

**KINETICS AND THERMODYNAMICS PROPERTIES OF CATALASE
ONTO DIATOMITE**

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ABSTRACT

In this study, the thermodynamic parameters and adsorption kinetic of catalase onto diatomite were investigated in aqueous solution for determining the effect of contact time, stirring speed, initial enzyme concentration, initial concentration of sodium phosphate buffer, temperature and pH. Maximum adsorption capacity values (q_m) showed a great dependence on pH. It was found that q_m -pH curves reached a maximum at around isoelectric point (iep) of catalase. It can be said that the maximum adsorption takes place because the electrostatic attraction is the most suitable at pH values close to the isoelectric point. Both structural and electrostatic effects must be invoked to explain the diminution of adsorbed catalase enzyme on either side of the iep. Also characterization of diatomite and catalase adsorbed diatomite were performed by SEM, FTIR-ATR, TGA and BET apparatus. Three different kinetic models, pseudo-first and -second-order and intraparticle diffusion were used to fit the kinetics data. The pseudo-second-order model best described the experimental data. The thermodynamic activation parameters, such as activation energy, enthalpy, entropy, and Gibbs free energy were determined.

KEYWORDS

Thermodynamics; Adsorption; Catalase; Diatomite; Kinetics

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, 5]. In the immobilization techniques adsorption have 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 [6-9]. Some materials such as porous glass, silica gels and cellulose are used for preparation of immobilized enzymes [10, 11]. Catalase is an enzyme containing metalloenzyme and is regarded as one of the most common enzymes in plant and animal tissues. Immobilization of catalase has useful applications in some industrial fields in removal of hydrogen peroxide used as oxidizing material [12]. Catalase can be obtained from various organisms and tissues, such as fungal or mamalian sources [13]. Many researchers maintain that catalase is immobilized

54 on a variety of support materials, providing both economical and efficient use of the
55 enzyme. [14]. Diatomite is a siliceous sedimentary rock composed of an amorphous
56 form of silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) containing a small amount of microcrystalline material. It
57 has a unique combination of physical and chemical properties such as high porosity,
58 high permeability, small particle size, large surface area and low thermal
59 conductivity. In addition, it is available in Turkey and in various locations around the
60 world [15, 16]. Diatomite in its natural state is a soft rocklike material consisting
61 essentially of the skeletal remains of a variety of singlecelled microscopic plants
62 known as diatoms. They are generally amorphous, hydrated or opaline silica, $\text{SiO}_2 \cdot x$
63 $n\text{H}_2\text{O}$, with various amounts of impurities such as silica sand, clay minerals, metal
64 salts and organic matter. Diatomite is a low-cost, environment-friendly and natural
65 micro/nanostructured material derived from sedimentary silica, and has cylindrical
66 and plate morphologies with well-developed mesoporous/macroporous structures
67 [17].

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69 The aim of this study was to determine the adsorption kinetics of catalase
70 enzyme on diatomite over a range of physicochemical conditions, which is important
71 to indentify various natural environmental systems. Samples of diatomite were
72 characterized by scanning electron microscope (SEM), attenuated total reflectance
73 combined with Fourier transform infrared spectroscopy (FTIR-ATR), thermal
74 gravimetric analysis (TGA) and Brunauer-Emmett-Teller surface area analysis (BET)
75 aparatus. A number of experimental parameters in this study are considered,
76 including the effect of stirring speed, initial catalase enzyme concentration, ionic
77 strength, pH and solution temperatures. The kinetic parameters were calculated after
78 the amount of enzyme adsorbed onto diatomite was determined. Then,

thermodynamic activation parameters such as activation energy, entropy, enthalpy and Gibbs energy of this process were calculated.

2. Materials and methods

2.1 Materials

Catalase from bovine liver was purchased from Sigma-Aldrich Co. In this research, sample of diatomite was obtained from the Aegean Region of Turkey and some analyzes were performed for its characterization. The specific surface area of diatomite was measured by BET N₂ adsorption by Micromeritics FlowSorb II-2300 equipment. The BET surface and pore volumes were calculated from the N₂ adsorption isotherms. The morphologies of diatomite samples were observed in (SEM) of Hitachi. (FT-IR) spectra were recorded on a Perkin Elmer FTIR spectrometer using ATR device. The (TGA) was obtained simultaneously using a Perkin Elmer instrument. All the chemicals were of analytical grade and were used without any further purification. The elemental analyzes of diatomite and catalase used in this work are shown in Table 1. Results containing some physicochemical properties of diatomite were given in Table 2.

2.2 Experimental procedure

Adsorption kinetic experiments were carried out using mechanic stirrer. All of the catalase enzyme solution was prepared with ultra pure water. Kinetic experiments were carried out by agitating 1000 mL of enzyme solution of initial concentration 0.20 mg/mL, 5.0×10^{-2} mol L⁻¹ in buffer solution (phosphate buffer) with enzyme of diatomite at a constant agitation speed of 700 rpm, 298 K and pH 7. In

this study, the experiments for catalase adsorption onto diatomite surface were carried out for 120 minutes at a constant agitation speed of 700rpm. This period is sufficient for the process to reach equilibrium. Preliminary experiments had shown the effect of the separation time on the adsorbed amount of enzyme. The initial tested concentration of enzyme solution was 0.10 mg/mL, 0.20 mg/mL and 0.25 mg/mL. The effect of pH on diatomite of catalase was analysed 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 phosphate ion concentrations was investigated at 0.01 – 0.075 M sodium phosphate concentrations. The effect of temperature at experiments were carried out at 288, 298, 309.5 and 318 K in a temperature bath. Samples of four milliliter 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 analysed using UV-Vis spectrophotometer (Cary 1E UV-Vis spectrophotometer, Varian) by monitoring the absorbance changes at a wave length 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)) [18].

