Adsorption of diastase over natural halloysite nanotubes (HNTs)

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Abstract. Adsorption of diastase over natural halloysite nanotubes is studied in order to evaluate the adsorption capacity of diastase. The halloysite surface characteristics were assessed using nitrogen adsorption, x-ray diffraction (XRD), thermal gravimetric analysis (TGA) and Fourier transformed infrared (FTIR). The surface area of the natural halloysite is found to be 51 m²·g⁻¹, with total pore volume of 0.106 cm³·g⁻¹. The natural halloysite has a basal spacing (d₀₀₁) of 10 Å confirming the structure of the natural halloysite material. TGA results indicated that halloysite loses its interlayer water in the range of 30 to 105 °C and the dehydration in the structural layer above 150 °C. The dehydroxylation of halloysite has occurred at approximately 460 °C. The FTIR result of the thermally treated halloysite sample indicated that the bands observed are assigned to Si-O and Al-O bonds. The effects of solution pH and temperature were studied on the adsorption capacity and percent removal of diastase from the solution. The adsorption kinetic found to fit well with both the Pseudo first-order and Pseudo second-order models, and the values of the kinetic constant were found to be 0.173 min⁻¹ and 0.00018 g·mg⁻¹·min⁻¹ respectively. The Langmuir isotherm model is found to fit well to the adsorption data and a kinetic value is found to be 0.00059 m³·g⁻¹. The maximum adsorption capacity was found to be 370 mg·g⁻¹, indicating the potential for applications of the natural nanostructured halloysite material as an effective adsorbent for diastase.

1. Introduction

1.1. Enzyme adsorption

Enzymes are able to work as a particularly specialized molecular machine to break down, ensemble or convert molecules [1]. Starch-converting enzymes α- and β-amylase produced by plants, animals and microorganisms belong to the group designation of diastase. Diastase is greatly used as a biocatalyst in the production of bioethanol from various sources such as malt and fungal. It is used to promote the hydrolysis of starch using yeast to a fermentable dextrose as shown in the chemical equation (1) [2],

\[(C₆H₁₀O₅)_n\text{(Starch)} + nH₂O \text{(Water)} \rightarrow nC₆H₁₂O₆\text{(Dextrose)}\] (1)

Normally, liquefaction and saccharification are the two separate steps involved in the hydrolysis of starch. Soluble oligosaccharides are produced from the hydrolysing of starch in the liquefaction step. Then, glucose is produced from the hydrolysis of soluble oligosaccharides in the following saccharification step [3]. However, it is difficult to recover the α-amylase from the reaction media once it is used. Thus, it is highly recommended to use immobilized α-amylase instead of a free α-
amylase [4]. There are numerous techniques for enzyme immobilization which can be separated into two types: physical techniques and chemical techniques. Physical techniques include adsorption and entrapment and encapsulation whereas chemical technique includes cross-linking [5]. Advancements in technology have allowed the enzyme immobilization onto a support material by physical methods to increase the stability of the enzyme and allow long-term operation in the industries. As the “binding mechanism” of enzyme is generally reversible, its recovery by using adsorption technique is simple and non-invasive. Therefore, physical adsorption is the recommended immobilization technique for numerous industrial applications because no chemical additives are necessary and the carrier can be generated for repetitive uses [6].

Inorganic supports materials exhibit benefits of high stability, resistibility against organic solvents and microbial attacks, and ease of disposal or reusability in contrast with organic supports materials [7]. Hydrotalcites and zeolite are the classic inorganic materials that have been studied in enzyme immobilization. However, these materials possess drawback of restriction of pore diameter and surface area, thus only the loading of certain specific enzymes is allowable. For instance, zeolite have been recommended as prospective supports for enzyme immobilization. Nonetheless, zeolite with pore diameter ranging from 0.5 and 1.1 nm, it proves that the access of large molecules to the microporous surface can be restricted by diffusion [8]. Due to the large pore diameters of HNTs, it appeared that HNTs are more attractive for enzyme immobilizations as compared to zeolites [7]. The amount of urease adsorbed onto the HNTs was determined to be 11.7 mg·g⁻¹ whereas for α-amylase was 15.4 mg·g⁻¹. The amount of enzymes adsorbed onto the HNTs is reported to be higher as compared to molecular sieve MCM-41 by applying the similar physical adsorption technique for enzyme immobilization. HNTs can offer sufficient space for large amounts of enzymes as compared to MCM-41 as it processes a larger pore size of than the diameter of α-amylase and urease.

