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V. ENVIRONMENTAL BIOTECHNOLOGY

CARBON MATERIALS FROM WASTE BIOMASS AS ANTI-BACTERIAL AIR FILTERS

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Abstract. Carbon materials from almond shells were developed for the adsorption of microorganisms from air. Samples were processed by means of a one-step process – high-temperature hydro-pyrolysis. Studies were carried out in a wide temperature range of 600-900 °C. As a result, carbonates with predominant micro- and mesopores were obtained. The samples were impregnated with zinc, silver, iron and copper 5% by weight. The obtained samples were characterized by XDR, BET, scanning electron spectroscopy and elemental analysis. The final products are distinguished by a moderate surface and the presence of nanosized metal particles. The antibacterial properties of the activated carbon composites were examined using standard methodology under dynamic contact conditions and *Escherichia coli* K12 as test microorganism. All tested composite materials exhibit strong antibacterial properties after 48 h of contact with microbial cells. Thus the application of these materials in filtering system will be possible solution for successful reduction of microbial cell number. It is assumed that a similar effect can be achieved in an air environment.

Keywords: activated carbon composites, antibacterial activity, waste almond shells

INTRODUCTION

Agents threatening human health are constantly emerging nowadays, such as air pollution, terrorist attacks with chemical and biological agents COVID-19 pandemic. There is a need to develop such facilities and materials to cope with this wide range of hazards in the air of public buildings, hospitals and even our homes. Activated carbon composites (ACC), containing metal nanoparticles are promising developments in this direction.

An important problem is the resistance of various microorganisms towards different antimicrobial agents. Antimicrobial-resistant pathogens are serious threat to human and animal health. Resistance of microorganisms towards antibiotics is constantly increasing, whereas many antimicrobial agents have demonstrated resistance against various types of microorganisms [6]. Certain metals could form a hostile environment for bacteria and fungi – these effects had been observed since ancient times and they had been applied successfully in surgery, water purification and everyday life. For example, copper and silver pots had been used in ancient Persia, Rome and Egypt. In this case the antibacterial impact is performed via oligodynamic effect of metals such as copper, iron, lead, mercury, zinc, aluminum, gold and especially silver. This leads to release of positively charged metal ions that interact with bacteria. The reason for antimicrobial activity of metals is the ability of their cations to inhibit enzymes, to cause damage to cell membranes, to prevent the absorption of vital trace elements by microbes. In addition, some metals may exhibit direct cytotoxic activity.

In the last decades there is a growing interest in metal nanoparticles and metal oxides as compounds with antibacterial potential. It has been found that metal nanoparticles (Ag, Cu, Fe, Zn) exhibit a wide range of antimicrobial activity against various types of microorganisms, including fungi and Gram-positive and Gram-negative bacteria [2, 3]. Copper has antibacterial properties similar to those of other precious metals, including silver and gold. In addition, copper nanoparticles have antimicrobial activity against a number of fungal and bacterial species [7]. It is well known that zinc oxide nanoparticles have antibacterial properties and inhibit the growth of microorganisms by penetrating the cell membrane, damaging lipids, carbohydrates, proteins and DNA [4]. Obviously, the lipid peroxidation is the most important process that leads to a change in the cell membrane, ultimately disrupting vital cellular functions [8]. Additionally, oxidative stress is associated with the incorporation of zinc oxide nanoparticles into *Escherichia coli* [10], whereas zinc oxide is amphoteric and reacts with both acids and bases to give Zn^{2+} cations.

The aim of the present study is to obtain innovative carbon materials with incorporated metals by new methods, and to investigate the antibacterial activity of the obtained composites.

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MATERIALS AND METHODS

Nanoporous carbon materials are synthesized by hydro-pyrolysis of almond shell at 750 °C. The obtained carbonizate was ground and separated into fractions, washed with distilled water and dried for 24 hours. Water-alcohol solutions of metal salts (nitrates of copper, silver, zinc and iron) are added to the samples. The resulting carbon matrix containing metal nanoparticles is subjected to high-temperature treatment at 600 ˚C in an inert atmosphere of N_2 for 120 min. with a heating rate of 5 ˚C / min.

Elemental analysis was performed using a Vario Macro Cube (Elementar Analyzensysteme GmbH) analyzer to determine the content of C, H, N, S. The oxygen content was determined differently.

The morphology study was performed using a FEI Quanta 250 FEG scanning electron microscope, in vacuum, at 10.0–15.0 kV.

X-ray diffraction analysis was performed using a Bruker D8 Advance diffractometer with CuKa radiation. The average crystallite size was calculated by Scherer equation.

The texture of the obtained carbon material was analyzed using low-temperature N_2 sorption, carried out in an automatic volumetric apparatus Quantachrome Autosorb iQ-C-XR/MP. Prior to nitrogen adsorption, the samples were subjected to outgassing in a vacuum at 300° C for 8 h.