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

where C_0 and C_t (mg mL^{-1}) are the initial and liquid- phase concentrations of enzyme solution at any time t (min), respectively; q_t (mg g^{-1}) is the amount of enzyme adsorbed per unit mass of diatomite at time t (min), V is the volume of the adsorption (mL), and m is the mass of the diatomite in the solution (g) [18].

3. Results and discussion

3.1 Effect of contact and equilibrium times and initial enzyme concentration

The adsorption of catalase onto diatomite 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. Measuring the concentration of catalase in solution at different incubation times generated in a time course of the adsorption. Fig.1. shows the plot of amount of enzyme adsorbed vs. time at different initial enzyme concentrations. According to Fig. 1, the time required to reach a stationary concentration is about 1 h. From the figure, it was observed that the amount of enzyme adsorbed gets increased from 0.069 to 0.098 mg g⁻¹ for an increase in initial enzyme concentration from 0.10 mg/mL to 0.25 mg/mL.

3.2 Analyzes of (FTIR) and (SEM) images

From the derived FTIR spectra of representative samples (Figure 2a₁, b₁ and c₁), we inferred the following results:

As seen in Figure 2(a₁), spectrum of diatomite, the band at ~1635 cm⁻¹ is due to OH bending vibrations of adsorbed water in sheet silicate minerals. The ~ 1038 cm⁻¹ band arises from the Si-O-Si vibration. The ~792 cm⁻¹ band occurs because of the OH translational vibration [19]

From Figure 2(b₁), FT-IR spectrum result of catalase showed strong peaks at ν =1620–1680 cm⁻¹, ν =1480–1580 cm⁻¹, and ν =1225–1300 cm⁻¹ mainly associated with amide-I (C=O stretching), amide-II (N–H bending vibration and C–N stretching vibration) and amide III stretching vibration bands, respectively [20, 21].

154• As shown in Figure 2(c₁), FTIR spectrum obtained for catalase adsorbed on
155 diatomite show the peaks of the amide I band at about 1657 cm⁻¹ and amide II band
156 at about 1525 cm⁻¹, which are typical of biomolecules [20].

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158 As can be seen clearly in Figures 2a, b and c, the surface of diatomite and
159 enzyme adsorbed diatomite show time-dependent morphological changes.
160 According to the figure, surface morphology of the catalase adsorbed diatomite
161 changed completely after 120 min. It can be concluded that the images are
162 consistent with experimental data and FTIR spectra.

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164 **3.3 Effect of solution pH**

165 The variation in the adsorption rate of catalase with respect to the pH can be
166 elucidated by considering the surface charge of the adsorbent materials. From the
167 Fig. 3, it was observed that the solution pH affected the amount of enzyme
168 adsorbed. The figure 3 demonstrates that the adsorption decreases with the
169 increasing of pH. When the net charge is zero at the isoelectric point in which
170 enzymes or proteins has a very stable structure. As can be seen in Figure 3, the
171 maximum adsorption capacity for diatomite is 0.17 mg / g at pH 5.5. But these
172 structures will start to decompose stable values below or above the isoelectric point
173 [22]. In this case, the amount of adsorption is effected. It was stated that the amount
174 of positive charge in the molecule at pH values above the isoelectric point of the
175 biomolecule is less than the amount of negative charge [23].

176

177 Therefore, biomolecules such as catalase are negatively charged at pH
178 values above and the positively charged at pH values below the isoelectric point.

When the pH values of the catalase solution is below the isoelectric point due to the reduction in the negative charge, the number of positive charges on the surface of diatomite increases. In this case, biomolecules of the positive charge excess causes 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 diatomite as it. Demirbaş et al stated this situation in a nice way (Figure 4) [24].

3.4 Effect of phosphate concentration

The presence of ionic solution had significantly influenced the adsorption rate of catalase. As seen in Fig.5, the adsorption was found to decrease with increasing concentration of sodium phosphate salts. 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 diatomite added to the sodium phosphate salt solution medium. On the other hand sodium phosphate salt, entering between catalase with diatomite leads to decrease electrostatic interaction. The decrease in the adsorption capacity of the adsorption process second of these two processes can be said that higher dominant effect. The same situation is observed in the interaction of dyes and some biomolecules with kaolinite clay surface [25-28].

3.5 Effect of temperature

Figure 6 shows the adsorption kinetics of catalase at 288, 298, 309.5 and 318K by plotting its uptake capacity, q_t , vs. time at the initial enzyme concentration of 0.20 mg/mL and pH 7. The speed of any reaction catalased 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 and the surface of diatomite. From the Figure 6, the maximum adsorption between the diatomite with the catalase were observed at 309.5 K (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 diatomite clay with the enzyme negatively. In parallel with this work, Vecchia et al found that immobilized from different sources have been found to be the optimum temperature 37°C [27], and Alkan, S. et al, maximum adsorption between montmorillonite clay with catalase were obtained at 35°C [29]. In another study Çetinus et al, catalase enzyme showed maximum activity at 35°C and it was decreased activity at temperatures above this temperature [30].

3.6 Thermal Gravimetric Analyzes (TGA)

As seen in Figure 7, the thermal gravimetric analyzes of diatomite, catalase and catalase adsorbed diatomite were measured by thermal gravimetric analyser (TGA). From the TG curves of a representative sample (Figure 7), we conclude that:

- In the temperature range from 25°C to 105°C, the weight loss due to absorbed water are 6.7% for diatomite (Figure 7a), 11.5% for catalase (Figure 7b) and 6.8% for catalase adsorbed diatomite (Figure 7c).
- As shown in Figure 7a, the second dehydration step observed in the temperature range 105-300°C, corresponds to the release of water molecules, which were in the interlayer space of sample [19]. In the temperature range from 400°C to 500°C, the rapid weight loss (2.26%) is documented by the steep slope of the TG curve. This is attributed to the dehydroxylation of the sample.

