

## ANTIBACTERIAL FIBERS BASED ON CELLULOSE AND CHITOSAN

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**Abstract:** Cellulose and chitin are the most abundant polysaccharides in nature. Chitin is the natural amino polysaccharide and is estimated to be produced annually almost as much as cellulose. These resources are renewable and inexhaustible if rationally utilised. Unique properties of chitin and chitosan (chitin derivative obtained by the deacetylation of chitin), such as antibacterial activity, biocompatibility, non-toxicity and bioresorptivity make these materials very suitable and important biomaterials. During our studies, we examined the possibilities of obtaining composite, biologically active cellulose-chitosan fibres. An effective two-stage procedure for obtaining antibacterial fibers based on cellulose and chitosan was developed. The first stage involves the formation of dialdehyde cellulose by potassium periodate oxidation of cellulose fibers, which is able to form Schiff's base with chitosan. In the second stage, chitosan coated cellulose fibers were prepared by subsequent treatment of oxidized cellulose fibers with a solution of chitosan in aqueous acetic acid. Maximum percentage of chitosan introduced into/onto the cellulose fibers was 0,51 % (w/w). Antibacterial activity of cellulose fibers coated with chitosan as the active component against bacteria *Escherichia coli* and *Staphylococcus aureus*, was confirmed by *in vitro* experiments.

**Keywords:** cellulose, chitosan, potassium periodate oxidation, antibacterial fibers.

### 1. INTRODUCTION

Cellulose is the most abundant natural polysaccharide and the most important component in plants that makes about 50% of biomass [1]. Amino polysaccharide chitin is the main component of exoskeleton of crustaceans and insects and the most immanent organic component in nature after cellulose. Today, shells of marine crustaceans (crabs, shrimps, lobsters) which represent the waste after the separation of eatable parts are most frequently used as commercial sources of chitin. Great potential source of chitin is zooplankton, especially antarctic krill. Since they are formed by biosynthesis in enzyme-catalyzed reaction, cellulose and chitin represent an inexhaustible source of raw material for chemical processing and obtaining new kinds of materials.

Primary structure of chitin is similar to that of cellulose and can be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group. Even though it has been neglected for a long time, its annual production is estimated to soon reach the production of cellulose [2]. Chitosan,  $\beta$ -(1-

4) linked 2-amino-2-deoxy- $\beta$ -D-glucopyranose, is a N-deacetylated derivative of chitin obtained by transforming the acetamido groups into primary amino groups. However, deacetylation of chitin is almost never complete and chitosan or deacetylated chitin still contains acetamido groups to some extent. Unlike cellulose, chitin and chitosan contain 5-8% nitrogen, which in chitin is in form of acetylated amino groups and in chitosan in form of primary aliphatic amino groups, which makes chitin and chitosan suitable for typical reactions of amines. The presence of amino groups in chitin and chitosan represents a great advantage because it enables distinctive biological functions as well as the application of modification reactions [2,3]. Excellent properties of these polysaccharides, such as biocompatibility, biodegradability, bioactivity, bioresorptivity, non-toxicity and good adsorption properties make these materials very suitable and important biomaterials and attract great industrial interest as possible substitutes for synthetic polymers [4,5].

In recent years, the number of diseases and clinical infections caused by microorganisms has increased, which led to an intense research of new

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natural fibres that would at the same time ensure permanent biological activity and complete security for a user [6]. Chitin and its derivatives have found a growing use in medicine. Recent studies have shown that chitosan can be used very successfully for the inhibition of bacterial growth, whereby antimicrobial characteristics of chitosan depend on its molecular mass, the degree of deacetylation and the type of bacterium [7, 8].

Since chitin and chitosan fibers, due to spontaneous crystallization of the rigid or partly rigid polymer chains, are distinctly brittle and crumble easily, they are not yet commercially produced on a mass scale worldwide. One way to increase their flexibility is to modify them with other polymers such as cellulose which has good physico-mechanical properties [9–11]. Functionalization of cellulose fibres additionally expands the potential use of this polymer. An extraordinary example of selective modification of cellulose is presented by periodate oxidation [12,13]. Oxidation with periodates opens the pyranose ring and leads to the introduction of aldehydes at both C-2 and C-3 positions. 2,3-dialdehyde cellulose (DAC) obtained in this way can be used for immobilisation of proteins, dyes or for reaction of other substances with its amino groups, or for exchange of ions after further oxidation of aldehydes to the corresponding carboxylic acid. A large number of studies [14,15] aim at obtaining antibacterial fibres based on cellulose and chitosan, with the use of cotton fibres as cellulose component, while there are no studies for obtaining antibacterial fibres with the use of chemical cellulose fibres as cellulose component. In this study lyocell fibres were used as cellulose component, because they are more homogenous in structure and properties than cotton, they have better sorption properties and because, among chemical cellulose fibres, they stand out for their unique properties such as very high strength in comparison to other chemical cellulose fibres, high crystallinity, specific brilliance and handle and excellent physiological properties.

Regarding the already mentioned, the aim of this study was to obtain two types of bicomponent biologically active fibers based on cellulose-chitosan composite, with chitosan as active component and to compare their properties. For obtaining composite fiber type fibril-matrix we used cellulose solutions from hemp fibre and solutions of commercially avail-

able chitosan in N-methylmorpholine-N-oxide (NMMO) as a common dissolving agent, which were shaped into cellulose-chitosan composite fibres. For obtaining fibre-type cover-core we applied a two stage procedure which includes oxidation of lyocell fibres with potassium periodate, followed by processing of oxidized fibres with the solution of chitosan in aqueous acetic acid. In this way, the fibers based on cellulose and hitizana type cover-core without the use of synthetic binders are obtained, which have shown antibacterial properties against bacteria *Escherichia coli* and *Staphylococcus aureus*.