The antimicrobial activity of the ACC materials was evaluated by using *Escherichia coli* K-12 (ATCC 25922) as test microorganism and ASTM Standard Test Method E 2149–10. Each ACC sample and the bacterial suspension are mixed in 1:10 (w/v) ratio. An aliquot from the mixture was taken at 1h, 24h and 48h and the remaining bacterial concentration was evaluated by performing serial dilutions and standard plate counting techniques. The number of colonies in the Petri dish after incubation (CFU/ml) was measured [5].

RESULTS AND DISCUSSION

XRD data (Fig. 1) show high degree of graphitization and presence of metal-oxide nanoparticles. The carbon materials obtained after hydro-pyrolysis is distinguished by moderately high BET surface area of 719 m^2/g. Micropores and macropores prevail. It contains 73.42 mass. % C, 0.52 mass. % H, 0.24 mass. % N, 2.78 mass. % S.

After metal deposition the surface area decrease, due to incorporation of metal nanoparticles in the pores: ACZn has surface area of 640 m^2/g, ACFe - 612 m^2/g, ACCu - 675 m^2/g.

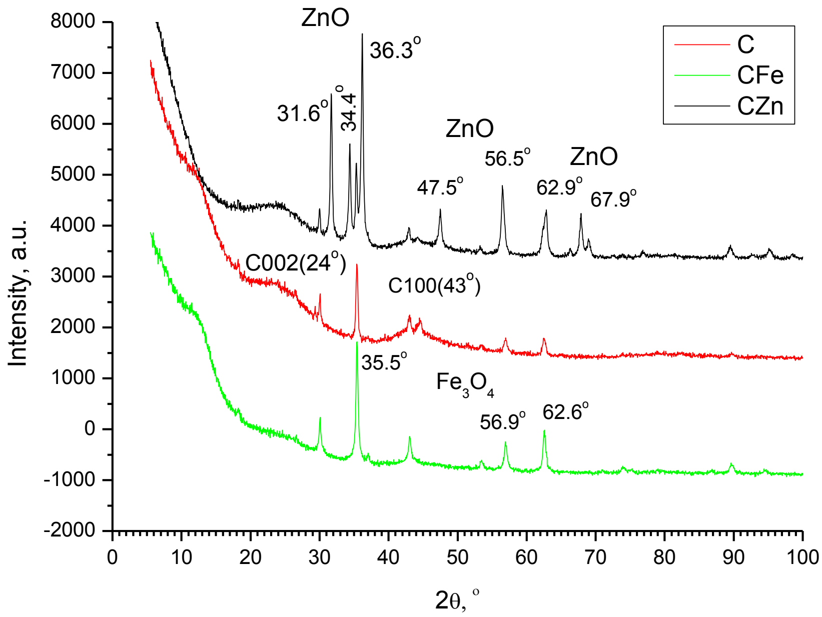

Fig. 1. XRD spectra of metal-loaded carbon materials.

SEM images (Fig. 2) show the comparatively uniform distribution of metal nanoparticles in the carbon matrix. Silver particles can be clearly seen as white spheres with different size and distribution (Fig. 2a). Zinc nanoparticles (ZnO, according to XRD) and copper nanoparticles (CuO and Cu_2O, according to XRD) are not well distributed. Numerous iron nanoparticles (Fe_3O_4, according to XRD) with irregular shapes are very well uniformly dispersed (Fig. 2d). Elemental analysis indicates a moderate degree of carbonation relative to the carbon to oxygen ratio (Table 1).

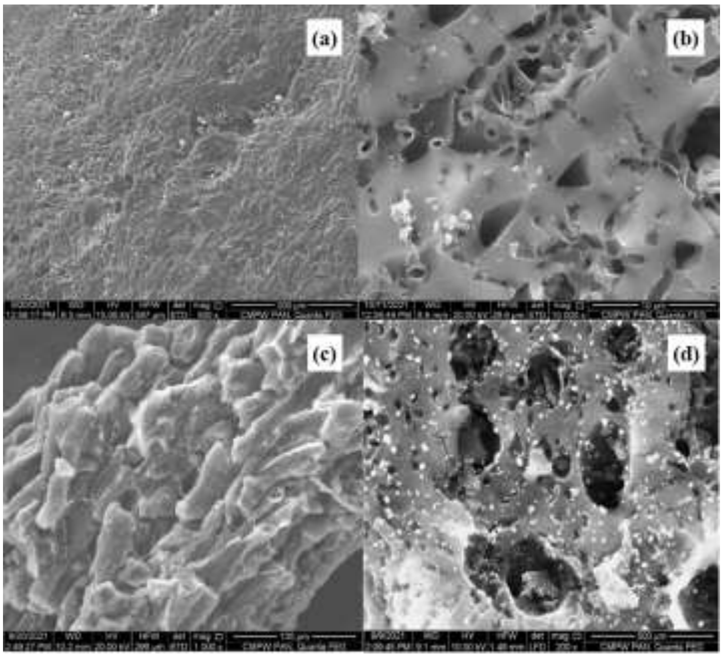

Fig. 2. SEM image of carbon composite containing silver Ag (a), zinc Zn (b), copper Cu (c), iron Fe (d).