It can be observed from the profiles of the TG curves, both catalase and catalase adsorbed diatomite are practically similar. As illustrated in Fig.7b and c curves, there are two weight loss stages at room temperature –200 °C and 200 – 500 °C, respectively. For the catalase and catalase adsorbed diatomite, the first stage is attributed to the structural water; the second stage is assigned to the decomposition of catalase [31]. Therefore, by comparing TGA scans of catalase adsorbed diatomite with diatomite, the extra weight loss of catalase adsorbed diatomite should be attributed to the decomposition of adsorbed catalase, and thus the loading ratio of catalase can be calculated.

3.7 Adsorption kinetics

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

3.7.1 Pseudo-first and second order models

The pseudo first-order equation is generally expressed as follows [32]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (2)$$

If the rate of the adsorption is a second - order mechanism, the pseudo – second -order equation is expressed by Eq. (3) [33]:

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \quad (3)$$

where q_e and q_t (mg g⁻¹) are the amount of adsorbed enzyme at equilibrium and any time t , respectively, and k_1 is the rate constant of pseudo - first- order adsorption

(min^{-1}). k_2 is the pseudo-second order rate constant ($\text{g mg}^{-1} \text{min}^{-1}$). The fitting results are given in Table 3.

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

$$t_{1/2} = \frac{1}{k_2 q_e} \quad (4)$$

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

$$h = k_2 q_e^2 \quad (5)$$

The adsorption rate constant k_1 and q_e were calculated from the plot of $\ln(q_e - q_t)$ against t and are not presented in Table 3 because the R^2 values are not very high, and is between 0.79 and 0.98.

The values of q_e and k_2 were estimated from the slope and intercept of plots of t/q_t vs t and the corresponding results are also presented in Table 3. The R^2 values for testing kinetic data were found in the range of 0.98-0.99. As shown in Table 3, experimental data can be explained by pseudo - second - order kinetic equations.

3.7.2 Intra-particle diffusion and mass transfer models

The initial rate of the intraparticle diffusion is calculated by the following Eq. (6) [23]:

$$q_t = k_{int} t_{1/2} + C \quad (6)$$

where k_{int} is the intraparticle diffusion rate constant ($\text{mg (g min}^{-1/2})^{-1}$) and is given in Table 4. The intraparticle diffusion coefficient for the adsorption of catalase was calculated from the slope of the plot of square root of time ($\text{min}^{1/2}$) 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 multi-linearity, which characterizes the two or more

steps involved in adsorption process [33, 34]. Fig. 8 shows the plot between q_t and $t^{1/2}$ for catalase onto diatomite particles. From Fig.8 (other figures not shown), it can be seen that the adsorption 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 adsorption process proceeds by first surface adsorption, 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 $k_{int,2}$ ($\text{mg} / (\text{g min}^{1/2})$) [35]. For mass transfer, a linear graphical relation between $\ln[(C_t/C_o)-1(1 + mK)]$ vs. t was not obtained (equation from [23]). 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.

3.8 Thermodynamic parameters

The second – order rate constants are used to estimate the activation energy of the catalase adsorption onto diatomite using Arrhenius Eq. (7)

$$\ln k_2 = \ln A - \frac{E_a}{R_g T} \quad (9)$$

where; E_a is the activation energy (J mol^{-1}), k_2 is the rate constant of adsorption ($\text{gmg}^{-1}\text{s}^{-1}$), A is the Arrhenius factor, which is the temperature - independent factor ($\text{g mg}^{-1}\text{s}^{-1}$), R_g is the gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$) and T is the solution temperature

(K). The slope of the plot of $\ln k_2$ vs. $1/T$ is used to evaluate E_a , which was found to be 4.54 kJmol⁻¹ physisorption (from Fig. 9). Low activation energies (<40 kJmol⁻¹) are characteristics for physisorption, while higher activation energies (40-800 kJmol⁻¹) suggest chemisorption [36]. Therefore, the thermodynamic activation parameters of the process, such as standard enthalpy (ΔH°), entropy (ΔS°) and Gibbs free energy (ΔG°) were determined using the Eq. (10) and (11) [36, 37]. Values of the ΔH° , ΔS° , ΔG° and E_a obtained from Eqs. (9) - (11) are listed in Table 5.

$$\ln \left(\frac{k_2}{T} \right) = \ln(k_b/h) + \frac{\Delta S^\circ}{R_g} - \frac{\Delta H^\circ}{R_g T} \quad (10)$$

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

where k_b is the Boltzmann constant (1.3807×10^{-23} JK⁻¹), h is the Planck constant (6.6261×10^{-34} J s) and R_g is the ideal gas constant (8.314 Jmol⁻¹K⁻¹). Fig. 10 shows the plot of $\ln \left(\frac{k_2}{T} \right)$ against $1/T$. The value of the standard enthalpy change ($\Delta H^\circ = 2.03$ kJmol⁻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 [34]. The negative standard entropy change (ΔS°) value (-235.58 JK⁻¹mol⁻¹) corresponds to a decrease in the degree of the adsorbed species. The negative values of ΔG° for catalase at various temperatures represent that the adsorption process is spontaneous and thermodynamically favorable.

As a result, adsorbed catalysis is used in many applications. For example, catalase adsorbed on solid surfaces is used effectively in the removal of hydrogen peroxide from food products in food industry [38]. In the literature, a number of solid support materials have been reported which are used in catalase adsorption [39]. The diatomite used in this study deserves to be a supporting material for catalase due to its high surface area and pore volume and stability.