Halloysite is a double-layered aluminosilicate clay mineral composing of one alumina octahedron sheet and one silica tetrahedron sheet in 1:1 stoichiometric ratio. It has similar chemical properties as kaolin, diverging primarily in the morphology of crystals [7]. Moreover, it has a structural formula of Al₂(OH)₄Si₂O₅·nH₂O, where a monolayer of water molecules splits up the unit layers in HNT, which owns a one-dimensional tubular porous structure of the mesoporous range (2-50 nm). Such property allows the multipurpose potential uses, including as a nano-sized support for the loading of functional guests [9]. In terms of length, HNTs differ from the submicron scale to various microns and some even greater than 30 µm. Its external diameter varies from about 30 to 190 nm, whereas its internal diameter varies from about 10 to 100 nm [10]. HNT is a natural occurring low cost material and available in abundance. Most significantly, when the pH is less than 8.5, positive charged is retained in the inner surface of HNTs which foster the loading of HNTs with a negatively charged macromolecules [11]. On the other hand, it is reported that a maximum adsorption capacity of 84.32 mg·g⁻¹ of methylene blue (MB) was accomplished in the adsorption of methylene blue onto HNTs which is much higher as compared to other clay minerals [12]. It has been proven in this study that HNTs is an effective nano-support in the aid in removing cationic dye through physical adsorption from aqueous solution. There was no leaching out of the enzyme α-amylase and urease occurred during the reaction and no free enzymes desorbed from the HNTs. Therefore, HNTs can be considered as nanoreactors as it offers as a stable support matrix for the enzyme immobilization.

A research conducted on the adsorption of diastase onto acid-treated bentonite between the concentrations of 0.05 to 0.5 g·mL⁻¹. It was found out that the increased in the initial diastase concentration increased the diastase adsorption which eventually saturated the initial α-amylase concentration beyond 0.5 g·mL⁻¹. It was claimed that a huge amount of diastase molecule moved towards the interface with increasing diastase concentration [13,14]. At the present time, the immobilization of diastase onto the natural halloysite nanotubes (HNTs) through physical absorption is not well reported. The immobilized diastase hinges on the features and efficiency of the HNTs and the diastase stability in immobilized state.

During a study on the immobilization of enzyme biocatalyst on HNTs conducted by Zhai et al. (2010) [7], it was found out that a tetrahedral layer of Si and O is condensed with an octahedral layer.
of A1 and OH. As the result of the surface potential of SiO₂ with a slight influence from the positive Al₂O₃ internal surface as the chemical properties of the internal cylinder core might be related with Al₂O₃, the zeta potential behaviour of HNTs particles is generally negative at pH 6 to 7 [15]. Due to the isoelectric point of urease is at pH 6 and α-amylase is at pH 4.6, α-amylase and urease retained negatively charged during the process of immobilization at pH 6. Hence, negatively charged α-amylase and urease were immobilized on the positively charged inner lumen of HNTs at pH 6 via ionic adsorption.

The effect of temperature on the adsorption of diastase onto acid-treated bentonite clay surfaces was reported [13]. The adsorption test was carried out by varying the temperature ranging from 5 to 45 °C. The results showed that as the temperature increased, the diastase adsorption increased. It was claimed that the motion of the diastase molecules increased with increasing temperature as adsorption is usually a process controlled by diffusion. It was pointed out that there are several other forces other than electrostatic forces granting towards the adsorption. It was suggested that hydrophobic forces might be the force taking affecting the process.

### 1.2. Adsorption kinetics

Kinetics and mechanism of adsorption onto the surface of the adsorbent are usually determined by numerous models. The rate of adsorption (dq/dt) is assumed to be proportional to the change amid the amount of the adsorption capacity of adsorbent and adsorption at time (t) which is \( (q_t - q_e) \) in an adsorption process and \( k_1 \) is the proportionality constant. Whereby \( q_e \) and \( q_t \) are the adsorption capacity at equilibrium and at time \( t \), respectively. Rate law is of arbitrary order \( n \) and \( q \) follows in (2),

\[
\frac{dq}{dt} = k_1(q_t - q_e)^n
\]  

(2)

The rate of occupation of sorption sites is proportional to the number of unoccupied sites in pseudo first-order power model. The Lagergren pseudo first-order is normally expressed as in (3),

\[
\frac{dq}{dt} = k_1(q_t - q_e)
\]  

(3)

