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Few-Layer Graphene Structures as a Promising Mycotoxin Sorbent

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Received February 15, 2022
Revised February 15, 2022
Accepted March 25, 2022

It has been experimentally established that samples of low-layer graphene, synthesized by carbonization of plant materials (lignin, cellulose, and spruce bark) under conditions of self-propagating high-temperature synthesis, are effective sorbents for mycotoxin T-2 under conditions simulating the environment in the gastrointestinal tract of mammals, and are capable of irreversibly sorb at least 94.6% of mycotoxin with a sorption capacity of 1 mg of mycotoxins per 1 g of sorbent. Key words: few-layer graphene, self-propagating high-temperature synthesis, specific surface area, mycotoxin sorption.

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DOI: 10.21883/TP.2022.07.54472.31-22

Introduction

Food security is a serious problem that the global economy is facing. A comprehensive interdisciplinary approach is required to solve this problem. In particular, an important food security objective is to reduce the loss of raw materials of biological origin and final processing products to the maximum possible extent. This objective includes one of the most difficult problems — food product damage by mold fungi whose metabolic byproducts include a wide range of toxic organic compounds — mycotoxins [1,2]. From chemical point of view, mycotoxin molecule structure varies from simple molecules containing heterocycles to more complex molecules, with 6–8 irregularly arranged heterocyclic rings [3]. Damage to the harvested crops with mycotoxins may occur at any stage: beginning from harvesting and up to crop sales. The inability to define the time when the initial biological raw materials are damaged with mycotoxins, makes mycotoxin removal or inactivation a very complicated problem. The problem becomes much more complicated if mycotoxins migrate from fresh raw materials into final products (stock feed, food products) [4].

Currently, multiple strategies, including physical, chemical, and biological methods, have been developed for the decontamination and detoxication of mycotoxins in damaged food products and stock feed (e.g. [5,6] and references therein). Nevertheless, until now, the problem of food products damage with mycotoxins is far from solution. This is an incentive for many research teams to look for new approaches to the solution of mycotoxin inactivation problem. Thus, mycotoxin immobilization using sorbents added to stock feed is a promising approach [7–9]. According to several recent publications, efficient mycotoxin sorbents include graphene nanostructures — graphene oxide (GO), reduced graphene oxide (rGO), and their composites [10–12]. The advantage of graphene nanostructures compared with traditionally used sorbents is their ability to immobilize a wide range of mycotoxins and their fundamental to regenerate mycotoxins for repeated use [13].

However, despite the shown future opportunity opportunity for introducing of graphene nanostructures for mycotoxin immobilization, their real application in practical biotechnology is doubtful. This is primarily a consequence of the insufficient performance of currently known synthesis methods. Even higher performance graphene nanostructure synthesis methods such as multiple Hummer's method options (e.g. [14] and references therein) make it possible to obtain GO and, therefore, rGO in the amounts sufficient only for laboratory experiments (dozens of per shift), which is obviously insufficient to solve such large-scale problem. In addition, Hummer's method (including its multiple modifications) for GO synthesis includes the use of corrosive acid and alkali agents for initial graphite processing, which cast doubt on its environmental friendliness and cost efficiency in case production capacity extension. Therefore, efforts

of many research teams are aimed at the development of graphene nanostructure synthesis technique meeting the requirements of environmental safety and at the same time enabling to produce graphene nanostructures in the amounts required for real application at a reasonable price.

Recently, we have proposed a high-performance and low-cost graphene nanostructure synthesis technique, i.e. a few-layer graphene — FLG, by carbonization of plant biopolymers in self-propagating high-temperature synthesis conditions (SHS process [15,16]. In terms of physics, SHS is a strongly exothermic reaction wave movement over a reagent mixture (oxidizer and reducer) where heat release is localized in a thin layer and transferred from layer to layer using transfer. Typical SHS process characteristics include: flame front propagation rate $\sim (0.1-20)$ cm/s; maximum combustion temperature $\sim (2300-3800)$ K; substance heating rate in wave $\sim (103-106)^\circ$ C/s. SHS process requires no unique equipment, and no fundamental large-scale restrictions are imposed. Also, afor the SHS process, no permanent energy input from external sources is required. In addition, SHS process may be carried out in any atmosphere or in a vacuum which allows to get the final product with pre-defined properties to a certain extent [17].

Few-layer graphene particles synthesized using this technique have shown their efficiency as a cathode material to achieve low-threshold field emission [18] and as a modifying additive for the creation of NBR-based polymer composites [19].

