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Copper-based nanostructured lignocellulose materials with antibacterial activity

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In this study we report for successfully preparation of copper-based lignocellulose nanocomposites. The wood fibers (WF) and technical hydrolysis lignin (THL) were used as lignocellulosic materials. To produce copper sulfide lignocellulose nanocomposites a two-component cupri reduction system at saturated steam were used. The copper content in the synthesized samples was determined by Inductive coupled plasma (ICP) analysis, thermal and acid decomposition methods. The linkage of copper ions with lignocelluloses was studied by Infrared (IR) spectroscopy. The IR bands observed at ~3400 cm^{-1} suggest that the main mechanism of interaction between lignocellulose materials and copper ions was realized by oxygen atoms of OH groups in cellulose and in the aromatic nucleus of the lignin macromolecule. SEM-EDS analyses showed the presence of copper particles on the surface of investigated samples. According TEM observations it was found the tendency to formation of clusters from CuNps in different shape. The size of the formed nanoparticles varies between 10-50 nm. The antibacterial activity of modified lignocelluloses was tested against Gram-positive (*Bacillus subtilis* 3562) and Gram-negative (*Escherichia coli* K12 407) bacteria. The results showed that copper-based THL has better antibacterial activity compared to WF material.

Key words: *lignocellulose nanomaterials, modification, copper sulfide, antibacterial activity*

INTRODUCTION

Glucose In recent years, owing to the increased environmental awareness, the usage of lignocellulosic materials as a potential replacement for synthetic fibers such as carbon, aramid and glass fibers in composite materials have gained interest among researchers throughout the world. Lignocelluloses are abundant in nature, renewable raw material and a low cost material. Owing to their low specific gravity, lignocellulose fibers are able to provide a high strength to weight ratio in plastic materials. The usage of lignocellulose materials also provides a healthier working condition than the synthetic fibers [1, 2]. The chemical compositions of lignocellulose materials vary according to the species, growing conditions, method of fiber preparations and many other factors. Lignocelluloses have a large number of reactive groups, a wide range of molecular weight, varying chemical composition, which contribute to their diversity in structure and property [3].

Copper sulfide ($Cu_{2-x}S$) has various phases [4,5]. All of these phases are p-semiconductor due to presence of copper vacancies in their lattice. Copper sulfides are used for solar cells, field emission device, nano switch, photo catalyst, biosensors, p-semiconductor, gas sensors and so on [4,5].

Antimicrobial effects of transition metal nanoparticles have attracted considerable interest from both medical and technological standpoints, because this kind of property plays a crucial role in applications such as protection of medical instruments and devices, water treatment and food processing [6]. Nano-form copper particles (CuNPs) have enhanced antimicrobial activity toward a broad spectrum of microorganisms, including pathogenic bacteria. Apart from their small size, the high surface to volume ratio and derived close interaction with microbial membranes, the source of cytotoxicity were also identified from leached copper–peptide coordination. This complex formation induces a multiple-fold increase in the generation of intracellular reactive oxygen species, reduces cell viability and inhibits overall biomass growth [6].

The purpose of this study is to clarify the modification of lignocelluloses materials (WF and THL) with cupri reduction system at saturated steam. We also examined the structure and antibacterial behavior of obtained copper-based lignocellulose materials.

EXPERIMENTAL

In this study the WF (produced in Lesoplast Inc., Troyan, Bulgaria) and THL (obtained by acid hydrolysis of wood in Pirinhart PLC, Razlog,

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Bulgaria) were used as lignocellulosic materials

The copper-based lignocellulose materials were prepared as follows. $CuSO_4.5H_2O$ was used as the metal precursor to produce CuNps. On the other hand $Na_2S_2O_3.5H_2O$ was used as reductor. The modification process was at hydromodule 1:6, and the ratio between the components ($CuSO_4.5H_2O$:$Na_2S_2O_3.5H_2O$) was 1:2 in quantity 45% in term of lignocellulose materials. To produce copper based lignocellulose nanocomposites a method at saturated steam were used [7,18]. All analytical grade reagents were used as without further purification for synthesis of Cu_2S nanoparticles and modification of lignocellulose materials.

