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 aparatus. 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 metalo enzyme 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 mammalian sources [13]. Many researchers maintain that catalase is immobilized on a variety of support materials, providing both economical and efficient use of the enzyme [14]. Diatomite is a siliceous sedimentary rock composed of an amorphous form of silica (SiO$_2$·nH$_2$O) containing a small amount of microcrystalline material. It has a unique combination of physical and chemical properties such as high porosity, high permeability, small particle size, large surface area and low thermal conductivity. In addition, it is available in Turkey and in various locations around the world [15,16]. Diatomite in its natural state is a soft rocklike material consisting essentially of the skeletal remains of a variety of singlecelled microscopic plants known as diatoms. They are generally amorphous, hydrated or opaline silica, SiO$_2$·nH$_2$O, with various amounts of impurities such as silica sand, clay minerals, metal salts and organic matter. Diatomite is a low-cost, environment-friendly and natural micro/nanostructured material derived from sedimentary silica, and has cylindrical and plate morphologies with well-developed mesoporous/macroporous structures [17].

The aim of this study was to determine the adsorption kinetics of catalase enzyme on diatomite over a range of physicochemical conditions, which is important to indentify various natural environmental systems. Samples of diatomite were characterized by scanning electron microscope (SEM), attenuated total reflectance combined with Fourier transform infrared spectroscopy (FTIR-ATR), thermal gravimetric analysis (TGA) and Brunauer-Emmet-Teller surface area analysis (BET) aparatus. A number of experimental parameters in this study are considered, including the effect of stirring speed, initial catalase enzyme concentration, ionic strength, pH and solution temperatures. The kinetic parameters were calculated after 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 N$_2$ adsorption by Micromeritics FlowSorb II-2300 equipment. The BET surface and pore volumes were calculated from the N$_2$ 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...
Table 1. Elemental compositions of diatomite and catalase

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Percentage present (%)</th>
<th>Constituent</th>
<th>Percentage present (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>48.40</td>
<td>O</td>
<td>46.5</td>
</tr>
<tr>
<td>O</td>
<td>36.80</td>
<td>C</td>
<td>34.1</td>
</tr>
<tr>
<td>Al</td>
<td>9.90</td>
<td>S</td>
<td>4.8</td>
</tr>
<tr>
<td>Mg</td>
<td>1.40</td>
<td>K</td>
<td>1.5</td>
</tr>
<tr>
<td>Fe</td>
<td>2.20</td>
<td>Na</td>
<td>0.9</td>
</tr>
<tr>
<td>K</td>
<td>0.70</td>
<td>Others</td>
<td>0.6</td>
</tr>
<tr>
<td>Ca</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

grade and were used without any further purification. The elemental analyzes of diatomite and catalase used in this work are shown in Table 1 above. Results containing some physicochemical properties of diatomite were given in Table 2.

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

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>0 - 105 µm</td>
</tr>
<tr>
<td>Colour</td>
<td>White</td>
</tr>
<tr>
<td>pH</td>
<td>7.63</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>165.7 m² g⁻¹ (Single point)</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>167.2 m² g⁻¹ (Multi point)</td>
</tr>
<tr>
<td>Total pore volume</td>
<td>0.524 cc g⁻¹</td>
</tr>
</tbody>
</table>

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, 5.0x10⁻² 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 700 rpm. 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 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 monitored by using a 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, qₜ, was calculated from the mass balance (Eq. (1)) [18].

\[ q_t = \frac{(C_0 - C_t) \times V}{m} \]

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 (Fig. 2a\(_1\), b\(_1\) and c\(_1\)), we inferred the following results:

- As seen in Fig. 2(a\(_1\)), spectrum of diatomite, the band at \(\sim 1635\) cm\(^{-1}\) is due to OH bending vibrations of adsorbed water in sheet silicate minerals. The \(\sim 1038\) cm\(^{-1}\) band arises from the Si-O-Si vibration. The \(\sim 792\) cm\(^{-1}\) band occurs because of the OH translational vibration [19].

- From Fig. 2(b\(_1\)), FT-IR spectrum result of catalase showed strong peaks at \(\nu = 1620-1680\) cm\(^{-1}\), \(\nu = 1480-1580\) cm\(^{-1}\), and \(\nu = 1225-1300\) 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].

- As shown in Fig. 2(c\(_1\)), FTIR spectrum obtained for catalase adsorbed on diatomite show the peaks of the amide I band at about 1657 cm\(^{-1}\) and amide II band at about 1525 cm\(^{-1}\), which are typical of biomolecules [20].

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

### 3.3 Effect of Solution pH

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

![Graph](image1.png)

**Fig. 1.** The effect of initial enzyme concentration to the adsorption rate of catalase on diatomite
Therefore, biomolecules such as catalase are negatively charged at pH values above and 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. [24] stated this situation in a nice way (Fig. 4).
Fig. 3. The effect of solution of pH to the adsorption rate of catalase onto diatomite

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

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

Fig. 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.