\( k_1 \) is the rate constant of pseudo first-order kinetic model in \( \text{min}^{-1} \). The integrated form of (3), after applying boundary conditions \( t = 0 \) to \( t = t \) and \( q_t = 0 \) to \( q_t = q_e \), is presented in (4),

\[
\ln(q_t - q_e) = \ln(q_e) - k_1 t
\]  

(4)

A plot of \( \ln(q_t - q_e) \) against \( t \) contributes a linear relationship whereby \( k_1 \) and \( q_e \) can be identified from the slope and y-intercept of the plot respectively [16]. The non-linear form of (4) is shown in (5),

\[
q_t = q_e \left[ 1 - e^{(-k_1 t)} \right]
\]  

(5)

The appropriateness of the pseudo first-order power equation to experimental values usually varies in two ways; the number of available sites is not represented by the parameter \( k_1(q_t - q_e) \). The parameter \( \ln(q_e) \) is a variable parameter and frequently determined to be unequal to the y-intercept of the plot \( \ln(q_t - q_e) \) against \( t \), while in the exact first order, \( \ln(q_e) \) must be equal to the y-intercept. In pseudo second-order power model that the rate-limiting stage can be chemical sorption relating to valence forces via sharing or exchange electrons amid adsorbent and adsorbate. The pseudo second-order adsorption kinetic rate equation (6) is expressed as follow,

\[
\frac{dq}{dt} = k_2(q_t - q_e)^2
\]  

(6)

whereby \( k_2 \) is the pseudo second-order kinetic rate constant \( (g \text{-adsorbent·mg-adsorbate}^{-1} \text{-min}^{-1}) \). The integrated form of equation (6), after applying the boundary conditions \( t = 0 \) to \( t = t \) and \( q_t = 0 \) to \( q_t = q_e \), is presented in (7) or in linear form equation (8),

\[
\frac{1}{q_t - q_e} = \frac{1}{q_e} - k_2 t
\]  

(7)
\[
\frac{(t/q_e)}{1}=h+\frac{1}{q_d t}
\]
where \(h = k_2 q_e^2\) is the initial adsorbate rate (mg-adsorbate/g-adsorbent-min). A plot of \((t/q_e)\) against \(t\) of (8), gives a linear relationship whereby \(q_e\) and \(k_2\) can be identified from the slope and \(y\)-intercept of the plot respectively [16].

Adsorption isotherm is commonly used to investigate the mechanisms of adsorption and the surface properties as well as the affinities of the absorbent [17]. Adsorption isotherm is known as the correlation between the quantities of a substance being adsorbed at constant temperature and its concentration in the equilibrium solution [18]. The reversible adsorption of an enzyme onto a solid surface is described by Langmuir theory, which can be applied to the adsorption from solution of diastase, \(D\) onto a surface site, \(S\) of HNMs [19]. To describe the adsorption mechanism by Langmuir model, the following assumptions are made; the adsorption is occurring only in physisorption mechanism; the adsorbate acts as an ideal solute in liquid phase; the adsorbate molecules stay at specific adsorption sites; the adsorbent surface is homogeneously dispersed with adsorption sites; each adsorption site has the same energy of interaction and, there are no interactions between the adsorbed molecules [20]. The adsorption and desorption kinetics are shown in (9) and (10).

\[
\text{Rate of adsorption, } r_{ad} = k_{ad} C_D C_v
\]
\[
\text{Rate of desorption, } r_d = k_d C_{(D\cdot S)}
\]
where \(C_D\) is the molar concentration of diastase, \(C_v\) is the molar concentration of free vacant sites (moles of \(S\) per g-HNMs), \(C_{(D\cdot S)}\) is the concentration of diastase-halloysite complex (moles/g-HNMs). \(k_{ad}\) is the forward adsorption rate constant (L·mole\(^{-1}\)·min\(^{-1}\)), \(k_d\) is the desorption rate constant (min\(^{-1}\)). At equilibrium, whereas the rate of adsorption is equivalent to the rate of desorption \((r_{ad}=r_d)\) and rearranging gives (11),

\[
C_{(D\cdot S)}/(1+K_{eq}) = k_{ad}/k_d = K_{eq}
\]
where \(K_{eq}\) is the adsorption equilibrium constant (volume of solution per mole of \(S\)). The total concentration of all sites, \(C = C_v + C_{(D\cdot S)}\) is combined with (11) result in (12),

