature T (K)	Concentration (mol L^{-1}) 10^2	pH	Stirring speed (rpm)	[I] (mol L^{-1})	R^2	$k_{int,1}$ (mg g^{-1})	R_1^2	$k_{int,2}$ (mg g^{-1})	R_2^2
288	2	7	700	5	0.66	1.126	0.99	0.234	0.95
298	2	7	700	5	0.8	1.016	0.97	0.515	0.93
309.5	2	7	700	5	0.8	0.878	0.99	0.393	0.98
318	2	7	700	5	0.75	0.008	0.95	0.003	0.97
298	1	7	700	5	0.61	0.852	0.98	0.14	0.99
298	2	7	700	5	0.8	1.016	0.97	0.515	0.93
298	2.5	7	700	5	0.89	0.56	0.98	0.17	0.97
298	2	5.5	700	5	0.55	1.697	0.99	0.218	0.97
298	2	7	700	5	0.8	1.016	0.97	0.515	0.93
298	2	9	700	5	0.77	0.714	0.95	0.32	0.87
298	2	7	700	1	0.75	0.8	0.96	0.274	0.97
298	2	7	700	5	0.8	1.016	0.97	0.515	0.93
298	2	7	700	7.5	0.89	0.931	0.99	0.49	0.86

sorption process proceeds first by surface sorption, and then intraparticle diffusion. The initial curved portion of the plot indicates boundary layer effect while the second linear portion is due to intraparticle or pore diffusion. The calculated intraparticle diffusion coefficient values, $k_{int,1}$ and $k_{int,2}$, at different conditions are shown in Table 4. Since $k_{int,1}$ values for the first part of the plot are high, this step is not a rate-limiting step. The slope of the second linear portion of the plot has been defined as the intraparticle diffusion parameter $k_{int,2}$ (mol g^{-1} $min^{-1/2}$) [29].

For mass transfer, a linear graphical relation between $\ln[(C_t/C_0) - 1(1 + mK)]$ vs. t was not obtained (equation from [17]). This result indicates that the model mentioned above for the system is not valid. The values of regression coefficient calculated from the equation mentioned above are given in Table 4.

3.6. Thermodynamic parameters

The second-order rate constants are used to estimate the activation energy of the catalase enzyme adsorption on krill clay using Arrhenius Eq. (7):

$$\ln k_2 = \ln A - \frac{E_a}{R_g.T} \quad (7)$$

where E_a is the activation energy (J mol^{-1}), k_2 is the rate constant of sorption (g mol^{-1} s^{-1}), A is the Arrhenius factor, which

is the temperature-independent factor (g mol^{-1} s^{-1}), R_g is the gas constant (J K^{-1} mol^{-1}) and T is the solution temperature (K). The slope of the plot of lnk_2 vs. 1/T is used to evaluate E_a, which was found to be 21.9 kJ mol^{-1} physisorption (Fig. 6). Low activation energies (5–40 kJ mol^{-1}) are characteristics for physisorption, while higher activation energies (40–800 kJ mol^{-1}) suggest chemisorption [30]. Therefore, the thermodynamic activation parameters of the process, such as enthalpy ΔH, entropy ΔS and free energy ΔG, were determined using the Eyring Eq. (8) [31–33]:

$$\ln(k_2 / T) = \ln(\frac{k_b}{h}) + \frac{\Delta S}{R_g} - \frac{\Delta H}{R_g T} \quad (8)$$

where k_B is the Boltzmann constant (1.3807 × 10^{-23} J K^{-1}), h is the Planck constant (6.6261 × 10^{-34} J s) and R_g is the ideal gas constant (8.314 J mol^{-1} K^{-1}. Fig. 7 shows the plot of ln(k_2/T) against 1/T. The value of the standard enthalpy change (19.42 kJ mol^{-1}) indicates that the adsorption is physical in nature involving weak forces of attraction and is also endothermic. At the same time, the low value of ΔH implies that there was loose bonding between the adsorbate molecules and the adsorbent surface [27]. The negative standard entropy change (ΔS) value (–176.35 J K^{-1} mol^{-1}) corresponds to a decrease in the degree of the adsorbed species.

3.7. Fourier transform infrared spectroscopy diagram of adsorption system

The diagram of pre-adsorption and post-adsorption of immobilization system was given in Fig. 8. The comparison between clay minerals and biomolecules, the characteristic peaks of clay mineral was seen in around 1,645 cm^{-1}. Therefore, it is not so much looking at the idea of whether this peak is adsorption. However, as seen in 1,540 cm^{-1} to the peak from the surrounding catalase enzymes to the krill clay clearly shows a portion of the adsorbed clay minerals. Also have the characteristic peaks in the region near the peak of the clay samples of catalase enzymes 1,660 cm^{-1} is difficult to express the adsorption of biomolecules in this region.

4. Conclusions

The present study shows that krill clay can be used as an effectively adsorbent to immobilized the catalase enzyme to clay from aqueous solutions. The amount of enzyme uptake was found to increase with increase in contact time, initial enzyme concentration and ionic strength and found to decrease with increase in pH and highest adsorption takes place at 36.5°C. Maximum adsorption capacity showed a great dependence on pH and initial enzyme concentration. It was found at pH 5.5, and initial enzyme concentration, 0.0075 g L^{-1} values.

