MME SEE CONGRESS 2023

5th Metallurgical & Materials Engineering Congress of South-East Europe
Trebinje, Bosnia and Herzegovina
7-10th June 2023

CONGRESS PROCEEDINGS

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CONGRESS PROCEEDINGS - MME SEE 2023
5th Metallurgical & Materials Engineering Congress of South-East Europe

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Published and printed by:
Association of Metallurgical Engineers of Serbia (AMES)
Kneza Miloša 9/IV,
11000 Belgrade
Serbia

For the publisher:

AMES president Dr. Miroslav Sokić

Circulation:

120 copies

ISBN 978-86-87183-32-2

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PREFACE

On behalf of the Scientific and Organizing Committee, it is a great honor and pleasure to wish all the participants a warm welcome to the Fifth Metallurgical & Materials Engineering Congress of South-East Europe (MME SEE 2023) which is being held in Trebinje, Bosnia and Hercegovina, 07 - 10 June 2023.

The MME SEE 2023 is a biannual meeting of scientists, professionals, and specialists working in the fields of metallurgical and materials engineering. The aim of the Congress is to present current research results related to processing/structure/property relationships, advances in processing, characterization, and applications of modern materials. Congress encompasses a wide range of related topics and presents the current views from both academia and industry: Future of metals/materials industry in South-East European countries; Raw materials; New industrial achievements, developments and trends in metals/materials; Ferrous and nonferrous metals production; Metal forming, casting, refractories and powder metallurgy; New and advanced ceramics, polymers, and composites; Characterization and structure of materials; Recycling and waste minimization; Corrosion, coating, and protection of materials; Process control and modeling; Nanotechnology; Sustainable development; Welding; Environmental protection; Education; Accreditation & certification.

The editors hope that Congress will stimulate new ideas and improve knowledge in the field of metallurgical and materials engineering. The Congress has been organized by the Association of Metallurgical Engineers of Serbia, with the co-organization of the Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia, Faculty of Technology and Metallurgy, University of Belgrade, Serbia, Faculty of Technology, University of Banja Luka, Bosnia and Herzegovina; the Faculty of Metallurgy, University of Zagreb, Sisak, Croatia; the Faculty of Natural Sciences and Engineering, University of Ljubljana, Slovenia; and the Faculty of Metallurgy and technology, University of Podgorica, Montenegro.

Financial support from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia to researchers from Serbia for attending the congress is gratefully acknowledged. The support of the sponsors and their willingness to cooperate have been of great importance for the success of MME SEE 2023. The Organizing Committee would like to extend their appreciation and gratitude to all sponsors and friends of the conference for their donations and support.

We would like to thank all the authors who have contributed to this book of abstracts and also the members of the scientific and organizing committees, reviewers, speakers, chairpersons, and all the conference participants for their support of MME SEE 2023. Sincere thanks to all the people who have contributed to the successful organization of MME SEE 2023.

On behalf of the 5th MME SEE Scientific and Organizing Committee

Miroslav Sokić, PhD

CLAY-CHITOSAN-SURFACTANT COMPOSITES AS EFFICIENT ADSORBENTS OF ZEARALENONE

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In this study bentonite was modified with two chitosans, commercial or chitosan synthesized from mushroom. Obtained products were subsequently treated with hexadecyltrimethyl ammonium bromide in the amount of 50% of the bentonite`s CEC value. Starting material and all prepared composites were characterized by FTIR-ATR spectroscopy in order to confirm modification with organic molecules. Adsorption of zearalenone (ZEN) by clay-chitosan composites and clay-chitosan-surfactant composites was investigated at pH 3. Results showed that adsorption of ZEN was higher for composites that had both chitosan and surfactant, confirming that surfactant ions are responsible for ZEN adsorption. Additionally, the effect of adsorbent mass on ZEN adsorption was investigated for clay-chitosan-surfactant composites (pH 3) and results have shown that adsorption increased with increasing the amount of both composites.

