Comparative adsorption of amylase, protease and lipase on ZnFe_2O_4 : kinetics, isothermal and thermodynamics studies

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Received: 7 April 2017 / Accepted: 19 June 2017 / Published online: 30 June 2017
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Abstract The role of enzyme engineering in biotechnology, biological and pharmaceutical process cannot be over emphasized. This study compared the adsorption of digestive enzymes; amylase, protease and lipase on to Zn-ferrite (ZnFe_2O_4). The metal ferrite was synthesized via a sol-gel technique and characterized with scanning electron microscopy (SEM), X-ray diffraction (XRD), Electron paramagnetic resonance (EPR) and Fourier transform infrared spectroscopy (FTIR). The adsorption was studied in a batch process and the data were subjected to kinetics and isotherm models. Characterization shows that the particle has a nanoporous structure, with pore sizes of about 5.4 nm and good magnetic properties. The FTIR data showed the presence of M–O bond, which is a characteristic of metal ferrites. The adsorption of the amylase, lipase and protease on ZnFe_2O_4 follow first-order kinetic model with rate constants increasing with concentration. The maximum adsorption capacities as revealed by the generalized adsorption isotherms are 7.20, 42.90 and 22.24 mg g^{-1} for amylase, lipase and protease, respectively, with cooperative binding. The Dubinin–Radushkevich model gave the maximum adsorption energies, E of 3.74 kJ mol^{-1} for amylase, 2.01 kJ mol^{-1} for lipase and 1.51 kJ mol^{-1} for the protease adsorption, showing that the process is physisorption dominated. The isotherms fit the adsorption data in the order of Freundlich > Generalized > Guggenheim–Anderson–de Boer > Tempkin

isotherm > Dubinin–Radushkevich. Thermodynamic study revealed a spontaneous adsorption process with increased entropy. ZnFe_2O_4 , therefore, is a very good adsorbent for the purification of enzymes and can be used as a supporter for enzymatic process that required immobilization of the enzymes.

Keywords Adsorption · Enzymes · Zn-ferrite · Kinetic and isotherm

Introduction

Adsorption of proteins on solid surfaces is of great importance to many applications in bionanotechnology. For instance, enzyme immobilizations on nanoparticles are important precursors for the design of bionanocatalysts, bionanosensors and many other bionanotechnological-based materials, taking into cognizance the large surface to volume ratio of nanoparticles (Nune et al. 2009; Umut 2013; McNamara and Tofail 2017). Surface functionalized nanoparticles of biomedical application are being designed to target a particular protein based on the vulnerability of protein to adsorption onto surfaces (Slocik and Naik 2010; Assarsson et al. 2016). Although, enzyme adsorption on the surface is a prerequisite for these important applications, reduced or total loss in activity may accompany such adsorption (MacCormack et al. 2012). Careful selection of immobilization strategies and adsorbent materials can increase the dexterity of the adsorbed enzymes to work in a much broader pH and temperature range with higher thermal stability than the native ones (Xu et al. 2014). Immobilized enzymes find applications in processes requiring mechanical strength, microbial resistance, thermostability, chemical durability, chemical functionality,

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low cost, hydrophilicity, regenerability and high capacity of enzyme (Burns 1976). Other advantages include; choice of batch or continuous processes, rapid termination of reactions, controlled product formation, ease of enzyme separation from the reaction mixture and adaptability to various engineering designs (Blanco et al. 1991; Huang et al. 1997).

Isolation and purification of proteins and peptides involved the use of chromatography, electrophoretic, ultrafiltration, precipitation and other procedures, affinity chromatography being one of the most important techniques (Bornhorst and Falke 2000; Kumar and Sharma 2015). Although these standard procedures are well established, their inability to cope with the samples containing particulate material in the early stages of the isolation/purification process is a big disadvantage. Since suspended solid and fouling components are present in the sample at this stage. In the cases of magnetic affinity, ion-exchange, hydrophobic or adsorption batch separation processes, applications of magnetically stabilized fluidized beds or magnetically modified two-phase systems have shown their advantage (Safarik and Safarikova 2004). The advantages of magnetic separation include; its simplicity with only few handling steps, inexpensive, inertness to the target peptides, which can also be used for the concentration of the peptides instead of ultrafiltration and precipitation. Moreover, the power and efficiency of magnetic separation procedures is useful at large-scale operations (Sarmiento et al. 2015). Compared with other techniques in enzyme immobilization/purification, adsorption has been considered to be the best due to its higher commercial potential, its simplicity and ability to retain high catalytic activity. Also, adsorption method offers the reusability of expensive supports after inactivation of immobilized enzyme (Gooding and Hall 1996; Yildiz and Gür 2007).