\[
C = C_{(D\cdot S)}/(1+K_{eq}) + C_{(D\cdot S)} = (1+K_{eq})C_{(D\cdot S)}/(K_{eq} C_{(D\cdot S)})
\]
Relating fraction of surface sites, \(\theta = C_{(D\cdot S)}/C\) gives Langmuir adsorption isotherm as shown in (13)

\[
\theta = (K_{eq} C_{(D\cdot S)})/(1+K_{eq} C_{(D\cdot S)})
\]
When \(\theta = q_e/q_{max}\), the alternative form of (13) is given in (14),

\[
q_e = (q_{max} \cdot K_l [D])/(1+K_l [D])
\]
where \(q_{max}\) and \(K_l\) are the maximum adsorption (mg·g\(^{-1}\)) and the Langmuir isotherm constant (mg\(^{-1}\)) respectively. \(q_e\) is the number of binding sites that are occupied by the adsorbate at the concentration \([D]\) [19].

To identify the best fitting isotherm, linear regression is widely used. The correlation coefficients are used to judge and compare the applicability of isotherm equations [21]. A swift increase in the amount of adsorbed adsorbate as the adsorbate concentrations increase at low concentrations can be predicted by Langmuir isotherm model. The amount of adsorbed adsorbate attains a maximum level which is equivalent to the number adsorption sites, \(q_{max}\) once the adsorption sites are fully filled. It is pointed out that the maximum adsorption capacity ought to concur with saturation of the monolayer coverage. As a result, it does not depend on temperature [22]. Langmuir model delivers details on uptake qualifications and is qualified of reflecting the common process behaviour of equilibrium adsorption even though it sheds no light on the mechanistic features of adsorption [23]. It is assumed by Langmuir model that chemical unsaturated atoms (sum of binding sites) exert forces that do not prolong more than the diameter of one adsorbed molecule. Consequently, adsorption is limited to a
monolayer. Commonly, equilibrium behaviour is not described precisely by Langmuir isotherm, particularly with heterogeneous adsorption systems where adsorption sustained further than a monolayer [23].

2. Experimental

2.1. Characterization

The natural halloysite nanotubes (HNTs, sigma-aldrich) were characterized using nitrogen adsorption-desorption using Quantachrome Autosorb Automated Gas Sorption System, thermal gravimetric analysis (TGA) using Mettler Toledo 851, Stare SW 10, Fourier transformed infrared (FTIR) and X-ray diffraction (XRD) using Bruker AXS D8 advance X-Ray Diffrectometer. The purpose of the characterization is to determine the surface area, pore sizes, crystal structures and thermal stability.

The nitrogen adsorption-desorption was employed for pore structure analysis and pore size distribution using BJH method. This technique determines over 0 – 1 range of relative pressure at a temperature of −196 °C. BET method is used for the measurement of the specific surface area of material. TGA analysis was performed by heating about 0.1 g of the halloysite sample in an alumina crucible with a heating rate of 10°C·min⁻¹, under high purity N₂ flow (50 mL·min⁻¹). FTIR spectra was recorded in the wavelength range of 4000 – 400 cm⁻¹. The XRD patterns of the halloysite sample were recorded at 2θ in the range of 2 – 90°.

2.2 Adsorption test

The dynamic batch process is applied in the current study for immobilizing diastase onto HNTs. Three parameters were tested in adsorption test which include the effect of contact time, solution temperature and pH. Standard solutions of 0.5, 0.25, 0.125 and 0.0625 g·L⁻¹ were prepared. The standard solutions were scanned at a wavelength of 280 nm to determine the UV-Vis spectrophotometer absorbance. The absorbance values for each standard solutions were read at the determined wavelength and the data were recorded. The concentration of diastase in solution was identified by measuring the absorbance at a single wavelength of 280 nm. The concentration of an absorbing diastase solution is linearly interrelated to the absorbance.

Two grams of HNTs were placed into an oven at 105 °C for drying overnight. A 500 mL of diastase solution was adsorbed using 0.5 g of HNTs. Samples were taken at pre-set time intervals of 5 min (for the first 20 min) and 10 min time intervals (for 30 to 120 min). The samples were filtered instantly after collection and the absorbance of the samples were measured in triplicate using UV-Vis spectrophotometer at 280 nm at once. Each adsorption experiment was repeated for 3 times and the average result was obtained.