## 2. EXPERIMENTAL

### 2.1. Materials

In this study, two samples of chitosan, manufactured by Aldrich (USA), were used: a sample of chitosan denoted as H<sub>1</sub> with Brookfield viscosity > 200 cP and deacetylation degree (DD) > 85% and chitosan denoted as H<sub>2</sub> with Brookfield viscosity > 800 cP and deacetylation degree (DD) > 75%. Some physicochemical characteristics (moisture, nitrogen and ash content, viscosimetric average molecular weight - M<sub>v</sub> and deacetylation degree-DD) of chitosan samples were precisely determined and summarized in Table 1.

The characteristics of cellulose obtained from short hemp fibers, in which noncellulose impurities were partially removed by pre-treatment, are shown in Table 2.

N-Methylmorpholine-N-oxide monohydrate (NMMOmh), melting point T<sub>i</sub>=71-73°C, molecular mass Mr=135, 16 g/mol, manufacturer Fluka, Switzerland.

Lyocell fibers (fineness: 1.3 dtex, length: 38 mm; without spin finishing) were obtained from Lenzing AG, Austria.

*Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), nutrient agar and tryptone were supplied by Virology and Immunology Institute “Torlak”, Belgrade.

All chemicals used for the following investigations were of analytical grade.

Table 1. Some physicochemical characteristics of used chitosan samples

Sample	Moisture (%)	Nitrogen (%)	Ash (%)	Viscosity (cP)	M <sub>v</sub> (Da)	DD (%)
H <sub>1</sub>	10,79	7,46	0,76	343,3	252 073,77	87,2
H <sub>2</sub>	10,58	7,35	0,29	1 042,9	366 204,17	76,5

Table 2. The characteristics of cellulose from short hemp fibers

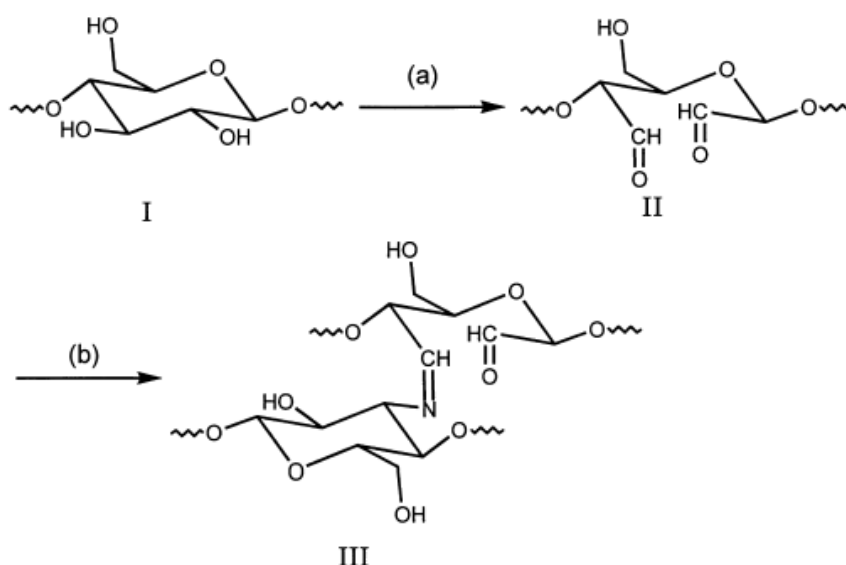
$\alpha$ - Cellulose content (%)	SP	Hemicelluloses (%)	Lignin (%)	Relative humidity (%)
91,12	1100	1,93	1,30	4,5

## 2.2. Method of obtaining the fibril-matrix type of fiber

The formation of composite fibers cellulose-chitosan, a fibril-matrix type of fiber, was performed according to the dry-wet procedure. Fibres were formed by pressing the solution of cellulose/chitosan mixture in NMMO at 120°C through a nozzle with an orifice of 0.3 mm.

## 2.3. Method of obtaining the cover-core type of fiber

For bicomponent fibres based on cellulose and chitosan type cover-core, an effective two-stage method was developed. The preparation process of bicomponent fibers is summarized in Scheme 1:



Scheme 1. Obtaining bicomponent fibers type cover-core

The first stage (a) involves oxidation of lyocell fibers with potassium periodate ( $\text{KIO}_4$ ). During oxidation, in glucose cellulose units (I) breaking of the connection between C-2 and C-3 atoms occurs, whereby 2, 3 dialdehyde cellulose is developed (II). The resulting aldehyde group on the cellulose fiber has an ability to couple with an amino group of chitosan. In the second stage (b), coating of chitosan on oxidized lyocell fibers occurs. By treating the fibers with the solution of chitosan in aqueous acetic acid, chitosan's free amino group reacts with the aldehyde group of cellulose and gives corresponding Schiff's base with a high degree of substitution, whereby bicomponent cellulose fibers coated with chitosan are obtained (III).

### 2.3.1. Oxidation of lyocell fibers with potassium periodate ( $\text{KIO}_4$ )

A sample of lyocell fibers was immersed in solutions of potassium periodate in 0.1 M acetic buf-

fer (ratio 1:50, w/v) at concentrations of 2,0 and 4,0 mg/ml, i.e. 0,2% and 0,4%, w/v. The mixture was stirred in the absence of light, at pH 4 and room temperature, for 15, 30, 45, 60, 120, 180, 300 and 360 minutes. After completion of oxidation, the lyocell fibers were washed with distil ice-cold water several times to remove the oxidant. These oxidized fibers were used for chitosan coating without drying.