In this study, ZnFe_2O_4 was synthesized via a sol-gel technique and characterized with scanning electron microscopy (SEM), electron diffraction analysis (EDAX), X-ray diffraction (XRD), Electron paramagnetic resonance (EPR) and Fourier transform infrared spectroscopy (FTIR). It was used for the adsorptions of 3 extremoenzymes (α -amylase, lipase and protease) given their industrial application potentials and the fact that their enzymatic activities increases with purity (Robinson 2015). The kinetics data from their adsorptions studies were subjected to pseudo first order, second order, elovich and intraparticle diffusion kinetic models. Similarly, data from equilibrium studies were analyzed with Guggenheim-Anderson-de Boer (GAB), generalized adsorption isotherms, Freudlich, Tempkin and Dubinin-Raduskevitch (D-R) adsorption isotherm models. The temperature dependent adsorption data were used for the estimation of the thermodynamic parameters.

Materials and methods

Materials

All chemical used in the study were reagent grade, they were used without further purification. Ferric nitrate nanohydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) and Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were purchased from Sigma Aldrich, while citric acid and ammonium were from ACS reagents. All other reagents were of analytical grade and MilliQ water was used for all the experiments.

Preparation of ZnFe_2O_4 nanoparticle

Zinc ferrite (ZnFe_2O_4) was synthesized via a sol-gel technique by weighing accurately 0.29748 g of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.80799 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and dissolved in 10 ml Milli Q water. The solutions of the salt were mixed and stirred at 50 °C for 12 h. Citric acid was added as a chelating agent and the mixtures were allowed to evaporate at 90 °C until a dark brown sol-gel was formed. A sudden increase in temperature led to the combustion resulting into the formation of ZnFe_2O_4 nanoparticles. The as-prepared nanoparticles were then calcinated for 4 h at 800 °C. The dried material was characterized by different physicochemical techniques.

Surface morphology and elemental composition of the material was analyzed using scanning electron microscopy (SEM) [VEGA3 TESCAN], X-ray diffraction (XRD) data were collected using a PAN Analytical X'Pert PRO X-ray diffractometer with Cu $K\alpha$ radiation ($\lambda = 1.5418\text{\AA}$). Fourier transform infrared (FT-IR) spectra were recorded from 400 to 4000 cm^{-1} in TENSOR 27 spectrometer (Bruker, Germany) using KBr pellet technique while the magnetic property were analyzed with BioSpin ESR EMX-8/2.7 system (Bruker, Germany).

Adsorption and kinetic studies of the enzymes

Crude enzymes were obtained from Microbiology Laboratory, Federal University of Agriculture Abeokuta, Nigeria. The enzymes were produced from *Aspergillus flavus* PW2961 strain obtained from the culture collection unit of the laboratory. Adsorptions of the enzymes (amylase, lipase and protease) onto the surface of ZnFe_2O_4 were carried out in a 10 ml vial by adding 5 ml of different concentrations (1.0–20.0 mg/L) of the crude enzyme to 0.2 g of the nanoparticle at room temperature and optimum activity pH (amylase 7.0, protease, 5 and lipase, 6.7). The final suspension was mixed for 30 s to disperse the nanoparticles and subsequently shaken for 2 h in an orbital shaker at 150 rpm. The adsorbed protein with nanoparticles

was separated from the mixture using an external magnetic field and the supernatant was analyzed for the protein content. The difference in protein concentration compared with the blank was the result of enzyme adsorption on to the adsorbent. Adsorption kinetics was studied by determining the amount of free protein in the supernatant by repeating the procedure above at different contact time. The amounts of enzyme adsorbed at time t , Q_t (mg g^{-1}) and at equilibrium Q_e (mg g^{-1}) were calculated using Eqs. 1 and 2 below:

$$Q_t = \frac{(C_o - C_t)V}{W} \quad (1)$$

$$Q_e = \frac{(C_o - C_e)V}{W} \quad (2)$$

where C_o (mg L^{-1}) is the initial concentration and C_t (mg L^{-1}) is the concentration of the crude enzyme at time t in the liquid-phase. C_e (mg L^{-1}) is the concentration of the enzyme in the supernatant at equilibrium, V is the volume of the solution (L), and W is the mass of ZnFe_2O_4 .