![Fig. 5. The effect of concentration of phosphate ions in the solution to the adsorption rate of catalase onto diatomite](image1)

![Fig. 6. The effect of temperature to the adsorption rate of catalase onto diatomite](image2)
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 Fig. 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. [27] found that immobilized from different sources have been found to be the optimum temperature 37°C, and Alkan S et al. [29] maximum adsorption between montmorillonite clay with catalase were obtained at 35°C. In another study Çetinus et al. [30] catalase enzyme showed maximum activity at 35°C and it was decreased activity at temperatures above this temperature.

3.6 Thermal Gravimetric Analyzes (TGA)

As seen in Fig. 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 (Fig. 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 (Fig. 7a), 11.5% for catalase (Fig. 7b) and 6.8% for catalase adsorbed diatomite (Fig. 7c).
- As shown in Fig. 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.

![Fig. 7. Thermal gravimetric analyzes of (a) diatomite (b) catalase and (c) catalase adsorbed diatomite](image-url)
Table 3. Parameters of pseudo-first order and pseudo-second order for the adsorption of various parameters of catalase on diatomite

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Kinetic models</th>
<th>Pseudo-second order</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial enzyme concentration (g L⁻¹)</td>
<td>288.0</td>
<td>2.00</td>
</tr>
<tr>
<td>pH</td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>Phosphate ion conc. (moll⁻¹)</td>
<td>309.5</td>
<td>2.00</td>
</tr>
<tr>
<td>qₑ (calculated) (mg g⁻¹)</td>
<td>318.0</td>
<td>2.00</td>
</tr>
<tr>
<td>qₑ (exp.) (mg g⁻¹)</td>
<td>298.0</td>
<td>1.00</td>
</tr>
<tr>
<td>k₂ (gmg⁻¹s⁻¹)</td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>h (mg min⁻¹g⁻¹)</td>
<td>298.0</td>
<td>2.50</td>
</tr>
<tr>
<td>t₁/2 (min)</td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>298.0</td>
<td>2.00</td>
</tr>
</tbody>
</table>
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]:

$$\ln(q_e - q_t) = \ln q_e - k_1t$$  \hspace{1cm} (2)

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

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2q_e^2}$$  \hspace{1cm} (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 (g mg$^{-1}$ 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):

$$t_{1/2} = \frac{1}{k_2q_e}$$  \hspace{1cm} (4)

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

$$h = k_2q_e^2$$  \hspace{1cm} (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]:

$$q_t = k_{int}t^{1/2} + C$$  \hspace{1cm} (6)

where $k_{int}$ is the intraparticle diffusion rate constant (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 (min$^{-1/2}$) vs. amount of enzyme adsorbed (mg g$^{-1}$). Previous studies by various researchers showed that the slope 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 $k_{int,2}$ (mg / (g min$^{-1/2}$)) [35].

For mass transfer, a linear graphical relation between ln [(C_t/C_p)-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)

$$\ln k_2 = \ln A - \frac{E_a}{R_gT}$$  \hspace{1cm} (7)

where: $E_a$ is the activation energy (J mol$^{-1}$), $k_2$ is the rate constant of adsorption (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 (8.314 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 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 (∆H°), entropy (∆S°) and Gibbs free energy (∆G°) were determined using the Eq. (8) and (9) [36,37]. Values of the ∆H°, ∆S°, ∆G° and E_a obtained from Eqs. (7) - (9) are listed in Table 5.

\[
\ln \left( \frac{k_2}{T} \right) = \ln \left( \frac{k_B}{h} \right) + \frac{\Delta H^\circ}{R_T} - \frac{\Delta H^\circ}{RT^2}
\]  

(8)

\[
\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ
\]

(9)

where \( k_B \) is the Boltzmann constant (1.3807x10⁻²³ JK⁻¹), \( h \) is the Planck constant (6.6261x10⁻³⁴ Js) and \( R_T \) 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

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

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

11
Fig. 10. Plot of ln(k2/T) vs. 1/T for the adsorption of catalase onto diatomite

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mechanism of adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>T / K</td>
<td>Initial enzyme concentration (g L(^{-1}))</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>288.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>309.5</td>
<td>2.00</td>
</tr>
<tr>
<td>318.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>1.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>3.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
<tr>
<td>298.0</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 5. Thermodynamic properties for adsorption of catalase onto diatomite

<table>
<thead>
<tr>
<th>T / K</th>
<th>ΔH(^\circ) (kJ mol(^{-1}))</th>
<th>ΔS(^\circ) (kJ mol(^{-1}) K(^{-1}))</th>
<th>ΔG(^\circ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>288.0</td>
<td>-69.88</td>
<td>2.03</td>
<td>-235.58</td>
</tr>
<tr>
<td>298.0</td>
<td>-72.23</td>
<td>-235.58</td>
<td>4.54</td>
</tr>
<tr>
<td>309.5</td>
<td>-74.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>318.0</td>
<td>-76.94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

change (ΔH\(^\circ\) = 2.03 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\(^\circ\) implies that
there was loose bonding between the adsorbate molecules and the adsorbent surface [34]. The negative standard entropy change ($\Delta S^\circ$) value (-235.58 JK mol$^{-1}$) corresponds to a decrease in the degree of the adsorbed species. The negative values of $\Delta G^\circ$ 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 Fig. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

37. Singh RP, Singh D. Effect of cosolvent (acetone) on the adsorption and movement of cypermethrin in Indian soils.


Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/17254