The effects of oxidation reaction of cellulose fibres with potassium periodate was studied through the consumption of periodate during the reaction. Iodometric titration was used to calculate the periodate consumption. In 20 ml of periodate solution, we added 10 ml of 10% potassium-iodine and 10 ml of 0.5 M  $\text{H}_2\text{SO}_4$ . Freed iodine is titrated with 0.01 M  $\text{Na}_2\text{S}_2\text{O}_3$  with starch as an indicator. The reduction of periodate content in the solution, expressed through the number of periodate molecules in 100 molecules of glucose, will hereinafter be referred to as periodate consumption [12].

### 2.3.2. Coating of chitosan onto the oxidized lyocell fibres

The above-mentioned oxidized lyocell fibers were immersed in chitosan solution with stirring for up to 6 h, at 60°C. Chitosan solution was prepared in this manner: chitosan (8.0 g) was placed in 100 ml 2% (v/v) aqueous acetic acid solution for 1 h swelling and then 300 ml acetic acid solution was added in the dispersion of chitosan and the dispersion was stirred during 1 h at 60°C. After the treatment, the fibers were washed with deionized water for several times, and soaked in deionized water (400 ml) for 20 h at ambient temperature. The resulting fibers were dried at 60°C during 6 h to produce the composite lyocell-chitosan fiber.

### 2.4. Determining the copper number and the content of carbonyl groups

Copper number of starting and periodate oxidized lyocell fibers, as a measure of the carbonyl group content, was determined according to the standard method SRPS H.N132 [16]. This method is based on the ability of cellulose to reduce the divalent copper into monovalent copper in alkaline medium. A linear relationship between the carbonyl group's content and copper number (Cu) reported by [17], was used to convert the experimentally measured copper number of the oxidized lyocell fibers in carbonyl group content as shown in equation:

$$\text{Carbonyl group's group content} \\ (\mu\text{mol/g}) = (\text{Cu} - 0,07)/0,06 \quad (1)$$

### 2.5. Chitosan content in the cellulose - chitosan composite fibers

The chitosan content in the composite fibers was calculated on the basis of nitrogen percentage and the calibration curve for the weight of chitosan and solution of HCl consumed in titration. The nitrogen content in the chitosan and cellulose-chitosan fibers was determined according to the standard method SRPS-ISO 937: 1992 [18].

### 2.6. Scanning electron microscopy (SEM) analysis

Scanning electron microscope (SEM) photographs were taken on a FE-SEM JEOL JSM-6330 F (Japan) to study the morphological characteristics of the fibres. Emission stream current during the measurement was 2μV after sputtering the samples with gold.

### 2.7. Infrared spectroscopy

Infrared spectra were recorded by using a BOMEM Hartman&Brayn MB-Series spectrophotometer in the wavenumber region of 4000-400 cm<sup>-1</sup>. The samples were prepared in a form of pellet, mixing the grained fibres and potassium bromide.

### 2.8. Assays for antibacterial activity

Gram-negative bacteria *Escherichia coli* ATCC 25922 and Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 were used to analyze the antibacterial activity of chitosan-coated lyocell fibers. Antibacterial activity of chitosan-coated lyocell fibers was assayed as follows: each of the bacterium (10<sup>5</sup>-10<sup>6</sup> log N/ml) was inoculated by 9 ml sterile potassium hydrogen phosphate buffer solution (pH 7.2) at 37°C for 24 h. Then the samples of modified lyocell fibers were added in solution and incubated at 37°C for 24 h, 48 h, 5 days and 15 days. Viable cells (log N/ml) were enumerated on TSA agar by pour plating 1 ml of serial dilutions of physiological solution followed by incubation at 37°C for 48 h. The grown colonies were counted, provided that each cell has given one colony. The percentage of bacteria reduction can be calculated by using the following equation:

$$R (\%) = (A-B) \times 100/A \quad (2)$$

where *A* and *B* are the bacteria amount per milliliter for the control (starting lyocell fibers) and chitosan coated lyocell fibers test samples, respectively. In this way, with direct comparing of the reference material with the treated sample, it is always possible to record the direct effect of antimicrobial treatment, because external factors (e.g. supply of nutrients) can be, to a large extent, excluded and, due to the characteristics of the sample and the reference materials, it can be assumed that any potential growth pattern will be the same [19].

## 3. RESULTS AND DISCUSSION

### 3.1. Obtaining and properties of fibres type fibril-matrix

During spinning of the composite fibres type fibril-matrix from the mixture of cellulose-chitosan solutions in NMMO, problems related to dissolving the chitosan and gelling the solution occurred. This caused frequent interruptions of polymer stream line; therefore, it was impossible to spin out a larger amount (length) of fibre. Obtained fibres, regardless of the ratio of the polymers, had a smooth surface

without any structural details and approximately round cross-section. Fineness and tensile strength of the obtained fibres were very uneven. The diameter of the fibres obtained from mixtures of cellulose and chitosan solutions in NMMO (concentrations 1 – 10 %) ranged from 10 µm to 200 µm. Even though these obtained fibres had poor and uneven physical-mechanical properties, they have shown good sorption and swelling characteristics, table 3.

Table 3. Swelling of the cellulose, chitosan and bicomponent cellulose-chitosan fibres in the distilled water (approx. fineness of fibres 3 dtex)

Time of swelling (min)	Swelling of the fibres (%)				
	Cellulose	Chitosan	Cellulose-chitosan ratio in the spinning solution		
			95:5	90:10	5:95
10	22,3	11,7	23,5	29,4	20,5
20	29,5	18,1	29,5	32,3	25,8
30	30,5	25,5	33,2	38,6	34,2
40	31,2	29,7	38,8	42,4	40,4
50	31,2	32,9	40,2	46,2	42,2
60	31,2	36,2	40,2	46,3	42,5

Sorption characteristics, as well as the swelling characteristics of bicomponent fibres were enhanced in comparison to the “pure” cellulose or chitosan fibres. The best results were achieved for the cellulose-chitosan ratio 90:10 for the examined times of swelling. With the cellulose-chitosan ratio 95:5 better results of swelling were noticed for shorter periods (up to 30 minutes) in comparison to the composite of cellulose-chitosan ratio 5:95 which has shown better results of swelling for longer periods (above 30 minutes). The same results can be noticed with cellulose and chitosan as well. This can be related to poor hydrophilic characteristics of the surface of chitosan which are shown in the initial period, after which its sorption properties and swelling are enhanced, while, with cellulose, the opposite occurs. After 50 minutes of swelling, the equilibrium state after which there are not any significant changes is maintained.