To investigate the mechanisms of the adsorption process, pseudo-first order, pseudo-second order, Elovich kinetic and intraparticle diffusion models (Eqs. 3–6) were applied to describe the kinetics of the enzymes adsorption. For all Eqs. 3–6, Q_e is the amount of enzyme in mg g^{-1} adsorbed at equilibrium while k_1 , and k_2 are the rate constant, K_{di} is intraparticle diffusion constant and C_i measures the thickness of the layer. The Elovich parameters α and β_{el} are initial adsorption rate ($\text{mg g}^{-1} \text{min}^{-1}$) and the desorption constant (g mg^{-1}) (Weber and Morris 1963; Lagergren 1996; Özacar and Şengil 2005; Adeogun and Balakrishnan 2016).

$$Q_t = Q_e(1 - e^{-k_1 t}), \quad (3)$$

$$Q_t = \frac{k_2 Q_e^2 t}{1 + k_2 Q_e t}, \quad (4)$$

$$Q_t = 1/\beta_{el} \ln(\alpha \beta_{el} \times t), \quad (5)$$

$$Q_t = K_{di} \times t^{0.5} + C_i. \quad (6)$$

Equilibrium data from the adsorption studies were also subjected to the Guggenheim–Anderson–de Boer (GAB), generalized adsorption isotherms, Freundlich, Tempkin and Dubinin–Raduskevitch (D–R) adsorption isotherm models (Kargi and Ozmihci 2004; Crini and Peindy 2006; Zheng et al. 2008; Adeogun and Balakrishnan 2016) represented by Eqs. 7–10).

GAB adsorption model proposed a multilayer adsorption with addition that the state of the adsorbate in the other layers being the same but different from the first layer. The model is a combination of two distinct adsorption states, i.e., equivalent adsorption site where the adsorbate bind strongly with the possibility of the occupied site

successively adsorbed weakly to the adsorbate. L_k and K_s are, respectively, the weak and strong state adsorption constants, while Q_o is the maximum adsorption in the inner wall of the adsorbent. This model will reduce to Langmuir equation for value $K_s = 0$ (Meissner et al. 2015). Generalized adsorption isotherm model is represented by Eq. 8, the model take into account the saturation of the binding site and cooperativity in the sites of adsorption. The value of $m > 1$ means an increase in affinity for other adsorbate molecule upon binding of adsorbate to one site, $m < 1$ mean binding in one site reduces affinity for others. Cooperative binding constant, $m = 1$ means independent binding of the adsorbent to available sites. K_G is the saturation constant in mg L^{-1} while Q_m is the maximum adsorption capacity (Kargi and Ozmihci, 2004). Freundlich isotherm described the heterogeneity in the surface. The K_F and n of Freundlich isotherm model (Eq. 9) are isotherm parameters characterizing adsorption capacity and intensity, respectively. Tempkin isotherm suggested a linear decrease in surface energy upon adsorption, taking into account the adsorbent/adsorbate interactions. Tempkin isotherm constants a_T and b_T in Eq. 10 are equilibrium binding constant (L/g).