4. Conclusions

The present study shows that diatomite can be used as an adsorbent for the catalase from its aqueous solutions. The amount of enzyme uptake was found to increase with increase in contact time, initial enzyme concentration and concentration of phosphate buffer and found to decrease with increase in pH and as indicated in Figure 6 the highest adsorption takes place at 309.5 K (36.5 °C). In order to investigate the mechanism of adsorption, pseudo-first- and second-order kinetic equations, and intraparticle diffusion model have been used to test the experimental data. The rate constants and the related correlation coefficients were determined in order to assess which model provides the best-fit predicted data with experimental results. Pseudo-second-order kinetic equation provided the best-fit to experimental data. The enzyme uptake process was found to be controlled by intraparticle diffusion. The negative value of the standard Gibbs energy change of the adsorption indicates that the adsorption is spontaneous. The positive value of the standard enthalpy change of the adsorption shows that the adsorption is an endothermic process. Consequently, experimental data obtained from this investigation reveal that physical adsorption is suitable for the attachment of enzyme into diatomite clay as support. Diatomite 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.

5. References

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Tables

Table 1. Elemental compositions of diatomite and catalase

Diatomite		Catalase	
Constituent	Percentage present (%)	Constituent	Percentage present (%)
Si	48.40	O	46.5
O	36.80	C	34.1
Al	9.90	S	4.8
Mg	1.40	K	1.5
Fe	2.20	Na	0.9
K	0.70	Others	0.6
Ca	0.60		

Table 2. Some physicochemical properties of diatomite used in this study

Particle size	0 - 105 μm
Colour	White
pH	7.63
Specific surface area (Single point)	165.7 $\text{m}^2 \text{g}^{-1}$
Specific surface area (Multi point)	167.2 $\text{m}^2 \text{g}^{-1}$
Total pore volume	0.524 cc g^{-1}

433 **Table 3.** Parameters of pseudo-first order and pseudo-second order for the adsorption of various parameters of catalase on
434 diatomite.

				Kinetic models						
Parameters				Pseudo –first order	Pseudo-second order					
T/K	Initial enzyme concentration (g L ⁻¹)	pH	Phosphate ion conc. (molL ⁻¹)	R^2	q _e (calculated) (mg g ⁻¹)	q _e (exp.) (mg g ⁻¹)	k ₂ (gmg ⁻¹ s ⁻¹)	R^2	h (mg min ⁻¹ g ⁻¹)	t _{1/2} (min)
288.0	2.00	7.0	0.050	0.791	0.073	0.077	1.292	0.998	0.099	10.060
298.0	2.00	7.0	0.050	0.962	0.085	0.088	1.312	0.997	0.115	8.665
309.5	2.00	7.0	0.050	0.955	0.110	0.113	1.426	0.996	0.161	6.207
318.0	2.00	7.0	0.050	0.974	0.099	0.103	1.548	0.999	0.159	6.273
298.0	1.00	7.0	0.050	0.986	0.069	0.071	2.213	0.998	0.157	6.363
298.0	2.00	7.0	0.050	0.968	0.085	0.086	1.975	0.998	0.169	5.885
298.0	2.50	7.0	0.050	0.971	0.098	0.098	1.521	0.997	0.149	6.708
298.0	2.00	5.5	0.050	0.982	0.170	0.172	0.633	0.988	0.109	9.177
298.0	2.00	7.0	0.050	0.967	0.085	0.086	1.975	0.996	0.169	5.885
298.0	2.00	9.0	0.050	0.922	0.061	0.061	2.606	0.995	0.159	6.289
298.0	2.00	7.0	0.075	0.916	0.048	0.048	4.022	0.996	0.193	5.179
298.0	2.00	7.0	0.050	0.962	0.085	0.086	1.975	0.994	0.169	5.885
298.0	2.00	7.0	0.001	0.901	0.098	0.102	1.286	0.998	0.131	7.620

435

436 **Table 4.** Parameters of mass transfer and intra – particle diffusion for the adsorption of various parameters of catalase on
 437 diatomite.

Parameters				Mechanism of adsorption				
				Mass transfer	Intra – particle diffusion			
				R^2	$k_{int,1}$ (mg g ⁻¹ min ^{-1/2})	R_1^2	$k_{int,2}$ (mg g ⁻¹ min ^{-1/2})	R_2^2
T / K	Initial enzyme concentration (g L ⁻¹)	pH	Phosphate ion conc. (molL ⁻¹)					
288.0	2.00	7.0	0.050	0.752	0.596	0.987	0.261	0.988
298.0	2.00	7.0	0.050	0.735	0.758	0.966	0.272	0.989
309.5	2.00	7.0	0.050	0.724	0.792	0.969	0.365	0.985
318.0	2.00	7.0	0.050	0.696	1.114	0.995	0.381	0.934
298.0	1.00	7.0	0.050	0.738	0.587	0.978	0.22	0.949
298.0	2.00	7.0	0.050	0.732	0.758	0.978	0.272	0.985
298.0	3.00	7.0	0.050	0.754	0.661	0.979	0.272	0.988
298.0	2.00	5.5	0.050	0.815	1.265	0.985	0.691	0.959
298.0	2.00	7.0	0.050	0.736	0.758	0.966	0.272	0.985
298.0	2.00	9.0	0.050	0.844	0.363	0.996	0.231	0.995
298.0	2.00	7.0	0.075	0.612	0.647	0.978	0.14	0.978
298.0	2.00	7.0	0.050	0.738	0.758	0.965	0.272	0.987
298.0	2.00	7.0	0.001	0.786	0.893	0.987	0.427	0.988

