

# BIOPOLYMER COMPOSITES BASED ON LIGNIN AND MICROCRYSTALLINE CHITOSAN

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## Abstract

*The article presents method of extracting lignin from hardwood and softwood after chemical treatment. Lignin fractions were extracted from black liquid (the by-product of pulp and paper industry), in a different reaction environment.*

*Biopolymer composites were obtained by combining extracted lignin fraction and microcrystalline chitosan, which can be used as preparations for innovative biopolymer materials that are applicable in medical and hygienic products. The studies were performed in order to evaluate MCCh/Lignin composites to suitability to construction of dressing materials in the form of sponges. The MCCh/Lignin composites were tested for different properties: mechanical, sorption and absorption.*

*The obtained lignin fractions and MCCh/Lignin composites were characterized by different structures and chemical purity as confirmed by FTIR spectra.*

**Key words:** *black liquid, lignin, microcrystalline chitosan, biopolymer composites, medical and hygienic products*

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## **1. Introduction**

At the present about 80% pulp manufactures kraft process is the main method of wood cooking in the world. During kraft process about half of the wood is dissolved and as the result of this process the black liquor contains inorganic chemicals and large quantities of organic materials, mainly lignin, hemicelluloses, poly-, oligo- and monosaccharides, and lignocellulosic fraction [1].

In order the maintain of profitability pulp and paper mills, producers have begun to research new opportunities to increase their income through the production not only pulp and paper as well as other biomaterials.

At the present also increased interest in renewable resources because all around the world expected shortages of access to oil and gas, and concerns about the accumulation of greenhouse gases in the atmosphere [1].

The concept of integrated forest products biorefinery now is interesting for many researchers who have decided to try to extract, hemicellulose and lignin from wood biomass and by-products from the pulp and paper industry [1]. The lignin is valuable product of the biorefining process of black liquor. The basic structural elements of lignin are phenylpropane derivatives. The main precursors of lignin are three monomers phenylpropanoids, namely p-coumaryl, coniferyl and sinapyl alcohols. The most common linkages in the lignin molecule are the  $\beta$ -O-4 ether linkages, followed by other types of ether and C-C linkages such as  $\alpha$ -O-4,  $\beta$ - $\beta$ .

Lignin is a polymolecular compounds with molecular weigh 1000-4000. Ability to lignin dissolve depends on the ability to form hydrogen bonds and depends on their molecular weight, structure. Reactivity and chemical properties of lignin depend on functional groups, ie.: -OCH<sub>3</sub> and OH and carbonyl groups [2,3].

Extracted polymer fractions from black liquor can be used in various industries. Lignin modification allows to obtain innovative polymeric materials with special properties (for example for medical applications or in plant protection products), lignin could be a substitute phenol in the production of plastic materials. Change structure of lignin can result for example in increase of content functional groups - OH - alcoholic or carbonyl and reduction of phenolic groups. The result is a polymer with completely new properties with a focus on thermoplastics or improvement of biodegradability in the case of a merger with other plastics [4-6].

It has been reported in literature that lignin, and its enzymatic products could be a material capable of forming complex with the chitosan. This results from the fact that lignin has a high negative charge, because of the presence of carboxyl groups in its structure, whereas the chitosan is polysaccharide with a cationic character. It was also found a possibility of formation of hydrogen bonds between hydroxyl and carbonyl groups in the lignin and between the amino groups from chitosan [7,8].

Occurring chemical interactions between the lignin and chitosan may promote the production of novel biopolymer materials such as micro- and nanostructures or fibroids, which are characterized by high thermal stability, mechanical strength, ability to controlled and adequate moisture sorption and antibacterial properties [8]. Accordingly, the materials made on the basis of the lignin-chitosan complex could find wide application in medical, hygiene products and in the packaging industry.

The aim of the study was to obtain lignin fraction from black liquor and production of biopolymer composites with participation of microcrystalline chitosan (MCCh) and isolated lignins, which are an ideal material for the construction of innovative biopolymer materials.

## 2. Materials and Methods

### 2.1. Materials

#### Black liquor

Black liquor (hardwood – HW and softwood – SW) by-product from pulp and paper industry was utilised for the purposes of this research.

