MYCOTOXINS ADSORPTION BY ORGANOMODIFIED NATURAL ZEOLITIC TUFF

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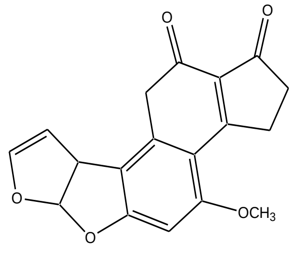
INTRODUCTION

Zeolites are crystalline hydrated tektoalumosilicates consisting of a three-dimensional framework of SiO_4^{4-} and AlO_4^{5-} tetrahedra linked through shared oxygen atoms. The heulandite group of zeolites including clinoptilolite are the most abundant zeolites in nature [1, 2]. Clinoptilolite is used in a wide range of industrial and agricultural applications, particularly in animal nutrition. Dietary inclusion of clinoptilolite in animal feeds improved average daily gain, and/or feed conversion and increased milk production in dairy cows and egg production in laying hens [3].

In the recent years contamination of cereal grain and animal feed with mycotoxins has become a global concern. Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminants of grain worldwide. Many of these mycotoxins can cause serious problems in livestock resulting in substantial economic losses. The most common mycotoxins found in animal feed are the aflatoxins, ochratoxins, trichothecenes, fumonisins, and zearalenone. One economical approach for reducing mycotoxicosis is the utilization of adsorbents (natural zeolites and clays) in animal feed to bind the mycotoxins efficiently in the gastro-intestinal tract and prevent their absorption. The natural forms of these minerals effectively bind aflatoxins both *in vitro* and *in vivo,* however, their negatively-charged surfaces are ineffective in binding other fairly nonpolar mycotoxins [4,5]. Chemical modification of phylosilicate clays and zeolites with long chain organic cations results in an increased hydrophobicity of the mineral surface providing a high affinity for hydrophobic organic molecules, like the majority of the mycotoxins [6].

The objective of this research was to investigate *in vitro* adsorption of aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) by natural zeolitic tuff modified with different amounts of octadecyldimethylbenzyl ammonium ions (ODMBA), at different pHs. Chemical structures of AFB_1 and FB_1 are presented at Figure 1.



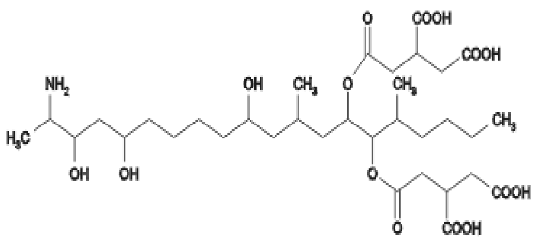


Figure 1. Chemical structures of AFB_1 and FB_1.

EXPERIMENTAL

The organozeolites were prepared from a natural zeolitic tuff from the Zlatokop deposit (Vranje, Serbia). The mineralogical composition of the natural zeolitic tuff was primarily clinoptilolite (minimum 85%) with smaller amounts of feldspar, quartz and pyrite as measured by X-ray powder diffraction analysis (XRPD). The cation exchange capacity (CEC) of the starting material was 139 mmolM^+/100g, while the external cation exchange capacity was 10 mmolM^+/100g.

The surfactant octadecydimethylbenzyl ammonium (ODMBA) chloride was purchased from Hoechst AG. For preparation of organozeolites, the zeolitic tuff was treated with ODMBA equivalents of 20, 50 and 100% of its ECEC (2, 5 and 10 mmolM^+/100g). The zeolitic tuff (5g) was mixed with 100 ml of each of the three ODMBA solutions in a mixer, at 10,000 rpm, for 3 min at 50°C, then rinsed with distilled water, and dried at 60°C. The samples were denoted OZ-2, OZ-5 and OZ-10.

In order to investigate AFB_1 and FB_1 adsorption by natural zeolitic tuff and the three organozeolites, duplicate aliquots of 0.1 M phosphate buffer (adjusted to pH 3, 7 and 9) containing 2 ppm AFB_1, or 2 ppm FB_1 in solution (10 ml) were added to 15 ml screw cap Falcon polypropylene tubes to which had been added 40 mg of each adsorbent. In order to eliminate exogenous peaks, controls were prepared by adding 10 ml of 0.1 M phosphate buffer (pH 3, 7, and 9) plus 40 mg adsorbent to test tubes. Test tubes were placed on a rotator shaker for 30 min at room temperature. Each mycotoxin test solution and control was centrifuged at 13,000 rpm for 5 min and 2 ml of the aqueous supernatant removed for mycotoxin analysis. AFB_1 and FB_1 concentrations in buffer solutions with and without mineral adsorbents were determined by HPLC method.

RESULTS AND DISCUSSION

Organozeolites OZ-2, OZ-5, and OZ-10 were prepared by ion exchange of zeolitic tuff with ODMBA up to the ECEC value of the starting material – 10 mmol M^+/100 g.

Aflatoxin B_1, a fairly nonpolar molecule, is a member of the dihydrodifuranocoumarin group and contains a β-keto-lactone functional group (Figure 1) [7]. Since AFB_1 is hydrophobic, it is assumed that it may partition into the hydrophobic phase created by surfactant tail groups at the zeolitic surface. The results of adsorption of AFB_1 by natural zeolitic tuff and the organozeolites are shown in Table 1

Table 1. AFB_1 adsorption by organozeolites, at pH 3, 7 and 9.

