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ANTIBACTERIAL ACTIVITY OF SELECTIVELY OXIDIZED LYOCELL FIBERS

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Abstract: The purpose of this research was to study antibacterial activity of selectively oxidized lyocell fibers with incorporated silver particles against gram (+) and gram (-) pathogens. Antibacterial properties were accomplished by incorporation of silver ions into modified lyocell fibers by chemisorption from aqueous silver nitrate solution. In order to improve sorption properties of lyocell fibers, the selective TEMPO-mediated oxidation, i.e. oxidation with sodium hypochlorite and catalytic amount of sodium bromide and 2,2´,6,6´-tetramethylpiperidine-1-oxy radical (TEMPO), was applied. The influence of oxidation conditions on the amount of sorbed silver, and thus on the degree of antibacterial activity was determined. It was found that the maximum amount of sorbed silver was 0.996 mmol/g cell. The antibacterial activity of the TEMPO-oxidized lyocell fibers with silver particles was confirmed *in vitro* against two strains: *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The silver-loaded TEMPO-oxidized lyocell fibers showed better antimicrobial activity against strain *Staphylococcus aureus.*

Key words: lyocell fibers, TEMPO-oxidation, silver sorption, antibacterial activity.

1. INTRODUCTION

In the circumstances of a modern way of life and striving for higher hygiene levels, production of materials with antibacterial properties is constantly increasing. Fibers with antibacterial properties should disable the growth or directly destroy a lot of pathogenic bacteria, which cause infections and many diseases [1, 2].

The purpose of this research was to study antibacterial activity of selectively oxidized lyocell fibers with incorporated silver particles against gram (+) and gram (-) pathogens. Antibacterial properties were accomplished by incorporation of silver particles into modified lyocell fibers by chemisorption from aqueous silver nitrate solution.

Silver was chosen as antibacterial agent, because, due to unique properties of silver particles, such as strong inhibitory, odor control, broad and long-term activity, bacteria are not able to develop their resistance to silver, as in the case of antibiotics [3]. These properties, together with high thermal stability and good fabric compatibility have established silver as a topical agent in several contemporary medical areas [4,5].

In order to improve sorption properties of lyocell fibers, the selective TEMPO-mediated oxidation, i.e. oxidation with sodium hypochlorite and catalytic amount of sodium bromide and 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO), was applied. TEMPO-mediated oxidation causes conversion of C6 primary hydroxyl groups of lyocell fibers into carboxyl groups via C6 aldehyde groups. Introduced carboxyl groups were used as reactive positions in further reaction, i.e. for incorporation of silver ions, because a hydrogen atom present in carboxyl groups could be easily replaced with silver ions. The most noticeable points of this TEMPO-mediated oxidation of polysaccharides are the following: highly regioselective oxidation of primary hydroxyl groups in polysaccharides to carboxyl groups can be achieved and this selective oxidation proceeds under aqueous mild conditions around room temperature at pH 10–11. [6,7].

Lyocell fibers were chosen for selective TEMPO-mediated oxidation because of the following: as man-made cellulose fibers they are more homogenous in structure and properties than cotton, with better sorption properties, and something that is very important among man-made cellu-

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lose fibers, they stand out due to some unique properties (very high strength in comparison with other man-made cellulose fibers, high crystallinity, specific lustre and handle, optimum conditions for the skin) [8]. Additionally, special features such as dissolving of cellulose without chemical modification, ability of solvent to attain exceedingly high concentration of cellulose (e.g., 35% w/w in DP 600), and nearly complete recovering of the nontoxic solvent NMMO, have established the lyocell process as an environmentally-friendly, relatively simple, economically viable, product-enhancing and highly flexible technology [9].

Antibacterial activity of TEMPO-oxidized lyocell fibers with incorporated silver particles was tested against test organisms: Gram-positive *Staphylococcus aureus* (ATCC 25.923) and Gram-negative *Escherichia coli* (ATCC 25.922).

