Adsorption study for the detoxification of aflatoxin B₁ by using the different clay minerals in Thailand

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Abstract

The objective of this research is to evaluate the ability of clay minerals to adsorb aflatoxin B₁ (AFB₁) *in vitro*. Fourteen clay minerals from different sources in Thailand and commercial adsorbents including bentonites and toxin binders were tested for AFB₁ adsorption capacities. Our results indicated that the clay minerals were capable of sequestering AFB₁ from aqueous solution differently. The experimental data were fitted to modified Freundlich model better than Langmuir model to investigate adsorption capacities and affinity constant. The S-shaped isotherms were observed for these clay minerals and commercial adsorbents. The clay minerals had maximum capacity ($Q_{\max}$) ranged from 7.10×10^{-4} mol/kg to 4.76×10^{-3} mol/kg whereas the commercial adsorbents had $Q_{\max}$ ranged from 8.21×10^{-4} mol/kg to 4.52×10^{-3} mol/kg. The distribution constant (K_d) for the clay mineral samples and commercial adsorbents were in the ranges of 2.15×10^5 - 6.70×10^7 and 2.52×10^5 - 4.71×10^5 , respectively. This *in vitro* method may be useful for accurately predicting the efficacy of adsorbents before testing *in vivo*.

Key words: adsorption, isotherm, aflatoxin B₁, clay mineral, Thailand

Introduction

Aflatoxins (AFs) are a group of structurally similar difuranocoumarin derivatives that are produced by the common moulds *Aspergillus flavus* and *parasiticus*. There are four major naturally occurring aflatoxins namely aflatoxins B₁, B₂, G₁, and G₂ which frequently found in foods and feedstuffs such as corn, rice, and peanuts. Aflatoxin B₁ (AFB₁) is widely known as the most acutely toxic and hepatocarcinogenic of the 16 naturally occurring aflatoxins¹⁾.

Chronic exposure to aflatoxins may not only significantly affect the productivity and farming trends, but also direct exposure to aflatoxin contaminated food commodities may impose a great risk to the consumers. Consequently, to reduce the economic lost and health risks from aflatoxin contamination, one of encouraging approaches is the addition of non-nutritive sorptive materials to feedstuffs to decrease the bioavailability of mycotoxin from the gastrointestinal tract²⁾. This attempt has been considered as practical and cost-effective methods to detoxify aflatoxins and remediate contaminated feed on a large

scale. Several *in vitro* tests showed that various sorbents, especially phyllosilicates were capable of binding aflatoxins in solution. Hydrated sodium calcium aluminosilicate (HSCAS) had reportedly a high affinity and high capacity for AFB₁ in aqueous solutions^{2,3)}. Not only natural clays such as smectite or bentonite which contains a large amount of montmorillonite but also activated charcoal have been proven for their effective adsorption of AFB₁ *in vitro* trials⁴⁻⁷⁾. Most studies of AFB₁ adsorption behaviors have involved the investigation of isothermal adsorption studies not only in aqueous but also in gastrointestinal conditions^{8,9)}.

The main objective of this work was to investigate the capacities and affinities of some clay minerals collected from different parts of Thailand compared to some commercial adsorbents for adsorption of AFB₁ by utilizing Langmuir and modified Freundlich isothermal adsorption models.

Materials and methods

Chemicals and reagents Standard AFB₁ was purchased from Sigma Chemical Co. (St. Louis, MO). Three commercial bentonites and four toxin binders were obtained from different suppliers. Clay minerals from various sources in Thailand were firstly prepared for having particle size $\leq 45\ \mu\text{m}$ to ensure uniformity prior to use in adsorption study. The high-purity water (18 M $\Omega\cdot\text{cm}$) used in this study was prepared by processing deionized water through a Milli-Q^{uf+} system.