However, it have to be considered that our graphene nanostructure technique is based on the „bottom-up" synthesis, in contrast existing graphene nanostructure techniques and, primarily, Hummer's method are based on „up-bottom" strategy. In this case, we cannot ensure that the morphological parameters of nanocarbon produced using our technique will correspond to the graphene nanostructures obtained using the most widely spread techniques and, thus, will be able to be successfully used for mycotoxin immobilization.

The purpose of the research was to ensure graphene nanostructure synthesis by carbonization of plant biopolymers in the self-propagating high-temperature synthesis process, characterization and check of their sorption properties against mycotoxins.

1. Experimental

1.1. Materials

1.1.1. Biopolymers

We may consistently suggest that a cycle of six carbon atoms — hexagon - is a native element that forms a graphene sheet honeycomb. Identification of an available source of such cycles and development of initiation technique for their „bottom-up" self-arrangement would allow the production of graphene structures in the amount required for application. We suggested that biopolymers containing cyclic structures in their backbone chain constitute a suitable source of hexagon. In this respect, the following materials were used as starting material for FLG synthesis: lignin (obtained by selection from dumps of Arkhangelsk pulp and paper plant); pine bark (from the Pacific National University's collection); microcrystalline cellulose (MKTs-101, Diapazon Pharm, Moscow).

1.1.2. mycotoxin

4,15-diacetoxy-8-(3-methylbyturyloxi)-12, 13-epoxitrichothecene-3-ol (mycotoxin *T*-2, „Fermentek Ltd.", Izrael) was used as a representative sample of mycotoxin.

1.1.3. Few-layer graphene synthesis

All biopolymers were preliminary dried, ground and sieved to produce a smooth paste. The prepared samples were mixed with oxidizer powder in a mass ratio of biopolymer:oxidizer $= 1:1$. Ammonia nitrate was used as an oxidizer. The laboratory setup consisted of a „pyrex"glass three-neck flask furnished with a thermocouple, vacuum gas separator, inert gas feeder and heating system. An accurately weighed reaction charge was placed in the flask and heating was actuated. The initiation of the carbonization process was marked by gas release. Then, heating was shut down and the gas release process completion was achieved which meant that the SHS was competed. The gas release start and end temperature was recorded by the thermocouple readings. The final powdered product was subjected to further study.

1.2. Methods

1.2.1. Electron microscopy

Electron images of FLG powder particles were made by TESCAN Mira-3M scanning election microscope.

1.2.2. Raman scattering spectroscopy

Raman scattering spectra (Raman spectra) obtained using Confotec-500, RB. Laser wavelength was equal to 532 nm. Before the measurement, the samples were deposited on silicone plates from water suspensions.

1.2.3. Specific surface and porosity measurement

Specific surface was measured using ASAP 2020 MR, USA, by BET method. Nitrogen was used as adsorbate gas. Before the measurement, the samples were held during 2 h in vacuum at 300°C.

1.2.4. IR Fourier spectroscopy

IR absorption spectra were made using „INTRAFLYUM FT-08", RF, IR Fourier spectroscope.

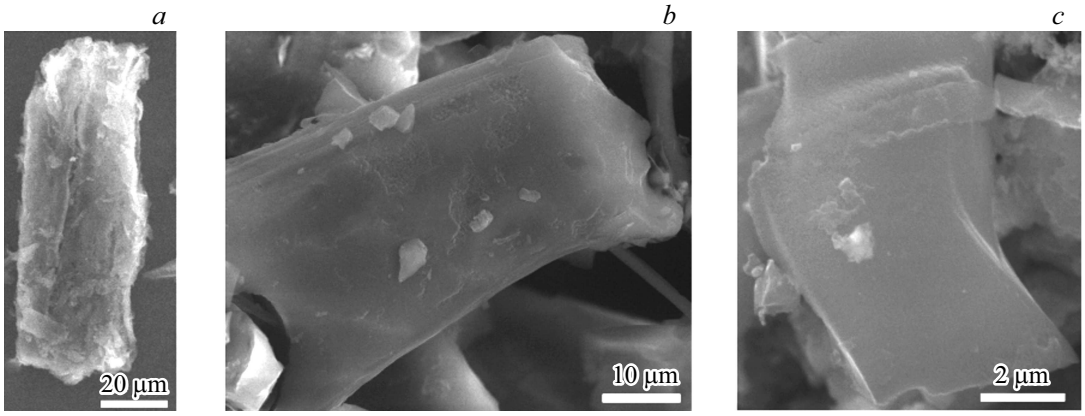

Figure 1. SEM images of FLG synthesised by SHS technique from linin (a), cellulose (b) and pine bark (c) , respectively.