Black liquor was obtained after wood chemical treatment (kraft pulping method).

#### Chitosan

Chitosan from Primex ehf. was used in the study. The characteristics of chitosan are: average molar mass ( $M_v$ ) = 373.0 kDa, deacetylation degree (DD) = 81.0 %, ash content – 0.31%, WRV=156.0%.

### 2.2. Preparation of microcrystalline chitosan (MCCh)

Microcrystalline chitosan (MCCh) was prepared by agglomeration from solution, by using a continuously method developed in Institute Biopolymer and Chemical Fibres (IBWCh) [9] with the use of a continuous reactor Dispax Reactor Labor-Pilot 2000/4 by IKA.

### 2.3. Extraction of lignin fraction

Lignin fractions were extracted from softwood and hardwood black liquor using sulphuric acid and sodium hydroxide. A defined amount of black liquor (30-50 cm<sup>3</sup>), was titrated with 1 N H<sub>2</sub>SO<sub>4</sub> to pH: 8.5; 8.0; 7.5; 7.0; 6.5; 6.0; 5.5; 5.0; 4.5, 4.0 and 2.0.

Next the resulting precipitate was centrifuged in a laboratory centrifuge at 500 G for 30 minutes. Fraction lignin and hemicellulose were separated. After centrifugation, precipitate was washed in 0.1 N H<sub>2</sub>SO<sub>4</sub> solution and centrifuged again. Next the solid was dissolved in 0.1 N NaOH solution and re-precipitated lignin by titration to pH-2 in 1 N H<sub>2</sub>SO<sub>4</sub> solution. The whole was again centrifuged and the resulting solid lignin was washed with 0.1 N H<sub>2</sub>SO<sub>4</sub> solution and centrifuged again. At the end the precipitate was washed with distilled water, centrifuged and air dried. The yield the lignin fraction was determined. The obtained lignin fractions were characterized by different structures and chemical purity as confirmed by FTIR spectra.

Extracting lignin fractions were based on the literature description [10].

### 2.4. Preparation of biopolymer composition

To prepare biopolymer composites, lignin fractions were added to 2,5% wt. suspension of microcrystalline chitosan in the form of aqueous solutions in a proportion of 5, 10, 15% wt. with respect to chitosan. Then to the polymer mixture was added glycerin in an amount of 0.5 part weights on 1 part weights of biocomposite (based on the dry weight of polymer). The mixture was homogenized for 10 to 15 min using a high speed stirrer type T 50 IKA at 2.000-3.000 r.p.m.

### 2.5. Method of preparation dressing materials in the form of sponges

The dressing material (lignin fractions and microcrystalline chitosan) was prepared in the form of a sponge (by drying techniques) in a laboratory lyophilizer ALFA Christ 1-4. Lyophilization was carried out in a temperature range of -20°C to 10°C and pressure of 0.1-0.7 mbar. Time of drying was 20-24 hours.

### 2.6. Determination of average molecular weight of chitosan

The average molecular weight of chitosan was determined on the basis of assessing the intrinsic viscosity. The Mark – Houwink equation was used to calculate the molecular weights; the constants  $K = 8.93 \times 10^{-4}$  and  $a = 0.71$  have been accepted.

The composition of the solvent: 4 M of urea, 0.2 M of acetic acid and 0.1 M of sodium chloride in 1000 cm<sup>3</sup> of distilled water.

Determination of average molecular weight of chitosan was made by gel chromatography SEC/GPC. The studies were carried out on a gel chromatograph HP-1050 (Hewlett - a Packard) equipped with refractive index detector HP 1047 and a set of columns of varying pore size of 300 Å and 4000 Å are filled in hard-filled based on silica gel. To develop the results of applied software: PL Caliber GPC Software (Polymer Laboratories Ltd.). Parameters of chromatographic analysis of chitosan:

- 1) column set: Plaquagel guard + 2x Plaquagel-OH Mixed (Polymer Laboratories)
- 2) temperature of columns : 30° C
- 3) mobile phase : 0.3M CH<sub>3</sub>COOH + 0.2 M CH<sub>3</sub>COONa + H<sub>2</sub>O
- 4) flow: 0.8 cm<sup>3</sup>/min
- 5) concentration of solution : 0.05%
- 6) volume of injection: 100µl

### **2.7. Determination of chitosan deacetylation degree**

Deacetylation degree of chitosan was determined by first derivative UV – spectrophotometry method. The 0.01 gram sample was dissolved in 10 ml of 0.1 mol/l aqueous acetic acid. The flask with solution is filled up to 100 ml with water. The UV absorption spectra was obtained using UV-VIS spectrophotometer. The contribution GlcNAc was obtained from calibration curve – plotting the maximum values of first derivatives against the corresponding GlcNAc concentration . The DD of sample was determined by formula.

