



*J. Serb. Chem. Soc.* 77 (12) 1759–1773 (2012)  
JSCS–4387

## Antimicrobial oxidized hemp fibers with incorporated silver particles

JOVANA MILANOVIĆ\*, TATJANA MIHAILOVIĆ, KATARINA POPOVIĆ  
and MIRJANA KOSTIĆ#

*Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4,  
11120 Belgrade, Serbia*

(Received 18 October, revised 10 December 2012)

**Abstract:** In this study, antimicrobial silver-loaded hemp fibers were prepared by selective TEMPO-mediated oxidation, *i.e.*, oxidation with sodium hypochlorite, catalytic amount of sodium bromide and the 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO), followed by silver sorption from aqueous silver nitrate solution. The most suitable experimental conditions for the selective TEMPO-mediated oxidation were determined by changing the oxidation conditions, *i.e.*, the concentration of sodium hypochlorite and the duration of oxidation. The obtained results showed that the maximum sorption capacity of 0.703 mmol of silver per gram of modified hemp fibers was obtained for the sample oxidized with 9.67 mmol NaClO per gram of fibers for 4 h. SEM Microphotographs of the modified hemp fibers with incorporated silver showed uniformly distributed silver particles on the surface of fibers, with isometric shapes and sizes from 10 to 100 nm, despite the fact that silver was sorbed from an ionic solution. The antibacterial activity of the TEMPO-oxidized hemp fibers with silver particles was confirmed *in vitro* against two strains: *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), and the antifungal activity against *Candida albicans* (ATCC 24433). The best antimicrobial activity of silver-loaded TEMPO-oxidized hemp fibers was showed against strain *S. aureus*.

**Keywords:** hemp fibers; TEMPO-mediated oxidation; silver sorption; antimicrobial textile.

### INTRODUCTION

Microorganisms are part of everyday life. They join humans in different forms. Especially the skin is home for many of these organisms. On the other hand, textiles are the interface between the skin and the environment and present

\* Corresponding author. E-mail: jovana@tmf.bg.ac.rs

# Serbian chemical Society member.

doi: 10.2298/JSC121018143M

the tissue with the longest contact with human skin. Therefore, textiles play a critical role, especially under skin conditions with an increased rate of pathogens infections. Production of materials with antimicrobial properties is constantly increasing. In order to protect customers, fibers with antimicrobial properties should disable the growth or directly destroy many pathogens that cause numerous diseases and infections in hospital environments as well as in daily life.<sup>1,2</sup>

The purpose of this research was to obtain antimicrobial hemp fibers with incorporated silver particles and evaluate their antimicrobial activity against different pathogens. The antimicrobial properties were accomplished by incorporation of silver into modified hemp fibers by chemisorption from aqueous silver nitrate solution. Silver was chosen as the antimicrobial agent because of the unique properties of silver, such as strong inhibitory, odor control, broad and long-term activity, and bacteria are unable to develop resistance to silver, as is the case with antibiotics. These properties, together with high thermal stability and good fabric compatibility, have been established silver as a topical agent in several medical areas of today.<sup>3,4</sup>

Furthermore, hemp fibers possess a range of specific properties, quick absorption of humidity accompanied with quick drying, good thermal and electrical properties, high tenacity, biodegradability and protection against UV radiation, which make them different from other textile fibers.<sup>5</sup> In order to prepare technical (multicellular) hemp fibers for textile applications, *i.e.*, to remove non-cellulosic substances (lignin, pectin, hemicelluloses)<sup>6</sup> and improve the sorption properties of hemp fibers,<sup>7</sup> selective TEMPO-mediated oxidation, *i.e.* oxidation with sodium hypochlorite, a catalytic amount of sodium bromide and the 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO), was applied. TEMPO-mediated oxidation causes the conversion of the C6 primary hydroxyl groups of hemp fibers into carboxyl groups *via* C6 carbonyl groups. The introduced carboxyl groups were used as reactive positions in the further reaction, *i.e.*, for the incorporation of silver ions, because the hydrogen atom present in carboxyl groups can easily be replaced with silver ions. The advantages of this TEMPO-mediated oxidation of polysaccharides are the following: highly regioselective oxidation of the primary hydroxyl groups in polysaccharides to carboxyl groups can be achieved under mild aqueous conditions at around room temperature at pH 10–11.<sup>8,9</sup>

The content of functional groups, moisture sorption and water retention were used to assess the changes in hemp fibers due to the oxidation. The influence of the oxidation conditions on the amount of sorbed silver, and thus on the degree of antimicrobial activity, was also determined. Changes in surface morphology and elemental composition of the TEMPO-oxidized silver-loaded hemp fibers were systematically investigated using Scanning Electron Microscopy–Energy Dispersive X-Ray analysis (SEM–EDX). The antimicrobial activities of silver-loaded hemp fibers against different pathogens: *Staphylococcus aureus* (ATCC 25923),

*Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC 24433) were evaluated *in vitro*.

## EXPERIMENTAL

### *Materials*

Domestic water-retted long hemp fibers from Bački Brestovac (Serbia) were used in this investigation. The chemical composition of these fibers was  $\alpha$ -cellulose, 76.10 %; lignin, 8.50 %; hemicelluloses, 11.90 %; pectin, 1.55 %; fats and waxes, 1.60 % and water-solubles, 0.35 %. All chemicals were obtained from commercial sources and were of *p.a.* grade.

### *Preparation of TEMPO-oxidized hemp fibers*

Hemp fibers (10 g) were suspended in water (750 mL) containing TEMPO (0.025 g) and sodium bromide (0.25 g). A 13 % NaClO solution (0, 0.30, 2.42, 4.84 and 9.67 mmol per gram of fibers) was added to the cellulose slurry under continuous stirring.<sup>10</sup> The pH of the slurry at room temperature was maintained at 10.5 by addition of 0.5 M NaOH during 1–4 h. After stirring for the designated time, the oxidation was quenched by addition of ethanol (*ca.* 5 mL). The oxidized hemp fibers were washed thoroughly with water and then with ethanol on filter paper using a Büchner funnel. The thus obtained water-insoluble fractions were then dried at room temperature for 48 h.