It is indicated in the study [20] that structural inhomogeneity of composite films shows as a consequence strong sorption properties, for example towards water. Extreme swelling of cellulose-chitosan films, caused by a high degree of its structural inhomogeneity, is in correlation with its reduced strength. The results obtained during this study confirm this relation in the examined cellulose-chitosan fibres. In the study [9], the effects of cellulose-chitosan composite on the mechanical characteristics of obtained fibres were tested. It is stated that

adding chitin (in smaller amounts) to cellulose can reduce molecular interactions on the interface of the structural elements, because acetamide groups of chitin will disturb steric effects of cellulose macromolecules. This can be seen as an explanation for the enhanced swelling of bicomponent fibres based on cellulose and chitosan, since because of the reduced molecular interactions on the interface of the structural elements, active groups will not be blocked by intermolecular bonds and will remain free and available to agents, which further affects enhanced swelling.

Because the obtained fibres type fibril-matrix had poor and uneven physical-mechanical properties, they were not suitable for conducting research as planned, so further research was directed towards obtaining antibacterial fibres type cover-core with cellulose lyocell fibre as the core and chitosan as the cover.

### 3.2. Obtaining and properties of the fibres type cover-core

#### 3.2.1. Obtaining the dialdehyde cellulose fibers

The first stage of obtaining biologically active cellulose-chitosan fibres type fibril-matrix involves the formation of 2,3-dialdehyde cellulose (DAC) by the potassium periodate oxidation of lyocell fibers. The effects of periodate oxidation on lyocell fibers were initially assessed by determining the periodate consumption, the carbonyl group content, weight loss and IC spectrum.

The rate of periodate consumption during the oxidation of lyocell fibers with 0.2% and 0.4% potassium periodate solution is shown in Table 4. The curves illustrating the course of the reaction for 0.2% and 0.4% KIO<sub>4</sub> show two distinct phases. For both solution concentrations the rate of periodate consumption is relatively high at first, but soon it decreases and becomes almost constant. Calvini et al. [21] considered much larger time frame (up to 264 h) and divided the reaction course into three distinct phases: fast initial phase with  $t_{1/2} = 120$  min, followed by a slower second reaction with  $t_{1/2} = 20$  h, and third phase, i.e. the process of stopping the reaction which includes the oxidation of the inner core of the crystalline regions with  $t_{1/2} = 36$  days. The first reaction phase is the fast process involving easy access to a portion of the molecule. According to Nevell [12] the initial fast rate of periodate consumption may be identified with complex formation. The oxidation reaction is thought to proceed via a cyclic diester of periodate with vicinal hydroxyls, which subsequently undergoes an intermolecular

redox process with C-C bond breaking according to a related mechanism.

As a result of periodate oxidation, lyocell fibers exhibited an increase in carbonyl group content ranging from 87% to 479% with the increase of oxidation time and concentration of  $\text{KIO}_4$ , Fig. 1a. During the first 60 minutes, there are no significant differences in carbonyl group content in the fibers oxidized with different concentration of  $\text{KIO}_4$ , the values are very similar. Lyocell fibers oxidized with 0.4%  $\text{KIO}_4$  from 60 to 300 min had higher carbonyl group content compared to the fibers oxidized with 0.2%  $\text{KIO}_4$ . And finally, lyocell fibers oxidized with 0.2% and 0.4%  $\text{KIO}_4$ , during 360 min had similar carbonyl group content with the different periodate consumption. This can be explained by the fact that cellulose chain-molecules containing oxidized units

are susceptible to scission, whereby new end-groups and soluble fragments are produced. The evidence of new end-groups formation can be obtained by comparing carbonyl group content against periodate consumption (Table 4 and Fig 1a.), where we can see a higher increase in carbonyl group content in relation to periodate consumption, especially in the case of higher periodate concentrations. The formation of soluble fragments during the oxidation can be demonstrated by measuring the weight loss of oxidized lyocell fibers, Fig. 1b. The weight loss of oxidized lyocell fibers is much higher for the higher periodate concentrations (0.4%  $\text{KIO}_4$ ), with values up to ~ 21%, while for the lower periodate concentration (0,2%  $\text{KIO}_4$ ) these values are in the range from 3.2% to 8.6%.