### **2.8. Determination of ash content**

Ash content chitosan is determined using a weight method – burning a sample at temperature of 800°C.

### **2.9. Determination of chitosan in the preparation**

Polymer content of chitosan was determined gravimetrically after drying (MCCh) or regeneration (modified chitosan salt) and next MCCh was dried to constant weight.

### **2.10. Determination of the water retention value (WRV)**

The water retention value of chitosan was determined gravimetrically based on the equation:

$$WRV = [(m_1 - m_0) : m_0] \times 100\%$$

where:

$m_1$  - weight sample of chitosan after storage in water for 20 hours and centrifugation (4000 rev/min) for 10 min

$m_0$  - weight sample of chitosan after drying at 105°C.

### **2.11. Determination of sorption dressing materials in the form of sponges**

The sorption of dressing materials was determined by weighted method. The sponge was placed in container with demineralized water and after specified time (0.17 h, 0.5 h, 3.0 h, 5.0 h and 24.0 h) sample were removed from the bath, next dried surface of sample and weighted. The ability of sorption biocomposites was expressed as sorption index [11].

The sorption index was calculated by the equation:

$$W(\%) = [(Mw - Ma) \times 100] : Ma$$

where:

W – sorption index (%)

Mw – weight of sponge with water (g)

Ma – initial weight of sponge (g).

## 2.12. Determination of physico-mechanical parameters of microcrystalline chitosan, composite materials in the form of sponge

The mechanical properties were determined in the accredited Laboratory of Metrology IBWCh, (certified accreditation AB 338). The basic mechanical parameters of composite materials were evaluated according to the following standards [12,13]: PN-EN ISO 4593:1999–thickness, in mm and PN-EN ISO 527-3:1998 - tensile strength in MPa and elongation at max. tension.

## 2.13. FTIR spectrum of lignin fractions and biocomposites

FTIR spectrums were performed on FTIR ATI Mattson type Genesis, with analysis software ATI Mattson i Peak Solve. Spectrums were made X-ray technology in the field of wave number 500-4000  $\text{cm}^{-1}$  with resolution of 4  $\text{cm}^{-1}$  and number of scan 32.

## 3. Results and discussion

### 3.1. Extraction of lignin fractions from black liquor

Lignin fractions were isolated from softwood and hardwood black liquor (the by-product of pulp & paper industry) using different reaction conditions. In the first step isolation of lignin was carried out in pH range from 2 to 8.5 with 1 N  $\text{H}_2\text{SO}_4$  addition. The second step of the isolation of lignin was carried out in pH 2. A two-step isolation of lignin from the black liquor was to purify it from non-lignin fractions, because of that purification it was allowed to obtain lignin fractions free of sulfate.

Usage of wide range of pH in the first stage of lignin separation had been performed in order to obtaining lignin fractions of varied structure. The differences in the structure of lignin may have a significant impact on the formation of polymer composites with chitosan.

The lignin fractions from hardwood black liquor were obtained with different yield depending on the pH value.

Whereas, lignin fractions from softwood black liquor were close to each other, wherein it should be noted that in both cases the highest yield was obtained for the fraction extracted at pH 2. (Table 1).

**Table 1.** The field of lignin fraction

pH	The yied of lignin fractions from hardwood, $\text{g}/\text{dm}^3$ black liquor	The yied of lignin fractions from softwood, $\text{g}/\text{dm}^3$ black liquor
8.5	18.50	45.43
8.0	20.00	42.44
7.5	21.73	49.06
7.0	21.23	53.61
6.5	23.07	41.73
6.0	23.47	44.65
5.5	24.18	47.47
5.0	25.00	48.38
4.5	30.02	42.28
4.0	31.17	42.04
2.0	35.26	41.30

This is confirmed by FTIR spectra for lignin fraction pH 2 and pH 8 present characteristic peaks typical for lignin structures (Figure 1, 2).

