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Kinetic and equilibrium studies of adsorption of β-glucuronidase by clinoptilolite-rich minerals

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ABSTRACT

The adsorption of the bacterial β-glucuronidase (GUS) enzyme, which is thought to be responsible for the production of reactive metabolites related to some diseases and cancer development, by clinoptilolite-rich mineral was investigated. Batch experiments were performed to analyze of the effects of the clinoptilolite amount and particle size, initial GUS concentration, shaking rate, pH and temperature on the adsorption equilibrium and kinetics. Adsorption equilibrium data were interpreted in terms of Langmuir and Freundlich isotherms; and they were well represented by the Langmuir isotherm model. The percentage of GUS removal by the clinoptilolite-rich mineral was changed in the range of 9.4–54.4% depending on its initial concentration. The kinetic data were analyzed using external film diffusion, intraparticle diffusion, pseudo-first-order and pseudo-second-order models and both external film and intraparticle diffusion appeared to be effective in GUS adsorption. Thermodynamic studies indicated that GUS adsorption is exothermic, physical and spontaneous at the temperatures investigated (288–310 K).

1. Introduction

Natural zeolites are crystalline porous aluminasilicates of group IA and IIA elements with pore and channel systems. These minerals consist of corner-sharing AlO_4 and SiO_4 tetrahedra. Their ion-exchange, adsorption, and molecular sieve properties make them unique and advantageous for use in different agricultural, chemical and environmental applications [1,2]. Recently the incorporation of organic molecules onto clinoptilolite and synthetic zeolites has attracted growing interest because their frameworks are suitable for the encapsulation and/or adsorption of different molecules. They are used for protein adsorption, separation or enzyme immobilization and have been suggested as drug support systems, antibacterial agent release matrices or bacteria loading supports [3–9]. Clinoptilolite and its modified forms were used to protect animals from feed-originating toxins by adsorbing toxic compounds in the gastrointestinal tract and preventing their passage into the circulatory system [10–14]. Purified natural clinoptilolite was reported to be harmless to the human body and recommended as an antidiarrheal drug and as antiacid agent for humans suffering from hyperacidity and showed no toxic or biological hazards [5,6,15]. Among the studies performed, those related to the use of clinoptilolite in cancer treatment, have special value; in animal models clinoptilolite treatment has improved health, prolonged life-spans and decreased tumor size [16–20]. Therefore, to identify the mechanism underlying the therapeutic effects of clinoptilolite-rich minerals, investigation of the interactions between clinoptilolite rich minerals and bioactive molecules related to health and diseases is crucial for the formulation of new materials for biomedical applications.

Considering the suggested positive role of clinoptilolite in animal health and cancer, in this study attention has been focused on an enzyme responsible for diseases. β-Glucuronidase (GUS) is an enzyme found in some mammalian and plant tissues. In the intestine, GUS activity is mostly bacterial in origin. This enzyme is responsible for drug metabolite detoxification and producing reactive metabolites related to diseases and cancer development [21–23]. Many exogenous (e.g., drugs, pesticides) as well as endogenous compounds (e.g., bilirubin, steroids, bile acids) are conjugated with glucuronic acid in the liver and excreted by the bile. The GUS enzyme catalyzes hydrolysis of glucuronides and liberates toxins and mutagens that are excreted into the gut after being glucuronated in the liver. Various carcinogens are produced by bacterial β-glucuronidase in the intestine, and the results of different studies indicate that GUS activity may be considered a cancer risk biomarker [24–27]. Food intake (such as yogurt and plants) and

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natural bioactive compounds (such as organic acids and plant extracts) were considered for the inhibition of GUS activity in various studies [28–31].

Although there are different studies on the use of zeolites as adsorbents for biomolecules, this is the first study that investigates the adsorption of bacterial β-glucuronidase enzyme by clinoptilolite rich mineral. In this study, adsorption of the bacterial GUS (from *Escherichia coli*) on to clinoptilolite-rich minerals from the Gördes basin (Western Anatolia, Turkey) was investigated by conducting experiments and analyzing data for different kinetic models.

2. Materials and methods

2.1. Sample preparation and characterization

Clinoptilolite-rich minerals were obtained from the Gördes region of Western Anatolia Turkey. The minerals were mainly made up of clinoptilolite (80–85%), and quartz (5–10%), analcime + mordenite (<5%) [32]. The clinoptilolite-rich mineral samples were ground, wet settled and sieved to remove soluble impurities. Thereafter the samples were dried in an oven at 200 °C for 3 h. Brunauer–Emmett–Teller (BET) surface area and micro and mezzo pore size distribution of the samples were determined by N_2 adsorption performed at 77.45 K with a Micromeritics ASAP 2010 static volumetric adsorption instrument after degassing at 350 °C and 10^{-5} mbar for 24 h. The surface morphology and macropore size of clinoptilolite-rich minerals were estimated with a Phillips XL30S FEG electron microscope. Chemical composition (by ICP–AES 96, Varian), mineralogy and crystallinity (by X-Pert Pro, Philips), IR characterization (by FTIR-8201, Shimadzu) and Thermogravimetric (by TGA-51/51H, Shimadzu) analyses were performed as previously described [17]. Zeta potential measurements of the mineral samples were measured using 5 mg/mL samples in potassium phosphate buffer solution at pHs 5.5, 6.8 and 8 with a Zetasizer 3000 HAS analyzer (Malvern Instruments), where pH 6.8 is the optimum pH for GUS activity (recommended by the purchaser instructions) and pH 5.5–8 is the pH range simulating the fasted and fed states of gastrointestinal media.

