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Graphene-type sorbents for the elimination of mycotoxin T-2

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A comparative study of sorbents based on several natural biopolymers and their carbonized products — few-layer graphenes obtained under the self-propagating high-temperature synthesis process — was conducted using the example of mycotoxin T-2 sorption. The studies show that the efficiency of mycotoxin T-2 sorption by few-layer graphenes in all indicators, including the irreversible sorption indicator, significantly exceeds similar characteristics for the original biopolymers (sulfate lignin, microcellulose, pine bark, birch lignin). The adsorbents were also studied using the low-temperature nitrogen adsorption method.

Keywords: mycotoxin sorption, mycotoxin T-2, lignin, few-layer graphene.

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Introduction

Food security is a serious problem faced by the global economy. A particular task of this global problem is to eliminate losses of raw materials of biological origin and final products of its processing resulting from contamination with mycotoxins [1]. Mycotoxins (MT) are secondary toxic metabolites of microscopic fungi — micromycetes. Modern intensive agriculture produces millions of tons of products that need to be stored and cleaned. It is impossible to exclude the ingress of MT into food products in practical applications as a result of the large volume of production, since the contamination can occur at any stage of harvesting and storage of crops. For instance, the intoxication of grain crops with micromycetes can reach up to 25% of the total harvest according to experts from the UN FAO (Food and Agriculture Organization of the United Nations) [2]. Both physical control methods (improvement of storage conditions, water washing, separation of clean product from contaminated) and methods of external treatment of contaminated product (heat treatment, ozonation) are used for detoxification of food affected by MT [3,4]. However, given that the chemical structure of most MT molecules varies from simple heterocycles to more complex molecules with 6–8 irregularly arranged heterocyclic fragments [5], MT are characterized by high chemical stability and thermal stability like all heterocyclic organic compounds. Accordingly, detoxification of a crop of plant origin intended for animal nutrition by thermal and/or chemical treatment

methods cannot provide the necessary degree of purification from MT and, therefore, does not exclude the ingress of toxins into the animal body [6]. Thus, it is also impossible to exclude the hazard of intoxication of people with MT (mycotoxicosis) through the formation of a food chain — transfer of toxins from animals to humans [7,8].

The main efforts of many research groups are focused on the creation of enterosorbents- sorbents that bind exo- and endogenous substances in the gastrointestinal tract of animals taking into account that it is almost impossible to completely exclude the detoxification of products.

When choosing an enterosorbent for detoxification of feed, it is necessary to take into account that the expansion of exports and imports of agricultural products between countries leads to an increase in the possibility of simultaneous contamination of fodder with various micromycetes and MT. In addition, due to global climate change, MT strains that were previously found only in countries with a tropical-subtropical climate are also registered in countries with a temperate climate, in particular in Russia [9,10].

Thus, modern enterosorbents should meet not only the basic requirements for enterosorbents, such as non-toxicity, specificity for the sorption of toxins, irreversible binding of toxins, their rapid elimination from the body and stability in the gastrointestinal tract (gastrointestinal tract), but they also should be polyvalent with respect to a number of the most common microtoxins [11]. And, although a number of enterosorbents based on inorganic and synthetic adsorbents have been developed to date (see, for example, [12,13] and

references therein), the search for new starting materials as the basis of enterosorbents, as well as new methods for the formation of enterosorbents, is still relevant.

Most of the requirements for modern enterosorbents are met by enterosorbents obtained on the basis of widespread natural biopolymers — lignins [14–16]. The disadvantage of lignin-based enterosorbents can be attributed to their low sorption capacity in variable (according to the acidity of the medium) conditions of the mammalian gastrointestinal tract, as evidenced by a low index of irreversible adsorption Q_s . This index is defined as the difference between the adsorption index at pH 2 (as in the stomach) and pH 8 (as in the colon).

The testing of the highly dispersed material synthesis technologies for their suitability as sorbents began with the development of such technologies [17,18]. In particular, papers on the study of graphene oxide (GO) as a polyvalent sorbent of MT (anatoxin, zearalenone, deoxynivalenol) have been published [19–21]. However, the productivity of the Hammers technique which is used for the synthesis of graphene oxide in the vast majority of cases is clearly insufficient for the transition from interlaboratory studies to advanced practical papers [22]. In addition, the synthesis technique itself does not meet modern stringent environmental safety requirements, as it requires the use of aggressive acids and active oxidants.

We proposed a method for producing few-layer graphene (FLG) obtained by carbonation of biopolymers in the process of self-propagating high-temperature synthesis, which, in terms of its performance and environmental safety, fully meets the requirements for modern technologies [23].