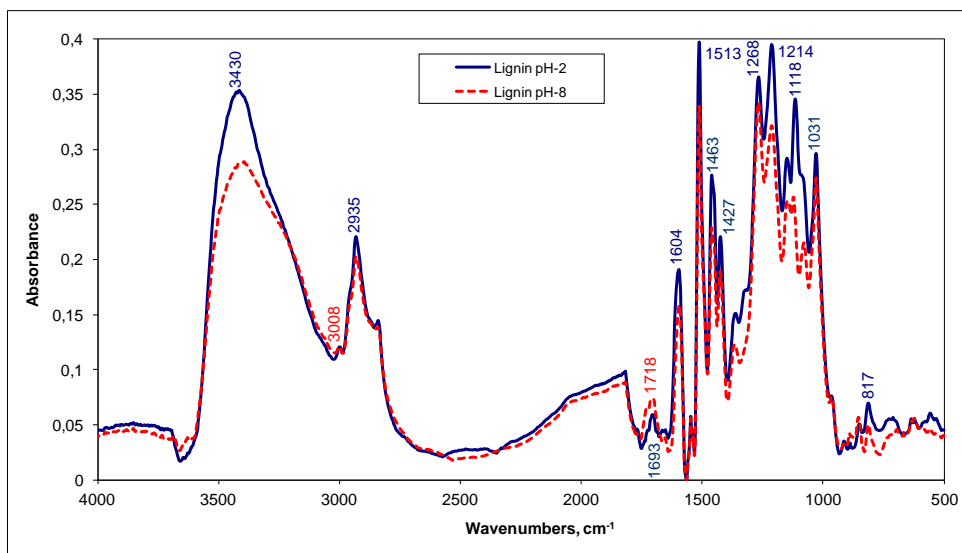


Figure 1. FTIR spectrum for softwood lignin fractions

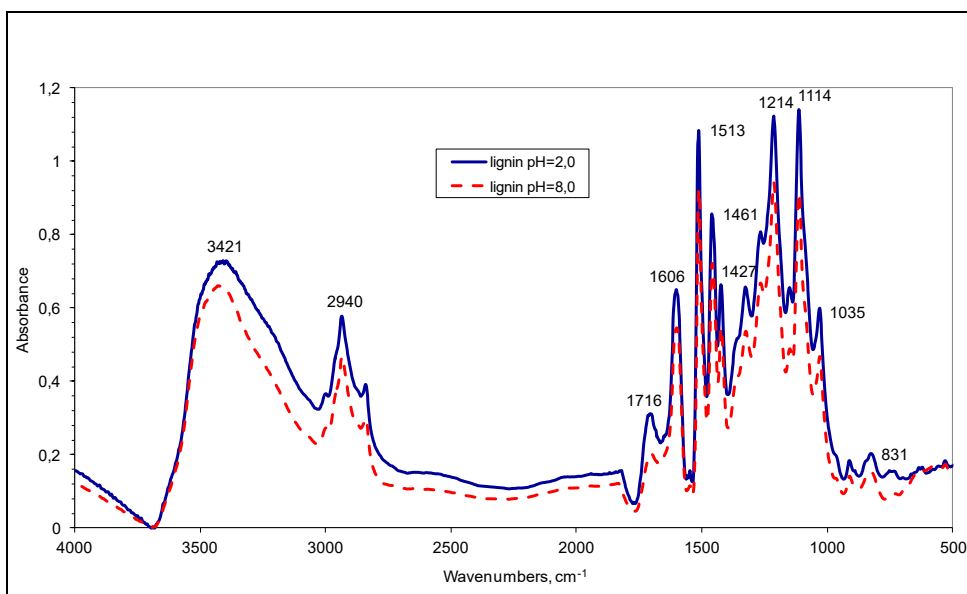


Figure 2. FTIR spectrum for hardwood lignin fractions

It was found that the obtained spectrum for the lignin fraction of pH 2 and pH 8 are typical lignin structures [14]. Tested samples are characterized by absorption curves at wavelengths of  $3400\text{ cm}^{-1}$  assigned to -OH stretching vibration (Figure 1 and 2).