Keywords: clays, chitosan, surfactants, zearalenone, adsorption

Introduction

Mycotoxins are secondary metabolites of fungi commonly found in cereal grains and animal feed. The most frequently found mycotoxins are: aflatoxins, ochratoxin A, zearalenone (ZEN) and fumonisins. Consumption of contaminated feed and food can cause serious health problems in animals and humans (Omotayo et al. 2019). Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by fungi of the genus *Fusarium*, that can produce a variety of toxic effects (miscarriage, infertility, or other reproductive problems) (Ropejko and Twarużek 2021). Various chemical, physical, and biological methods have been used to remove mycotoxins from contaminated animal feed. The most extensively studied physical method is adsorption by various types of compounds, mainly minerals (like natural aluminosilicates- zeolites and/or clays) because they are abundant, inexpensive, and environmentally acceptable. Among clays, bentonites have proven to be excellent adsorbents only for aflatoxin B1 (Wang, Hearon, and Phillips 2020;-Marković et al. 2016; Geofrey et al. 2022; Gan 2019). However, the adsorption of other less polar mycotoxins - like ZEN by natural minerals is low, so to increase its adsorption, these minerals are usually modified with long-chain cationic surfactants (Spasojević et al. 2021; Marković et al. 2017; Daković et al. 2007; Zhang et al. 2022). Recently, composites of natural biopolymers (chitosan and/or alginate) and clays have been extensively studied as adsorbents for many low polar molecules, dyes, phenols, and even aflatoxin B1 (Lian et al. 2019; Wang et al. 2020). Chitosan is a particularly interesting biomaterial due to its biocompatibility, biodegradability, antimicrobial activity and adsorption properties (Samal et al. 2012). Chitosan is a natural cationic biopolymer composed of randomly distributed N-acetyl glucosamine and D-glucosamine units, varying in composition, sequence and molecular chain length (Samal et al. 2012). It is obtained by deacetylation of chitin - the second most abundant organic polymer in nature after cellulose. Chitin can be found in the epidermis or exoskeletons of crustaceans (crabs, shrimp, insects, etc.) as well as in the cell wall of mushrooms and bacteria (Wong 2009). Recently, the use of biopolymer – clay mineral nanocomposite has attracted wide attention in pharmaceutical and biomedical field as well as in animal nutrition and wastewater treatment.

In this work, composites of bentonite with two chitosans - commercial low molecular weight chitosan and chitosan isolated from the mushroom in the laboratory were prepared. Composites were additionally modified with surfactant – hexadecyltrimethyl ammonium (HDTMA) bromide. The natural bentonite and both types of composites were characterized by FTIR-ATR analysis and tested as adsorbents of ZEN, under *in vitro* conditions. The adsorption of ZEN was followed at different amounts of each adsorbent in suspension, at pH 3.