### *Determination of the content of carboxyl groups in the TEMPO-oxidized hemp fibers*

The carboxyl groups of the oxidized hemp fibers react with the salts of weaker acids such as calcium acetate, forming a salt of the oxidized cellulose fibers and releasing an equivalent amount of the weaker acid. Based on this and a modification of the calcium acetate method<sup>11</sup> for determining the acidity in modified hemp fibers, the following method of carboxyl group estimation was developed. Hemp fibers (0.5 g) were treated with 0.01 M HCl for 1 h and then washed thoroughly with water. In the next step, 50 mL of distilled water and 30 ml 0.25 M of calcium acetate solution were added to the oxidized hemp fibers. After standing for 2 h with frequent shaking to facilitate completion of the interchange, 30 mL portions of the liquid were titrated with 0.01 M sodium hydroxide using phenolphthalein as indicator.<sup>12</sup>

### *Determination of the content of carbonyl groups in the TEMPO-oxidized hemp fibers*

The content of carbonyl groups in the oxidized hemp fibers was measured according to a method described in the literature.<sup>10,13</sup> The oxidized hemp fibers were further oxidized with sodium chlorite at pH 4–5 to selectively convert the carbonyl groups in the samples to carboxyl ones, and then the total content of carboxyl groups was determined by the calcium acetate method described above. Thus a cellulose slurry with 10 % consistency was prepared and then this slurry (20 g) was added to a mixture containing NaClO<sub>2</sub> (1.81 g), 5 M CH<sub>3</sub>COOH (20 g), and water (57 mL). Oxidation was performed by stirring the mixture at room temperature for 48 h, followed by washing thoroughly with water and filtration. The carboxyl groups formed by the NaClO<sub>2</sub> oxidation were regarded as carbonyl groups present in the original oxidized hemp fibers.

### *Determination of moisture sorption*

Moisture sorption of oxidized hemp fibers was determined according to the standard ASTM D 2654-76, 1976.<sup>14</sup> Fibers were exposed to a standard atmosphere (20±2 °C and 65±2 % relative humidity) for 24 hours (ASTM D 1776-74, 1974).<sup>15</sup> The moisture sorption was calculated as the weight percentage relative to absolute dry material.

#### *Determination of water retention value*

The water retention of the hemp fibers was determined by the standard centrifuge method. ASTM D 2402-78, 1978.<sup>16</sup>

#### *Silver sorption by TEMPO oxidized hemp fibers*

Silver ions were incorporated into the previously TEMPO-oxidized hemp fibers and control ones by chemisorptions under the following previously optimized conditions:<sup>17</sup> fibers (0.1 g) were immersed in 100 mL of 0.01 mol dm<sup>-3</sup> AgNO<sub>3</sub> solution and shaken at room temperature for 240 min in the dark. The concentration of Ag<sup>+</sup> after sorption was determined by NH<sub>4</sub>SCN titration employing Fe(NH<sub>4</sub>)(SO<sub>4</sub>)<sub>2</sub> as an indicator, according to the Volhard Method.<sup>18</sup>

#### *Surface morphology and elemental composition – scanning electron microscopy with energy dispersive X-ray analysis (SEM–EDX)*

Investigation of the fiber morphology was investigated by scanning electron microscopy using a JEOL JSM 6610LV microscope operating at 20 kV after sputtering the samples with gold. The elemental composition was analyzed using an INCA Energy 350 Dispersive X-ray Microanalysis System.

#### *Determination of the antimicrobial activity of the silver-loaded TEMPO-oxidized hemp fibers*

The agar diffusion test<sup>19</sup> was used to assess the antimicrobial activity of the TEMPO-oxidized hemp fibers with incorporated silver particles. Three test organisms were used: Gram-positive *S. aureus* (ATCC 25923), Gram-negative *E. coli* (ATCC 25922), and the yeast *C. albicans* (ATCC 24433). The agar diffusion test consists in placement of 1.0 cm×1.0 cm samples (0.05 g of parallelized and pressed fibers) onto an agar support inoculated with the test microorganisms and, after 24 h incubation at 37 °C, measuring the width of the zone of inhibition (clear) or suppression (diffuse) of growth against the indicator organisms in comparison to a control sample.