Study of isothermal adsorption A series of aqueous solutions of AFB₁ having concentrations from 0.50 to 8.00 mg/L was employed. Each isotherm study employed 4.00 mL of AFB₁ solution at each concentration and 10.0 mg of each adsorbent. Also three controls for each adsorbent were prepared: a purified water control containing no toxin, toxin control containing AFB₁ without adsorbent, and a control containing purified water and adsorbent. The sample and control tubes were capped and placed on an electronic shaker at 200 $\times$ g for 24 hr in an incubator at 25 °C (Innova 4060, New Brunswick Scientific, Edison, NJ). After shaking, the samples were centrifuged (Allegra 64R, Beckman Coulter, Fullerton, CA) at 12,000 $\times$ g for 30 min to separate the adsorbents from the supernatants which was further analyzed to determine the concentrations of AFB₁ which the absorbance at 362 nm using UV-Visible spectrophotometer⁸⁾ (Lambda 25, Perkin Elmer, Wellesley, MA). Each experiment was performed in triplicate.

Data calculations and curve fitting The UV/Visible absorption data were firstly introduced into an Excel spreadsheet (Microsoft, Redmond, WA) to calculate the amount of AFB₁ left in solution (C_e), and the amount AFB₁ adsorbed (q) for each data point. The AFB₁ concentrations left in the supernatants were calculated from the calibration curves obtained from the external standard method obeying Beer-Lambert's law. The adsorption data were then transported to Table Curve 2D v 3 (Jandel Scientific, San Rafael, CA) to fit adsorption isotherm equations. The Langmuir and modified Freundlich adsorption models were utilized as user defined functions. The maximum adsorption capacity (Q_{max}) and distribution coefficient (K_d) were calculated and compared for each adsorbent^{8,10,11)}.

Results and discussion

In the present study, fourteen selected clay minerals and commercial adsorbents including three bentonites and four toxin binders were evaluated for their ability to adsorb AFB₁ from aqueous solution. Isothermal adsorption study of AFB₁ on various adsorbents using a series of different concentrations of AFB₁ was investigated. The S-shaped isotherm

Table 1. Comparison of fitted parameters obtained from two isotherm models for AFB₁ adsorption onto different adsorbents at 25 °C.

Adsorbent	Langmuir Model (LM) ^{a)}			Modified Freundlich Model (MFM) ^{b)}			
	r ²	Q _{max}	K _d	r ²	Q _{max}	K _d	n
BN1	0.69209	0.99	1.12E+03	0.97903	4.52E-03	2.52E+05	2.82
BN2	0.60207	0.87	1.24E+03	0.93621	2.80E-03	2.84E+05	3.95
BN3	0.84760	0.49	1.44E+03	0.88581	1.61E-03	3.60E+05	1.25
COM1	0.58865	0.82	1.64E+03	0.96648	1.53E-03	4.62E+05	2.70
COM2	0.58123	0.71	1.71E+03	0.93575	1.09E-03	4.26E+05	3.34
COM3	0.61409	0.72	1.10E+03	0.98803	1.11E-03	3.04E+05	3.15
COM4	0.69948	0.67	1.45E+03	0.89760	8.21E-04	4.71E+05	2.25
S1	0.69005	1.03	1.03E+03	0.98905	4.76E-03	2.37E+05	2.68
S2	0.63963	1.05	9.42E+02	0.98864	4.68E-03	2.31E+05	2.97
S3	0.93847	0.44	1.35E+03	0.98369	1.64E-03	2.90E+05	1.32
S4	0.87867	0.55	8.56E+02	0.97249	1.66E-03	2.15E+05	1.56
S5	0.96311	0.17	3.35E+03	0.96626	1.96E-03	2.76E+05	1.05
S6	0.89882	0.73	1.24E+03	0.97004	3.18E-03	2.54E+05	1.47
S7	0.91695	0.50	1.53E+03	0.96719	1.87E-03	3.32E+05	1.32
S8	0.67158	1.02	9.82E+02	0.98963	2.95E-03	2.72E+05	2.80
S9	0.55549	1.09	1.53E+03	0.86450	3.12E-03	4.41E+05	2.43
S10	0.77967	0.65	8.94E+02	0.97383	9.61E-04	2.98E+05	2.09
S11	0.74603	0.57	1.96E+03	0.84637	1.34E-03	4.79E+05	1.82
S12	0.84396	0.67	2.06E+03	0.95424	1.15E-03	6.70E+07	1.66
S13	0.94328	0.15	2.11E+03	0.94789	7.89E-04	3.54E+05	1.07
S14	0.66661	0.52	6.99E+02	0.80178	7.10E-04	2.38E+05	2.07

a) $q = Q_{\max}[K_d C_e / (1 + K_d C_e)]$ and b) $q = Q_{\max}[K_d C_e]^n$ where q = adsorbed amount (mol/kg), C_e = equilibrium concentration (mol/L), K_d = distribution coefficient, and n = heterogeneity factor.