Materials and methods

Clay - Ca-bentonite (clay) from Beretnica deposit in the Republic of Serbia (B) was used as a starting material. Bentonite was modified with commercial low molecular weight chitosan (LMW) (Sigma-Aldrich Co., Germany) and chitosan isolated from fruit bodies of commercially grown mushroom *Agaricus bisporus* (1.5H) (Delta Danube d.o.o., Kovin, Republic of Serbia). For modifications, chitosan solutions (0.5 g/L) were prepared in 1% (v/v) acetic acid (Centrohem, Republic of Serbia) and slowly added to B suspension (100 g/L). Experiments were performed using a mixer at 6000 rpm with an activation time of 30 min. Composites were separated in filtration process and dried at 60°C for 24 h. The obtained materials were subsequently modified with long chain organic cation (surfactant) – hexadecyltrimethyl ammonium bromide (S) (Sigma-Aldrich Co., Germany). The amount of surfactant used for modification of clay-chitosan composites was equal to 50% of CEC of starting clay (120.33 mEq/100g). The starting B, clay-chitosan composites (BLMW and B1.5H), as well as clay-chitosan-surfactant composites (BLMWS and B1.5HS) were characterized by Fourier transform infrared (FTIR) spectroscopy using Nicolet iS50 spectrophotometer (Thermo Fisher Scientific, United States) with a diamond ATR accessory in the range 4000–400 cm^{-1} and with the resolution of 2 cm^{-1} and 32 scans. All composites were preliminary tested as adsorbents for ZEN (Sigma-Aldrich Co., Germany). The stock solution of ZEN (1000 mg/L) was prepared in acetonitrile and then used to prepare working solutions of the ZEN in 0.1 M phosphate buffer. The adsorption of ZEN was investigated with the initial ZEN concentration of 4.0 mg/L, with 10 mg/10 mL of each adsorbent, at pH 3. Additionally, adsorption of ZEN by clay-chitosan-surfactant (BLMWS and B1.5HS) composites was further studied with the same initial ZEN concentration and with different amounts of each adsorbent (20, 10, 5 and 2 mg/10 mL) in suspension, at pH 3. In all experiments, suspensions were shaken for 30 min at room temperature using laboratory shaker (Heidolph Unimax 1010, Germany) with speed adjusted at 300 rpm, then centrifuged at 13000 rpm for 3 min. Zearalenone initial and concentrations in supernatants were determined by high performance liquid chromatography – HPLC (LC-20, Shimadzu, Japan) with a fluorescence detector at λ_{ex} = 274 nm and λ_{em} = 465 nm. An Inertsil® ODS-4 (150 x 4.6 mm, C_{18}, 5 µm) column was used for analysis, while the mobile phase, acetonitrile:water:acetic acid (60:40:1), was pumped at a flow rate of 1 mL/min, using isocratic elution.

Results and discussion

FTIR-ATR spectra of B and composites are shown in Figure 1. These results provided information on B modification with biopolymer and surfactant.

In spectra of B, absorption bands characteristic for bentonites were observed. The band at 3620 cm^{-1} corresponds to stretching vibrations of structural OH groups, while band at 3398 cm^{-1} and band at 1631 cm^{-1} correspond to the OH stretching and bending vibrations of water molecules adsorbed by B. The most intense band with a peak at 992 cm^{-1} is attributed to Si-O stretching vibrations, while bands at 914 and 840 cm^{-1} correspond to Al-Al-OH and Al-Mg-OH bending vibrations, respectively. Band at 794 cm^{-1} corresponds to Si-O stretching and Si-O-Al stretching vibrations, while bands at 695 and 512 cm^{-1} correspond to Si-O-Mg/Fe, and Al-O-Si stretching vibrations, respectively (El Miz et al. 2017; Salil, Shrivastava, and Pattanayak 1997; Slaný, Jankovič,

and Madejová 2019). Bentonite characteristic bands were also present in FTIR-ATR spectra of all composites, suggesting that modification did not significantly alter B structure.