1.2.5. Particle dispersion measurement

Particle dispersion was measured by dynamic light scattering (DLS) using Mastersizer 2000, USA. For measurement, 0.05 mass.% MG particle water suspensions were prepared.

1.2.6. Study of sorption-desorption properties

For absorption, 50 μg of mycotoxin T-2 in solution was used with toxin : adsorbent ratio 1 : 1000, medium pH 2.0, temperature 37°C. Then the solution was centrifuged, toxin was re-extracted from the centrate into chloroform three times 20 ml each. Chloroform extracts were combined and evaporated to dryness in a rotary evaporator. Quantitative determination of residual amounts of mycotoxin T-2 in dry residue was carried out by thin-layer chromatography with bioautographic completion using *Candida pseudotropicalis* strain 44 PK. Mycotoxin absorption at pH 2 (Q, %) and desorption at pH 8 (D_S) were determined. Q is an average adsorption in percentage of the total mycotoxin taken for the experiment. Amount of firmly absorbed mycotoxin (Q_S, %) were calculated according to the difference of absorption Q and desorption D_S.

2. Results and discussion

The general view of FLG particles synthesized from lignin (FLG_lg), cellulose (FLG_cel) and spruce bark (FLG_b), and their distribution by sizes were assessed using complementary scanning electron microscopy (SEM) (Figure 1) and DLS (Figures 2) techniques.

Figure 1 shows an example of microphotographs of particles produced by means of lignin carbonization in the self-propagating high-temperature synthesis conditions. Figure 1 demonstrates lamellar flake-like microstructure formed by tightly bound individual graphene sheets, which is typical of few-layer graphene particles (e.g. [20,21]).

DLS data is shown in Figure 2.

Data in Figure 2 shows that planar particle sizes and the nature of size distribution curve depend on the initial biopolymer macromolecule architecture (precursor). The largest planar sizes were obtained for FLG_lg, for which all particles are in the micron range (number-average planar size 2 μm). For FLG_cel and FLG_b, the maximum planar particle size is shifted toward the submicron region and is equal to 0.73 and 0.90 μm, respectively. A comparison of DLS and SEM data allows to suggest that, though the synthesized particles may have linear sizes up to several dozens of microns, the number of particles with such linear sizes is not high.

To assess the quality of graphene nanostructures, Raman spectra were obtained (Figure 3).

All obtained Raman scattering spectra have D- and G-peaks, and combined regions of $2D$- and $D+G$-peaks. Similar spectra were obtained in many publications devoted to Raman spectra of graphene structures (e.g. [22,23]). The calculated intensity ratio of D- and G-peaks for lignin, cellulose and pine bark samples were equal to 0.78, 0.95 and 1.13, respectively. According to theoretical assumptions, the area ratio of D- and G-peaks is proportional to the number of graphene layers in the stack. Therefore, if we assume that the intensity ratio of D- and G-peaks for single-layer graphene is 0.26, we will easily calculate that the number of graphene layers in the stack is within 3–5 in our case.

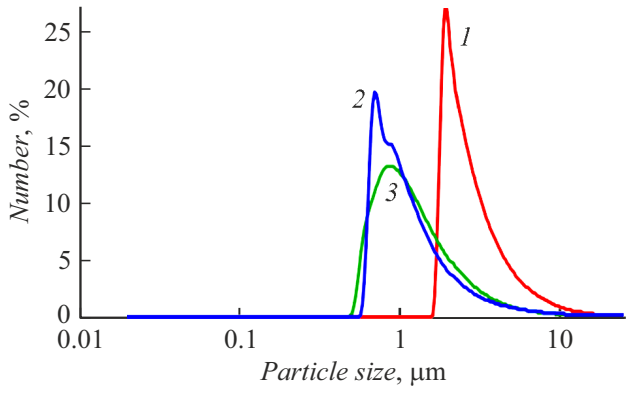

Figure 2. Size distribution of FLG particles synthesized in SHS process conditions from lignin (1), cellulose (2), pine bark (3).