438

Table 5. Thermodynamic properties for adsorption of catalase onto diatomite

T/K	ΔG° (kJmol^{-1})	ΔH° (kJmol^{-1})	ΔS° ($\text{Jmol}^{-1}\text{K}^{-1}$)	E_a (kJmol^{-1})
288.0	-69.88	2.03	- 235.58	4.54
298.0	-72.23			
309.5	-74.94			
318.0	-76.94			

Figures

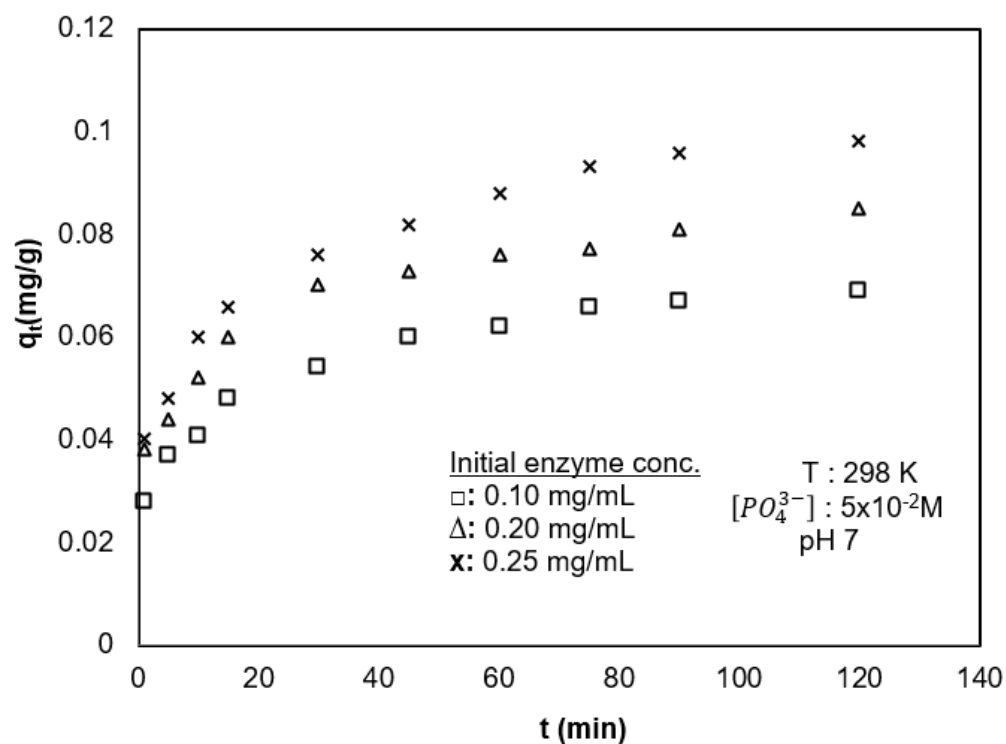


Fig. 1. The effect of initial enzyme concentration to the adsorption rate of catalase on diatomite

