CHAPTER 2

EXPERIMENTAL

2.1 Instruments and apparatus

1) UV-VIS spectrophotometer, Lamda 25, Perkin Elmer, USA
2) Incubator shaker, innova 4080, New Brunswick Scientific, USA
3) Incubator shaker, Orbital Incubator, Gallenkamp, UK
4) Centrifuge, J2-MC centrifuge, Beckman Coulter, USA
5) Centrifuge, UniCen 15 D, Herolab, Germany
6) Centrifuge, Allegra 64 R, Beckman Coulter, USA
7) Analytical balance, AB 204-S, Mettler Toledo, Switzerland
8) Analytical balance, MX 5, Mettler Toledo, Switzerland
9) Milli-Q water purification system, Millford, USA
10) CHNS/O Analyzer, PE 2400 Series II, Perkin Elmer, USA
11) Oven, Modell 400, Memmert, Germany
12) Particle size analyzer laser, Mastersizer S, Malvern Instruments, UK
13) Surface area analyzer, Autosorb1 MP, Quantachrome, USA
14) pH meter, Model 5986-25, Cole-Parmer, USA
15) Conductivity meter, Model 1671, Jenco electronic, China

2.2 Chemicals

1) Acetone, 99.7%, AR, LAB-SCAN, Ireland

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2) Ethanol, 99%, AR, Merck, Germany

3) Sodium hypochlorite, 10% Com., Carlo Erba, Italy

4) Potassium hydroxide, AR, BDH, England

5) Cystine, OAS, Elemental Microanalysis, UK

6) Oxygen gas, 99.7%, HP, Lanna industrial gas, Thailand

7) Helium gas, UHP, Lanna industrial gas, Thailand

8) Zeolite, Biochemika, Fluka, Switzerland

9) Activated carbon, Merck, Germany

10) Standard aflatoxin B_1, Supelco, USA

11) Bentonite, Aldrich, USA

12) Synthetic Na-X zeolites and synthetic sodalite zeolite samples, Inorganic Materials Research Unit, Chiang Mai University, Thailand

13) Novasil Plus, Trouw Nutrition International, USA

2.3 Preparations of the standard aflatoxin B_1

2.3.1 Stock standard aflatoxin B_1 solution

Standard aflatoxin B_1 was produced by *A. parasiticus* solution NRRL 2999 cultured in rice media and then extracted with a series of solvent before calibrated with the standard obtained from Supelco Company by high performance liquid chromatography.

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2.3.2 Standard aflatoxin B_1 solution

A series of standard aflatoxin B_1 solution was prepared by diluting the stock solution in aqueous medium to obtain the aflatoxin B_1 concentrations of 0.50, 1.00,

1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.50, and 8.00 ppm, respectively. Milli-Q water was used for the preparation of all solutions.

2.4 Preparations of other chemical solutions

2.4.1 Potassium hydroxide solution 10%

Potassium hydroxide solution was prepared by dissolving potassium hydroxide in ethanol at the ratio of 10% w/v. This reagent was used to clean glassware.

2.4.2 Sodium hypochlorite solution 1%

The 1% NaOCl solution was prepared by diluting the 10% stock solution. This solution was used for cleaning all toxin containers.

2.5 Adsorption studies

2.5.1 Construction of the aflatoxin B_1 calibration curves

The standard curves for aflatoxin B_1 determination were constructed by using a series of aflatoxin B_1 solution having the concentration of 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.50, and 8.00 ppm, respectively. Each standard solution was measured for absorbance at 362 nm by UV-VIS spectrophotometer, and subsequently the calibration graph was obtained by plotting the absorbance versus aflatoxin B_1 concentration.

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2.5.2 Study of adsorption isotherms

Six different adsorbents including the commercial toxin binder, the commercial bentonite, activated carbon, the commercial zeolite, synthetic Na-X zeolite, and

synthetic sodalite zeolite were used in this study. A 10.0 mg of adsorbent was weighed out in a clean test tube. To each tube, the 4 ml of aflatoxin B_1 solution with each concentration of 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.50, and 8.00 ppm were added to the adsorbent, giving a final concentration of 0.25% w/v. Toxin control containing 4 ml of each aflatoxin B_1 concentration without adsorbent were also prepared. All experiments were performed in triplicate. The samples and controls were shaken at 200 rpm for 24 hr at 25 ^{o}C. After equilibration, the samples and controls were centrifuged at 12000 rpm for 20 min to separate the adsorbent from the supernatant. The adsorbed amount of aflatoxin B_1 on each adsorbent was investigated from the concentration of aflatoxin B_1 left in the solution determined by using UV-VIS spectrophotometry at a wavelength of 362 nm. These data were used to fit isotherm equations to characterize the adsorption behavior of aflatoxin B_1 onto adsorbent.

2.5.3 Study on the temperature effect

To investigate the effect of temperature, the toxin/adsorbent mixtures were incubated at different temperatures: 25, 37, and 45 ^{o}C, respectively. Other experimental conditions were the same as conducted in a study of adsorption isotherms. All experiments were done in triplicate.

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2.6 Isotherm fitting by 2D Table Curve program

The absorption data were used to calculate the amount of aflatoxin B_1 left in solution and the amount adsorbed for each data point. The experimental data were transferred to Microsoft Excel® program, fitted with linearized Langmuir model and

linearized Freundlich model compared to the generalized Langmuir model (GLM), the generalized Freundlich model (GFM), and the modified Freundlich model (MFM) fitted by 2D Table Curve program, which could to characterize the adsorption behavior of aflatoxin B_1 on each adsorbent.

2.7 Characterizations of the adsorbents

2.7.1 Determination of particle size

The particle size of the commercial toxin binder, the commercial bentonite, the commercial zeolite, synthetic Na-X zeolite, and synthetic sodalite zeolite were determined by particle size analyzer, Mastersizer S, Malvern Instrument, UK

2.7.2 Determination of total surface area and pore size distribution

The commercial toxin binder, the commercial zeolite, synthetic Na-X zeolite, and synthetic sodalite zeolite were subjected to the determination of total surface area and pore size distribution. The technique used to determine such properties is the Brunauer, Emmett and Teller (BET) method using an Autosorb1 MP, gas sorption system, USA. The adsorption surface area and desorption surface area of each adsorbent were measured. The total surface area was calculated based on desorption surface area and the dried weight of the sample after analysis.

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2.7.3 Determination of total carbon content

Six adsorbents including the commercial toxin binder, the commercial bentonite, activated carbon, the commercial zeolite, synthetic Na-X zeolite, and synthetic sodalite zeolite were investigated for the determination of total carbon

content. They were analyzed by CHNS/O Analyzer, PE 2400 Series II, Perkin Elmer, USA. The sample was dried in oven and cooled down in the desiccator before used. The 1.50-2.00 mg sample was weighed out in aluminium foil and then wrapped it up before subsequently analysis for total carbon content.

2.7.4 Determination of pH, conductivity, and cation exchange capacity (CEC)

The pH and conductivity values of six adsorbents including the commercial toxin binder, the commercial bentonite, the commercial zeolite, synthetic Na-X zeolite, and synthetic sodalite zeolite were investigated followed Greenland and Hayes [58]. For determination of cation exchange capacity was done by following the method of Hesse [59].

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