2. EXPERIMENTAL

2.1. Material

Lyocell fibers, obtained from "Lenzing AG" Austria, were used in this study. The fiber fineness is 1.3 dtex and fiber length is 38 mm. Lyocell fibers are without spin finishing. All used chemicals obtained from commercial sources are p.a. grade.

2.2. Methods

2.2.1. Preparation of TEMPO-oxidized lyocell fibers

Oxidation procedure was based on the literature methodology [10, 11]. Lyocell fibers (10 g) were suspended in water (750 ml) containing TEMPO (0.025 g) and sodium bromide (0.25 g). Subsequently, the pre-determined amount of NaClO solution containing 13 % available chlorine, corresponding to 0; 0.30; 2.42; 4.84 and 9.67 mmol/g fibers, was added to the cellulose slurry under continuous stirring. The pH value of the slurry was maintained at 10.5 at room temperature by adding 0.5 M NaOH for 0.25-4 h. Duration of oxidation was chosen according to the literature data [11] suggesting that there is no significant increase in carboxyl content in the modified fibers when oxidation is longer than 4 hours. After stirring for a pre-determined time, oxidation was quenched by adding ethanol (ca. 5 ml). Oxidized fibers were washed thoroughly with water and then ethanol on a filter paper set in a Büchner funnel. The oxidized samples were then dried at room temperature for 48 h.

2.2.2. Determination of carboxyl groups (COOH) in the TEMPO-oxidized lyocell fibers

The potentiometric titration, as a direct method, was used for determination of carboxyl groups in modified lyocell fibers. Cellulose samples (0.5 g) were added to 100 ml of 0.5M NaCl. The titration was started with a neutral fibers suspension to which 10 ml of 0.1M HCl in 0.5M NaCl was added using a precision burette. Titration was carried out by adding of 0.1M NaOH in 0.5M NaCl from a precision burette. During titration, the solution was stirred with a glass propeller and kept in airtight titration vessel. All experiments were carried out under thermostatically controlled conditions at 25°C. Inert atmosphere was maintained by continuous flow of argon. After each addition, the potential was recorded automatically with Methrohm 848 titrino plus. The stability criterion for recording of the potential after each addition was a drift of less than 0.5 mV/min. The amount of impurities not stemming from the fibers was determined by performing blank titrations. All presented values are the mean values of five parallel measurements [12].

2.2.3. Silver sorption by TEMPO-oxidized lyocell fibers

Silver particles were incorporated into TEMPO-oxidized lyocell fibers by chemisorptions under the following conditions: fibers (0.1 g) were immersed in 100 mL of 0.01 mol/dm^3 $AgNO_3$ solution, and shaken at room temperature for 240 min in the dark [13]. The change in concentration of silver after sorption was determined by NH_4SCN titrations employing $Fe(NH_4)(SO_4)_2$ as an indicator according to Volhard's method [14].

2.2.4. Determination of antibacterial activity of TEMPO-oxidized lyocell fibers with incorporated silver particles

Agar diffusion test [15] was used to assess the antibacterial activity of TEMPO-oxidized lyocell fibers with incorporated silver particles. Two test organisms were used: Gram-positive *S. aureus* ATCC 25,923 and Gram-negative *E. coli* ATCC 25,922. The agar diffusion test consists of placement of 1.0 cm × 1.0 cm samples (0.05 g of parallelized and pressed fibers) onto an agar support inoculated with tested 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 compared to a control sample.

3. RESULTS AND DISCUSSION

3.1. TEMPO-oxidation and introduction of COOH groups

Applied TEMPO-mediated oxidation causes conversion of the C6 primary hydroxyl groups of lyocell fibers into carboxyl groups via C6 aldehyde groups (Figure 1). The nitroxyl radical affects the oxidation from the alcohol to the aldehyde oxidation state, while hypobromite generated *in situ* from hypochlorite and bromide performs the further oxidation of aldehyde to carboxylic acid [6,7].