Table 1. Elemental analysis of almond shell carbon

Wt %	C	H	N	S	O
Alsh	78.11	3.47	0.83	1.12	16.47

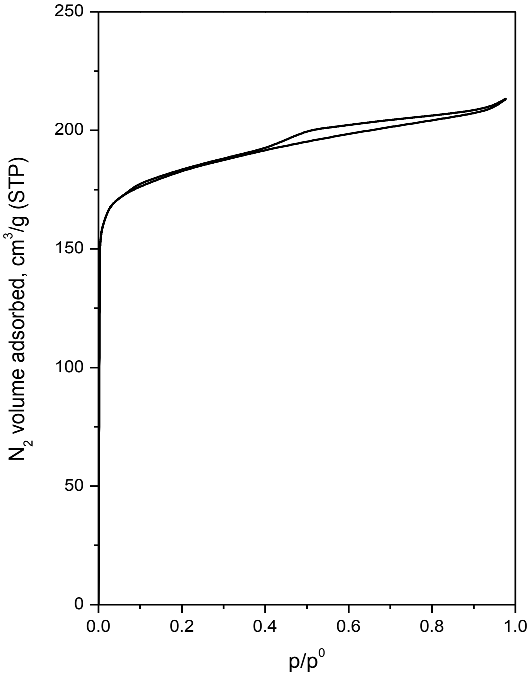

Fig. 3 Nitrogen adsorption isotherm (-196°C) of activated carbon from almond shell

The carbon materials obtained after hydro-pyrolysis is distinguished by moderately high BET surface area of 719 m²/g (Fig. 3). Micropores and macropores prevail. BET surface are decreases after metal loading, most probably due to incorporation of metal particles into mesopores. ACZn has BET surface area of 640 m^2/g, ACFe - 612 m^2/g, ACCu - 675 m^2/g, ACAg – 601 m^2/g. Macropores contribute to almost complete bacterial uptake, whereas metal and metal oxide nanoparticles act like antibacterial agents.

The antibacterial effect of ACC materials was evaluated in two aspects: effect of metal nanoparticles on bacterial growth and whether it depends on ACC particle size. The antibacterial effect was evaluated against Gram negative *Escherichia coli* K12 bacterium as the bacterial suspension with cell density of about $1.5 \div 3.0 \mathrm{x} 10^{6}$ CFU were placed in contact with the respective ACC sample. The number of viable cells was determined as a sample from each of the incubated variants was subjected to serial dilutions and plating on Petri dishes. In parallel with the samples, the *Escherichia coli* K12 suspension was cultivated as positive control (Fig.4). To demonstrate the effect (mainly cell absorption) of activated carbon on bacterial cells, an equivalent amount of activated carbon sample without metal nanoparticles was incubated with the same bacterial suspension. The ACC supplemented with copper nanoparticles exhibit the strongest antibacterial effect after 24 hours of cultivation reducing the microbial cell number at about 99.99%, while the ACC supplemented with silver and zinc nanoparticles exhibit similar effect after 48 h of incubation. A similar effect of expressing better antibacterial properties of copper nanoparticles compared to zinc nanoparticles is reported by other autors [1]. The generally reported [9] stronger antibacterial effect of silver nanoparticles in our studies is significantly weaker and comparable to the antimicrobial properties of zinc. The cultivation of *E. coli* K12 with activated carbon without any metal nanoparticles also demonstrates some reduction in the CFU, as entry of bacterial cells in the activated carbon pores seems to be the possible explanation for the effect.