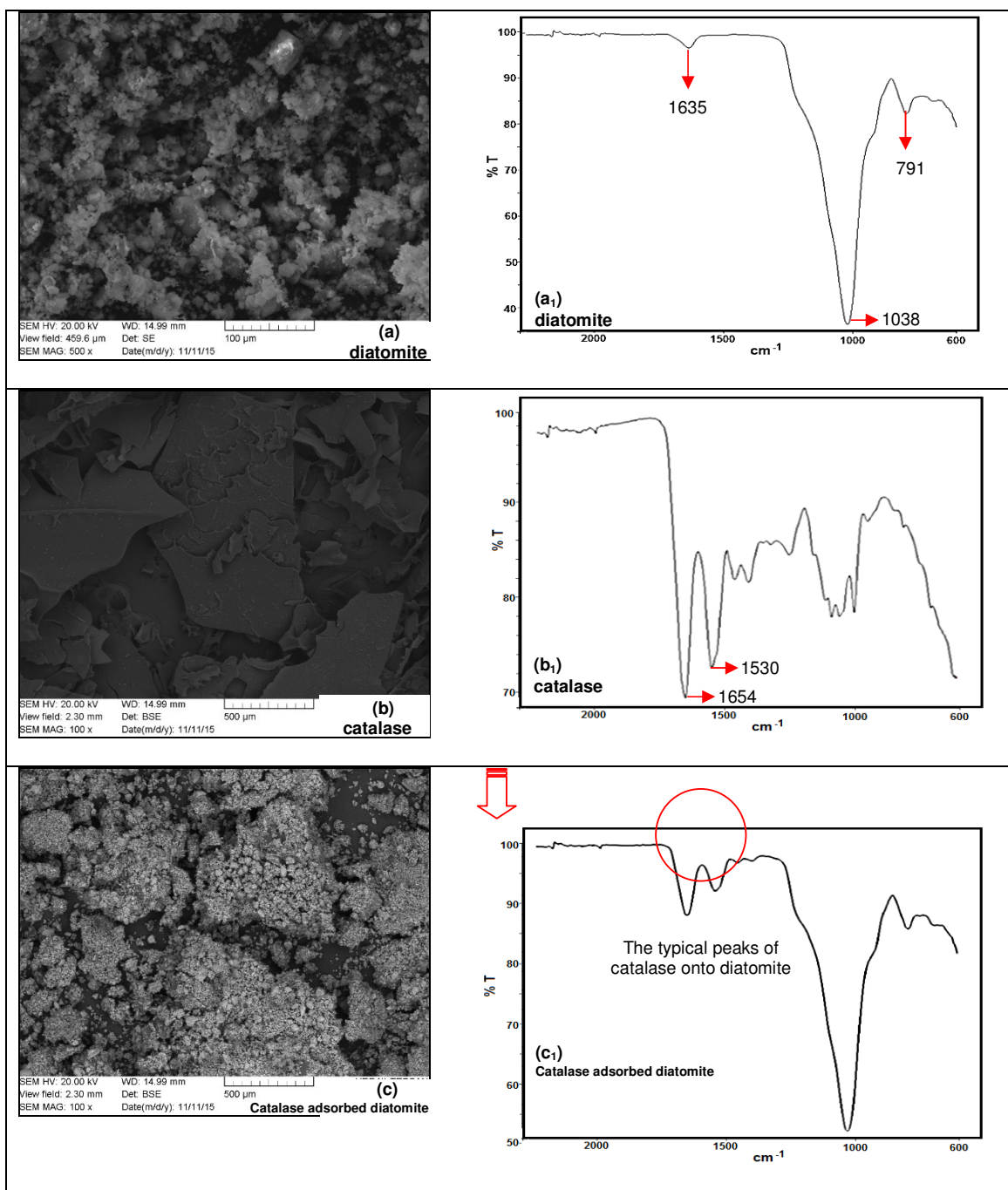
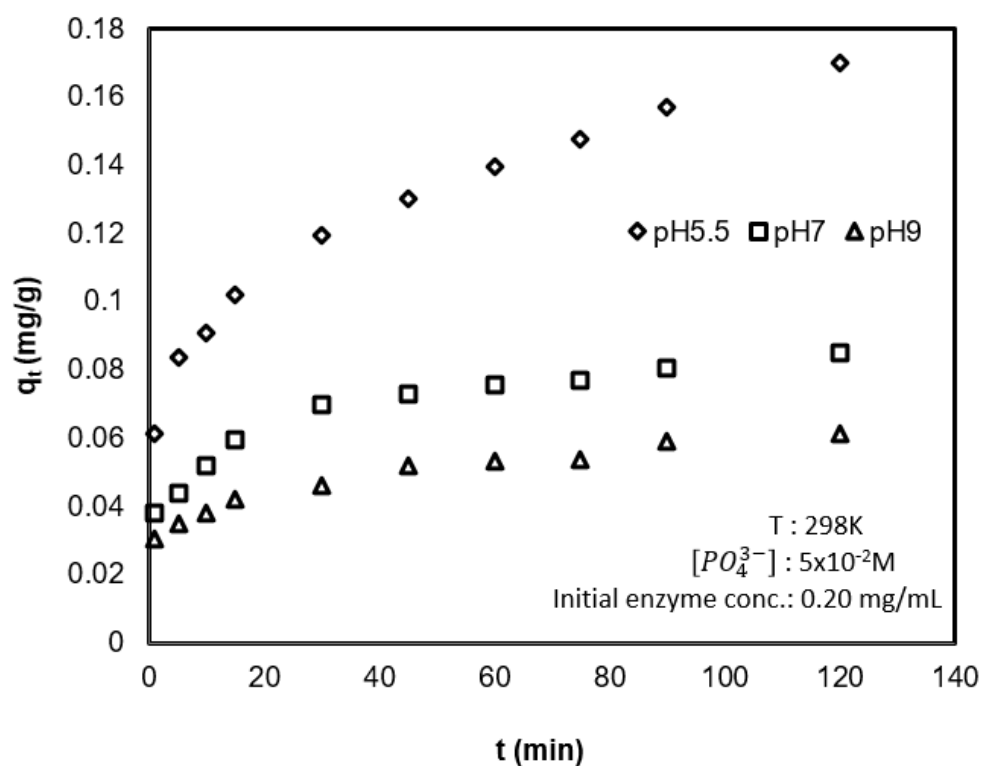


Fig. 2. SEM microphotographs and FT-IR spectra of (a, a₁) diatomite (b, b₁) catalase and (c, c₁) catalase adsorbed diatomite after 120 min.

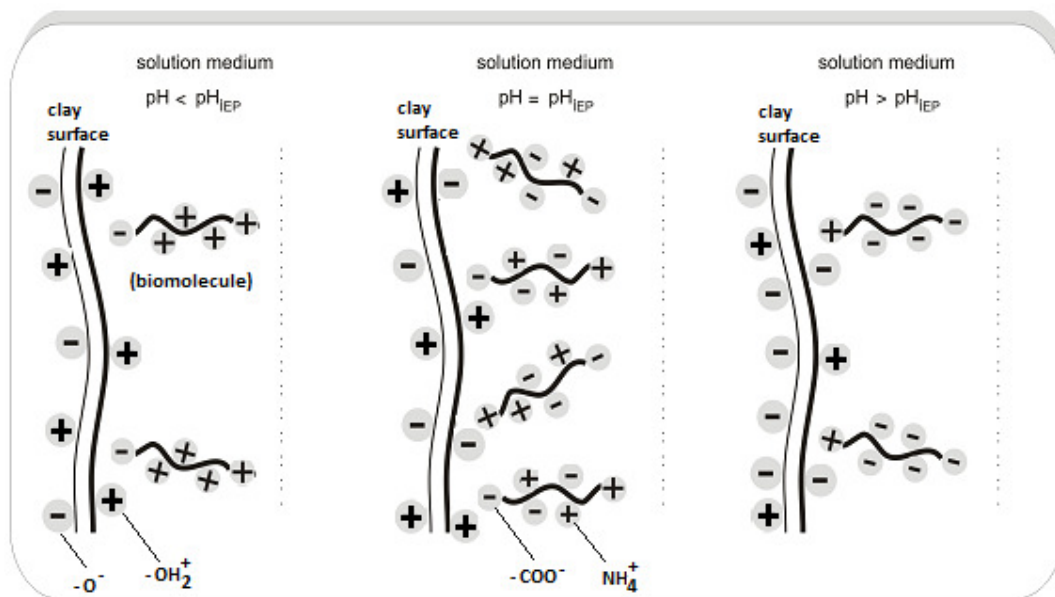
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460 **Fig.3.** The effect of solution of pH to the adsorption rate of catalase onto diatomite.