A comparative study of the effectiveness of the processes of sorption of MT T-2 by these two types of sorbents was conducted in this paper for determining the prospects of replacing enterosorbents based on natural biopolymers with their carbonized analogues.

1. Experimental part

1.1. Precursors

Biopolymers of various origins were used as FLG precursors:

1) technical lignin, sulfate lignin JSC „Mondi SLPK“; elemental composition: C — 65.9%, H — 5.8%, O — 26.3%; functional composition: OCH_3 — 11.3%, phenolic groups OH_{ph} — 5.1%, S — 1.5%; $COOH$ — 4.3%; designation of the resulting carbonized product — FLG-1;

2) microcrystalline cellulose [24]; obtained from LLC „Chempharmproduct Pharmacy Warehouse“; particle size — 50 μm ; pH 5–7; designation of the obtained carbonized product — FLG-2;

3) pine bark; obtained from the collection of the Far Eastern Research Institute of Forestry (FGBI DALNIILKh, Khabarovsk); designation of the obtained carbonized product — FLG-3;

4) natural lignin — dioxanlignin isolated by Pepper's method from birch wood *Betula verrucosa*; elemental composition: C — 60.7%, H — 6.8%, O — 34.8%; functional composition: OH_{ph} — 4.4%, $COOH$ — 4.5%; designation of the obtained carbonized product — FLG-4.

1.2. Obtaining of FLG

Biopolymers were carbonized under the conditions of the method of self-propagating high-temperature synthesis (SHS). SHS uses the internal heat of an exothermic chemical reaction between mixed powdery components.

The main advantages of the SHS method include:

(1) possibility of carrying out the process without using permanent external heating sources, in any atmosphere or in a vacuum, the absence of significant energy costs, since the process proceeds through the use of internal energy contained in the initial reagents;

(2) possibility of using simple technological equipment;

(3) high speed of the SHS process (several orders of magnitude higher than everything achieved so far in similar technological processes);

(4) The SHS equipment is compact and does not require large areas.

Cyclic biopolymers such as cellulose (BDA), glucose (BDA) and hydrolysis lignin were used as a carbon source. The carbon source was mixed with an oxidizer (ammonium nitrate, BDA) in a ratio of 1:1. The feed mixture was loaded into the reactor, where the SHS process was started by heating to 300°C. The yield of the final product from the mass of the initial biopolymer was 25–30%.

The synthesis process is described in more detail in [25].

1.3. Sorption experiment

The experiment was conducted as follows. At the first stage, the adsorption index of MT (Q) was determined, for which the toxin and adsorbent were incubated in a ratio of 1:1000 in a solution containing 50 μg of toxin at a pH of 2 and a temperature of $37 \pm 0.2^\circ C$ (modeling of the *in vitro* environment of the stomach). A crystalline MT produced by Fermentek ltd. (Israel) was used. Then the solution was centrifuged, and the toxin was extracted from the liquid fraction with 70% aqueous methanol solution. The residual amounts of MT T-2 in the dry residue were determined by enzyme immunoassay using the „RIDASCREEN“ T-2 Toxin kit. (R-Biopharm). At the second stage, the desorption of MT (D) was determined, for which the solid fraction after centrifugation was incubated in solution at pH 8 (modeling of environmental conditions *in vitro* in the colon). Then the toxin content in the liquid fraction was centrifuged again and determined after its desorption at pH 8. Q is the average value of the sorption index, expressed as a percentage of the total number of micrograms taken in the experiment. The amount of strongly (irreversibly) sorbed MT ($Q_s, \%$) was determined

based on the difference between the adsorption index Q and desorption index D .

1.4. Determination of the characteristics of the porous structure

The characteristics of the porous structure of adsorbents were determined by low-temperature nitrogen adsorption on the ASAP 2020 mp specific surface area and porosity analyzer (Micromeritics, USA). The adsorbent samples were pre-degassed at 100°C to a residual pressure of $7 \cdot 10^{-4}$ Pa with holding under specified conditions for 2 h. The sample weight ranged from 20 to 160 mg, depending on the assumed structure of the sample. Next, gas adsorption and desorption were carried out in the pressure range from 0 to 101.3 kPa. The parameters of the porous structure were calculated from the obtained isotherms using the software of the device. The total pore volume V_{Σ} (cm^3/g), width and specific pore surface area (SPSA) (m^2/g) were determined using the Brunauer–Emmett–Teller method (BET). The width of the micropores was calculated using the Horvath–Kawazoe method, [nm], micropore volume V_{micro} , [cm^3/g], calculated using the Dubinin–Radushkevich method, the volume of mesopores V_{meso} , [cm^3/g], was determined using the Broekhoff-de Boer method.

2. Results and discussion

Figure 1 shows the adsorption–desorption indices of MT T-2 under conditions simulating the *in vitro* mammalian gastrointestinal tract for FLG samples.