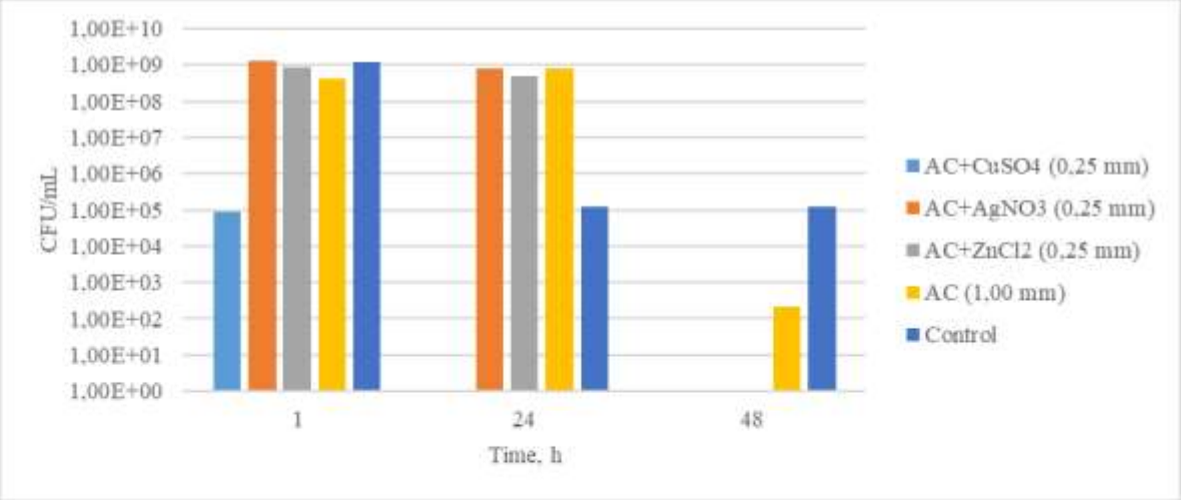

Fig. 4. Antibacterial effect – influence of the nature of the metal additive.

It might be expected that the reduction in the activated carbon particle size will result in larger active surface and therefore the antibacterial effect will be affected. The results from our investigation using AC-Zn particles with three different sizes of 0.25 mm, 0.40 mm and 1.00 mm showed that the application of AC-Zn in these concentrations does not affect the antibacterial effect (Fig. 5).

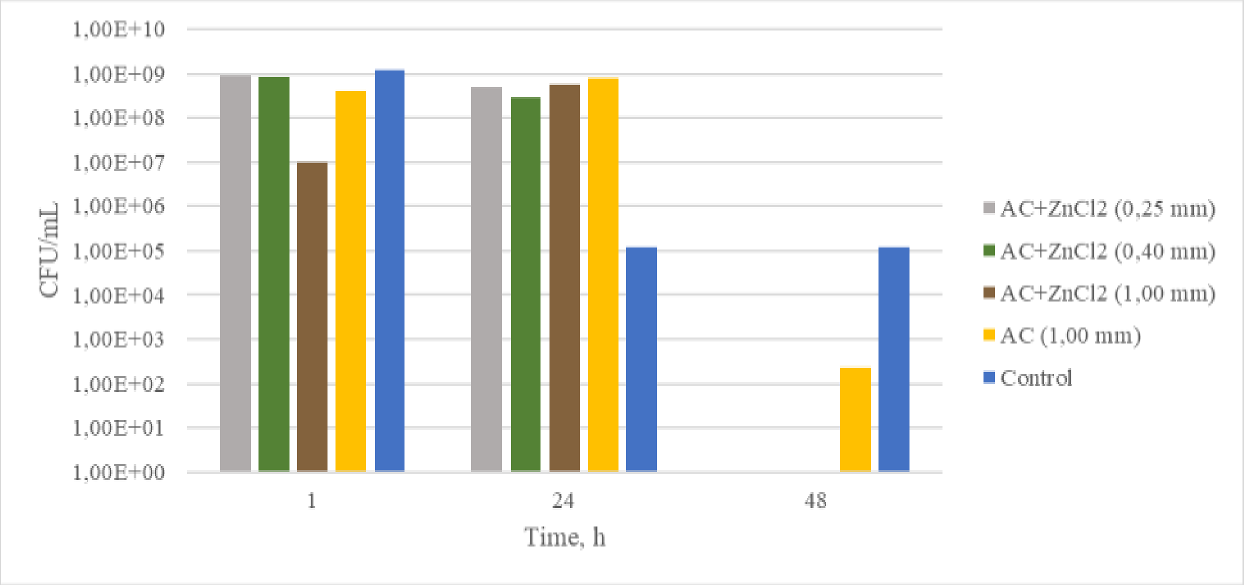

Fig. 5. Antibacterial effect – influence of the size of the particles in the composite.

CONCLUSIONS

Preparation of metal nanoparticles incorporated in activated carbon composites provides a novel material with antibacterial activity for further development and optional application in hygiene devices and individual masks.

A study was conducted to study the influence of the synthesis conditions (temperature, ratio of raw materials, time, etc.) on the structure of the obtained material. The obtained carbon materials are characterized by a structure in which micro- and macropores predominate, which allows efficient absorption of bacteria and viruses from water and air. With the help of added various metals, complete inhibition of the growth of Gram-negative bacteria is achieved after 48 h in dynamic contact conditions irrespective of the type of ACC.

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