The GUS enzyme (EC 3.2.1.31, with a molecular weight of 290 kDa from Sigma-Aldrich Chemical Company, Germany) used in this study was of bacterial origin (*E. coli*). Enzyme working solutions were prepared from the GUS enzyme with a 100 μg/mL concentration in potassium phosphate buffer solution at pHs 5.5, 6.8 and 8. The size distribution of the enzyme was measured with a Zetasizer 3000 HAS analyzer (Malvern Instruments).

2.2. Batch adsorption studies

To study the possibility of the eliminating the GUS enzyme by clinoptilolite-rich minerals in the gastrointestinal system, batch adsorption experiments, which were designed to simulate gastrointestinal conditions (37 °C, pH 5.5–8), were performed. Optimum conditions for GUS activity (from an *E. coli* source), 37 °C and pH 6.8, were also considered. The experimental parameters, including the amount and particle size of clinoptilolites, initial enzyme concentration, shaking rate, pH and temperature are shown in Table 1. Potassium phosphate buffer solution (10 mL) of was placed into a 25 mL conical flask, and experiments were performed for the specified GUS concentrations and clinoptilolite amount. Flasks were placed in an orbital shaking water bath for 150 min which was more than ample time for equilibrium. 100 μL of sample was removed from each flask to estimate the enzyme concentration at specific time intervals; mineral free samples were used as control. Samples were analyzed spectrophotometrically using a microplate reader (Varioscan, Thermo) at 255 nm which is the wavelength of the maximum absorbance of GUS. To determine the GUS concentration, a standard curve (GUS concentration vs. absorbance) was plotted for various GUS concentrations in the range of 0–500 μg/mL. Adsorption experiments were performed in triplicate, and mean values were used for adsorption analyses. The amount of enzyme adsorbed was calculated by mass balance as follows:

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

where C_o is the initial enzyme concentration (μg/mL), C_t is the enzyme concentration in the solution at any time t (μg/mL), q_t is the concentration of GUS (μg/mg) at time t in the adsorbed phase, V is the volume of the solution (mL) and m is the mass of the clinoptilolite (mg).

2.3. Modeling of adsorption kinetic and equilibrium data

The transport of GUS from the gastrointestinal media to the clinoptilolite particle surface through an external film and, subsequent diffusion from the external surface to internal sites through the pores of the particle (intraparticle diffusion; pore diffusion surface diffusion) and adsorption of GUS on the mineral active sites are three consecutive steps during the process. Although all of these steps are effective during the process, the overall rate of adsorption may be controlled by the one that is the slowest. Theoretical analysis of adsorption by particle yields complicated mathematical relationships that were provided and solved by Barrer [33], Boyd [34] and Crank [35]; for some special cases. Considering the complexity of the adsorption process, various simplified models have been reported in the literature and each model has its own limitations due to their simplifying assumptions. Spherical adsorbent particles in an infinite volume of dilute solutions (linear isotherm equilibria) with constant temperature and diffusivity are usually used in simplified models such as in existing study.

With a substantial understanding of the kinetic and equilibrium constants, one can identify the ideal temperatures for system and predict the rates of process for the elimination of GUS, which will lead to optimize design outcomes (higher yield, faster rate) under simulated intestinal conditions. Therefore, the adsorption equilibria and kinetic models constants are critical design variables to estimate the performance and to predict the mechanism of an adsorption process. In this study to investigate the mechanism of adsorption, rate constants were determined by external film diffusion, intraparticle diffusion and pseudo 1st and 2nd order reaction models. In the description of adsorption equilibria Langmuir and Freundlich isotherm models were applied.

2.3.1. Diffusion models

2.3.1.1. External mass transfer or surface resistance control. If diffusion within an adsorbent particle is very rapid or if the adsorbent is nonporous, adsorption can be considered to occur only on the external surface, while diffusion through the laminar film surrounding a particle controls diffusion. Thus, in this analysis, the relationship given by Boyd [34] may be used:

$$\frac{\bar{q}}{q_\infty} = 1 - e^{[-(3k_f t)/(KR_p)]} \quad (2)$$

where the value of average adsorbed phase concentration q is presented as $\bar{q}$ at any time t (μg/mg), q_∞ is the equilibrium adsorbed phase concentration (μg/mg), k_f is the external mass transfer coefficient (m/min), R_p is the average particle diameter (m), K is the initial slope of the isotherm at the specified temperature (mL/mg). For cases in which mass transfer is controlled by surface/skin resistance, an analogous relationship in which the external film diffusion coefficient k_f is replaced by $k_s = D_s/\delta$, the ratio of the