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The fungicidal properties of the carbon materials obtained from chitin and chitosan promoted by copper salts

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ABSTRACT

Renewable raw materials chitin and chitosan (*N*-deacetylated derivative of chitin) were subjected to action of different copper modifiers that were carbonized in the atmosphere of the N_2 inert gas. As a result of the novel manufacturing procedure, a series of carbon materials was obtained with developed surface area and containing copper derivatives of differentiated form, size, and dispersion. The copper modifier and manufacturing procedure (concentration, carbonization temperature) influence the physical–chemical and fungicide properties of the carbons. The received carbons were chemically characterized using several methods like low-temperature adsorption of nitrogen, X-ray diffraction analysis, scanning electron microscopy, cyclic voltammetry, elemental analysis, and bioassay. Besides chemical testing, some biological tests were performed and let to select carbons with the highest fungicidal activity. Such carbons were characteristic of the specific form of copper derivatives occurring in them, i.e., nanocrystallites of Cu^0 and/or Cu_2O of high dispersion on the surface of carbon. The carbons may find an application as effective contact fungistatic agents in cosmetology, medicine, food industry, etc.

1. Introduction

Nowadays, much of the research focuses on synthesizing new carbon materials which could help in reclaiming of the production waste. Chitin, i.e., poly-(1-4)-β-N-acetyl-D-glucosamine, is present in the shells of crustaceans, insects, spiders, and bat guano [1–4].

The original studies involving the use of chitosan as a precursor for the preparation of activated carbons appeared relatively recently and were carried out at the Faculty of Chemistry, Nicolaus Copernicus University in Torun [5–9]. The studies have confirmed that the proposed chemical activation leads to an effective improvement of the structural parameters compared to carbons obtained by carbonization of non-activated chitosan: surface area S_{BET} is in the range of 440–2000 $m^2\ g^{-1}$, high nitrogen content up to 6.5% by weight, steerable pore structure and volume.

Literature reviews deliver a description of some on the biocidal activity of silver, silver derivatives, and silver containing materials [10–12]. High price and therefore a limited accessibility of silver justify the search for other metals which could exhibit similar activity [13,14]. Recently, several research attempts have been made towards the application of metallic clusters containing metals at 0 oxidation degree as well as at intermediate oxidation states like copper(I) derivatives. Moreover, some papers [15,16] have reported that the biocidal activity of such metallic materials depends strongly on their degree of dispersion. For instance, microporous carbon fibers decorated with highly dispersed metal clusters are a good biocidal material [17].

It is known from ancient times that some metals like copper, silver, and gold exhibit bactericidal and fungicidal [13,18–23]. According to these studies, metallic copper may have a broad spectrum of antimicrobial properties if applied in contact mode [24], like the inhibition of growth of some health hazard pathogens: bacteria (*Escherichia coli*, *Staphylococcus*, *Legionella pneumophila*), viruses (influenza type A), and fungi. Copper(I) oxide is a derivative having fungicidal contact properties.

According to the assumptions of the HSAB theory, soft acids and bases will easily react with DNA. Consequently, copper ions have a particular affinity to the DNA and can bind and disrupt its helical structure by cross-linkage within and between the DNA strands [25–27]. Thus, the insertion of copper derivatives (Cu^0- and Cu^{+1}-based) into carbon matrix seems to be crucial regarding the mentioned conditions. The amine groups which are present in chitosan chains are definitely essential for the adsorption of copper ions due to chelating properties. Fig. 1 demonstrates the formation of the chelate chitosan–Cu(II). Chitosan itself can be applied for the capture of heavy metals in the process of water and waste water purification, recovery of precious metals from solutions, and the removal of radioactive ions. The chelation effect has been proven especially in the case of transition metal ions [4,28].

In our studies we are going to employ copper(II) complexation by amino groups to enable their retention in the carbon matrix after the carbonization of chitin and chitosan containing copper compounds. To our knowledge so far, the application of chitin and chitosan has not been investigated and there are no reports published on this topic. The chief aim of our work is to elaborate the method of manufacturing new carbon–copper materials from chitin and

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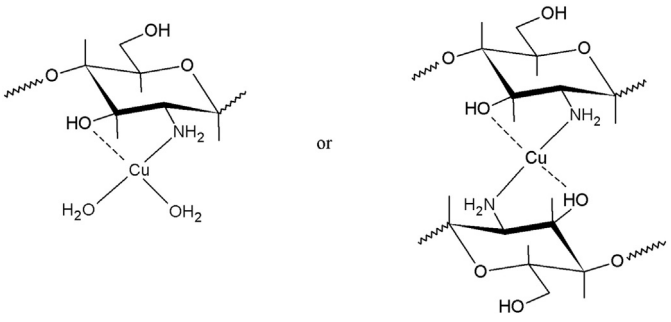

Fig. 1. The formation of the chelate chitosan–Cu(II) [28].

chitosan and to study the fungicidal/fungistatic effect of the carbon materials.