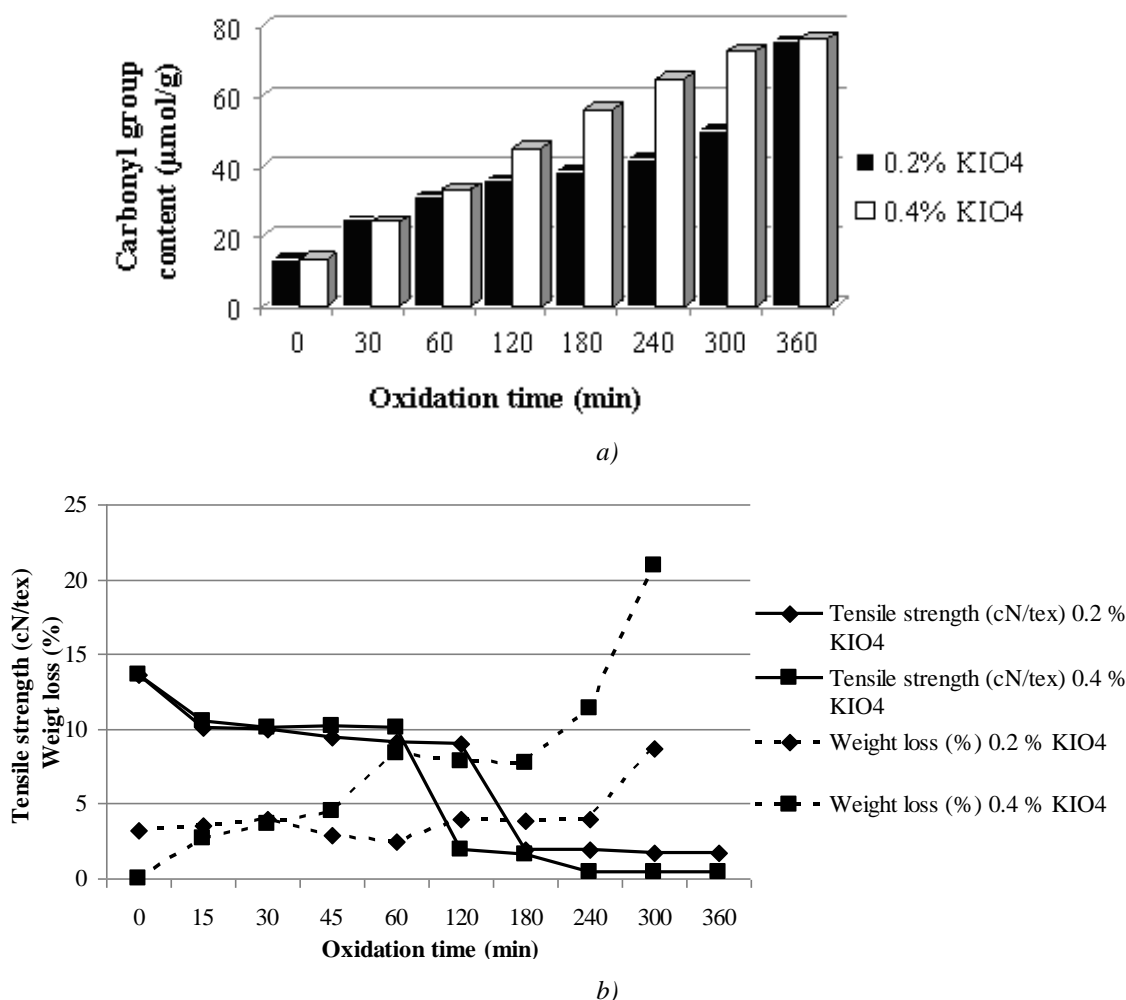


Figure 1. The dependence between carbonyl group content (a), weight loss and tensile strength of oxidized lyocell fibres (b) and oxidation time and concentration of  $\text{KIO}_4$  used for oxidation

The oxidation process has a significant influence on the physical-mechanical properties of oxidized lyocell fibers. As it could be seen from Fig. 1b, tensile strength of the oxidized lyocell fibers

decreased with increasing the oxidation time and concentration of  $\text{KIO}_4$ . The tensile strength of the oxidized lyocell fibers did not change remarkably for the oxidation time in the range of 0-60 min.

However, it significantly decreased when the oxidation time was over 60 min, probably due to the destruction of the crystalline structure of cellulose, while at the same time the risk of weight loss of the oxidized fibers is increased (see Fig. 1b). It is well known from the literature [22, 23] that periodate attacks the crystalline regions of cellulose already at low degrees of oxidation, which affects its chemical and physical properties.

Table 4. The dependence between periodate consumption and oxidation time and concentration of  $KIO_4$  used for oxidation

Oxidation time (min)	Periodate consumption (molecules/100 g glucose units)	
	0.2 % $KIO_4$	0.4 % $KIO_4$
15	0.0344	0.0291
30	0.0411	0.0163
45	0.0361	0.0517
60	0.0444	0.0551
125	0.0688	0.0751
180	0.0741	0.0951
240	0.0740	0.1094
300	0.0755	0.1254
360	0.1031	0.1257

### 3.2.2. Coating of chitosan on oxidized lyocell fibres

Coating of chitosan on oxidized fibres represents a second stage in obtaining the fibres type cover-core. The dependence between the content of chitosan in the obtained bicomponent fibres and the time of immersion of the oxidized fibres in chitosan coating solution, as well as the time of oxidation of starting fibres are shown in Figures 2 and 3. As shown in Figure 2, the amount of incorporated chitosan is increased with the time of processing of oxidized fibres in the chitosan solution from 0-120 minutes. When the time of immersion was over 120 min, the chitosan content became nearly constant at 0.45% for fibres oxidized by 0.2%  $KIO_4$  during 60 minutes. Therefore, chitosan coating time of 120 minutes was chosen for further investigations.

The aldehyde groups content of the oxycellulose reflects not only the oxidative extent of the cellulose fibers oxidized by periodate but also the extent of incorporated chitosan. The amount of incorporated chitosan increased with the oxidation time during the initial stage (60 minutes), while it decreased with longer oxidation time, Fig. 3. The chitosan content was higher for fibers oxidized by

0.2%  $KIO_4$  in relation to fibers oxidized by 0.4%  $KIO_4$ , and for fibers immersed in the solution of chitosan with higher molecular weight ( $H_2$ ) in relation to solution of chitosan with lower molecular weight ( $H_1$ ). The maximum amount of fixed chitosan was 0.51% of the weight of fibers and it was determined for the sample oxidized with 0.2 %  $KIO_4$  during 60 minutes and coated with chitosan  $H_2$ . When the reaction time of oxidant was over 180 min, the chitosan content became nearly constant, regardless of the content of the carbonyl groups. This phenomenon can be explained by considering the differences in the ways in which the oxidation takes place and in formation of Schiff's base in the cellulose [11,23]. During the oxidation, small periodate ions are able to enter the interior of cellulose fiber, so the glucose units inside and on the surface of the cellulose fiber can be oxidized. On the other hand, a huge chitosan molecule cannot access aldehyde groups formed in small pores of the fiber. Furthermore, a single chitosan molecule can react with many aldehyde groups. All this affects the limitation of fixed chitosan.