	AFB1 adsorption index, %		
	pH 3	pH 7	pH 9
Natural zeolitic tuff	95	87	87
OZ-2	87	53	57
OZ-5	75	53	57
OZ-10	67	70	65

Results presented in Table 1 showed that AFB_1 adsorption by organozeolites OZ-2, OZ-5 and OZ-10 decreased with increasing levels of organic phase at the zeolitic surface. The highest AFB_1 adsorption indexes were obtained for natural zeolitic tuff at all investigated pHs. The fact that nonionizable low polar AB_1 had a high affinity for the natural zeolitic tuff, at all investigated pHs, suggests that inorganic cations at the hydrophilic zeolitic surface may be the active sites on which this molecule is adsorbed. As can be seen from Table 1, the differences in AFB_1 adsorption at different pHs were observed for all three organozeolites. The highest adsorption indexes were obtained, at pH 3, for organozeolite OZ-2, in which 20% of inorganic cations were exchanged with ODMBA, indicating that at low surface coverage, the remaining uncovered surface of the organozeolite still has enough active sites for AFB_1 adsorption. The decrease in the adsorption with increasing pH for OZ-2 and OZ-5, was noticed, although AFB_1 is not ionizable. In case of OZ-10, pH almost does not have an influence on adsorption of AFB1.

Fumonisin B_1 is a large organic molecule, and it contains carboxylic, hydroxyl, and amino functional groups (Figure 1) [7]. The carboxylic and amino functional groups suggest that FB_1 in solution exist in cationic or anionic form at different pHs. Preliminary results have shown FB_1 adsorption on unmodified natural zeolitic tuff (C_{0FB1} = 2 ppm, and $C_{suspension}$ = 10 g/l) was 90.3, 2.0 and 6.2% at pH 3, 7 and 9, respectively. The unmodified surface of the zeolitic tuff with its net negative charge on the surface, has little or no affinity for anionic species, suggesting that FB_1 may exist in the anionic form at pH 7 and 9. Results of FB_1 adsorption by the three organozeolites, at different pHs are presented in Table 2.

Table 1. FB_1 adsorption by organozeolites, at pH 3, 7 and 9.

	FB_1 adsorption index, %		
	pH 3	pH 7	pH 9
OZ-2	75	66	46
OZ-5	93	96	74
OZ-10	93	96	97

As can be seen from Table 2, the presence of ODMBA ions at the zeolitic surface greatly improved adsorption of FB_1, especially at pH 7 and 9 and FB_1 adsorption increased with increasing amounts of ODMBA in the organozeolite. Results suggest that ODMBA ions at the zeolitic surface may be the active sites relevant for FB_1 adsorption. For OZ-2, FB_1 adsorption decreased with increasing the pH of solution, while for organozeolites with higher amounts of ODMBA differences in adsorption with solution pH are less visible. Thus, the lowest adsorption of FB_1 was achieved on organozeolite OZ-2 in which 20% of inorganic exchangeable cations were replaced with ODMBA, while the highest adsorption of FB_1 was achieved when the zeolite surface was totally covered with organic cations (sample OZ-10). It is well known that the sorption properties of organic compounds from water to organomineral adsorbents are closely related to solute properties such as water solubility and polarity [8]. FB_1 is a large organic molecule containing one NH_2-group, three hydroxyl and four carboxylic groups. Compared to AFB_1, FB_1 is more polar and water soluble [7]. Since OZ-2 has 20% of inorganic cations exchanged with ODMBA, the lower FB_1 adsorption on this organozeolite at pH 7 and 9 may result from the presence of the anionic form of FB_1 that can not be adsorbed at the uncovered negatively charged surface of the modified zeolitic tuff.

CONCLUSION

Results demonstrated that natural zeolitic tuff modified with different levels of ODMBA is effective in adsorbing the ionizable mycotoxin FB_1 at pH 3, as well as at pH 7 and 9. However, the adsorption of nonionizable AFB_1 was greatly reduced with the presence of organic cations at the zeolitic surface. The unmodified zeolitic tuff showed the highest affinity for AFB_1.

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ADSORBCIJA MIKOTOKSINA NA ORGANOMODIFICIRANI PRIRODNI ZEOLITNI TUF

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Apstrakt

U ovom radu su prikazani rezultati ispitivanja adsorpcije aflatoksina B_1 (AFB1) i fumonizina B_1 (FB1) na prirodnom zeolitu modifikovanom sa različitim količinama oktadecil dimetil benzil amonijum jona (ODMBA), na pH 3, 7 i 9. Organska komponenta je dodavana u različitim količinama, a maksimalno do vrednosti spoljašnjeg kapaciteta katjonske izmene zeolitiskog tufa (10 mmol M^+/100g). Dobijeni rezultati su pokazali da adsorpcija AFB1 na organozeolitima opada sa porastom sadržaja organske faze na površini klinoptilolita, na svim ispitivanim pH vrednostima. Najviša adsorpcija AFB1 je postignuta na prirodnom zeolitiskom tufu, kako na pH 3, tako i na pH 7 i 9. Za razliku od AFB1, prisustvo ODMBA jona na površini klinoptilolita značajno povećava adsorpciju FB1, na pH 3, 7 i 9. Određeno je i da adsorpcija ovog toksina raste sa porastom sadržaja organske komponenete u organozeolitima. Najviša adsorpcija FB1 je postignuta na organozeolitu kod koga je površina pokrivena monoslojem organske komponente.

Ključne reči: zeoliti, organozeoliti, mikotoksini, adsorpcija