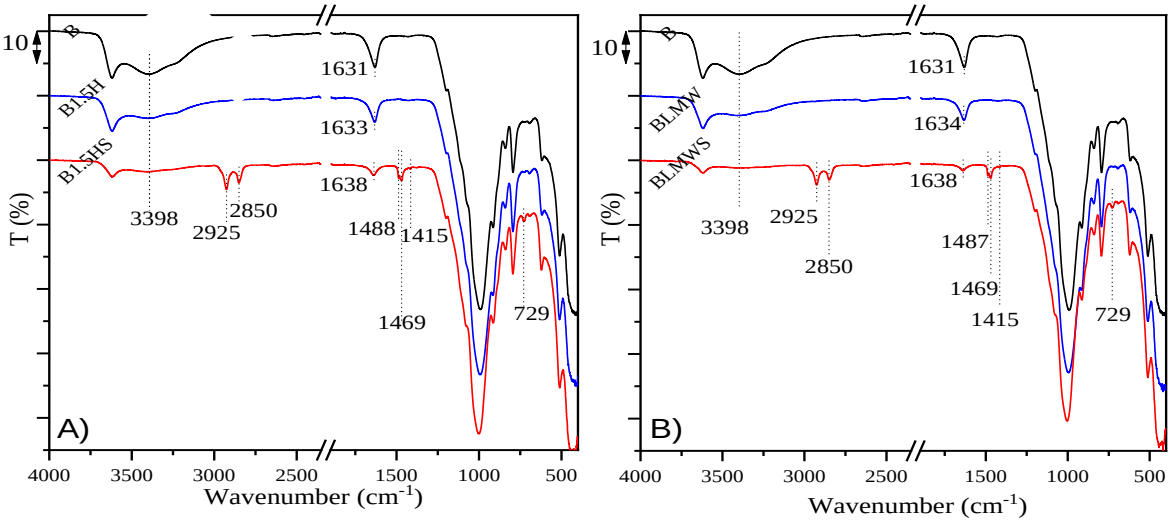

Figure 1 FTIR-ATR spectra of: A) starting material-B, clay-1.5H chitosan composite (B1.5H), clay-1.5H chitosan-surfactant composite (B1.5HS); B) starting material-B, clay-LMW chitosan composite (BLMW), clay- LMW chitosan-surfactant composite (BLMWS)

However, it is noticed that after modification with chitosan and surfactant the intensity of bands at 3398 cm^{-1} and 1631 cm^{-1} gradually decreased. Additionally, the band at 1631 cm^{-1} (in the spectrum of B) was slightly shifted to 1633 cm^{-1} in the case when 1.5H chitosan was used for modification (composite B1.5H) and to 1634 cm^{-1} when LMW chitosan was used (composite BLMW). Subsequent modification with S additionally shifted this band to 1638 cm^{-1} in spectra of B1.5HS and BLMWS. The decrease of the intensity of bands at 3398 cm^{-1} and 1631 cm^{-1}, as well as shifts of the band at 1631 cm^{-1} toward higher wave numbers, confirmed presence of chitosan and/or surfactant in B and suggested that modification with chitosan and subsequent modification with surfactant caused progressive loss of water molecules that coordinate cations in the interlayer space of B. These results confirmed increase in the hydrophobicity of the composites. Furthermore, from Figure 1 it can be seen that new bands, characteristic for surfactant S, appear in the spectra of clay-chitosan-composites (B1.5HS and BLMWS). Namely, the bands at 2925 cm^{-1} and 2850 cm^{-1}, which correspond to the asymmetric and symmetric stretching vibration of CH_2 groups of the alkyl chain of the surfactant, as well as the bands at 1488, 1469, 1415, and 729 cm^{-1} corresponding to C-H bending (scissoring and rocking) vibrations in the alkyl chains (Bezrodna et al. 2009). The appearance of these IR bands confirmed surfactant presence in composites B1.5HS and BLMWS and successful modification of clay-chitosan composites with HDTMA ions.

The results of preliminary experiments on adsorption of ZEN by chitosan-clay composites and chitosan-clay-surfactant composites are given in Table 1.

Results showed that both clay-chitosan-surfactant composites have high adsorption of ZEN (90.75% for BLMWS and 91.75% B1.5HS), while adsorption of this mycotoxin by clay-chitosan composites was low (4.18% for BLMW and 7.25% for B1.5H). Since, it is well known, that surfactant ions in organomodified minerals (zeolite, kaolin, etc.) are responsible for adsorption of ZEN (Spasojević et al. 2021; Marković et al. 2017), low adsorption of ZEN observed for both clay-chitosan composites suggested that additional modification of chitosan-clay composites with surfactant ions is necessary to create active sites at composites surfaces at which ZEN will be adsorbed.

Table 1 Preliminary ZEN adsorption by clay-chitosan and clay-chitosan-surfactant composites

Adsorbent	Adsorption %
B1.5	7.25
B1.5S	91.75
BLMW	4.18
BLMWS	90.75

Clay-chitosan-surfactant composites were efficient in removal of ZEN in the preliminary experiment, thus, B1.5HS and BLMWS composites were further studied as adsorbents for ZEN by using the same initial concentration of ZEN and different amounts of each adsorbent (20, 10, 5 and 2 mg/10 mL) in suspension, at pH 3. Results of effect of adsorbent mass on ZEN adsorption are given in Figure 2.