The copper content in the samples was determined by ICP (Prodigy High Dispersion ICP Spectrometer – Teledyne Leeman Labs) analysis used thermal and acid decomposition methods. The linkage of copper ions with lignocelluloses was studied by Infrared (IR) spectroscopy (MATSON 7000 Fourier Transforming Infra-Red spectrometer). The shape and size of formed copper nanoparticles was observed by TEM (JEOL 2100). The surface morphology was verified by SE M (JEOL JSM 6390). Energy-dispersive X-ray spectroscopy (EDS) was used as quantitative analysis of elemental composition of samples.

For investigation of antibacterial behavior of lignocelluloses materials bacterial strains used in this study were obtained from the culture collection of Bulgarian National Bank of Industrial Microorganisms and Cell Cultures and included Gram-positive bacteria - *Bacillus subtilis* 3562 and Gram-negative - *Escherichia coli* K12 407.

The cultures growth, bacterial growth-inhibiting effect and the optical density of the culture was described previously [9]. The measurement of antibacterial activity of hybrid materials was calculated according to N. Rangelova *et al.* [9], between the control sample and the test sample.

RESULTS & DISCUSSION

In our previous investigations [7,10] we described the scheme of Cu^{2+} reduction process and Cu_2S formation. The results for copper content are shown on Fig. 1. As can be seen the copper content is higher in the modified THL than the wood fibers. Probably lignin adsorbed in a greater extent the metal ions in comparison with the fibers. As it is known, lignin is very often used as an adsorbent because of its large internal surface area. In this case the metal ions may be adsorbed by both physical sorption or through coordination bonding.

Fig. 2 presents the infrared spectra of lignocellulose and copper-based lignocellulose materials. In these spectra four characteristic absorption regions can be distinguish: from 3700 to 3000 cm^{-1}, 3000 to 1800 cm^{-1}; 1800 to 700 cm^{-1}; and below 700 cm^{-1}. The bands in the first area (3700-3000 cm^{-1}) corresponding to stretching vibration of free (unsubstituted) and hydrogen bonding OH groups in polymer backbone and water [2, 11]. The second area consist intense band at ~ 2940 cm^{-1} and may be attributed to the asymmetric and symmetric stretching vibrations of C-H linkage due to CH, CH_2 or CH_3, and the methyl groups (O-CH_3) in the lignocellulose materials. The area from 1800 to 700 cm^{-1} is unique to each of carbohydrate polymers – “fingerprint region”. These include vibrations of the skeletal (C-O-C and C-C) glucosidic bonds, as well as deformation and stretching vibrations of the C-OH linkages in the pyran ring. Here also include the C–H, C–O, C–O–C, C–OH asymmetric, symmetric and deformations stretching vibrations of aromatic skeletal or glycosides linkages and primary alcohol groups [11-13]. The bands at 1600 and 1637 cm^{-1} are

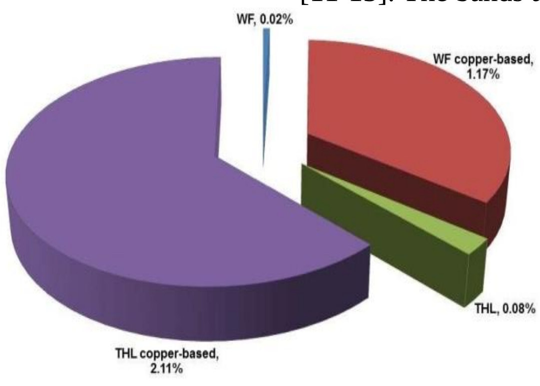

Fig. 1. Copper content in lignocellulose and copper-based lignocellulose materials.