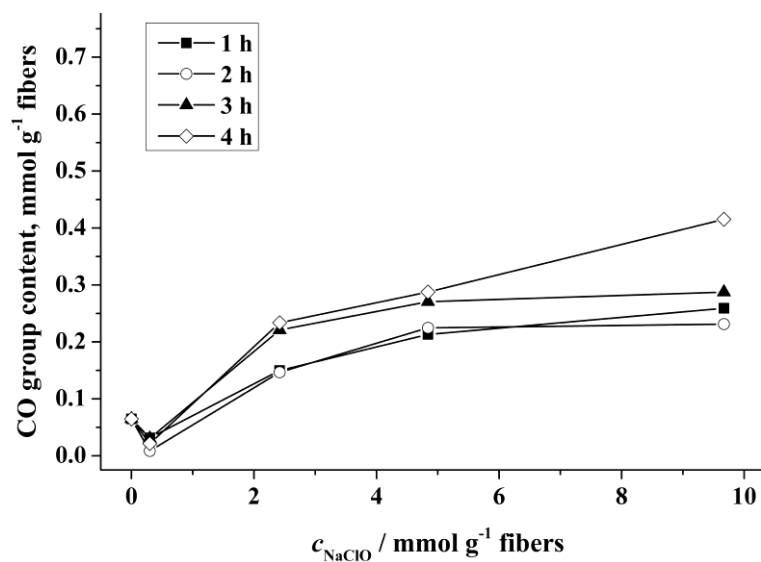
## RESULTS AND DISCUSSION

### *Obtaining silver-loaded hemp fibers*

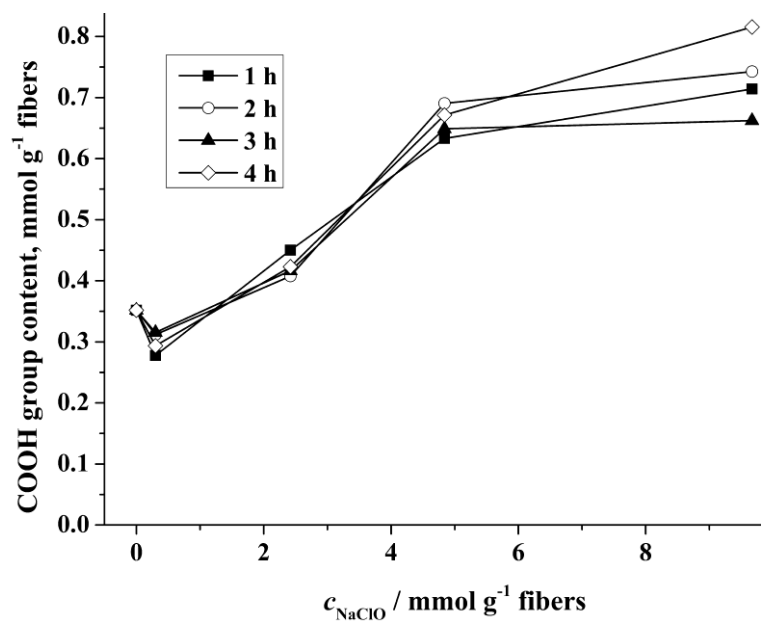
The hemp fibers were first oxidized by TEMPO-mediated oxidation in order to prepare fibers for silver deposition, *i.e.*, to improve the sorption properties by introduction of functional groups, which are necessary for silver sorption. Several oxidations with different concentration of sodium hypochlorite (0.30, 2.42, 4.84 and 9.67 mmol per gram of fibers) and different oxidation time (1–4 h) were investigated. The influences of the oxidation time and concentration of NaClO on the amount of introduced carbonyl and carboxyl functional groups, and silver sorption are shown in Fig. 1.

As can be seen Figs. 1a and 1b, the TEMPO-mediated oxidation with the lowest concentration of NaClO (0.30 mmol per gram of fibers) caused a slight decrease in the content of both the CHO and COOH functional groups, compared with corresponding value for the unmodified hemp fibers. The reason for these decreases could be the consumption of most of the oxidizing agents on oxidation and removal of lignin, hemicelluloses, and other accompanying components in hemp fibers, while the remaining amount of the oxidizing agent was not suffi-

cient to enable significant conversion of hydroxyl groups to carbonyl and further to carboxyl groups.<sup>7</sup>



(a)



(b)

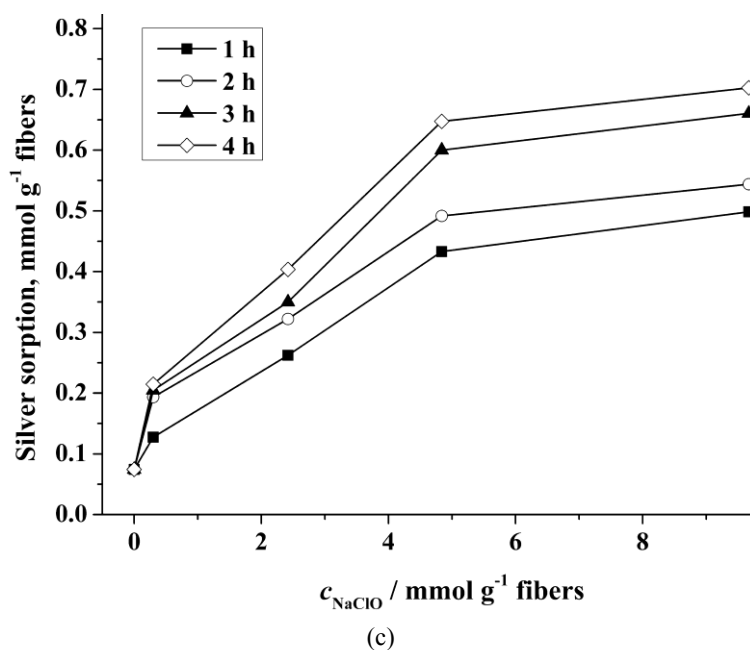


Fig. 1. Relationship between the amount of NaClO (0.30, 2.42, 4.84, and 9.67 mmol g<sup>-1</sup> fibers) used in TEMPO-mediated oxidation of hemp fibers and a) carbonyl group content, b) carboxyl group content and c) silver sorption. The oxidations were performed for 1–4 h at room temperature and pH 10.5.

Oxidation in the presence of higher concentrations of NaClO (2.42–9.67 mmol per gram of fibers) led to slight increases in the carbonyl and significant increases in the carboxyl group content. Obtained increase of the content of functional groups is predictable results, because TEMPO-mediated oxidation presents the selective oxidation at C6 of the anhydroglucose units of cellulose to carboxyl groups *via* the intermediate carbonyl stage, finally producing water-soluble polyglucuronic acids. The carboxyl groups introduced by the TEMPO-oxidation are present on the crystal surfaces and in disordered regions of celluloses, without any introduction inside of the cellulose crystallites. The nitroxyl radical affects the oxidation from the alcohol to the carboxyl oxidation state, while the hypobromite generated *in situ* from hypochlorite and bromide performs further oxidation of the carbonyl to the carboxylic acid.<sup>8,9</sup> The maximum carbonyl (0.415 mmol per gram of fibers) and carboxyl group content (0.815 mmol per gram of fibers) were obtained for the hemp fibers oxidized under the most severe conditions (4 h, 9.67 mmol NaClO per gram of fibers).

The hydrophilic carboxyl groups present in the TEMPO-oxidized hemp fibers enable the incorporation of silver, which was realized in the present study from aqueous silver nitrate solution. These groups are reactive sites for the

incorporation of silver and the predicted mechanism is that one  $\text{COO}^-$  group reacts with one  $\text{Ag}^+$  by the ion-exchange process; *i.e.*, by partial cation exchange of  $\text{H}^+$  (from the  $\text{COOH}$  groups) by  $\text{Ag}^+$ .<sup>20</sup> According to the protocol proposed by Ifuku *et al.*,<sup>21</sup> after the ion-exchange process, reduction of the silver salt on the surface of the TEMPO-oxidized fibers can lead to fine silver particles (Fig. 2).