Plots were observed for all sorptive materials. Fig 1 showed the example of isotherm plots of three representatives of each group: bentonite 1 (BN1), commercial toxin binder 1 (COM1), and clay mineral 1 (S1). Typically, the S-type curve appears when the solute molecule does not have a strong affinity to the surface until there is a significant amount of solute adsorbed; hence the slope increases with the affinity. This usually occurs when the previously adsorbed solute molecules modifying the surface, and further bind to the incoming solute in

Fig. 1 The S-shaped adsorption isotherm plots for AFB₁ to the different adsorbents: BN1, COM1, and S1 were achieved; where BN = bentonite, COM = commercial toxin binder, and S = clay mineral, respectively. The standard deviation on each data point of the adsorbed AFB₁ amount obtained from the triplicate experiment of each adsorbent was slightly small.

the solution. Furthermore, the possibly vertical arrangement in the adsorbed layer and the competition with other molecules for the substrate sites could result in the S-shaped isotherm appearance. The outcome obtained in this study showed that each sorbent could adsorb AFB₁ differently at each condition especially at high toxin concentration. The Langmuir isotherm model ($q = Q_{\max}[K_d C_e / (1 + K_d C_e)]$) and modified Freundlich isotherm model ($q = Q_{\max}[K_d C_e]^n$): where q is the amount of AFB₁ adsorbed, $Q_{\max}$ is the maximum amount of AFB₁ adsorbed, C_e is the equilibrium concentration of AFB₁ in solution, K_d is the distribution constant (affinity parameter), and n is heterogeneity factor, were employed to compare fitting characteristics of the adsorption data. The adsorption data of all sorptive materials were better fitted to modified Freundlich model since it presented high correlation coefficients (r^2) for all adsorbents in the range of 0.80178-0.98963 and allowed a quantitative comparison of maximum adsorption capacities ($Q_{\max}$) and affinity constants (K_d) for

adsorbents as expressed in Table 1. The fitted result of modified Freundlich model showed that the clay mineral S1 had the highest maximum adsorption capacity (4.76×10^{-3} mol/kg) whereas the clay mineral S12 possessed the highest distribution constant (6.70×10^7). This study also offered that several clays were capable of sequestering AFB₁ from aqueous solutions in which the extents of AFB₁ adsorption could depend upon their physicochemical properties. Generally, adsorption capability of adsorbents for AFB₁ could occur at original edge sites, external surface, interlayer surface, interlayer exchangeable cations, or previously adsorbed molecules. Small particles with large specific active sites are able to enhance AFB₁ adsorption. Previous studies demonstrated that smectite and other montmorillonite-containing clay minerals exhibited high adsorption capacity for AFB₁ due to their high cation exchange capacity (CEC) and high selectivity. Our supporting information for this study was obtained for the interesting clay minerals, especially S1. The S1 which showed the highest adsorption capability comprised moderate CEC of 44 meq/100 g comparable to minerals in smectite group. In addition, the X-ray diffraction (XRD) analysis indicated that S1 contained the large composition of montmorillonite.

Finally, our studies confirmed that some clay minerals such as S1 which collected from a part of Lobburi Province, Thailand, could be effective for detoxification of AFB₁ *in vitro*. However, further research study on the efficiency and safety of clay should be verified by testing *in vivo*. Generally, the criteria of selection of any proper sorbents useful for the detoxification of AFB₁ shall be based on: high capacity, high affinity, high selectivity for only the toxin, without binding any nutrients and produce toxic residues left in animals fed the contaminated feeds^{9, 12}.

Acknowledgements

This study was supported by the Thailand Research Fund (TRF) and Center for Innovation Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIS), Thailand.

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