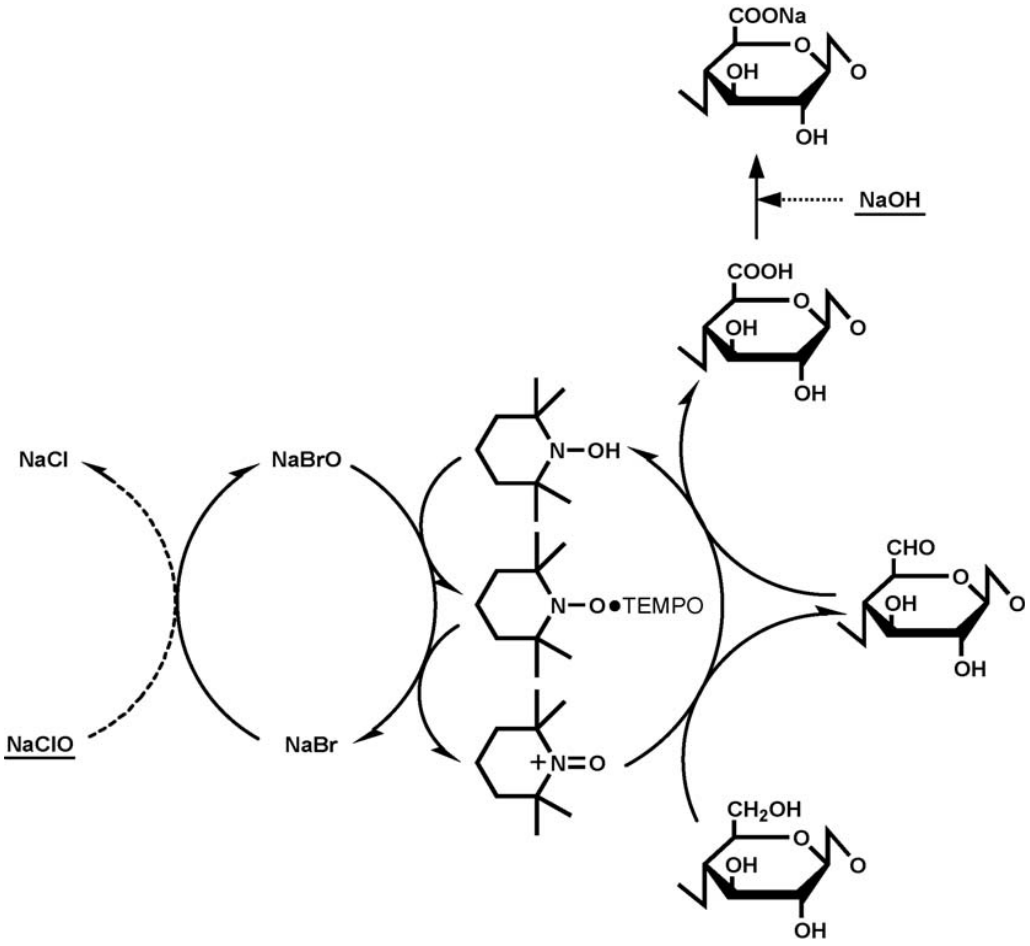


Figure 1. TEMPO-mediated oxidation of cellulose to form C6-carboxyl groups via C6-aldehyde groups [7]

The effect of oxidation time and amount of added NaClO on carboxyl group content in the unmodified and modified lyocell fibers is shown in Figure 2. The carboxyl group content in unmodified lyocell was 0.035 mmol/g fibers. During the TEMPO-mediated oxidation carboxyl contents in oxidized lyocell fibers increased in all cases by the addition of oxidation agent (NaClO) and with increasing oxidation time, compared to unmodified samples.