As follows from the data obtained, all FLG samples obtained from precursors demonstrate a very high sorption capacity with respect to MT T-2. The FLG-2 (FLG_cel) and FLG-4 (FLG_lgnat) samples can be singled out in particular, for which the values of Q and Q_s are almost equal. This means that losses due to the desorption of MT T-2 in conditions simulating the large intestine of the gastrointestinal tract are practically not observed for these FLG samples ($< 0.1\%$).

If we consider the prospects of using technical lignin and pine bark as a precursor, it can be noted that, despite slightly worse sorption indicators (by 3–4%), from the point of view of practical use, such parameters as raw material availability and the possibility of improvement are some advantages in their choice environmental conditions in places of long-term storage of technical lignin and pine bark, representing waste from the processing of wood-based biomass [26,27].

Since work is still underway to find natural sources for use as MT sorbents (see, for example, [28] and references therein), it is of undoubted interest to compare the sorption properties of the initial biopolymers used in this work as precursors and their carbonized derivatives — FLG (Fig. 2).

As can be concluded from the data in Fig. 2 that carbonized biopolymers FLG demonstrate significantly better

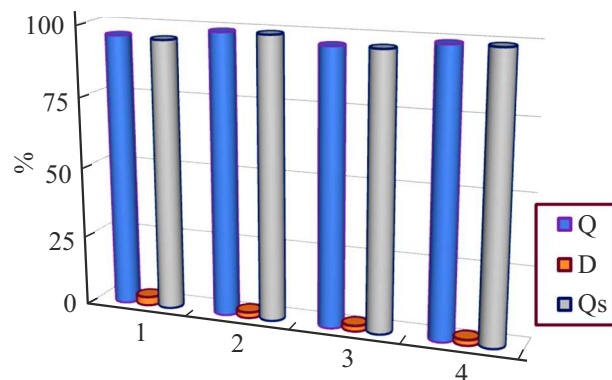


Figure 1. Sorption-desorption characteristics of FLG samples obtained from various precursors with respect to MT T-2: Q — sorption at pH 2, D — desorption index, Q_s — irreversible sorption index.

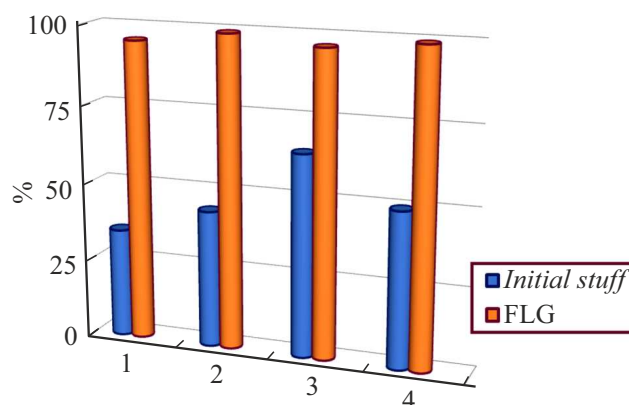


Figure 2. Indicators of irreversible adsorption with respect to MT T-2 for samples of feedstock (blue) and their carbonized derivatives — FLG (red). 1 — technical sulfate lignin, 2 — microcrystalline cellulose, 3 — pine bark, 4 — natural birch lignin.

sorption characteristics relative to MT T-2 compared with the initial biopolymers.

We applied the method of low-temperature nitrogen adsorption for a better understanding of the observed discrepancy in the sorption properties of the initial biopolymers and their carbonized analogues.

The adsorption isotherms for samples of biopolymers—of precursors 1–4 are shown in Fig. 3, and their carbonized analogues are shown in Fig. 4.

It is necessary to consider the features of the isotherms of lignins (precursors) and graphene samples synthesized from them. To interpret the results on the sorption of MT T-2. Firstly, it should be noted that the isotherms for all lignin samples are qualitatively the same. Secondly, it can be argued that the isotherms should be classified as type IV(a) according to the IUPAC classification based on the analysis of the shape of these isotherms. The characteristic features of the type IV isotherm are a well-defined hysteresis loop, which is caused by capillary condensation, which

Characteristics of the surface-porous structure of FLG samples.