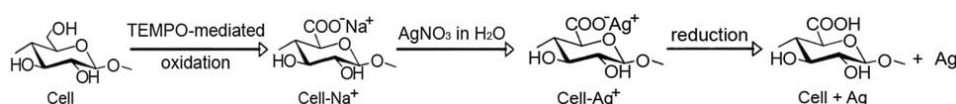


Fig. 2. Synthesis of silver particles on TEMPO-oxidized cellulose fibers.<sup>21</sup>

If Figs. 1b and 1c are compared, it is obvious that in the case of modification with higher concentrations of  $\text{NaClO}$  (2.42–9.67 mmol per gram of fibers), increases in both the carboxyl group content and silver sorption were obtained. However, for each sample, the number of sorbed silver ions was smaller than the number of introduced hydrophilic carboxyl groups, *i.e.*,  $\text{Ag}^+ < \text{COO}^-$ . According to the literature,<sup>22</sup> a difference in the “affinity” of various metal cations for the carboxyl groups of cellulose was noted by Heymann and Rabinov, and examined in detail by Davison. The order of increasing affinity found by this author was  $\text{N}(\text{CH}_3)_4^+ < \text{Li}^+ < \text{Na}^+ < \text{K}^+ < \text{Tl}^+ < \text{Ag}^+ < \text{Ca}^{2+}, \text{Ba}^{2+}$ . The applied Ca-acetate method<sup>11</sup> for the determination of  $\text{COOH}$  groups and the stronger affinity of  $\text{COOH}$  groups for  $\text{Ca}^{2+}$  than for  $\text{Ag}^+$  could be the reason for the obtained result that the number of  $\text{Ag}^+$  sorbed was smaller than the number of  $\text{COO}^-$ .

In spite of the fact that number of silver ions incorporated was smaller than the number of introduced  $\text{COOH}$  groups for all the TEMPO-oxidized hemp fibers, the amount of silver sorbed increased from 0.1273 to 0.7026 mmol silver per gram of fibers with increasing  $\text{NaClO}$  concentration used in the oxidation of the hemp fibers. The corresponding value for the unmodified sample is 0.0743 mmol silver per gram of fibers. Additionally, in the case of silver sorption, it was noticed that duration of TEMPO-mediated oxidation had a greater influence on amount of sorbed silver than the reaction time had on the amount of introduced  $\text{COOH}$  groups. To clarify this statement, the oxidation with 4.84 mmol  $\text{NaClO}$  per gram of fibers is given as an example. When the modification was performed with 4.84 mmol  $\text{NaClO}$  per gram of fibers, the amounts of introduced  $\text{COOH}$  groups were 0.633 and 0.690 mmol per gram of fibers for oxidation times of 1 and 4 h, respectively, while the corresponding silver sorptions increase from 0.433 to 0.648 mmol per gram of fibers, respectively.

All these above-mentioned results infer that, besides the introduced of  $\text{COOH}$  groups, the TEMPO-mediated oxidation caused other changes in the hemp fibers that had an influence on silver deposition.

During the TEMPO-mediated oxidation, together with the introduction of functional groups, the chemical composition and fibrous morphology of hemp fibers changed, depending on the oxidation conditions. The TEMPO-mediated oxidation removes non-cellulosic substances and makes the hemp fibers finer, cleaner, softer, and more suitable for further processing and textile applications.<sup>9,12,23,24</sup> Changes in the crystallinity and void system (diameter, volume and inner surface of voids)<sup>25</sup> of hemp fibers caused by TEMPO-mediated oxidation affect their sorption properties. In a previous article,<sup>7</sup> the influence of TEMPO-mediated oxidation on the properties of hemp fibers were given in detail. In order to understand the influence of changes in fiber structures and properties caused by TEMPO-mediated oxidation on silver sorption better, the relationship between silver sorption and the sorption properties of TEMPO-oxidized hemp fibers is presented herein. Sorption properties of oxidized hemp fibers were evaluated by determination of water retention power and moisture sorption.

From the correlation between the content of incorporated silver into TEMPO-oxidized hemp fibers and their water retention values (*WRV*) given in Fig. 3, it is obvious that, generally, with increasing water retention values, the silver sorption also increased. Sample oxidized under the most severe conditions (9.67 mmol of NaClO per gram of fibers for 4 h) exhibited maximum values for both, *i.e.*, a sorption capacity for Ag ions of 0.703 mmol per gram of fibers and a *WRV* of 125.41 %. The obtained correlation, *i.e.*, increase in both Ag sorption and *WRV*, can be explained by the fact that when fibers sorb more water, they swell more

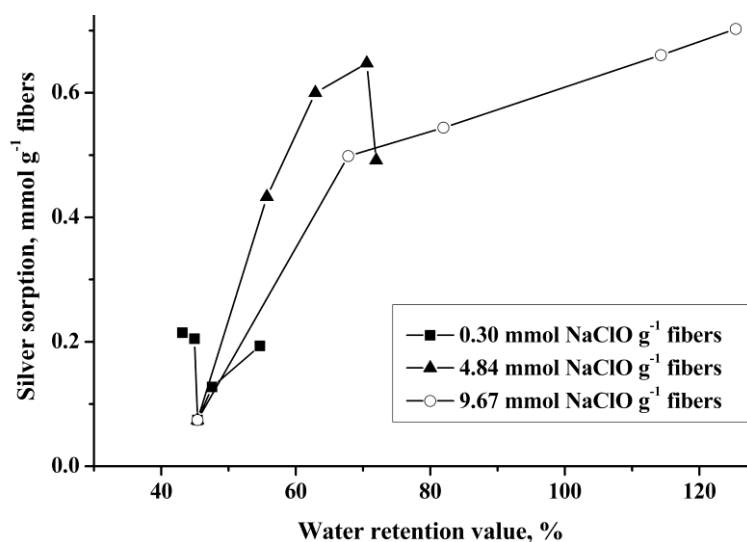


Fig. 3. Relationship between the water retention value, %, and silver sorption, mmol g<sup>-1</sup>, on TEMPO-oxidized hemp fibers. The oxidations were realized using 0.30–9.67 mmol NaClO g<sup>-1</sup> fibers, for 1–4 h at room temperature and pH 10.5.



and the fiber structures become “open” and accessible for Ag sorption. Additionally, in the case of hemp fibers, an effective elimination of hydrophobic impurities from the surface of the fibers and morphological changes of the fibers lead to additional water sorption into the fibers, as well as silver ions from aqueous solution.<sup>7</sup> For the samples oxidized with the highest amount of NaClO for 1–4 h, the relationship between the moisture sorption values and the Ag<sup>+</sup> sorption capacity for TEMPO-oxidized hemp fibers is shown in Fig. 4.