The carboxyl group content in the modified lyocell fibers was within the range of 0.049-0.618 mmol/g fibers. For the lyocell fibers oxidized in the presence of the lowest amount of NaClO (0.30 NaClO mmol/g fibers) the carboxyl group content increased with prolonged oxidation time. In the case of oxidation in the presence of higher amount of oxidative agent, i.e. 2.42 and 4.84 mmol NaClO/g fibers, carboxyl group content increased with prolonged oxidation up to 3 and 1 h, respectively. The maximum increase of carboxyl content in the oxidized lyocell fibers of 17.6 times ($COOH_{mod}/COOH_{unmod}$) was obtained for the sample oxidized in the presence of 2.42 mmol NaClO/g fibers during 3 h. After reaching the maximum, the carboxyl group content decreased slightly in the case of oxidation with 2.42 mmol NaClO/g fibers, and very sharply in the presence of 4.84 mmol NaClO/g fibers. A decrease obtained in carboxyl content in the oxidized lyocell fibers can be explained by dissolution of highly oxidized cellulose fractions, i.e. the oxidized products turn out to have higher water-

solubility and thus are removed to the filtrates during the washing process. Applying severe conditions (9.67 mmol NaClO/g fibers) in the case of TEMPO-mediated oxidation of lyocell fibers, results in the loss of a fibrous structure and for textile applications only oxidation under mild conditions should be used [6,7].

Introduced carboxyl groups were used as reactive positions in the further reaction, i.e. for incorporation of silver, because a hydrogen atom present in carboxyl groups could be easily replaced with silver ions.

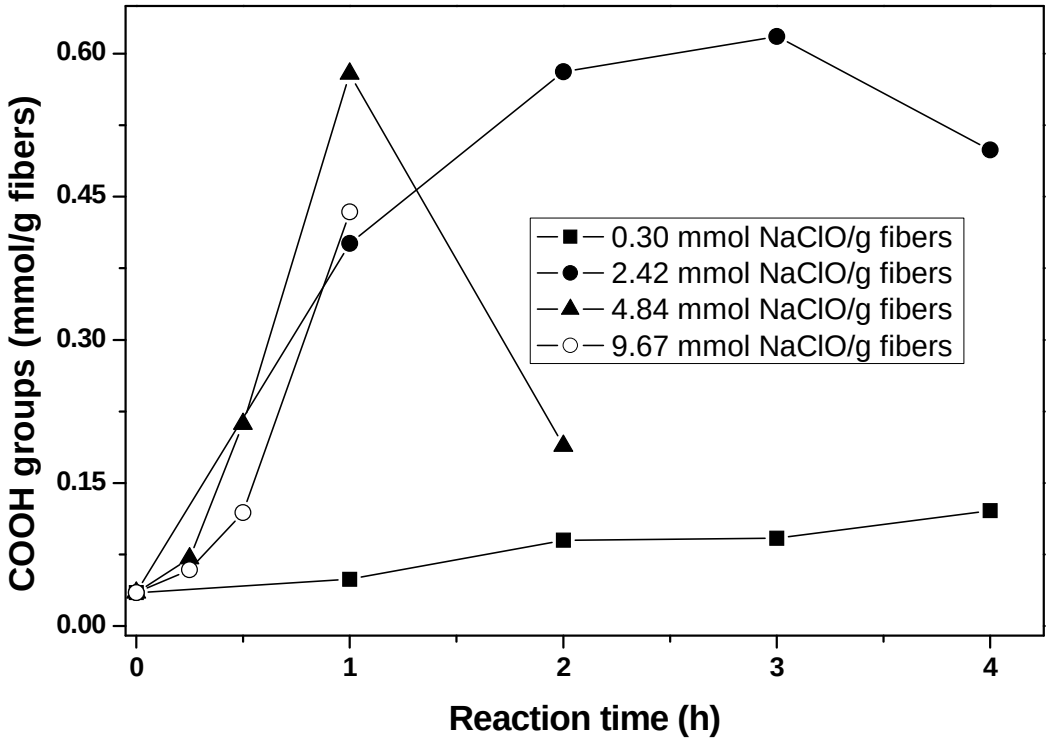


Figure 2. Relationships between oxidation time and carboxyl groups in the TEMPO-oxidized lyocell fibers, where 0.30; 2.42; 4.84 and 9.67 mmol NaClO (per gram of fibers) was applied, at room temperature and pH 10.5.