Structural changes in the fibres occurred during the oxidation and coating can be followed via IC spectrum, Figure 4. The occurrence of the peak on the IC spectrum of the oxidized lyocell fibre at about  $1730\text{ cm}^{-1}$ , suggests an increase of the carbonyl group content as well as the aldehyde groups. According to the study [11], it is suggested that the change at  $1726\text{ cm}^{-1}$  on the spectrum of the oxidized cellulose agrees with the vibration  $C=O$  of the double aldehyde group. In the study [10], where cellulose oxidation also occurred, the increase in the intensity of tape at  $1729\text{ cm}^{-1}$  is stated as the evidence for the increase in the content of the carbonyl groups. The change in the intensity of the spectrum at  $1700$  [14] and  $1740\text{ cm}^{-1}$  [24] also suggests the change in the content of the carbonyl groups.

The intensity of the tape is changed at about  $1630\text{ cm}^{-1}$  with bicomponent fibres in relation to the oxidized fibres, which agrees with incorporating the chitosan to the fibres. The study [15] states that the peak at  $1640\text{ cm}^{-1}$  could also suggest incorporation of chitosan. Besides that, the literature [11] states that after treating oxidized cellulose with chitosan, the distinctive peak on the absorption tape shifts towards  $1716\text{ cm}^{-1}$ , which is characteristic for the  $C=N$  double bond, which further suggests that the Schiff's base is formed by reaction between aldehyde group of the oxidized cellulose and the amino group of chitosan. Another study [10] suggests that after treating oxidized cellulose with chitosan, the absorption tape appears at about  $1628\text{ cm}^{-1}$  on the IC spectrum, which is typical for the amino groups.

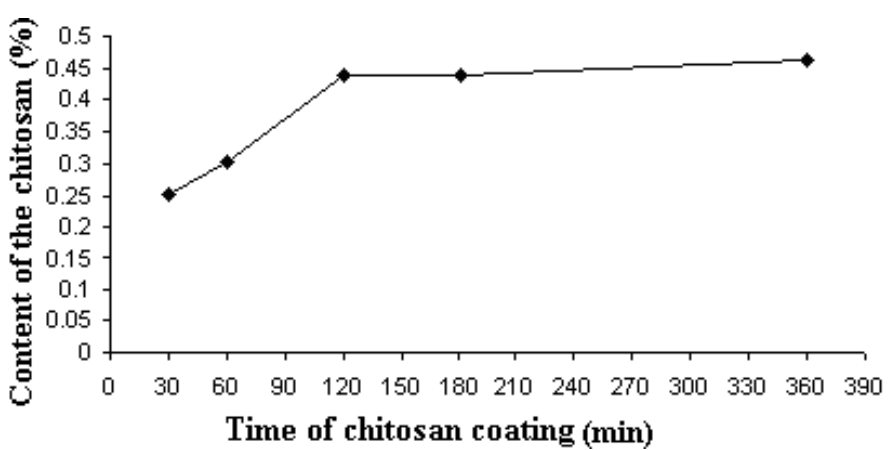


Figure 2. The dependence between content of the  $H_1$  chitosan incorporated into the oxidized fibres (the conditions of oxidation: 0.2%  $KIO_4$ , 60 min) and the time of chitosan coating

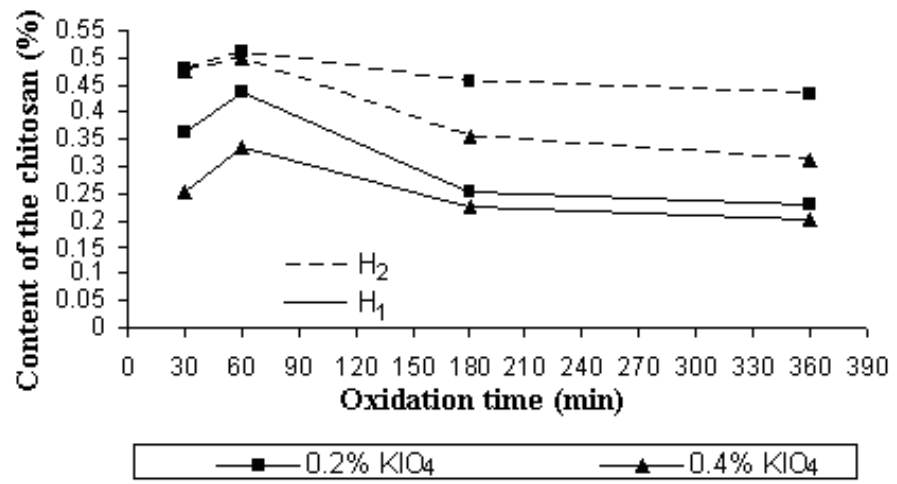


Figure 3. The dependence of content of chitosan incorporated into the fibres and molecular mass of chitosan and the time of oxidation of the starting fibres with  $KIO_4$  (conditions of chitosan coating: 120 minutes, 60°C)