$$Q_{eq} = \frac{Q_o L_K C_e}{(1 + L_K C_e - K_s C_e)(1 - K_s C_e)}, \quad (7)$$

$$Q_{eq} = \frac{Q_m C_e^m}{(K_G + C_e^m)}, \quad (8)$$

$$Q_{eq} = K_F C_e^{1/n}, \quad (9)$$

$$Q_e = \frac{RT}{b_T} \ln a_T C_e, \quad (10)$$

$$Q_e = Q_s \text{Exp}(-\beta \varepsilon^2). \quad (11)$$

The D–R sorption isotherm is based on ideal assumptions such as equipotent of the sorption sites, absence of stoic hindrance between adsorbed and incoming particles and surface homogeneity on microscopic level. D–R isotherm is represented by Eq. 11, where Q_m is the theoretical saturation capacity (mol g^{-1}), β is a constant related to the mean free energy of adsorption per mole of the adsorbate ($\text{mol}^2 \text{J}^{-2}$), and ε is the Polanyi potential given by the relation; $\varepsilon = RT \ln(1 + 1/C_e)$. C_e is the equilibrium concentration of enzyme (mg L^{-1}), R ($\text{J mol}^{-1} \text{K}^{-1}$) is the gas constant and T (K) is the absolute temperature. The constant β gives an idea about the mean free energy E (kJ mol^{-1}) of adsorption per molecule of the adsorbate when it is transferred to the surface of the solid from relationship $E = (2\beta)^{-0.5}$. If the magnitude of E is between 8 and 16 kJ mol^{-1} , the process is chemisorption, while for values of $E < 8 \text{ kJ mol}^{-1}$ suggests a physical process. Non-linear regression analysis

Fig. 1 The scanning electron micrograph and energy diffraction of ZnFe₂O₄

method was used to obtain the least square fit for all the models, using a program written on Micro Math Scientist 3.0 software (Salt Lake City, Utah).

Statistical test

The acceptability and hence the best-fit of the kinetic data were based on the square of the correlation coefficients R^2 and the percentage error function which measures the

differences (% SSE) in the experimental data and the values predicted by the models. The validity of each model was determined by the sum of error squares (SSE, %) given by:

$$\%SSE = \sqrt{\frac{((Q_{(exp)} - Q_{(cal)}) / Q_{exp})^2}{N - 1}} \times 100 \quad (12)$$

N is the number of data points. The higher is the value of R^2 and the lower the value of % SSE; the better fitted the

data. A model is adjudged best-fit and selected based on statistical parameters.

Results

Characterizations of ZnFe₂O₄

The scanning electron micrograph and energy diffraction are presented in Fig. 1a–c. Figure 2 shows the typical XRD patterns of ZnFe₂O₄. The EDAX analysis showed that the surface is mainly constituted by the elements in the desired ratio. The EPR spectra of ZnFe₂O₄ were obtained at 9.859876 GHz at room temperature to determine its magnetic properties. EPR study of ZnFe₂O₄ is presented in Fig. 3 and its parameters were determined using $g = \frac{h\nu}{\beta H}$. Figure 4 shows FT-IR spectra of the ZnFe₂O₄ before (a) and after (b) the adsorption of the enzyme on the surface of ZnFe₂O₄.

Kinetics and mechanism of adsorption

The adsorption kinetic data of α -amylase, lipase and protease enzymes on ZnFe₂O₄ were subjected to kinetic model given by the Eqs. 3–6, Figs. 5, 6, 7 were obtained by the least square fit of the kinetic models using non-linear least square fit with the program written on Micro Math Scientist software (Salt Lake City, Utah) with the parameters shown in Table 1.

Adsorption isotherms

The amount of a substance adsorbed into the solid phase of adsorbent is related to the concentration of the substance in bulk solution at a particular temperature in equilibrium using the adsorption isotherm. Equilibrium adsorption data

Fig. 2 XRD patterns of the ZnFe₂O₄

of α -amylase, lipase and protease enzymes on ZnFe₂O₄ were subjected to isotherm models given by the Eqs. 7–11, Fig. 8a–c were obtained by the least square fit of the models with the parameters shown in Table 2.

Thermodynamics of adsorption process

The thermodynamics parameters, i.e., ΔG^0 , ΔH^0 and ΔS^0 were estimated using the following relation:

$$\Delta G^0 = -RT \ln K_d \quad (13)$$

$$\ln K_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \quad (14)$$

The equilibrium constant, K_d , was estimated from the value of Q_e/C_e obtained at different temperature studies of equilibrium. Van't Hoff plot of $\ln K_d$ against the reciprocal

Fig. 3 EPR study of ZnFe₂O₄

Fig. 4 FT-IR spectra of the ZnFe₂O₄ before (a) and after (b) the adsorption

Fig. 5 Kinetic fits for the adsorption of α -amylase on ZnFe_2O_4 **a** pseudo-first order model fits, **b** pseudo-second order model fits, **c** Elovich and **d** Intraparticle diffusion model fits

of temperature ($1/T$), according to Eq. 14 produced straight lines (Fig. 9) with intercept as $\Delta S^0/R$ and slope as $\Delta H^0/R$.