3.2. Silver sorption by TEMPO-oxidized lyocell fibers

Relationships between TEMPO-oxidation conditions and incorporated silver in the oxidized lyocell fibers are shown in Figure 3. Introduced COOH groups and other changes in lyocell fibers caused by TEMPO-oxidation gave rise to an increase of silver sorption. The amount of incorporated silver into TEMPO-oxidized lyocell fibers was in the range from 0.018 mmol/g fibers to 0.809 mmol/g fibers, while for unmodified lyocell fibers the amount of sorbed silver was 0.014 mmol/g fibers.

In the case of modification with the lowest concentration of oxidative agent NaClO (0.30 mmol/g fibers), with prolonged oxidation time, a slight increase of the silver sorption, up to 6 times, was obtained (Figure 3a). Modification with 2.42 mmol NaClO/g fibers, during 2 h, led to an increase in the amount of sorbed silver, while prolonged oxidation time (3 and 4 h) caused a slight decrease of silver sorption (Figure 3b). A decrease obtained of the amount of sorbed silver can be explained by dissolution of highly oxidized cellulose fractions, which leads to a decrease of the amount of COOH groups. For lyocell fibers oxidized with higher concentrations of NaClO (4.84 and 9.67 mmol/g fibers), the amount of sorbed silver slightly increased during the first 30 min (Figures 3c, d). This slight increase can be explained, according to the literature [6, 16], by the fact that the TEMPO-oxidation presents conversion of OH groups into COOH groups via the intermediate CHO stage, [7] and in the first 30 min, there was not enough COOH groups that can react with silver. With prolongation of oxidation time up to 1 h, a considerable amount of silver was introduced into lyocell fibers. A maximum increase of the amount of sorbed silver was obtained for the sample oxidized in the presence of 4.84 mmol NaClO/g fibers during 1 h (0.809 mmol/g fibers). Oxidation

with 4.84 and 9.67 mmol NaClO/g fibers, longer than 2 h and 1 h, respectively, led to a decrease of silver sorption, as a consequence of the dissolution of highly accessible fractions and degradation of fibrous form of lyocell. As we mentioned previously, applying too severe conditions should be avoided, because of losing fibrous structure of oxidized fibers [6, 7].