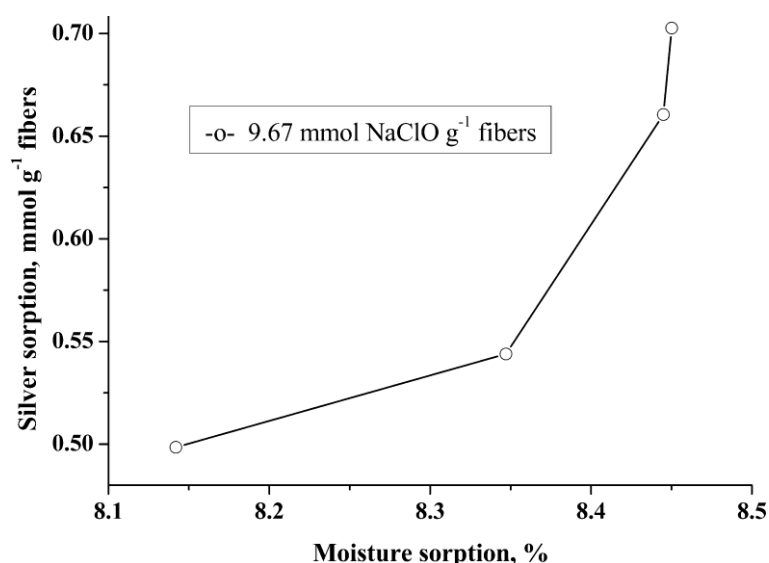


Fig. 4. Relationship between moisture sorption, %, and silver sorption, mmol g<sup>-1</sup>, by TEMPO-oxidized hemp fibers. The oxidations were performed using 9.67 mmol NaClO g<sup>-1</sup> fibers for 1–4 h at room temperature and pH 10.5.

The surface morphologies of the TEMPO-oxidized hemp fibers modified with the highest concentration of 9.67 mmol NaClO per gram of fibers for 2 h both without and with loading of Ag<sup>+</sup> were examined by SEM and the obtained microphotographs are presented in Figs. 5a and 5b, respectively. The SEM analysis revealed a large number of small and evenly distributed particles on the surface of the silver-loaded TEMPO-oxidized hemp fibers that were not present before the loading. A careful study of these two samples using the SEM–EDX technique was performed in order to identify elemental composition of the particles. The EDX spectra acquired for the before and after silver loading, presented in Figs. 5c and 5d, respectively, clearly show that the “white dots” present on the surface of the silver-loaded TEMPO-oxidized hemp fibers have strong silver peaks, while no silver observed on the unloaded sample. An EDX analysis over a 2 μm×2 μm area of the silver-loaded sample showed the presence of silver on the

fiber surface in a concentration of about 8 %. Gold peaks (Au) are present in the EDX spectra because the samples were gold-sputtered before SEM analysis.