For effect of temperature study, 50 mL of 1 g·L⁻¹ of the diastase solution in distillate water (pH 7) was used with 0.05 g of HNTs. The mixtures were stirred slowly at temperatures of 25, 35 and 45 °C respectively, for 2 h until equilibrium is achieved. The solutions were filtered and the absorbance of the supernatant for remaining diastase was measured in triplicate by a UV-Vis spectrophotometer at 280 nm at once. Each adsorption experiment was repeated for 3 times in order to obtain the average result. Similar experiments were conducted at 25 °C by varying the pH of a distillate water in the range of (3 – 8) and diastase concentration of 1 g·L⁻¹. The pH was adjusted by adding a few drops of 0.1 M NaOH or 0.1 M HCl before each run.

The amount of diastase adsorbed onto the HNTs at any time, q (mg·g⁻¹) was calculated by using mass balance relationship as shown in (15).

\[ q_t = (C_0 - C_t)(V/W) \]

where \( C_0 \) and \( C_t \) are the initial and concentration of diastase solution at time t respectively (mg·L⁻¹), \( V \) is the volume of the solution (L) and \( W \) is the dry weight (g) of the HNT. \( \ln(q_e - q_t) \) against time (t) was plotted for pseudo first-order power model. Likewise, \((t/q_t)\) against time (t) was plotted for pseudo
second-order power model. The kinetic parameters, $k_1$ and $k_2$ and regression coefficients were determined from the plots.

3. Results and discussions

3.1. Characterization

The textural characterization of untreated halloysite was carried out using nitrogen adsorption-desorption at −196 °C. According to the International Union of Pure and Applied Chemistry (IUPAC), type IV classification with H3 hysteresis loop is identified. The hysteresis involves filling and emptying of the mesopores by capillary condensation. Such behaviour does not exhibit any limiting adsorption at high $P/P_0$ because it results from the existence of non-rigid aggregates of plate-like particles or assemblages of slit-shaped pores [24]. This explains that the natural halloysite exhibits the characteristic of conventional micro- and mesoporous materials such as MCM-41 as shown in figure 1. Using the BET method, the surface area of the natural halloysite was calculated to be 51 m$^2$·g$^{-1}$. Meanwhile, the total pore volume of the natural halloysite was found to be 0.11 cm$^3$·g$^{-1}$ using BJH method. This reviewed that the untreated halloysite possesses a relatively high specific surface area and total pore volume.

TGA was used to determine the mass loss of the natural halloysite as a function of temperature at a constant heating rate. To evaluate the thermal stability of the natural halloysite compound, the TGA analysis is presented in figure 2 in the temperature range of 30 to 700 °C. The first peak is detected in the temperature ranging from 30 to 105 °C accompanied with mass loss of 2.1%. This is due losing adsorbed water on the external surfaces of the halloysite. The second and third peaks are observed at approximately 150°C and 260 °C respectively. Such weight losses are attributed to the thermal dehydration of halloysite in the structural layer. Small deviation theoretical values were observed at the temperatures ranging from 300 to 400 °C because of the presence of intercalated water, eliminated slowly from the interlayer spaces of halloysite. Dehydroxylation is where the hydroxyl group in the structural layer of halloysite is being removed when continuous heat above 480 °C. This contributes to 12.4 % of the total mass of the sample.

Fourier transformed infrared (FTIR) spectra provides an evidence of the changes of the structure due to thermal effects. The FTIR was characterized for halloysite at 400 °C. Figure 3 reports the FTIR spectrum of the halloysite which has shown three bands in the region of 3700 – 3600 cm$^{-1}$. Through the FTIR spectrum, the bands are located at 3695 and 3626 cm$^{-1}$. This region is assigned to the stretching vibrations of hydroxyl groups. It can be observed that there are no significant changes in the functional group thermal treatment of halloysite at 400 °C. This is because the band at 3695 and 3626 cm$^{-1}$ are attributed to the inner surface hydroxyl groups. The deformation of H–O–H is observed in the region of 1700 – 1500 cm$^{-1}$. The interlayer water is indicated by the vibration band at 1653 cm$^{-1}$ in the natural halloysite. The band observed 1036 cm$^{-1}$ is resulted by the stretching vibrations of Si–O–Si. The intensity of the vibration of O–H deformation of the inner Al-OH groups are found at 913 cm$^{-1}$ of the thermally treated halloysite. The bands at 753 cm$^{-1}$ exhibits the typical OH translational vibrations.

XRD was used to characterize the natural halloysite as shown in figure 4. The XRD patterns of the HNTs show the diffraction peaks which are indexed to the characteristics of the HNTs. The natural halloysite has a basal spacing ($d001$) of 10 Å confirming the structure of the natural halloysite material.