Discussion

SEM analysis showed a well-defined porous structure of the ZnFe_2O_4 without a secondary phase. Figure 2 shows the typical XRD patterns of ZnFe_2O_4 , all diffraction peaks compared favorably to cubic spinel (JCPDS No. 22-1012) structure of ZnFe_2O_4 . No other impurity peaks were noted which is a clear indication of pure product. Using Debye–Scherrer equation, $D = \frac{k\lambda}{\beta \cos \theta}$ for the strongest peaks, the mean grain sizes of ~ 5.42 nm was obtained. The magnetic properties by Lorentzian fit of EPR data analysis gave

resonance width (ΔH) of 257.88 Gauss, resonance field of 3851 (Gauss) and the effective g-factor was estimated to be 2.06. These values compare favorably with ZnFe_2O_4 prepared by other methods the high g-factor is due to the presence of Zn^{2+} in the ferrite structure. The FTIR analysis revealed the prominent absorption bands between 390 and 503 cm^{-1} which slightly shifted to higher wave number upon adsorption could be attribute to Fe–O and Zn–O bonds vibrations. Upon adsorption, the intensity of the peak at 1690 cm^{-1} reduced, which could be attributed to the interaction of the enzyme with nanoparticle.

Studying the adsorption kinetics and mechanism of the enzymes on ZnFe_2O_4 to estimate the optimum operational conditions and efficiency for full-scale processes is desirable. The kinetic of adsorption, revealed the rate constants

Fig. 6 Kinetic fits for the adsorption of lipase on ZnFe_2O_4 **a** pseudo-first order model fits, **b** pseudo-second order model fits, **c** Elovich and **d** Intraparticle diffusion model fits

while the mechanism reveals the steps involved in the adsorption processes. Figures 5, 6 and 7 showed that the quantity of enzyme adsorbed (Q_t) increases with time and enzyme concentrations. This trend is attributed to the gradual occupation of the empty sites as the adsorption progressed and become saturated at equilibrium. The first-order kinetic model best fitted the kinetic data with initial increase in rate constant as the initial enzyme concentration increases. Although average values of R^2 obtained for both first- and second-order kinetic model are almost the same, first order was adjudged as the best fit owing to the values of Q_e obtained ($Q_{e \text{ calc}}$), which are consistent with the experimental values ($Q_{e \text{ exp}}$), statistical analysis with lower values of the %SSE is also in favour of first order model. The data fitted well with Elovich model ($R^2 > 0.9$) as shown in Table 1, increase in adsorption rate, α is noted with increasing enzyme concentrations. This may be as a

result of increased probability of collision of enzyme with the adsorbent surface as the concentration increases. The desorption rate (β) on the other hand decreases with concentration for all the enzymes, since β is linked to the distribution of activation energies of adsorption, therefore, adsorption of the enzymes at higher concentrations may be limited by energetic barriers Zhang and Stanforth (2005). The mechanisms of the adsorption of enzymes to ZnFe_2O_4 were investigated using intraparticle diffusion model. The intraparticle diffusion constant, K_{di} increases the enzyme concentration, this is as result of the influence of increase in driving force and corresponding resistance of the surface boundary to the concentration gradient as the enzymes accesses the available sites on ZnFe_2O_4 . The non-zero positive value of the intercepts C_i showed that adsorption is not solely determined by intraparticle diffusion, rather mass transfer of the enzymes into the internal structure of

Fig. 7 Kinetic fits for the adsorption of protease on ZnFe_2O_4 **a** pseudo-first order model fits, **b** pseudo-second order model fits, **c** Elovich and **d** Intraparticle diffusion model fits

the adsorbent also played significant roles (Tütem et al. 1998).

The Langmuir isotherm fit which proposed a monolayer adsorption on surfaces with identically homogeneous sites could not properly fit the equilibrium data, hence, the adsorption of related isotherms. It is obvious from the plot that the adsorption capacity increases initially, owing to the presence of active sites which got saturated with increase in enzymes concentrations. Guggenheim–Anderson–de Boer isotherm was used for the analysis of the adsorption of amylase, lipase and protease onto ZnFe_2O_4 , respectively. The values of K_s is far less than L_k for all the enzymes, is a clear indication of predomination of strong adsorption of the enzymes onto the ZnFe_2O_4 structure than being weakly adsorbed, which means that the heat of adsorption of the second layer is identical to the higher layers, but heat of

adsorption of the second layer and subsequent layers is less than the heat of fusion (Do 1998; Lemus 2011). The Generalized adsorption isotherm gives reasonable adsorption values of the maximum adsorption capacities because its assumptions are similar to that of Langmuir except that it includes cooperative binding of macromolecules with multiple binding sites. This isotherm gives maximum adsorption capacities of 7.02, 42.90 and 22.24 mg g^{-1} , respectively, for amylase, lipase and protease. The values of $m > 1$ obtained for all the enzymes suggested that binding of the enzymes were cooperative. Freundlich isotherm parameters, n values of greater than 1 obtained for all the enzymes showed that the process of adsorption is highly favored. Tempkin isotherm parameters obtained showed that protease is adsorbed with the least energy, hence the maximum adsorption capacity obtained for this