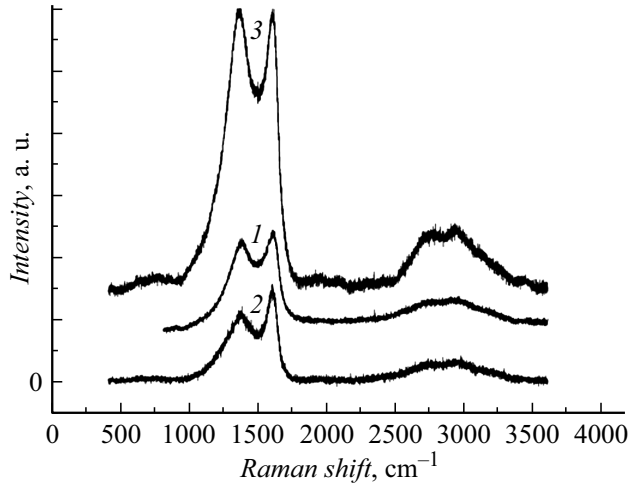

Figure 3. Raman spectra of FLG synthesized by SHS method from lignin (*1*), cellulose (*2*), pine bark (*3*).

Table 1. Surface characteristics of few-layer graphene synthesized from various biopolymers

Type of pores	Pore volume, cm^3/g	Specific surface, m^2/g
FLG_*lg*		
Micropores (< 2 nm)	0.1671	497.00
Mesopores (2–50 nm)	0.0164	1.93
Macropores (> 50 nm)	0.0099	0.27
Total	0.1934	499.20
FLG_*cel*		
Micropores (< 2 nm)	0.2134	658.07
Mesopores (2–50 nm)	0.0451	13.88
Macropores (> 50 nm)	0.0004	0.05
Total	0.2589	672.00
FLG_*b*		
Micropores (< 2 nm)	0.1490	540.58
Mesopores (2–50 nm)	0.0415	13.13
Macropores (> 50 nm)	0.0508	0.44
Total	0.3413	554.15

Table 1 summarizes the measurements of the specific surface of few-layer graphene synthesized from various biopolymers. Data analysis (Table 1) suggests that the precursor's nature also obviously influences the specific surface of the carbonized product. In this case, micropores provide the major contribution to the total specific surface. Thus, the maximum specific surface and the maximum amount of micropores were obtained for FLG_*cel*.

Figure 4 shows the study of composition of surface groups of samples synthesized by IR Fourier spectroscopy method.

As shown in Figure 4, the composition of terminal groups of all groups of carbonized products is identical. IR curves contain bonds C–O–C (945 cm^{-1}), C–H (2920 cm^{-1}) and C=C (1627 cm^{-1}) typical of graphene materials. 2*D*-graphene materials synthesized by us feature a recordable bond C–N (2233 cm^{-1}), which occurs due to the use of ammonia nitrate as an oxidizer.

Thus, the nature of the precursor mainly influences the morphological parameters of the final FLG particles, with the set of terminal functional groups unchanged.

The next important research stage involved experiments for mycotoxin *T*-2 sorption by the obtained FLG samples. Earlier, for sorption activity assessment of potential intestinal sorbents, Kryukov et al. developed a new method *in vitro* simulating the sorbent/sorbate relationship in gastrointestinal tract of mammals ($T = 37°C$). The method is based on the increase in acidity typical of the gastral tract (pH = 2), up to the acidity typical of the intestinal tract (pH = 8) [24]. Sorption activity or irreversible adsorption index Q_S was assessed by the difference of sorption indexes with different pH. Our experiments carried out according to this approach allowed us to determine Q_S for the test FLG samples (Table 2).

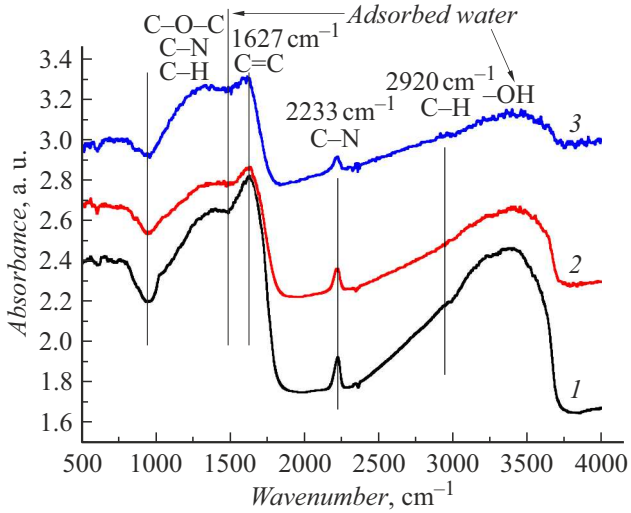

Figure 4. IR Fourier spectra of MG synthesized by SHS method from lignin (*1*), cellulose (*2*), pine bark (*3*).