3.2. Adsorption test

The results and discussions of the kinetic by pseudo first-order and pseudo second-order models are presented in this section. Diastase uptake rate controls with its residence time uptake at solid and solid interface is described by this sorption kinetics study. The contact time between adsorbate and adsorbent is extremely significant as it hinges on the nature of the system used. Figure 5 shows the variation of adsorption intake of diastase by HNTs with contact time. It is observed that the diastase
adsorption intake was rapid in the initial 20 min, thereafter proceeded at a negative adsorption rate and finally achieved equilibrium. The abundant number of vacant adsorption sites available on the surface of the HNTs at the initial stage is the main cause of higher rates at the beginning. The high driving force for the relocation of diastase molecules from solution to the HNTs’ surface which is resulted from the high concentration gradient in the beginning of adsorption process. Nonetheless, as time proceeds, it is noticed that the concentration gradient decreases because of the increases in the occupation of vacant sites by the diastase molecules in the solution. At 20 min, the adsorption process reached saturation where the vacant site on the HNTs were fully occupied with maximum adsorption capacity of 169 mg-diastase per g-HNT. Meanwhile, the forward adsorption rate is zero at this stage.

From 20 to 40 min, the diastase adsorption capacity decreases gradually to 136 mg-diastase per g-HNT and with further small decreases from 40 to 60 min. This implies that desorption commences in the solution where the diastase molecules release from the surface of HNTs in order to achieve the state of sorption equilibrium. Concurrently, the forward adsorption rate is negative at this stage as the adsorption became less efficient due to the active binding sites were being used up with time, thus limited free sites for diastase molecules to attach to. At about 60 min onwards, the adsorption process attained equilibrium where the adsorption capacity remains approximately constant.

Figure 1. Nitrogen adsorption-desorption isotherms of NHNs.

Figure 2. TGA analysis of NHNs.

Figure 3. FTIR spectra of thermal-treated halloysite at 400°C.

Figure 4. XRD scans of NHNs.

The rate of adsorption of diastase onto the HNTs is said to be equal to the rate of desorption off the HNTs surface. In other words, the amount of diastase being adsorbed onto the HNTs was equivalent to
the amount of diastase being desorbed from the HNTs. Consequently, it is observed from the contact
time curve that the state of sorption equilibrium is reached at 60 min. From this outcome, in order to
make sure the state of sorption equilibrium is accomplished, the agitation time was fixed at 120 min of
time for the rest of the batch experiments. Potential monolayer coverage of the diastase on the HNTs
surface is denoted as the contact time curve attained is smooth and continuous, leading to saturation.
The rapid diastase intake is particularly significant in process design and operation in practical usages.

![Figure 5. Effect of contact time on the adsorption of diastase by HNTs (diastase concentration: 1 g·L⁻¹; T: 25 °C; pH 7).](image)

3.3. Kinetic study
In order to examine the controlling mechanism of the adsorption process, pseudo first-order and
pseudo second-order power models were used to test the kinetic data. The best fit values of (qₑ)ₑ and k₁ of pseudo first-order equation (4) and h, (qₑ)ₑ and k₂ of the pseudo second-order equation (8)
determined from figures 6 and 7 and are presented in table 1 along with their corresponding
correlation coefficients respectively. For the pseudo second-order power model, it is perceived that the
(qₑ)ₑ varies significantly from (qₑ)ₑ, while the values are much closer to that of the pseudo first-order
kinetic than pseudo second-order kinetics. Nevertheless, the kinetic data were said to fit well with both
the pseudo first-order and pseudo second-order power models with a correlation coefficient of 0.97
and 0.95 respectively.

| Kinetics Model       | Parameters          | Values       |
|----------------------|---------------------|--------------|
| Pseudo first-order   | (qₑ)ₑ (mg·g⁻¹)     | 200          |
|                      | k₁ (min⁻¹)          | 0.1732       |
|                      | (qₑ)ₑ (mg·g⁻¹)     | 169          |
|                      | R²                  | 0.97         |
| Pseudo second-order  | (qₑ)ₑ (mg·g⁻¹)     | 323          |
|                      | k₂ (g·mg⁻¹·min⁻¹)   | 0.00018      |
|                      | h (mg·g⁻¹·min⁻¹)    | 19           |
|                      | (qₑ)ₑ (mg·g⁻¹)     | 169          |
|                      | R²                  | 0.95         |