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462 **Fig.4.** Effect on the diatomite surface at different pH medium of the catalase

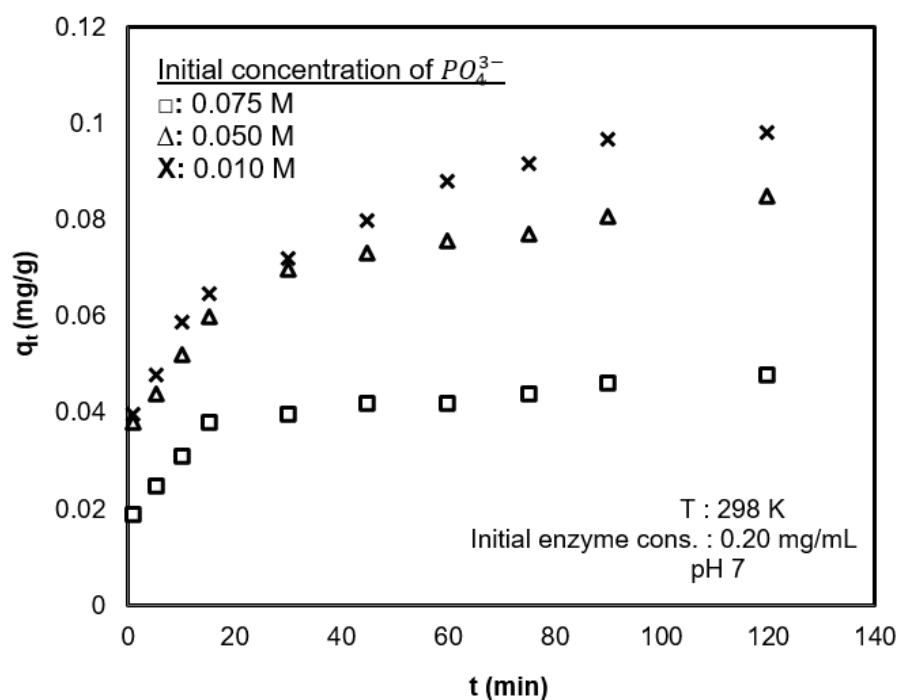


Fig. 5. The effect of concentration of phosphate ions in the solution to the adsorption rate of catalase onto diatomite.

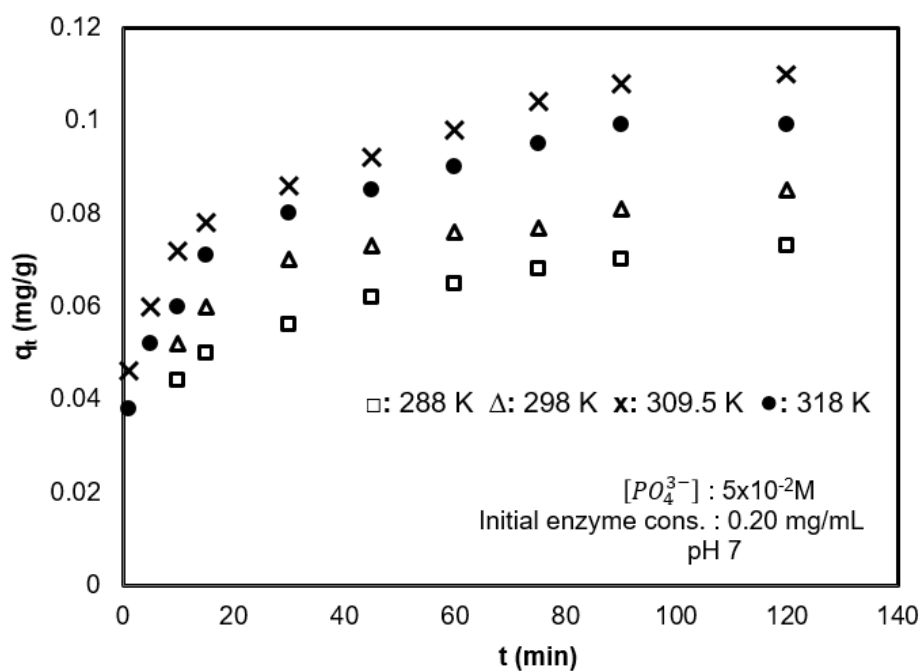
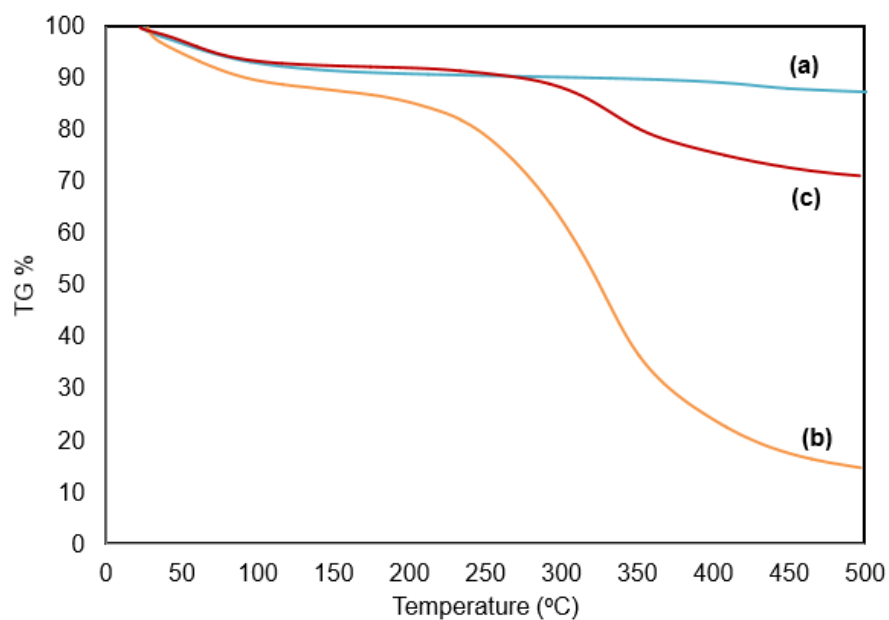


Fig.6. The effect of temperature to the adsorption rate of catalase onto diatomite.