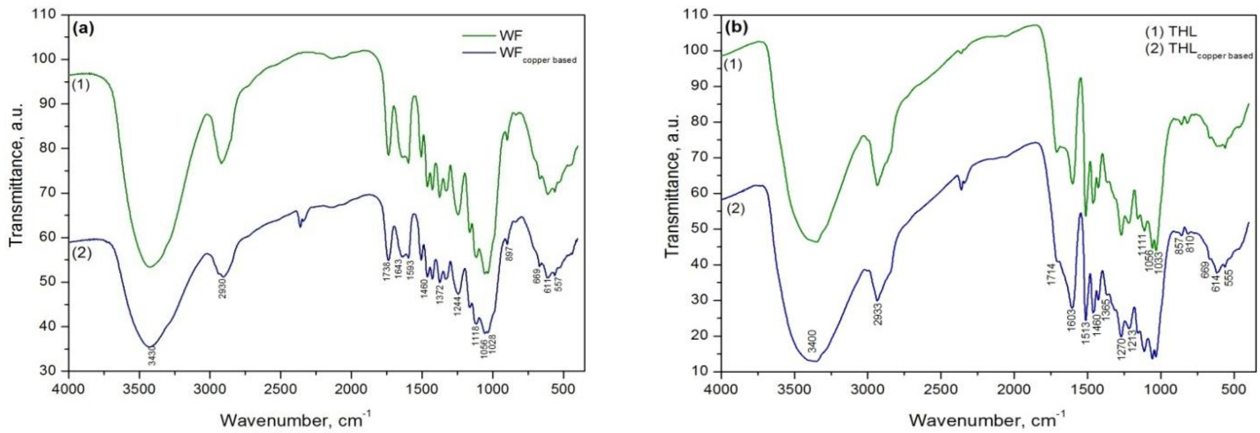

Fig. 2. IR spectra of lignocellulose(a) and copper-based (b) lignocellulose materials.

assigned to the absorbed water and β-glucosidic linkages between the sugar units, respectively. In last area (700-400 cm^{-1}) can be found the characteristic absorption bands of C–OH out-of-plane bending mode in cellulose, skeletal deformation of aromatic rings, substituted groups and side chains in lignin, some heavy atoms stretch in cellulose macromolecules and others [12-14].

The FTIR spectra of lignocellulose and copper-based lignocellulose materials are similar. The main differences in the IR spectra (Fig. 2 a, b) were observed in the range 3500-3200 cm^{-1}. A decrease in the intensity of the O–H absorption band at 3430 cm^{-1} was observed. This fact can be connecting with reduced of content of hydroxyl group in lignocelluloses after reaction. Many authors reported that the bands above 3400 cm^{-1} can be attributed to the vibration of coordination bonding between the copper ions by oxygen atoms from the OH groups of lignocellulose materials [15,16]. On the base of above mentioned results and literature data can be suppose that the probable mechanisms of interaction between copper ions and lignocellulose materials by oxygen atoms of OH groups in cellulose and in the aromatic nucleus of the lignin macromolecule. In details we described previously these results in several papers [7,10].

The SEM images of raw materials and modified lignocelluloses are shown on Fig. 3. On the surface of wood fibers (Fig. 3, a) can be seen the presence of pore with diameter 50 nm. After the modification (Fig. 3, b) on the WF surface are observed bright particles, due to existence of copper nano particles. Moreover as can be seen that the pores disappear on the surface which can be explain with penetration of copper particles in the presented hollows. The micrograph images of THL (Fig. 3, c and d) have shown the heterogeneous, compact structure with rough and disordered surface.

After modification the existence of copper ions on the samples surface is confirm by EDS analysis for both type of copper based lignocelluloses materials. The results of EDS analysis which are

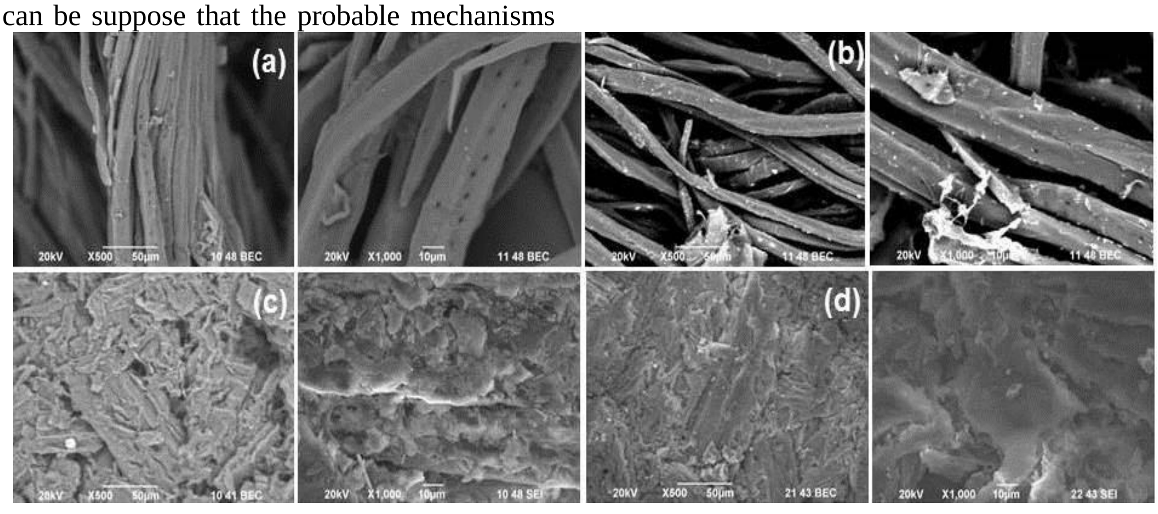

Fig. 3. SEM images of WF (a), WF copper based (b), THL (c) and THL copper based (d).

connected with the present elements in the samples are summarize in Table 1.