The mechanism of the diastase adsorption process in which a single site on the surface of HNT is
liable for acquiring a single molecule of the diastase on its surface is supported by pseudo first order
power model [16]. As a result, the HNTs’ surface area processes can be expounded as mono-site-
occupancy adsorption mechanism. In addition, the physisorption process by which weaker interactions
such as electrostatic forces and van der Waals forces necessitate between the adsorbate and the surface
of the adsorbent is supported by pseudo first-order power model. Furthermore, the chemical properties
of both adsorbate and the adsorbent do not change and the binding is not strong in physisorption.
Hence, the physisorption mechanism is rapid and absolutely reversible through desorption (Bulut et al.
2008). On the other hand, pseudo second-order power model supports the mechanism that
Chemisorption dominates the comprehensive rate of diastase adsorption process by which two-site occupancy adsorption mechanism is followed. Stronger particular forces encompassing in the development of chemical linkage such as a single ionic and covalent bond are involved in chemisorption. Chemically adsorbed molecules are unable to transfer to the surface within an interface, thus chemisorption mechanism is completely irreversible. Ultimately, the good agreement of both models points out that the adsorption process can be controlled by both physiosorption and chemisorption.

3.4. Langmuir adsorption isotherm
The initial diastase concentration has an effect on the adsorptive intake of diastase by HNTs as shown in figure 8. The adsorption capacity at equilibrium, $q_e$, increased from 126 to 128 mg-diastase per g-HNT with an increase in the initial diastase concentration from 900 to 1200 mg·L$^{-1}$, signifying that the higher the adsorbate to adsorbent ratio, the superior will be the quantity of diastase adsorbed. The high diastase concentration gradient generating a high driving force to transport the diastase molecules from solution to the surface of HNTs. Henceforth, the adsorption process was conducted much more sufficient, resulting in the rise in the diastase adsorption capacity. Langmuir adsorption isotherm was proposed for a quantitative understanding of the interaction between diastase and specific surface of HNTs. The best fit values of $q_{max}$, $K_L$ of Langmuir adsorption model equation (14) determined by plotting the linear correlation $1/C_e$ against $1/q_e$ and are presented in table 2. The Langmuir adsorption capacity, $q_{max}$ was obtained as 370 mg·g$^{-1}$ from the experimental data. The correlation coefficient shows a strong positive proof that the adsorption of diastase onto HNTs follows the Langmuir adsorption isotherm whereby a monolayer coverage occurs on the surface of HNTs and only physisorption occurs. This proposes that the adsorption happens to the active sites of the adsorbent, confirming the suitability of the Langmuir adsorption model. Hence, it can be presumed that the molecules are absorbed at a fixed number of distinct sites with each site accept only one molecule of
adsorbate. Additionally, the vacant sites are also expected to be enthusiastically equivalent and distant to one another. Therefore, there are certainly no interactions between adsorbed molecules occur in the adjacent regions [4].

![Graph](image)

**Figure 8.** Effect of initial diastase concentration on the adsorption over HNTs (T: 25°C; pH 7).

| Parameters     | Values |
|----------------|--------|
| q_{max} (mg·g⁻¹) | 370    |
| K_L (L·mg⁻¹)    | 0.0006 |
| R²              | 0.98   |

**Table 2.** Langmuir adsorption isotherm constants of diastase onto HNTs.

### 3.5. Effect of temperature

The adsorption studies were performed over a temperature range of 25 to 45 °C in order to determine the effect of temperature on the diastase adsorption. As illustrated in figure 9, it is observed that the equilibrium adsorption capacity was clearly affected by temperature, with the amount of diastase adsorbed increasing from 127 to 160 mg-diastase per g-HNT when the temperature was raised from 25 to 35 °C. This can be described by the rise in the rate of diffusion of the adsorbate molecules crosswise the exterior boundary layer as well in the interior pores of the adsorbent molecules which results from the decline in the viscosity of the solution as well as the alteration in the equilibrium adsorption capacity of the adsorbent for a specific adsorbate [25]. It is worth proclaiming that a lower adsorption capacity is expected as the electrostatic attractions are generally weakened at higher temperature and this would be only occurred at temperatures above 35 °C. However, a higher adsorption capacity at 35 °C absolutely designate that other than electrostatic forces, there are numerous other forces such as hydrophobic forces ought to be contributing towards the adsorption [13]. This can be evidenced by the reported data that protein retention in hydrophobic interaction is improved with increasing in temperature [14]. Apart from that, the increase is due to the increasing in van der Waals interactions with increasing temperature [4]. Furthermore, the amount of active sites increases on the HNTs surface with increasing temperature and consequently, the amount of adsorbed diastase increased. It is postulated that the motion of the diastase molecules increased with increasing temperature since adsorption is generally a diffusion controlled process, thus causing in higher adsorption whereby simplifying the development of surface monolayers [13]. The decline in adsorption capacity was observed at temperatures over 35 °C is due to the conformational alterations to the surface of diastase or structural distortion of HNTs [14]. Hydrogen bonds identifies the shape of proteins and they are effortlessly disrupted via alterations of temperature. Below 35 °C, the bonds which define the shape of protein are inflexible enough to allow the shape alterations essential for fitting into a site. Above 35 °C, the bonds are excessively weak to clutch the protein in proper position as well as sustain its shape. Eventually, denaturation occurs as the proper shape of protein is lost and enzyme is demolished.
3.6. Effect of pH