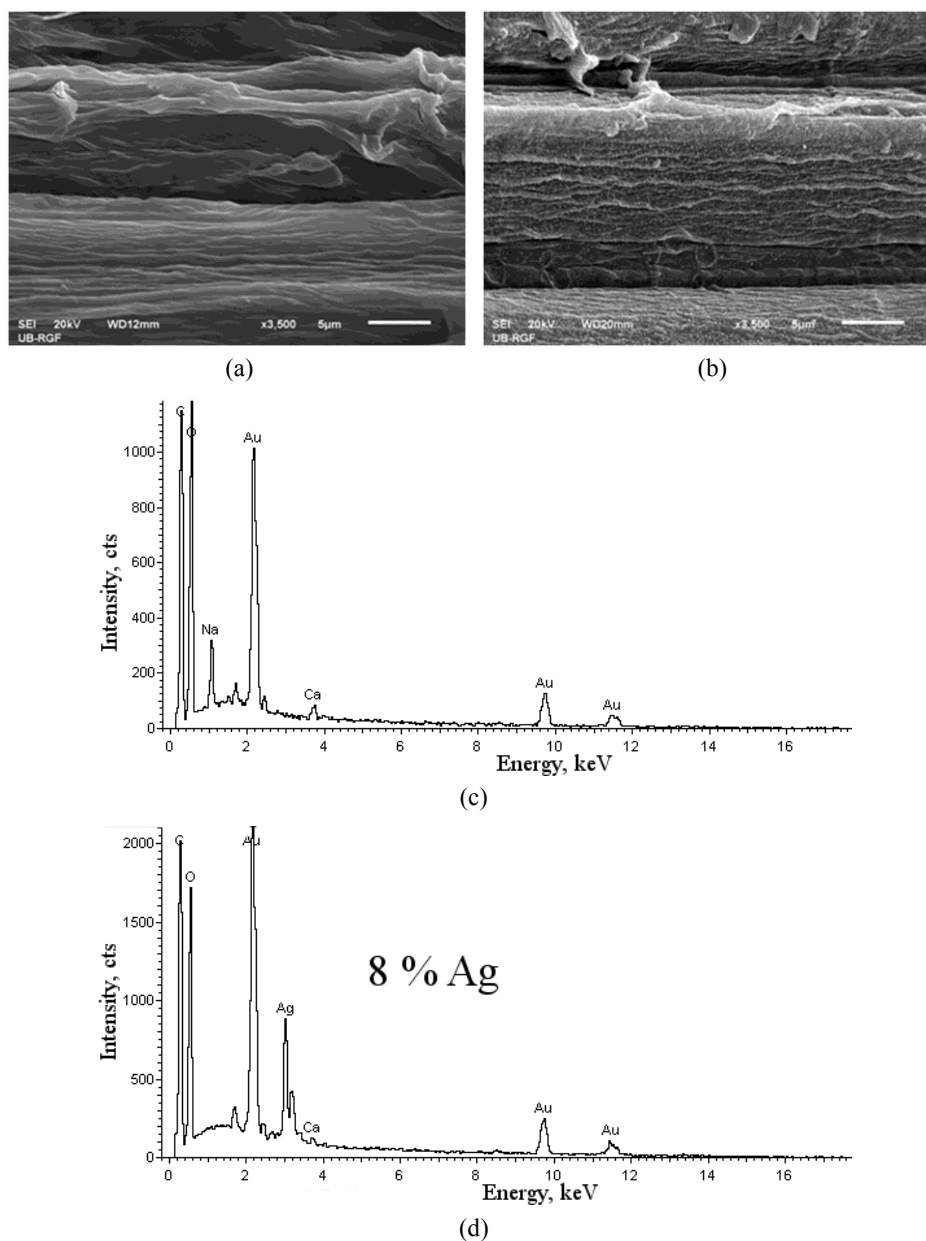


Fig. 5. SEM Images and EDX spectra of the TEMPO-oxidized hemp fibers modified with 9.67 mmol NaClO g<sup>-1</sup> fibers for 2 h (a and c) and silver loaded TEMPO-oxidized hemp fibers under the same conditions (b and d). Magnification: 3500×; bar: 5 µm.

SEM Images (Fig. 6) recorded under higher magnification (13000, 50000 and 100000 times) were employed to determine the dimensions of the silver particles. Silver particles, with isometric shapes and sizes ranging from 10 to 100 nm, were uniformly distributed on the surface of the silver-loaded TEMPO-oxidized hemp fibers, despite the fact that the silver deposition was realized from an aqueous silver nitrate solution by the ion-exchange technique, without any subsequent reduction step. According to the literature,<sup>21</sup> cellulose fibers represent unique nanoreactors for *in situ* synthesis of metal nanoparticles from ionic solution using mild reducing agents. This ability of cellulose is the consequence of its specific morphology and available oxygen-rich functional groups on the surface of the fiber. The important role of cellulose and the reducing groups in formation of silver nanoparticles was also demonstrated by Pinto *et al.* and Jiang *et al.*<sup>26,27</sup>

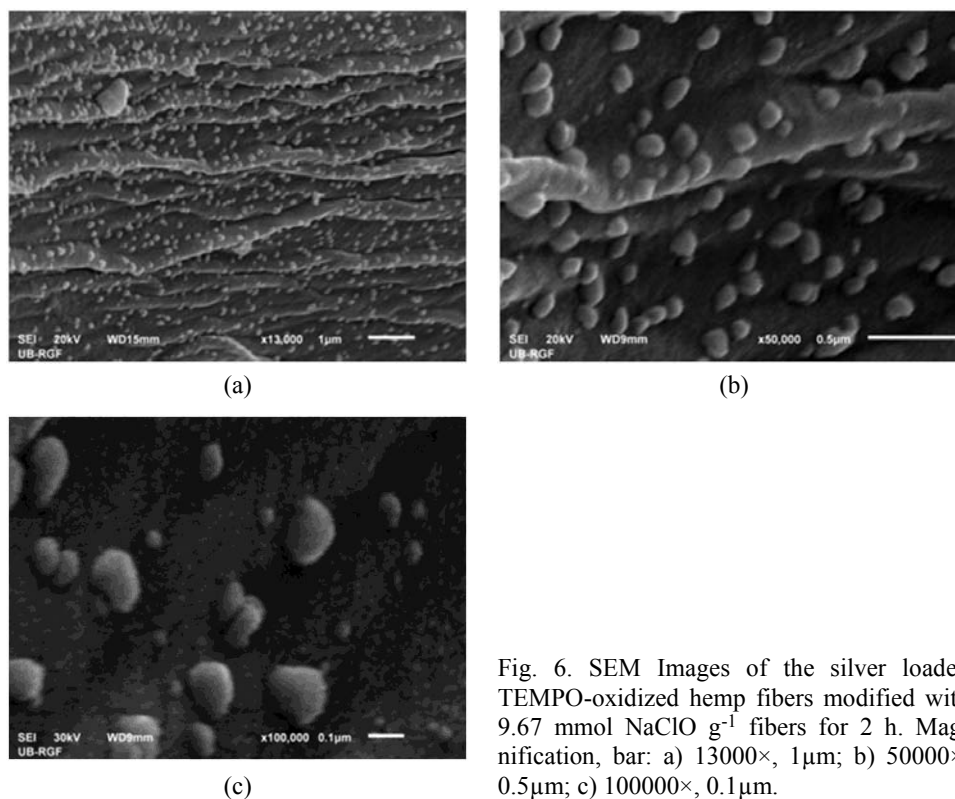


Fig. 6. SEM Images of the silver loaded TEMPO-oxidized hemp fibers modified with 9.67 mmol NaClO g<sup>-1</sup> fibers for 2 h. Magnification, bar: a) 13000 $\times$ , 1 $\mu$ m; b) 50000 $\times$ , 0.5 $\mu$ m; c) 100000 $\times$ , 0.1 $\mu$ m.

In the present study, the direct synthesis and growth of nanoparticles from an ionic solution (without the use of any kind of reducing agent) can be explained by the formation of highly reductive aldehyde groups, as an intermediary product in the TEMPO-oxidation, which were able to reduce silver ions to metallic silver. This statement is supported by the fact that more severe oxidation conditions

resulted in both higher numbers of aldehyde groups (Fig. 1a) and higher amounts of more evenly distributed nanoparticles (Fig. 7). The color of the silver-loaded TEMPO-oxidized hemp fibers changed drastically from cream yellow for oxidized fibers to brown in response to silver particle deposition.