2. Materials and methods

2.1. Materials

Chitosan (CH) was purchased from Sigma Aldrich (chitosan physical form >75% deacetylation, medium molecular weight CAS Number: 9012-76-4). Chitin (CA) was purchased from Sigma Aldrich (chitin from shrimp shells CAS Number: 1398-61-4) as purified natural product in the form of loose flakes. $Cu(NO_3)_2 \cdot 3H_2O$ of analytical purity was applied for preparation of water solution for further modification of the carbons. *Rhizopus nigricans*, a mold typical for food products, was selected for biological tests.

2.2. Method of preparation of carbons

Activated carbons were modified with copper in the following way. Chitin or chitosan was treated with the 0.1 or 0.05 M aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ (ACu0.1 or ACu0.05), then for selected samples a chemical activator $ZnCl_2$ (Zn) was added, according to the method previously developed by the authors [9]. The mixture was heated under the flow of nitrogen at the temperature of 700 °C for 1 h. In the previous studies [5–9] it was found that the carbonization temperature of 700 °C was optimal for the preservation of nitrogen atoms on the carbon surface and development of structural parameter (surface area and total pore volume). Therefore, carbonization temperature was not considered as a parameter influencing the investigated phenomena. Carbons obtained with the activator were additionally washed in a hot ultrasonic bath. The washing was performed using successive portions of distilled water until the neutral pH was reached. Next, the rinsed carbons were dried. Furthermore, the unmodified reference carbons were also prepared after the annealing of raw chitin or chitosan. Further in text these materials are called CA and CH, respectively.

2.3. Surface area and pore structure

The structural parameters of the carbon materials were characterized by low-temperature nitrogen adsorption. The relevant isotherms of all the samples were measured at 77 K on a Gemini VI volumetric adsorption analyzer (Micromeritics, USA). Before each adsorption measurement, the sample was outgassed under vacuum at 200 °C.

2.4. Elemental analysis

The activated carbons were analyzed (Vario MACRO CHN, Elementar Analysensysteme GmbH) for their total carbon and nitrogen contents.

2.5. Morphology

The carbons were examined by scanning electron microscopy (SEM, 1430 VP, LEO Electron Microscopy Ltd.) with an energy dispersive X-ray spectrometer (EDX, Quantax 200; detector: XFlash 4010, Bruker AXS).

2.6. X-ray diffraction

X-ray diffraction (XRD) spectra were measured by means of a CuKα source in the range of 2Θ from 10° to 70° (X-Pert PRO Systems, Philips).

2.7. Electrochemical studies

Cyclic voltammetry (CV) curves were recorded using a computer-controlled Autolab (Eco Chemie) modular electrochemical system equipped with a PGSTAT128N potentiostat, controlled by NOVA software. The measurements were carried out using a three-electrode electrochemical cell presented in one of our earlier papers [29–34]. The working, counter and reference electrodes were, respectively, a powdered carbon electrode (PACE), a Pt wire and a Ag/AgCl (3 mol L^{-1} KCl) electrode. After vacuum desorption (10^{-2} Pa), the carbon sample (mass = 50 mg) was placed in an electrode container and drenched with a deaerated solution to obtain a 2–3 mm sedimentation layer. The potentiometric responses of the carbon electrodes were measured in oxygen-free electrolyte solutions (1 M KCl; usually after 24 h). The relevant sweep rates and amplitudes are given each time in the figure captions (20 to 200 mV s^{-1}). All measurements were carried out in the thermostated system at room temperature (293 K).

2.8. Microbiological test

The fungicidal properties of synthesized carbon materials were tested. First, thermal (2 h, 160 °C) and chemical (ethanol 70%, Meliseptol) sterilization of laboratory equipment was performed. Microfungi (molds) were grown on a solid carbohydrate microbiological substrate (pepton K/agar–agar/glucose) during incubation at 20–22 °C. Some grains of unmodified carbon materials (CA, CH) and carbon–copper materials under testing were placed in the middle of the microbiological substrate to ensure contact with growing molds. Inoculation was performed by spraying spores of mold over the test sample.

2.9. Porosity characterization and surface area

Table 1 shows the results gathered by means of low-temperature nitrogen adsorption of carbons obtained by carbonization of raw precursors (chitin and chitosan), modified with copper and activated by zinc chloride. Specific surface area was determined by the Brunauer–Emmett–Teller (BET) method (S_{BET}). Carbons obtained from the unmodified chitosan (CH) are non-porous, as evidenced by the low value of S_{BET} equal to 3 $m^2\ g^{-1}$. It is different in the case of chitin, where the

Table 1
Specific surface area (S_{BET}) and elemental content for carbon materials obtained from chitin (CA) and chitosan (CH).

Sample	S_{BET} ($m^2\ g^{-1}$)	Elemental content (wt.%)		
		N	C	H
CA	360	5.7	87.9	1.3
CA–ACu0.05	455	5.1	74.3	1.5
CA–ACu0.1	303	5.0	64.6	1.4
CA–ACu0.05–Zn1.00	1184	5.6	66.2	1.4
CA–ACu0.1–Zn1.00	1287	5.6	70.2	1.3
CH	3	6.9	83.1	1.1
CH–ACu0.05	123	7.8	70.7	1.5
CH–ACu0.1	102	7.9	66.7	1.3
CH–ACu0.05–Zn1.00	905	5.8	67.4	1.4
CH–ACu0.1–Zn1.00	1159	5.0	67.8	1.2