Sample	SPSA, m ² /g	V _Σ , cm ³ /g	V _{meso} , cm ³ /g	V _{micro} , cm ³ /g	Width of micropores, nm	Width of pores, nm
FLG-1	261	0.142	0.007	0.123	1.13	2.2
FLG-2	288	0.236	0.109	0.132	1.12	3.3
FLG-3	367	0.421	0.103	0.314	1.14	2.5
FLG-4	283	0.194	0.050	0.119	2.74	2.7

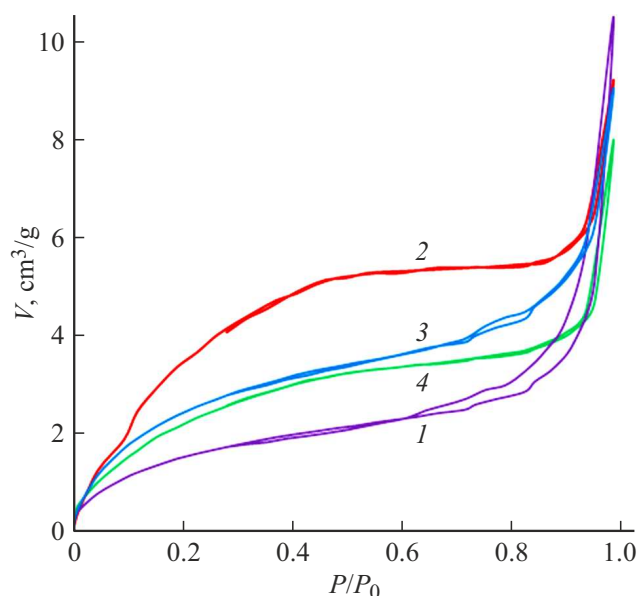


Figure 3. Nitrogen adsorption isotherms for precursor biopolymer samples: 1 — microcrystalline cellulose, 2 — birch lignin, 3 — pine bark, 4 — sulfate lignin.

usually occurs in mesopores. It should be noted that type IV isotherms are observed for many industrial mesoporous adsorbents.

Considering the direction (almost vertical) of the sorption and desorption branches, the hysteresis loops shown in Fig. 3 should be attributed to the type H1. Capillary condensation of the adsorbate occurs in the region starting at high values of the pressure index $P/P_0 \approx 0.7-0.8$, which also indicates a developed mesoporous structure of the studied samples. Some features of the shape and width of the hysteresis loop for different lignin samples may be related to the nature of the mesopore distribution. As the analysis of the literature data shows, H1 type isotherms are often observed for adsorption proceeding by a chemisorption mechanism.

Interpreting the type of sorption isotherms obtained for carbonized products, they can be correlated with sorption isotherms of type H4 (Fig. 4). This type of isotherms indicates that the studied carbon nanomaterials have a surface-porous structure formed mainly by narrow slit-like micropores with a width of 2–3 nm. A complete calculation

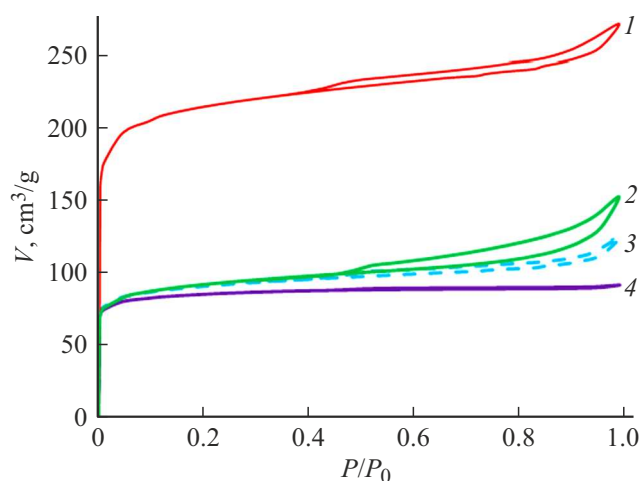


Figure 4. Isotherms of adsorption of FLG samples: 1 — FLG-3, 2 — FLG-2, 3 — FLG-4, 4 — FLG-1.

of the specific surface area, total pore volume and partial contributions of meso- and micropores is provided in the following table.

Comparing the characteristics of the surface-porous structure of FLG samples with their sorption parameters, it can be noted that some advantage in the sorption characteristics of FLG-2 and FLG-3 can be associated with an additional partial contribution of mesopores.

Comparing the data on the sorption characteristics of lignins and their carbonized analogues, it can be concluded that the significant advantage of FLG is attributable to the features of their morphometry — the presence of a surface-porous structure formed mainly by narrow slit-like micropores characteristic of FLG particles formed by a stack of 2–5 graphene layers.

Conclusions

The efficiency of sorption of MT T-2 by natural biopolymers of plant origin and their carbonation products — FLG obtained by the SHS method was studied. Adsorbents were studied by low-temperature nitrogen adsorption, and it was found that the initial plant materials have a mesoporous structure, whereas graphenes are characterized by the presence of narrow slit-like micropores. The data obtained

indicate a higher sorption capacity of graphenes in comparison with the original biopolymers, due to differences in the capillary-porous structure of the sorption materials under study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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