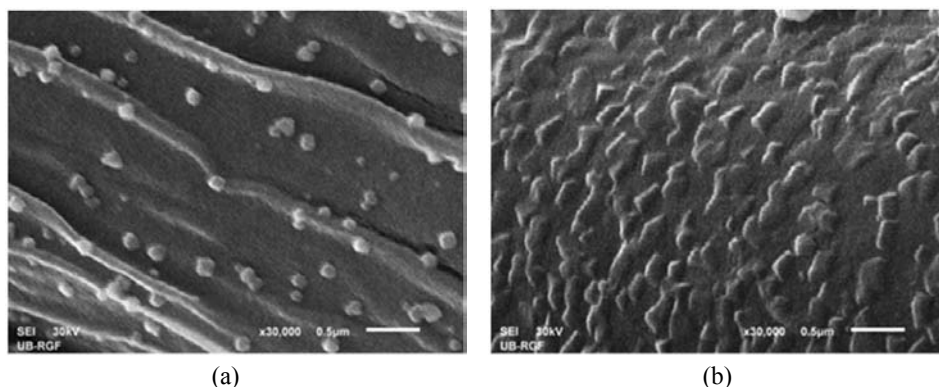


Fig. 7. SEM Images of the silver loaded TEMPO-oxidized hemp fibers modified with  $9.67 \text{ mmol NaClO g}^{-1}$  fibers for a) 2 and b) 4 h. Magnification:  $30000\times$ ; bar:  $0.5 \mu\text{m}$ .

#### *Antimicrobial activity of TEMPO-oxidized hemp fibers with incorporated silver particles*

Antibacterial activities of the TEMPO-oxidized hemp fibers with incorporated silver particles were tested against the Gram-positive bacterium *S. aureus* (ATCC 25923) and the Gram-negative bacterium *E. coli* (ATCC 25922), and the antifungal activity was tested against a fungus from the *Candida* family, *C. albicans* (ATCC 24433). From the results presented in Table I, it could be seen that the TEMPO-oxidized hemp fibers with incorporated silver particles generally inhibited the growth of all the tested pathogens. *S. aureus* was more sensitive than strain *E. coli*. For the bacterium *S. aureus*, the silver-loaded hemp fibers containing  $0.433$  and  $0.648 \text{ mmol Ag per gram of fibers}$  (entries 7 and 9, in Table I) were the most effective, whereas for the bacterium *E. coli*, the sample with  $0.262 \text{ mmol Ag per gram of fibers}$  (entry 3) was the most effective. Most of the tested samples showed good antifungal activity against the fungus *C. albicans*. The sample with the maximum amount of sorbed silver ( $0.703 \text{ mmol Ag per gram of fibers}$ ) (entry 12) did not show maximum antimicrobial activity against any of the tested microorganisms. The obtained results showed that there was no clear correlation between the amount of sorbed silver and antimicrobial activity. However, the quantity of incorporated silver particles was, in all cases, sufficient to inhibited the growth of tested pathogens, probably because, according to the literature,<sup>28</sup> silver does not attack microorganisms directly; it operates as a catalytic agent and moreover, it is not consumed in this process.

TABLE I. Antimicrobial activity of TEMPO-oxidized hemp fibers with incorporated silver particles

Oxidative conditions			Entry No.	Width of the inhibition zone after 24 h, mm		
$c_{\text{NaClO}} / \text{mmol g}^{-1}$ of fibers	Reaction time, h	Sorbed silver, mmol per gram of fibers		<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
0.00	0	0.074	1	1.0–1.5	1.0–1.5	1.0–1.5
0.30	4	0.214	2	1.0–1.5	1.5	1.0–1.5
2.42	1	0.262	3	2.5–3.0	2.5–3.0	2.5–3.0
		0.322	4	3.0	1.0	2.5–3.0
	3	0.350	5	2.5	1.0–1.5	3.0
		0.404	6	2.5–3.0	1.0–1.5	3.0
4.84	1	0.433	7	3.5–4.0	1.0–1.5	2.5–3.0
		0.600	8	3.0	1.0–1.5	2.5–3.0
	4	0.648	9	4.0	1.5–2.0	3.0–3.5
9.67	1	0.498	10	2.5–3.0	1.0	2.0
		0.660	11	2.0	1.0	2.5–3.0
	4	0.703	12	3.0	1.0	2.0

## CONCLUSIONS

The introduced hydrophilic carboxyl groups (0.278–0.815 mmol per gram of fibers) and other changes in hemp fibers caused by TEMPO-mediated oxidation influence an increased silver sorption. The amount of sorbed silver onto the modified hemp fibers was in the range 0.214–0.703 mmol per gram of fibers. SEM microphotographs of the modified hemp fibers with sorbed silver showed uniformly distributed silver particles on the surface of the fibers, with isometric shapes and sizes from 10 to 100 nm, and the EDX spectra showed the presence of silver at a concentration of about 8 %. The obtained direct synthesis and growth of nanoparticles from ionic solution (without the use of any kind of reducing agent) could be explained by the formation of highly reductive aldehyde groups, as an intermediary product in the TEMPO-oxidation, which are able to reduce silver ions to metallic silver. The TEMPO-oxidized hemp fibers with sorbed silver showed good antibacterial activity against the tested bacterium strains: *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922), and the fungus *C. albicans* (ATCC 24433), while the best antimicrobial activity of the silver-loaded TEMPO-oxidized hemp fibers was shown against the Gram-positive bacterium *S. aureus*.

*Acknowledgments.* This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project OI 172029). The authors also thank ITES Odzaci (Serbia) for supplying the hemp fibers and Aleksandar Pacevski, PhD (Faculty of Mining and Geology, University of Belgrade) for obtaining the SEM images.

## ИЗВОД

АНТИМИКРОБНА ТЕМПО-ОКСИДИСАНА ВЛАКНА КОНОПЉЕ СА  
ИНКОРПОРИСАНИМ ЧЕСТИЦАМА СРЕБРА

ЈОВАНА МИЛАНОВИЋ, ТАТЈАНА МИХАИЛОВИЋ, КАТАРИНА ПОПОВИЋ И МИРЈАНА КОСТИЋ

*Технолошко-металуришки факултет, Универзитет у Београду, Карнегијева 4, 11120 Београд*