Table 2. Sorptio/desorption properties of FLG samples in respect of mycotoxin *T*-2,%

Sample	*Q*	D_S	Q_S
FLG_*lg*	96.0 ± 0.8	0.17 ± 0.02	95.3 ± 0.9
FLG_*cel*	> 99.0	< 0.1	> 99.0
FLG_*b*	96.4 ± 0.6	< 0.1	96.4 ± 0.6

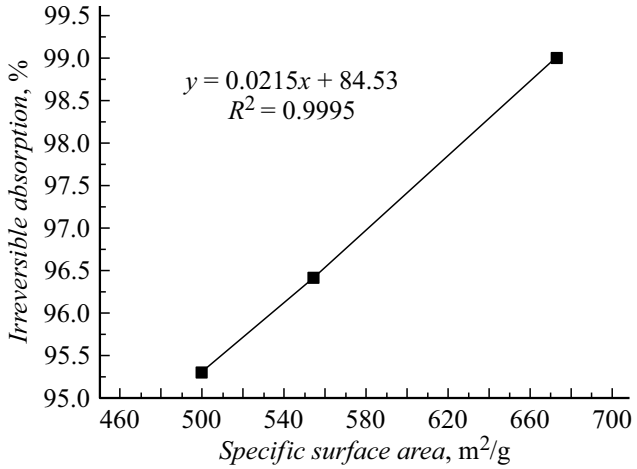

Figure 5. Dependence of absorption capacity Q_S on specific surface of FLG samples.

A comparison of the obtained results shows that FLG_*cel* sample synthesized from cellulose is characterized by the highest absorption value at pH = 2 (more than 99%). FLG_*lg* and FLG_*b* samples also show rather high Q values (not lower than 96%). Low desorption ($< 0.1-0.2\%$) of mycotoxin T-2 at pH = 8 is an important result in terms of the potential practical use of few-layer graphene.

Thus, it can be stated that the test FLG samples show their ability of strong mycotoxin bonding on the surface in rather high amounts — about 1 mg/g. It should be noted that the authors of [25] used graphene oxide/chitosan composite as sorbent and obtained a sorption capacity of max. 3.85 ng/g, much lower than in the experiments with FLG samples. In other publication [26] graphene oxide was used as sorbent and max. 69% mycotoxin was adsorbed. Thus, the synthesized FLG samples shall be considered as promising by mycotoxin T-2 adsorbents. Surface and porosity properties of absorbents are known to play a key role in sorption processes. As has been shown above, FLG_*cel* sample has the highest specific surface and it is logical to assume that this sample has the highest sorption capability against mycotoxin T-2.

Figure 5 shows dependence of absorption capacity Q_S on specific surface of FLG samples.

As shown in Figure 5, the relation between the specific surface and sor[tion capacity Q_S for the test samples is expressed by dependence $y = 0.0215 + 84.53x$, where y is Q_S, x is the total specific area, [m^2/g], and linear correlation coefficient R between these variables is equal to 0.999 (very high positive correlation). It should be noted that relationship between the specific surface of micropores and sorption capacity is also characterized by the correlation coefficient R equal to 0/99.

Conclusion

Carbonization of biopolymers (lignin, cellulose) and plant biomass in the form of bark was carried out in the self-propagating synthesis conditions. It has been identified that carbonization products include 2*D*-graphene nanostructures, which correspond by their morphometric parameters to few-layer graphene with 3–5 layers.

The sorption capacity of the samples was studied in relation to 4,15-diacetoxy-8-(3-methylbyturyloxi)-12, 13-epoxitrichothecene-3-ol (mycotoxin T-2) in conditions simulating gastrointestinal tract media of mammals. Presence of tight correlation relationship between specific surface and sorption capacity of samples in relation of mycotoxin T-2. The obtained data allows to suggesting that it is important to continue on the sorption properties of the synthesized carbon nanomaterials concerning other mycotoxin classes.

Acknowledgments

The authors express their gratitude to professor L.T.Krupskaya (Pacific National University, Irkutsk) for the provided softwood bark samples.

Funding

The research has been carried out within the national subject study of the Ioffe Research Institute (subject № 0040-2014-0013) and Institute of Biology of Komi NTs UrO RAN (subject „Effect of ionizing emission and non-radiation factors on biological objects and biogenic migration of heavy natural radionuclides“ 122040600024-5).

Conflict of interest

The authors declare that they have no conflict of interest.

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