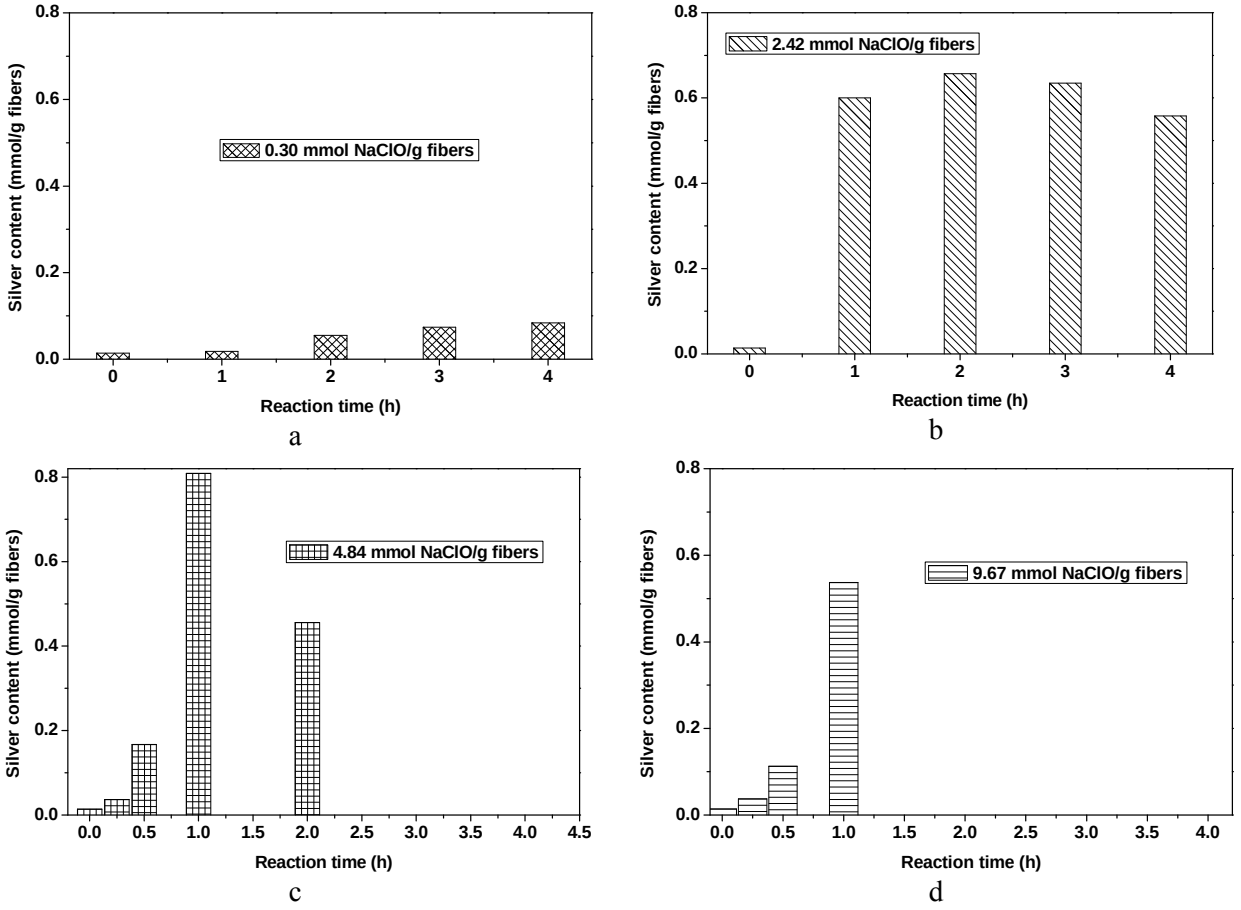
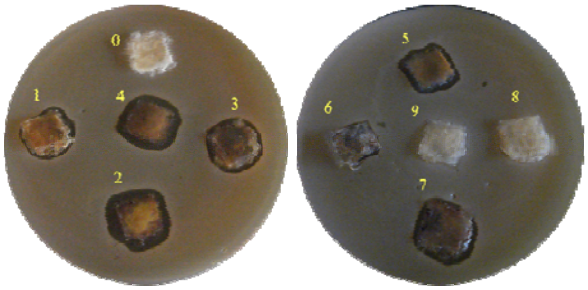
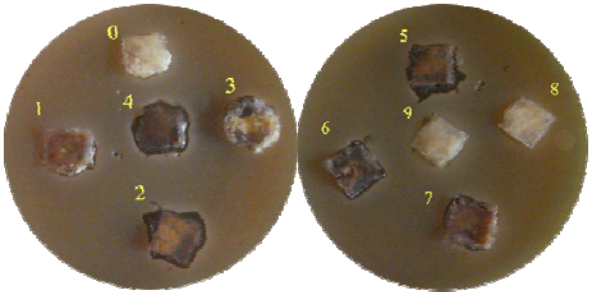


Figure 3. Relationships between oxidation time (h) and incorporated silver (mmol/g fibers) in the TEMPO-oxidized lyocell fibers, where a) 0.30; b) 2.42; c) 4.84 and d) 9.67 mmol NaClO (per gram of fibers) was applied, at room temperature and pH 10.5.