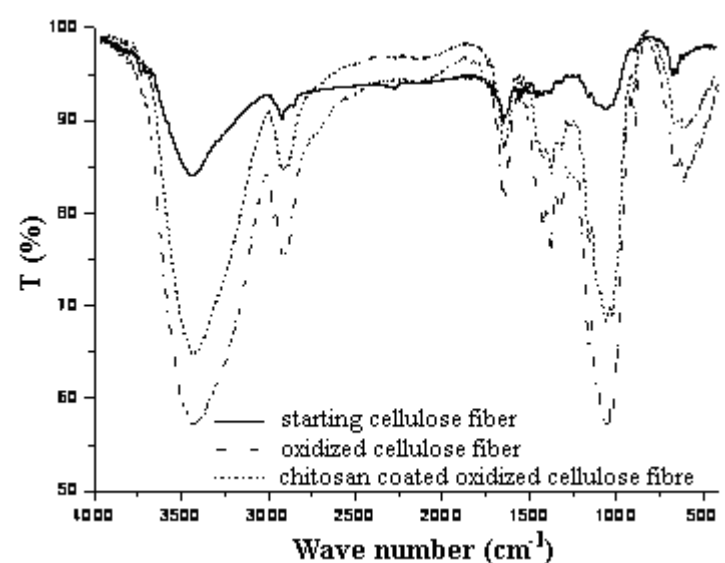


Figure 4. IR spectrum of the starting, the oxidized and the chitosan coated oxidized cellulose (lyocell) fibre

The coating of oxidized lyocell fibers with chitosan has a significant influence on physico-mechanical properties of the obtained bicomponent fibers. As it can be seen from Table 5, tensile



strength of several tested chitosan coated lyocell fibers is lower in relation to the starting lyocell fibers, but it is higher in comparison to the oxidized fiber. Here, chitosan acts as a coupling agent to bind fibrils to the fiber's "body" and fills in the roughness in the surface made during the oxidation and thereby enhances the fiber's mechanical properties. These can be seen on SEM images of the surface of the oxidized, and the surface of the oxidized and chito-

san coated lyocell fiber, Fig. 5. The surface of the oxidized fiber is very rough, which is caused by oxidation (Figure 5.b), while the surface of chitosan-coated lyocell fiber is very smooth, indicating that after treatment with chitosan, the surface of fibers was covered with a layer of chitosan. Also small grains, which are agglomerates of chitosan, can be seen on the surface of chitosan-coated lyocell fibers as shown in Fig.5.c.

Table 5. Tensile strength of oxidized and chitosan-coated lyocell fibres

Concentration of KIO <sub>4</sub> (%)	Oxidation time (min)	Chitosan coated	Tensile strength (cN/tex)	CV* (%)
Starting lyocell fibres	0	0	27,32	11,49
0,2% KIO <sub>4</sub>	30	--	20,02	15,82
		H <sub>2</sub>	23,24	11,94
	180	--	3,96	16,61
		H <sub>2</sub>	19,70	11,65
0,4% KIO <sub>4</sub>	60	--	20,10	9,68
		H <sub>2</sub>	19,86	16,31

\* CV- coefficient of variation

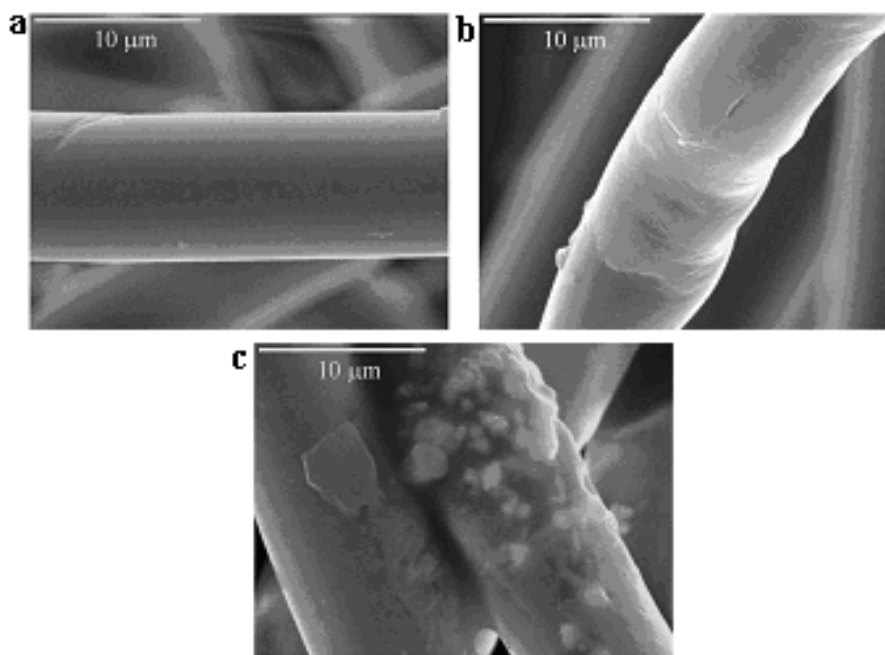


Figure 5. SEM images of the surface: the starting lyocell fiber (a), the oxidized lyocell fiber (b) and the oxidized and chitosan H<sub>2</sub> coated lyocell fiber (c)

### 3.2.3. Antibacterial activity of the chitosan coated lyocell fibres

The results of this study, shown in the Table 6, indicate that chitosan-coated lyocell fibres significantly inhibit the growth of tested bacteria. Chitosan-coated lyocell fibres generally show stronger bacteriacidal effects towards gram-positive bacteria *Staphylococcus aureus* than towards gram-negative bacteria *Escherichia coli*, which is in agree-

ment with several studies [8, 25]. The most effective were chitosan-coated lyocell fibers with the chitosan content above 0.35%, whereby samples denoted as L60-0.4-2, L30-0.2-2 and L30-0.2-1 were the most effective for the gram-positive bacteria *Staphylococcus aureus*, while for gram-negative bacteria *E. coli*, sample denoted as L30-0.2-1 was the most effective. The results indicate that the lyocell fibers coated with both chitosan H<sub>1</sub> and chitosan H<sub>2</sub> show activities against *S. aureus* and *E. coli*

bacteria, while chitosan with higher molecular weight ( $H_2$ ) is more effective than chitosan with a lower molecular weight ( $H_1$ ), particularly for samples L60-0.4-2, and L30-0.2-2. Chitosan with the higher molecular weight has a tendency to be adsorbed on the surface of the fiber and because of its large molecules it only slightly penetrates into the fibre. The chitosan amino groups are more accessi-

ble and able to react with the microorganisms, which is in agreement with the results obtained in the literature [15]. Samples of oxidized and chitosan-coated fibres denoted as L360-0.2-2, and L360-0.2-1, show very low or hardly any antibacterial activity (sample L360-0.2-1 against *E. coli*). Products that have negative influence on the antibacterial activity occur here, probably due to the long oxidation time.