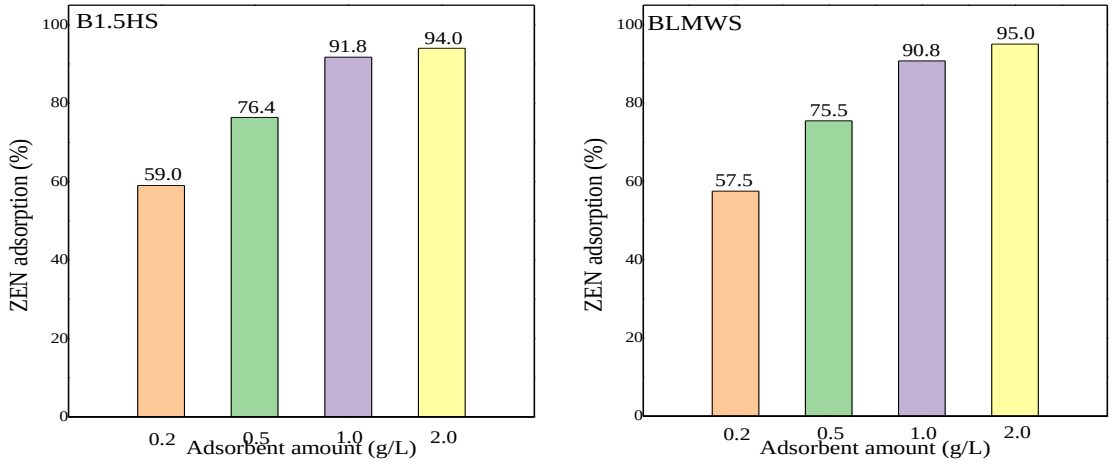

Figure 2 Adsorption of ZEN (%) at different amounts (0.2, 0.5, 1.0, and 2.0 g/L) of adsorbents: A) clay-1.5H chitosan-surfactant composite (B1.5HS); B) clay-LMW chitosan-surfactant composite (BLMWS)

Results presented in Figure 2, showed that ZEN adsorption increased with increasing of the amount of each composite in suspension. With the lowest amount of composites (0.2 g/L), adsorption of ZEN was 59.0% for B1.5HS and 57.5% for BLMWS, while with the highest amount of each adsorbent in suspension (2.0 g/L), the following ZEN adsorption indexes were obtained: 94.0% for B1.5HS and 95.0% for BLMWS. From obtained results, it is obvious that ZEN adsorption by chitosan-clay-surfactant composites increased with increasing amount of surfactant in composites. The increased ZEN adsorption with increasing amounts of each adsorbent is related to the increasing number and availability of adsorption sites in chitosan-clay-surfactant composites.

The facts that chitosan is a biomaterial with known antimicrobial activity and that adsorption of ZEN by composites containing commercial chitosan and chitosan isolated from mushroom is similar, opens the possibility for using the fungi in preparation of clay-chitosan-surfactant composites as new animal feed additives with antimicrobial properties.

Conclusion

The natural bentonite was modified with two chitosans of different origin, and the resulting composites were additionally modified with cationic surfactant. Characterization of the products by using FTIR-ATR spectroscopy confirmed successful modification with chitosan and surfactant, and their presence in the composites. Zearalenone adsorption experiments showed that clay-chitosan-surfactant composites are excellent adsorbents compared to clay-chitosan composites, confirming that surfactant molecules are the active sites relevant for adsorption of this toxin. The high ZEN adsorption achieved by both clay-chitosan-surfactant composites, composites simple modification process, the abundance and low cost of the starting bentonite as well as biocompatibility of chitosan make these materials suitable for potential application for decontamination of animal feed contaminated with ZEN.

Acknowledgement

This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 7748088, Project title: Composite clays as advanced materials in animal nutrition and biomedicine - AniNutBiomedCLAYs.

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