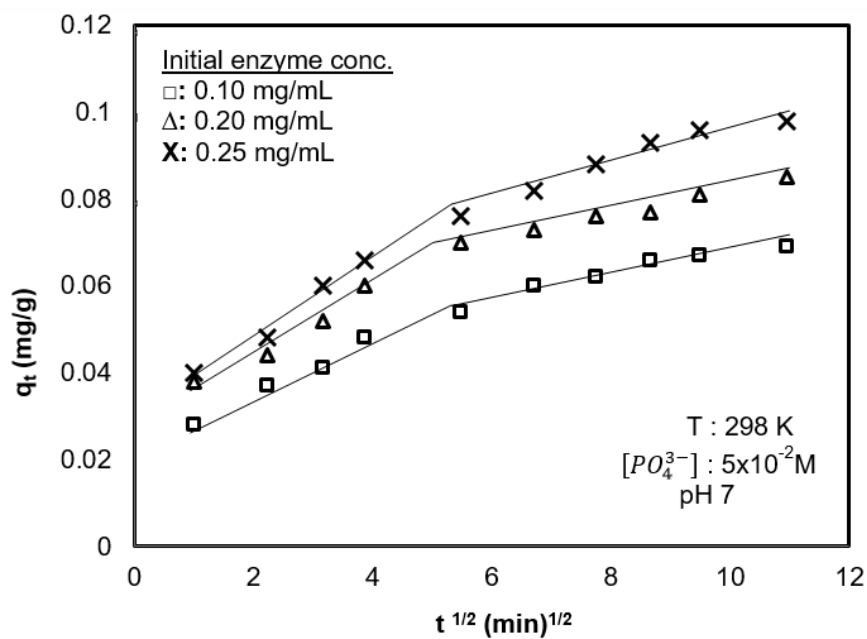
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471 **Fig.7.** Thermal gravimetric analyzes of (a) diatomite (b) catalase and (c) catalase
472 adsorbed diatomite.

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475 **Fig.8.** Intra-particle diffusion plots for different initial concentration of enzyme.

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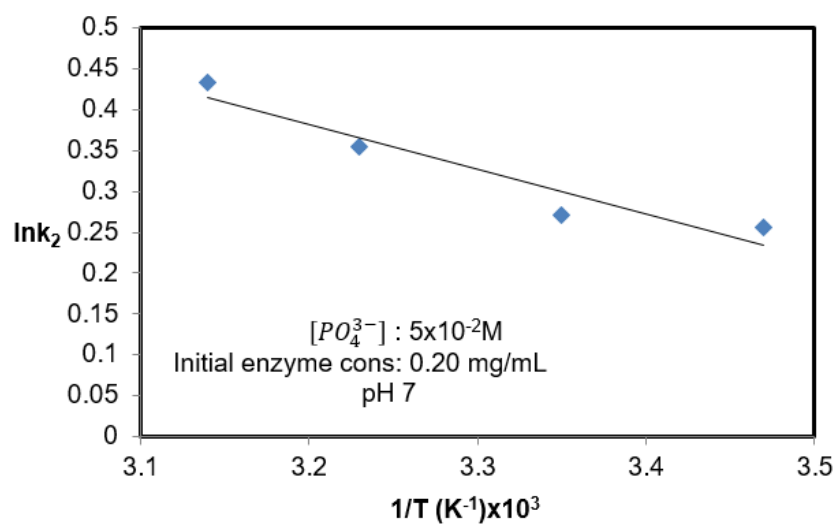


Fig. 9. Arrhenius plot for the adsorption of catalase onto diatomite.

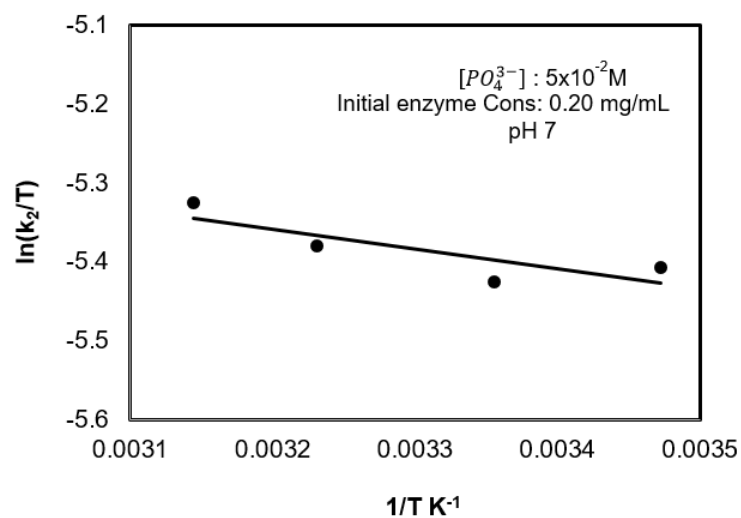


Fig. 10. Plot of $\ln(k_2/T)$ vs. $1/T$ for the adsorption of catalase onto diatomite.