The two main factors influencing the solution pH dependence of adsorption are the dispersal of adsorbate in the solution phase and the overall charge of the adsorbent. On the other hand, the factor that is responsible for the bonding or enzyme immobilization includes the carrier’s surface activity in league with a functional moiety or characteristic group on the enzyme’s protein surface. The existence of the bonds such as ionic, hydrogen and covalent hydrophobic amid the enzyme protein and the support hinges on the nature of the surface of enzyme. Enzyme can be immobilized either externally or internally on the support’s surface via bonding. The hydrophilic residues in the diastase are usually discovered on the surface of the molecule. The type of interaction suggested are the hydrophobic interactions amid hydrophobic siloxane layer (Si-O-Si) of the surface of clay and enzyme molecule and the electrostatic interactions amid positive charged aluminols (Al-OH) of the internal surface of clay and negatively charged amino acid residues of the enzyme [13].

The effect of solution pH on the adsorption of diastase by HNTs is shown in figure 10. The adsorption capacity of the diastase onto HNTs increased with decrease solution pH. This is due to the electrostatic attraction of negatively charged amino acid residues of diastase with positive charged surface of HNTs at low pH. The amount of hydroxyl groups (OH) which are the key reactive groups that are able to interact with charges is contained in HNTs. The zeta potential of HNTs was identified from 2.2 to 11 and the pH_{zpc} (pH at the zero point of charge) was discovered to be 2.7. Due to Al-OH groups experience protonation, the surface of HNTs is positively charged under pH 2.7 whereas a negatively charged is acquired beyond this pH. The generation of the positive or negative charge surface is due to the attribution. Plentiful of Si-OH and Al-OH are contained on the surface of HNTs that undergo ionization in (16)

\[
\text{Si/Al} \quad \text{OH}_2^+ \quad \text{Si/Al} \quad \text{OH} \quad \text{Si/Al} \quad 0^-.
\]

The reaction on the right hand side of equation (16) is caused by silica, an acidic oxide to be leading over a great range of pH values whereas an amphoteric behavior is displayed by alumina. The surface is negatively charged over a wide range of pH values as the surface of the HNTs is primarily occupied by silica. Consequently, negatively charged diastase is attached readily onto HNTs in aqueous solution. The positive charged surface of HNTs is saturated by electrons with the increasing of pH > pH_{zpc}. Competition of negatively charged diastase with hydroxide ions for the adsorption sites occurs and thereby decreases the adsorption capacity of diastase. An increase in the adsorption capacity of diastase is caused by the increasing of electrostatic attraction amid adsorption sites and negatively charged diastase with decreasing solution pH.
4. Conclusions

The adsorption kinetic and mechanism of diastase over natural halloysite nanotubes (HNTs) was studied and found to be reliant on the pH and temperature. The kinetic data were found to fit well with both the pseudo-first order and pseudo-second order model with kinetic constants of 0.1730 min⁻¹ and 0.00018 g·mg⁻¹·min⁻¹ respectively. An agreement of both model points out that the adsorption process can be controlled by both physiosorption and chemisorption. The fit of the Langmuir adsorption model is achieved, indicating that a monolayer coverage occurs on the surface of HNTs and only physisorption occurs. The maximum adsorption capacity is 0.370g diastase per gram of halloysite, indicating the potential for a possible use of the natural nanostructured material as an effective immobilizing support for diastase. The reversible adsorption of diastase onto HNTs is well described by Langmuir theory.

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