Table 1 Parameters for the kinetic fits for the adsorption of amylase, lipase and protease on ZnFe_2O_4

Pseudo First Order			Second order					Intraparticle diffusion				Elovich			
mg/L	$Q_{e(\text{exp})}$ (mg g ⁻¹)	Q_e (mg g ⁻¹)	k_1 (min ⁻¹)	R^2	%SSE	Q_e (mg g ⁻¹)	k_2 (g mg ⁻¹ min ⁻¹)	R^2	%SSE	k_{id} (mg g ⁻¹ min ^{-0.5})	C_{id} mg g ⁻¹	R^2	α (mg (g min) ⁻¹)	β_{el} (g mg ⁻¹)	R^2
Amylase															
1.0	0.163	0.205	0.014	0.991	0.025	0.313	0.031	0.969	0.087	0.014	0.006	0.947	0.006	12.956	0.948
2.0	0.559	0.625	0.020	0.998	0.038	0.863	0.019	0.990	0.175	0.053	0.001	0.979	0.026	4.621	0.964
2.5	1.126	1.113	0.035	0.998	0.008	1.325	0.032	0.998	0.115	0.104	0.072	0.960	0.157	3.830	0.994
3.0	2.097	2.037	0.040	0.996	0.034	2.376	0.022	0.994	0.161	0.193	0.156	0.945	0.426	2.310	0.968
4.0	3.299	3.235	0.041	0.998	0.037	3.747	0.015	0.998	0.259	0.307	0.264	0.939	0.790	1.510	0.998
Lipase															
5.0	0.409	0.741	0.007	0.858	0.192	1.342	0.003	0.856	0.539	0.030	0.044	0.796	0.012	3.777	0.871
7.0	1.008	1.455	0.011	0.912	0.258	2.412	0.003	0.908	0.811	0.081	0.074	0.867	0.032	1.753	0.887
10.0	2.040	2.337	0.019	0.982	0.172	3.304	0.004	0.976	0.730	0.190	0.022	0.961	0.087	1.179	0.930
12.0	3.475	3.884	0.020	0.989	0.236	5.356	0.003	0.984	1.086	0.331	0.001	0.971	0.162	0.737	0.943
15.0	5.472	6.026	0.023	0.977	0.320	8.086	0.002	0.969	1.509	0.531	0.064	0.948	0.285	0.485	0.868
Protease															
5.0	0.766	0.741	0.055	0.994	0.014	0.823	0.111	0.998	0.033	0.071	0.081	0.897	0.877	9.002	0.989
8.0	1.891	1.789	0.065	0.986	0.059	1.958	0.061	0.993	0.039	0.174	0.216	0.879	6.415	4.387	0.845
10.0	3.976	3.867	0.060	0.996	0.063	4.234	0.026	0.999	0.149	0.373	0.461	0.881	10.886	1.980	0.998
15.0	7.206	6.978	0.064	0.995	0.132	7.584	0.016	0.999	0.218	0.674	0.869	0.872	36.913	1.196	0.988
20.0	11.111	10.924	0.091	0.999	0.108	11.373	0.025	0.999	0.151	1.048	1.669	0.817	202.693	1.572	0.931

Fig. 8 Isothermal fits for the adsorption of Enzymes onto ZnFe_2O_4 : **a** amylase, **b** lipase, **c** protease

adsorbent. Dubinin–Radushkevich model gave theoretical saturation capacities values Q_s , of 1.62, 9.99 and 8.45 mg g^{-1} , respectively, for amylase, lipase and protease, while the maximum adsorption energies E , ranges between 1.51 and 3.74 kJ mol^{-1} , showing that the process is physisorption dominated process. The overall comparison of the Isotherms using the average values of R^2 shows that the isotherm fit are in the order Freundlich > Generalized > Guggenheim–Anderson–de Boer > Tempkin isotherm > Dubinin–Radushkevich.