TEM observations of WF sample containing copper are shown in Fig. 4. As can be seen the fibers are coated a cluster-forming CuNPs with dimension in the range between 30-50 nm (Fig. 4a) and their shape vary from spherical to elliptical. At higher magnification (Fig. 4b) of the image the present tendencies towards aggregation of copper particles preserve. On the Fig. 4b can be found separated cupper nanoparticles less than 30 nm (from 10 to 30 nm) distributed in the main matrix.

The antibacterial properties of obtained modified lignocelluloses were tested against *B. subtilis* and *E. coli* K12. Bacterial growth of *B. subtilis* was totally suppressed by both materials - WF copper-based materials and THL copper-based materials – 97.42% and 100% respectively (Table 2). This was confirmed by plating a diluted aliquot from each sample onto a LB agar plate and observing bacterial colony after incubation of 24 hours (Fig. 5).

Table 1. EDS elemental analysis of samples

Lignocellulose material	Elemental Composition, wt.%			
	Carbon	Oxygen	Copper	Sulphur
WF	51.14±0.80	48.84±0.80	---	---
$WF_{copper\ based}$	50.09±1.07	46.11±1.07	3.5±0.63	0.3±0.11
THL	59.15±0.76	40.72±0.76	----	----
$THL_{copper\ based}$	58.9±1.47	33.84±1.43	6.12±0.97	1.14±0.18

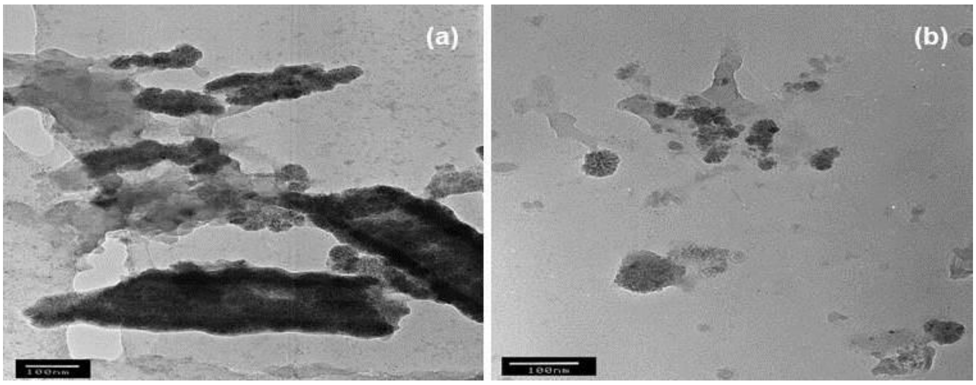

Fig. 4. TEM images of copper-based wood fibers.

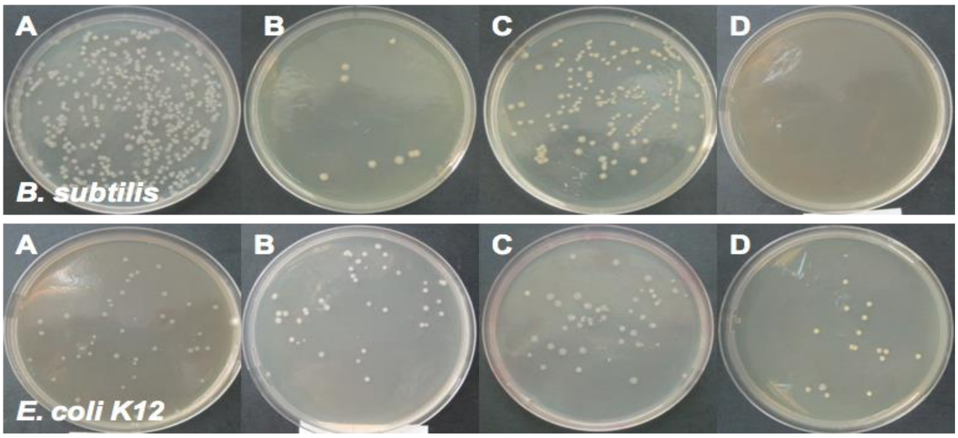

Fig. 5. Test results of *B. subtilis* (upper panel) and *E. coli* K12 (lower panel) for: WF (A), WF copper-based (B), THL (C) and THL copper-based (D).