Циљ овог рада је добијање антимикробних влакана конопље са сорбованим сребром. Модификовање влакана конопље у циљу повећања сорпционих својстава, а тиме и што бољег сорбовања сребра, постигнуто је селективном ТЕМПО-оксидацијом, тј. оксидацијом помоћу катализатора 2,2,6,6-тетраметилпиперидин-1-окси радикала (ТЕМПО) у систему натријум-хипохлорит/натријум-бромид, док је сребро сорбовано из раствора сребро-нитрата. Променом параметара оксидације, тј. концентрације натријум-хипохлорита и времена оксидације, одређени су најпогоднији услови извођења ТЕМПО-оксидације. Добијени резултати показали су да је максимални сорпциони капацитет (0,703 mmol сребра по граму влакна) модификованих влакана конопље добијен код узорка који је оксидисан помоћу 9,67 mmol NaClO по граму целулозе, током 4 сата. SEM микрофотографије модификованих влакана конопље са сорбованим сребром показале су, на површини влакана, равномерну дистрибуцију честица сребра изометричног облика и нано величине (од 10 до 100 nm), упркос чињеници да је сребро сорбовано из јонског раствора. Антибактеријска активност ТЕМПО-оксидисаних влакана конопље са инкорпорисаним честицама сребра тестирана је *in vitro* на две врсте бактерија: грам-позитивна *Staphylococcus aureus* (ATCC 25923) и грам-негативна *Escherichia coli* (ATCC 25922), док је антифунгална активност испитана према гљивици *Candida albicans* (ATCC 24433). Најбољу антимикробну активност ТЕМПО-оксидисана влакна конопље са инкорпорисаним честицама сребра, показала су према грам-позитивној бактерији *S. aureus*.

(Примљено 18. октобра, ревидирано 10. децембра 2012)

## REFERENCES

1. U. C. Hipler, P. Elsner, J. W. Fluhr, in *Biofunctional Textiles and the Skin; Current Problem in Dermatology*, Vol. 33, U. C. Hipler, P. Elsner, Eds., Karger, Basel, 2006, p. 165
2. D. Hofer, in *Biofunctional Textiles and the Skin; Current Problem in Dermatology*, Vol. 33, U. C. Hipler, P. Elsner, Eds., Karger, Basel, 2006, p. 42
3. A. B. G. Lansdown, in *Biofunctional Textiles and the Skin; Current Problem in Dermatology*, Vol. 33, U. C. Hipler, P. Elsner, Eds., Karger, Basel, 2006, p. 17
4. U. Wollina, M. B. Abdel-Nase, S. Verma, in *Biofunctional Textiles and the Skin; Current Problem in Dermatology*, Vol. 33, U. C. Hipler, P. Elsner, Eds., Karger, Basel, 2006, p. 1
5. R. Kozłowski, S. Manys, J. Kozłowska, *Sovremennoe polozenie u perspektivy na budushchee dlya l'na i pen'ki na rubezhe XX i XXI vekov*, Tezisy dokladov nauchno-prakticheskai konferentsii "Len – na poroge XXI veka". Vologda, Russia, 2000 (in Russian)
6. H. M. Wang, R. Postle, R. W. Kessler, W. Kessler, *Text. Res. J.* **73** (2003) 664
7. J. Milanovic, M. Kostic, P. Milanovic, P. Skundric, *Ind. Eng. Chem. Res.* **51** (2012) 9750
8. P. S. Chang, J. F. Robyt, *J. Carbohydr. Chem.* **15** (1996) 819
9. T. Saito, Y. Okita, T. T. Nge, J. Sugiyama, A. Isogai, *Carbohydr. Polym.* **65** (2006) 435
10. T. Saito, A. Isogai, *Biomacromolecules* **5** (2004) 1983
11. E. C. Yackel, W. O. Kenoyon, *J. Am. Chem. Soc.* **64** (1942) 121
12. J. Praskalo, M. Kostic, A. Potthast, G. Popov, B. Pejic, P. Skundric, *Carbohydr. Polym.* **77** (2009) 791

13. E. J. Parks, R. L. Hebert, *Tappi. J.* **55** (1972) 1510
14. *ASTM D 2654-76: Moisture Content and Moisture Regain of Textiles*, in *Annual Book of ASTM Standards*, American Society for Testing and Materials, Philadelphia, PA, 1978
15. *ASTM D 1776-74: Conditioning Textiles and Textile Products for Testing*, in *Annual Book of ASTM Standards*, American Society for Testing and Materials, Philadelphia, PA, 1978
16. *ASTM D 2402-78: Standard Test Method for Water Retention of Fibers, Centrifuge Method*, in *Annual Book of ASTM Standards*, American Society for Testing and Materials, Philadelphia, PA, 1978
17. M. M. Kostic, J. Z. Praskalo, S. I. Dimitrijevic, M. V. Baljak, B. M. Pejic, P. D. Skundric, in *Proceedings of the 7th Annual Textile Conference by AUTEX – From Emerging Innovations to Global Business*, Tampere, Finland, 2007, p. 1267
18. P. Patnaik, *Handbook of Environmental Analysis*, Lewis Publishers, CRC Press, Boca Raton, CA, 1997, p. 39
19. B. G. Joiner, in *Bioactive Fibers and Polymers*, J. V. Edwards, L. T. Vigo, Eds., American Chemical Society, Washington DC, 2001, p. 201
20. J. Z. Praskalo-Milanovic, M. M. Kostic, S. I. Dimitrijevic-Brankovic, P. D. Skundric, *J. Appl. Polym. Sci.* **117** (2010) 1772
21. S. Ifuku, T. Manami, M. Minoru, S. Hiroyuki, H. Yano, *Biomacromolecules* **10** (2009) 2714
22. A. M. Sookne, M. Harris, in *Cellulose and Cellulose Derivatives*, Part I, E. Ott, H. M Spurlin, M. W. Grafflin, Eds., Interscience Publishers, New York, 1954, p. 213
23. A. E. de Nooy, A. C. Besemer, H. van Bekkum, *Carbohydr. Res.* **89** (1995) 269
24. Z. Dang, J. Zhang, A. Ragauskas, *Carbohydr. Polym.* **70** (2007) 310
25. T. Kreze, S. Jeler, S. Strnad, *Mater. Res. Innov.* **5** (2002) 277
26. R. J. B. Pinto, P. A. A. P. Marques, C. P. Neto, Y. Trindade, S. Daina, P. Sadocco, *Acta Biomater.* **5** (2009) 2279
27. T. Jiang, L. Liu, J. Yao, *Fibers Polym.* **12** (2011) 620
28. R. L. Davis, S. F. Etris, *Catal. Today* **36** (1997) 107.