The adsorption system studied belongs to the second-order kinetic model. The enzyme uptake process was found to be controlled by intraparticle diffusion. The negative value of the Gibbs energy change of the adsorption indicates that the adsorption is spontaneous. The positive value of the enthalpy change of the adsorption shows that the adsorption is an endothermic process. Thus, the temperature 36.5°C leads to higher catalase enzyme adsorption at equilibrium.

The nature of the carrier selected influences for immobilization methods. Krill clay has a high potential to adsorb these enzyme from aqueous solutions. Therefore, it can be effectively used as an adsorbent for the adsorption of this enzyme. Consequently, experimental data obtained from this study reveal that physical adsorption is suitable for the attachment of enzyme into krill clay as a support.

Acknowledgement

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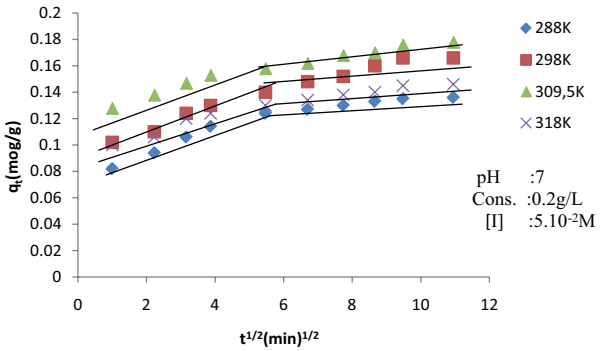

Fig. 5. Intraparticle diffusion plots for different temperatures.

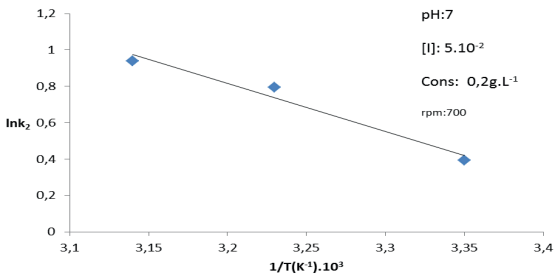

Fig. 6. Arrhenius plot for the adsorption of catalase enzyme on krill clay.

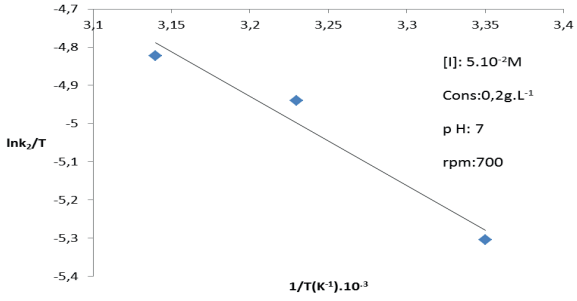

Fig. 7. Plot of ln(k_2/T) vs. 1/T for adsorption of catalase enzyme on krill clay.

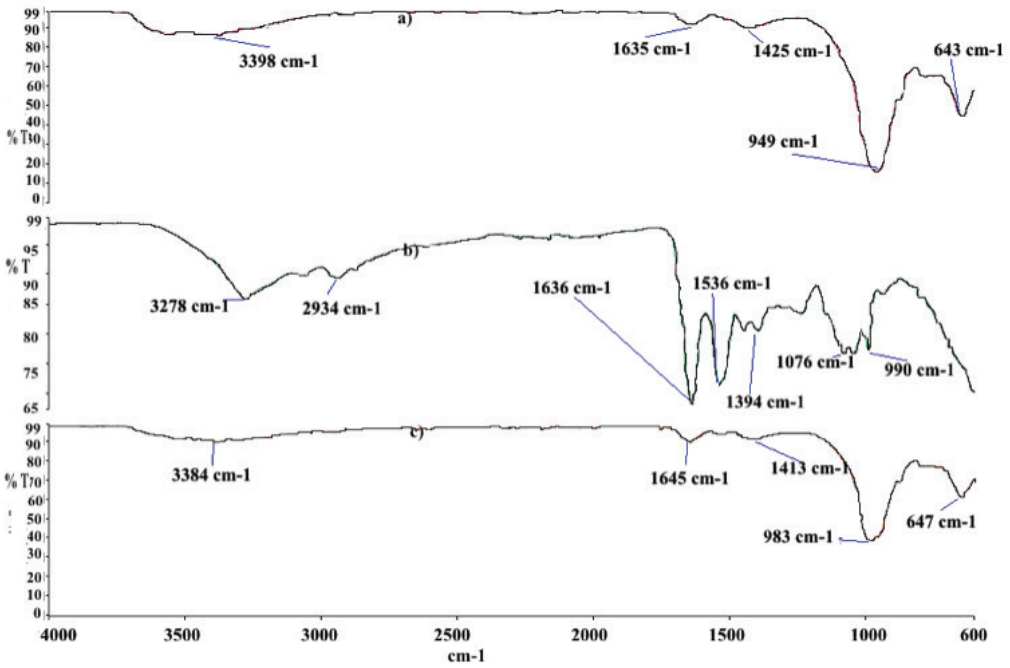

Fig. 8. The diagram of: (a) krill clay, (b) catalase enzymes and (c) post-adsorption of systems.

Table 5
Thermodynamic function data obtained by adsorption of krill clay surface with catalase enzyme

Parameters				
Temperature T (K)	ΔG (kJ mol^{-1})	E_a (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS J K^{-1} mol^{-1}
288	–70.21			
298	–71.97	21.9	19.42	176.35
309.5	–74.04			
318	–75.50			

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