The free energy change, ΔG is obtained from Eqs. (13, 14) according to the Van't Hoff linear plots of $\ln K_d$ versus $1/T$ plot in Fig. 9. The thermodynamic parameters are presented in Table 3. From Fig. 9 and the parameters presented in the Table, it is found that the negative value of ΔG indicates the spontaneous nature of adsorption. Positive value of enthalpy change indicates that the adsorption process is endothermic in nature, while the entropy

changes, ΔS showed increased randomness at the adsorbate–adsorbent inter phase during the adsorption process.

Conclusion

ZnFe_2O_4 nanoparticles was successfully synthesized and confirmed by characterization. The adsorption of the amylase, lipase and protease on ZnFe_2O_4 follow first order kinetic model when compared with second order kinetic model. The adsorption energy increases with concentration as shown by the Elovich model, while the intraparticle diffusion model suggested mass transfer as one of the factors affecting adsorption. The maximum adsorption capacities as revealed by the generalized adsorption isotherms are 409.03, 103.56 and 73.67 mg g^{-1} , respectively, for amylase, lipase and protease, respectively, with cooperative binding. The Dubinin–Radushkevich model gave

Table 2 Parameters for the Isotherm fits for the adsorption of amylase, lipase and protease on ZnFe₂O₄

	Guggenheim–Anderson–de Boer (GAB)				Generalized Adsorption Isotherm			Freundlich Isotherm			Templin Isotherm			Dubinin–Radushkevich Isotherms			
	Q_0 (mg g ^{−1})	$L_k \times 10^7$ (L mg ^{−1})	K_s (L mg ^{−1})	R^2	Q_m (mg g ^{−1})	K_G (L mg ^{−1}) ^{1/<i>m</i>}	<i>m</i>	K_F (mg g ^{−1} min ^{−1/<i>n</i>})	<i>n</i>	R^2	b_T	a_T (L g ^{−1})	R^2	Q_s (mg g ^{−1})	$\beta \times 10^8$ (mol J ^{−1}) ²	E (kJ mol ^{−1})	R^2
Amylase	1.35	7.444	0.081	0.982	7.02	0.33	4.39	1.294	3.676	0.996	7606.59	57.60	0.982	1.62	3.58	3.737	0.842
Lipase	8.57	1.868	0.052	0.999	42.90	0.48	6.38	5.793	2.559	0.999	912.58	9.14	0.986	9.99	12.4	2.009	0.937
Protease	7.55	1.257	0.023	0.999	22.24	0.47	4.15	4.404	3.064	0.998	1187.75	7.51	0.994	8.45	22.0	1.507	0.939

Fig. 9 Thermodynamics fits for the adsorption of Enzymes onto ZnFe₂O₄**Table 3** Thermodynamic parameters for the adsorption of amylase, lipase and protease on ZnFe₂O₄

	Temp (K)	ΔG (kJ mol ⁻¹)	ΔH (kJ mol ⁻¹)	ΔS (kJ mol ⁻¹ K ⁻¹)	R^2
Amylase	298	-9.3	4.4	46.0	0.886
	308	-9.8			
	313	-10.0			
	323	-10.5			
Lipase	298	-12.1	5.7	59.7	0.92
	303	-12.4			
	318	-13.3			
	323	-13.6			
Protease	298	-11.0	1.9	43.1	0.988
	303	-11.2			
	308	-11.4			
	323	-12.0			

the maximum adsorption energies, E , ranges between 0.162 and 0.567 kJ mol⁻¹ showing that the process is physisorption dominated process. Thermodynamic study revealed a spontaneous adsorption process with increase in entropy. ZnFe₂O₄, therefore, is a very good adsorbent for the purification of enzymes and can be used as a support for enzymatic process that required immobilization of the enzymes.

Acknowledgements This work benefitted immensely from the financial support in the form of grants from CSIR, for 12 months TWAS-CSIR Postdoctoral Fellowship, FR Number: 3240275035, awarded to Abideen Idowu Adeogun that enables part of this work to be carried out at the CECRI Pollution Control Division Laboratory. We are also he is thankful to the authority of the Federal University of

Agriculture, Abeokuta, Nigeria for Granting the study leave to honor the fellowship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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