3.3. Antibacterial activity of TEMPO-oxidized lyocell fibers with incorporated silver ions

Antibacterial activity of TEMPO-oxidized lyocell fibers with incorporated silver particles was tested against test organisms: Gram-positive *Staphylococcus aureus* (ATCC 25.923) and Gram-negative *Escherichia coli* (ATCC 25.922). From the results presented in Table 1, it can be seen that there was no antibacterial activity of unmodified lyocell (mark 0) and of TEMPO-oxidized lyocell fibers (mark 8 and 9), while the incorporation of silver in TEMPO-oxidized lyocell fibers generally inhibited the growth of tested bacteria (marks 1 - 7). *Staphylococcus* aureus, gram-positive bacteria, was more sensitive to the TEMPO-oxidized lyocell fibers with silver then strain *Escherichia coli*, gram-negative bacteria. For bacteria *Staphylococcus aureus*, the silver loaded TEMPO-oxidized lyocell fibers containing 0.600 mmol Ag/g fibers and 0.167 mmol Ag/g fibers, (samples mark 2 and 4), were the most effective, whereas for bacteria *Escherichia coli*, samples marked as 2 and 5, were the most effective. The last sample (5) also showed the maximum value for silver sorption capacity (0.809 mmol/g fibers). The results obtained showed that, there was no clear correlation between the amount of sorbed silver and antibacterial activity. However, the quantity of incorporated silver, in all cases, was enough to inhibit the growth of tested bacteria, probably, due to the fact that, according to literature [17], silver does not attack microorganisms directly; but operates as a catalytic agent, and moreover, it is not consumed in this process.

Table 1. Antibacterial Activity of TEMPO-oxidized Lyocell Fibers with Incorporated Silver against Strains: Staphylococcus Aureus and Escherichia Coli

Modification conditions	**marks on Petri dishes**	**width of the inhibition zone after 24 h (mm)**		
		S. aureus	***E. coli***	
unmodified	0	0	0	 *Staphylococcus aureus*
0.30 mmol NaClO/g fibers, 1h + Ag^+	1	2.0 - 2.5	1.5	
2.42 mmol NaClO/g fibers, 1h + Ag^+	2	3.5 - 4.0	2.5	
4.84 mmol NaClO/g fibers, 0.25h + Ag^+	3	2.0	1.5	
4.84 mmol NaClO/g fibers, 0.50h + Ag^+	4	3.5	2.0	 *Escherichia coli*
4.84 mmol NaClO/g fibers, 1h + Ag^+	5	2.5	2.5	
4.84 mmol NaClO/g fibers, 2h + Ag^+	6	1.5	1.5	
9.67 mmol NaClO/g fibers, 1h + Ag^+	7	2.5	1.5	
0.30 mmol NaClO/g fibers, 1h	8	0	0	
9.67 mmol NaClO/g fibers, 1h	9	0	0	

4. CONCLUSIONS

The results obtained showed that selective TEMPO-oxidation of primary hydroxyl groups provides introduction of carboxyl groups into modified lyocell fibers. The carboxyl group content in the oxidized lyocell fibers was within the range of 0.049-0.618 mmol/g fibers, depending on oxidative conditions. The maximum increase of carboxyl content in oxidized lyocell fibers of 17.6 times ($COOH_{mod}/COOH_{unmod}$) was obtained for the sample oxidized in the presence of 2.42 mmol NaClO/g fibers during 3 h.

The introduced COOH groups and other changes in lyocell fibers caused by TEMPO-oxidation influenced an increase of silver sorption. The amount of incorporated silver into TEMPO-oxidized lyocell fibers was in the range from 0.018 mmol/g fibers to 0.809 mmol/g fibers.

The TEMPO-oxidized lyocell fibers with incorporated silver showed good antibacterial activity against tested pathogens *Staphylococcus aureus* and *Escherichia coli*, while better antibacterial activity was shown against gram-positive strain *Staphylococcus aureus.*

5. ACKNOWLEDGMENT

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6. REFERENCE

[1] D. Hofer, *Current Problem Dermatology, In Biofunctional Textiles and the Skin*, Karger: Basel, 2006, 42–67.

[2] U.-C. Hipler, P. Elsner, J. Fluhr, *Current Problem Dermatology, In Biofunctional Textiles and the Skin*, Karger: Basel, 2006, 165.

[3] A.B.G. Lansdown, *Silver in Health Care: Antimicrobial Effects and Safety in Use*, *In Biofunctional Textiles and the Skin*, Karger, Basel, 2006, 17–34.

[4] U. Wollina,; M. B. Abdel-Nase, S. Verma, *Current Problem Dermatology, In Biofunctional Textiles and the Skin,* Karger: Basel, 2006, 1.