Table 2. Antibacterial test results of *B. subtilis* and *E. coli* K12 after 24 h cultivation.

Materials	*B. subtilis*		*E. coli K12*	
	CFU/ml	Reduction of bacteria (%)	CFU/ml	Reduction of bacteria (%)
WF	11.6 x10^8	-	9 x10^{10}	-
$WF_{copper\text{-}based}$	0.3 x10^8	97.42%	3.9 x10^{10}	56.7%
THL	27 x10^8	-	4.2 x10^{10}	-
$THL_{copper\text{-}based}$	n.d.	100 %	1 x10^{10}	76.2 %

These materials caused approximately 56.7% and 76.2 % reduction of *E. coli* K12 growth, indicated that *B. subtilis* is more sensible to CuNPs than *E. coli* K12. Our results demonstrate that THL copper-based materials have more promising effect towards investigated bacteria, compared to WF copper-based materials. The mechanism of biocidal action of copper nanoparticles may be explained with the fact that CuNPs release Cu (II) ions on contact with moisture. These copper ions bind with the COOH and SH groups of protein molecules of bacterial cell wall [17,18].

CONCLUSIONS

Copper-based nanostructured lignocellulose materials (wood fibers and technical hydrolysis lignin) with antibacterial properties were prepared. The quantity of copper is higher in the modified THL than the wood fibers. It was suggested that the most probable mechanism of interaction between lignocelluloses and copper ions is the coordinative binding with the oxygen atoms of OH groups in cellulose and in the aromatic nucleus of the lignin macromolecules. SEM-EDS analyses confirm the presence of copper particles on the samples surface. TEM observation showed cluster-forming CuNPs. The size of the formed nanoparticles varies between 10-50 nm. Antibacterial properties of the copper based lignocelluloses against *B. subtilis* and *E.coli* K12 were demonstrated. The results revealed that the *B. subtilis* strain was more sensitive because it showed larger killing in comparison to *E.coli* K12.

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МЕДНИ НАНОСТРУКТУРИРАНИ ЛИГНОЦЕЛУЛОЗНИ МАТЕРИАЛИ С АНТИБАКТЕРИАЛНО ДЕЙСТВИЕ

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(Резюме)

В представената изследователска работа са показани резултати за получаване на медни лигноцелулозни нанокомпозити. Дървесните влакна (ДВ) и технически хидролизен лигнин (ТХЛ) са използвани като лигноцелулозни материали. За получаването на медно-сулфидни лигноцелулозни нанокомпозити са използвани двукомпонентна медно-редукционна система и лигноцелулозни материали. Модифицирането е проведено по метода на наситена пара. Съдържанието на мед в пробите е определено чрез термичен и киселинен метод на разлагане с последващ ICP анализ (индуктивно свързана плазма), Връзката на медните йони с лигноцелулозния материал бе изследвана чрез инфрачервена (ИЧ) спектроскопия. ИЧ ивиците, наблюдавани при 3400 cm^{-1} показват, че основният механизъм на взаимодействие се осъществява чрез координационно свързване на медните йони с кислородни атоми на ОН групи в целулозата и в ароматно ядро на лигниновата макромолекула. СЕМ-EDS анализите показват наличието на медни частици върху повърхността на изследваните проби. От ТЕМ изображенията бе установена склонността към образуване на клъстери от CuNps с различна форма. Размерът на образувалите се наночастици варира в интервала между 10-50 nm. Антибактериалната активност на модифицираните лигноцелулози бе тествана срещу Грам-положителни (*Bacillus subtilis* 3562) и Грам-отрицателни (*Escherichia coli* К12 407) бактерии. При сравняване на двата материала резултатите показват, че модифицираният ТХЛ, на медна основа, има по-добра антибактериална активност спрямо дървесно-влакнестия материал.