Table 6. Reduction of bacteria (R, %) achieved by chitosan coated lyocell fibres

Sample	Oxidation with $KIO_4$		Chitosan type. <sup>a)</sup>	<i>S. aureus</i> <sup>b)</sup>				<i>E. coli</i> <sup>c)</sup>			
	Time (min)	Conc. (%)		Incubation period				Incubation period			
				24 h	48 h	5 days	15 days	24 h	48 h	5 days	15 days
L0	0	0	--	0	0	0	0	0	0	0	0
L60-0,4-2	60	0,40	$H_2$	100	100	100	100	24,1	61,7	67,0	100
L60-0,4-1			$H_1$	5,8	18,2	14,3	0	1,3	16,6	16,6	0
L30-0,2-2	30	0,20	$H_2$	100	100	100	100	22,2	43,5	100	100
L30-0,2-1			$H_1$	100	100	100	100	100	100	100	100
L360-0,2-2	360	0,20	$H_2$	35,9	3,6	6,7	0	1,6	7,8	0	0
L360-0,2-1			$H_1$	40,7	30,4	11,0	2,3	0	0	0	0

L<sub>0</sub> – control sample (starting lyocell fibres without coated chitosan)

<sup>a)</sup> Chitosan coating conditions: 120 min, 60 °C

<sup>b)</sup> Starting number of bacteria 4.5 log N/ml

<sup>c)</sup> Starting number of bacteria 4.23 log N/ml

#### 4. CONCLUSION

Antibacterial fibres based on cellulose and chitosan type cover-core were obtained by two-stage procedure during which the lyocell fibres were oxidized by periodate to increase their aldehyde content or, to be more precise, to obtain dialdehyde cellulose, after which coating of chitosan on the oxidized fibre occurs. Aldehyde group of cellulose reacts with free amino groups of chitosan to form the Schiff's base, while the chitosan is incorporated into the fibres without using the synthetic binders. Unlike the fibril-matrix type of fibres, cover-core type of fibres showed good physical-mechanical properties and because of that, further analyses were focused on this type of fibre.

By increasing the concentration and the time of periodate activity, the degree of oxidation or aldehyde group content in the cellulose fibres increases. At the same time that leads to partial oxidized destruction of the cellulose and weakening of the tensile strength of the oxidized fibres in relation to the starting fibres. However, the tensile strength of oxidized and then chitosan-coated lyocell fibres is better in relation to the tensile strength of oxidized fibres, because chitosan acts as a binding component which joins the fibrils with the "bodies" of the fibre,

and it fills in any damage on the surface of the fibre caused by oxidation, thus strengthening their structure by creating cellulose-chitosan composite.

The amount of incorporated chitosan increased with the oxidation time during the initial stage (60 minutes), while it decreased with longer oxidation time regardless of the increase in the number of aldehyde groups. In addition, the amount of incorporated chitosan increased with the time of immersion in the chitosan solution (coating time) during the first 120 min, and then became nearly constant.

Chitosan-coated cellulose fibres significantly inhibit the growth of tested bacteria. They show stronger bacteriicidal effect towards gram-positive bacteria *S. aureus* than towards gram-negative bacteria *E. coli*. Fibres coated with chitosan with higher molecular mass ( $H_2$ ) have shown better bacteriicidal effects in relation to the fibres coated with chitosan with lower molecular mass ( $H_1$ ).

The results obtained by this study expand the potential manufacturing area and the usage of cellulose and chitosan in bicomponent fibres production as well as in the production of other antimicrobial products based on natural polymers.

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## АНТИБАКТЕРИЈСКА ВЛАКНА НА БАЗИ ЦЕЛУЛОЗЕ И ХИТОЗАНА

**Сажетак:** Целулоза и хитин су најраспрострањенији полисахариди у природи. Хитин је природни аминополисахарид и процјењује се да ће његова годишња производња достићи производњу целулозе. Ови ресурси су обновљиви и неисцрпни уколико се рационално користе. Јединствена својства хитина и хитозана (дериват добијен деацетиловањем хитина), као што су антибактеријска активност, биокомпатибилност, нетоксичност и биоресорптивност чине ове материјале веома погодним и важним биоматеријалима. Током нашег рада, истраживали смо могућности добијања композитних, биолошки активних влакана целулоза – хитозан. Развијен је ефикасан двостепени поступак за добијање антибактеријских влакана на бази целулозе и хитозана. Први степен обухвата оксидацију целулозних влакана са калијум-перјодатом, при чему настаје диалдехидна целулоза, која је способна да формира Шифову базу са хитозаном. У другом степену су, обрадом оксидисаних целулозних влакана с раствором хитозана у сирћетној киселини, добијена целулозна влакна наслојена хитозаном. Максимална количина хитозана уведена у/на целулозна влакна износила је 0,51 %, мас. Антибактеријска активност целулозних влакана наслојених хитозаном, као активном компонентом, према бактеријама *Escherichia coli* и *Staphylococcus aureus*, потврђена је *in vitro* експериментима.

**Кључне ријечи:** целулоза, хитоза, оксидација калијум-перјодатом, антибактеријска влакна.