[5] M. Heide, U. Mohring,; R. Hansel,; M. Stoll,; U. Wollina, B. Heinig, *Current Problem Dermatology, In Biofunctional Textiles and the Skin,* Karger: Basel, 2006; p 179.

[6] P. S. Chang & J. F. Robyt, *Oxidation of primary alcohol groups of naturally occurring polysaccharides with 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion*, Journal of Carbohydrate Chemistry, Vol. 15 (1996) 819–830.

[7] T. Saito, et al., *TEMPO-mediated oxidation of native cellulose: Microscopic analysis of fibrous fractions in the oxidized products,* Carbohydrate Polymers, Vol. 65 (2006) 435–440.

[8] P. A. White, *Lyocell: The production process and market development in regenerated cellulose fibres* Woodhead Publishing Ltd, Cambridge, England, 2001, 62–87

[9] S. Zikeli, *Current Problem Dermatology, In Biofunctional Textiles and the Skin*, Karger: Basel, 2006, 110.

[10] T. Saito, et al., *Distribution of carboxylate groups introduced into cotton linters by the TEMPO-mediated oxidation*, Carbohydrate Polymers, Vol. 61 (2005) 414–419.

[11] T.Saito & A. Isogai, *TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water insoluble fractions,* Biomacromolecules*,* Vol. 5 (2004) 1983–1989.

[12] L. Fras, et al., *Determination of Dissociable Groups in Natural and Regenerated Cellulose Fibers by Different Titration Methods,* Journal of Applied Polymer Science, Vol. 92, (2004) 3186–3195.

[13] M. Kostic, J. Praskalo, S. Dimitrijevic, M. Baljak, B. Pejic, P. Skundric, *Silver-Loaded Hemp Fibers With Antimicrobial Activity*, In Proceedings of 7th Annual Textile Conference by AUTEX – From Emerging Innovations to Global Business, 26–28 June, Finland 2007, 1267.

[14] Lj. Rajakovic, T. Djurkic, A.Peric-Grujic, *Analiticka hemija, Kvantitativna hemijska analiza, Hemijski praktikum (in Serbian)*, TMF Beograd, 1999, 192.

[15] B. G. Joiner, *Bioactive Fibers and Polymers*, American Chemical Society, Washington, DC, 2001, 201.

[16] C. Tahiri & M. R. Vignon, *TEMPO-Mediated Oxidation of Native Cellulose. The Effect of Oxidation Conditions on Chemical and Crystal Structures of the Water-Insoluble Fractions,* Cellulose, Vol. 7 (2000) 177–188.

[17] R. L. Davis & S. F. Etris, *The development and functions of silver in water purification and disease control,* Catalysis Today, Vol. 36 (1997) 107–114.

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АНТИБАКТЕРИЈСКА АКТИВНОСТ СЕЛЕКТИВНО ОКСИДИСАНИХ ВЛАКАНА ЛИОЦЕЛА

Сажетак: У овом раду је проучавана антибактеријска активност селективно оксидисаних влакана лиоцела са сорбованим сребром, према грам (+) и грам (-) патогеним микроорганизмима. Антибактеријска активност је постигнута инкорпорирањем сребра на модификована влакна лиоцела, хемисорпцијом из раствора сребронитрата. У циљу повећања сорпционих својстава влакна лиоцела су оксидисана помоћу 2,2´,6,6´-тетраметилпиперидин-1-окси радикала (ТЕМПО). Показан је утицај услова модификовања влакана на количину везаног сребра, а тиме и на степен антибактеријске активности. Максимална количина сорбованог сребра износила је 0,996 ммол/г влакна. Антибактеријска активност испитана је *in vitro* стандардним микробиолошким методама према патогеним микроорганизмима *Staphylococcus aureus* (ATCC 25923) и *Escherichia coli* (ATCC 25922). Резултати су показали бољу антибактеријску активност ТЕМПО-оксидисаних влакана лиоцела са честицама сребра према грам (+) бактерији *Staphylococcus aureus*.

Кључне речи: лиоцел, ТЕМПО-оксидација, сорпција сребра, антибактеријска активност.

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