The bands at  $2940\text{ cm}^{-1}$  correspond to methyl, methylene and methoxyl groups in guaiacyloxy units of lignin. In the test samples characteristic of lignin bands at a wavelength of  $1600$  and  $1510\text{-}1514\text{ cm}^{-1}$  were observed. The obtained spectrums showed minor difference between lignin obtained at pH-2 than at pH 8. The bands  $1461$  and  $1427\text{ cm}^{-1}$  are associated with the presence of the test samples of asymmetrical deformations of the C-H bonds in guaiacylopropane and guaiacyloxy units.

The wavelength at  $1214\text{ cm}^{-1}$  correspond to guaiacyl aromatic rings, which are characteristic for lignin. The bands at  $1114$  and  $1035\text{ cm}^{-1}$  confirm the presence of syringyl groups.

In conclusion depending on the used reaction medium the lignin fractions with different chemical structures and purity can be isolated from black liquor .

### 3.2. Formation MCCh/Lignin polymer biocomposites

As a result of the study various MCCh/lignin polymer biocomposites were prepared as described in 2.4 and 2.5 section. The physico-chemical parameters of chitosan were shown in (Table 3).

**Table 3.** Physicochemical parameters of microcrystalline chitosan (MCCh)

Symbol of chitosan	$\bar{M}_v$ kDa	SD %	Concentration of polymer %	WRV %	pH
MCCh	309.0	79.6	4.39	510.0	7.2 – 7.4

The quantitative composition of the produced composites were shown in (Table 4).

**Table 4.** Quantities composition of MCCh/Lignin biocomposites

Symbol of sample	Composition
G/MCCh + 5 % Lig	Microcrystalline chitosan - 63.93% wt. Lignin - 3.20% wt. Plasticizer (glycerin) - 32.87% wt.
G/MCCh + 10 % Lig	Microcrystalline chitosan - 61.95% wt. Lignin - 6.20% wt. Plasticizer (glycerin) -31.85% wt.
G/MCCh + 15% Lig	Microcrystalline chitosan - 60.10% wt. Lignin - 9.00% wt. Plasticizer (glycerin) -30.9% wt.

Prepared biocomposites were assessed for their suitability for the construction of dressing and hygiene materials in the form of a sponge.

### 3.3. Physical and mechanical properties of MCCh/Lignin composites in the form of sponges

The strength parameters, sorption and absorbent properties of MCCh/Lignin composites were tested. The amount of added lignin to the chitosan was 5, 10, 15% by weight. (Table 5).

**Table 5.** Physical and mechanical properties of MCCh composites in the form of sponges with HW (hardwood) lignin fraction addition

Symbol of sample	Thickness, mm	Tensile strength MPa	Elongation at max. tension %	WRV, %	Sorption time, h	Sorption index Ws %
G/MCCh	2.03 ± 0.07	0.040 ± 0.003	4.16 ± 0.8	141	0.17	211
					0.5	299
					3.0	445
					5.0	552
					24.0	690
G/MCCh + 5% Lignin pH 2	2.22 ± 0.25	0.022 ± 0.004	7.90 ± 0.88	168	0.17	244
					0.5	387
					3.0	542
					5.0	646
					24.0	785
G/MCCh + 10% Lignin pH 2	1.98 ± 0.6	0.026 ± 0.003	8.25 ± 1.68	145	0.17	271
					0.5	401
					3.0	572
					5.0	712
					24.0	753
G/MCCh+ 15% Lignin pH 2	2.03 ± 0.19	0.025 ± 0.002	5.56 ± 1.19	144	0.17	267
					0.5	424
					3.0	591
					5.0	689
					24.0	838
G/MCCh + 5% Lignin pH 4	1.83 ± 0.12	0.023± 0.001	9.01 ± 2.59	150	0.17	241
					0.5	439
					3.0	458
					5.0	753
					24.0	884
G/MCCh + 10% Lignin pH 4	1.97 ± 0.09	0.017 ± 0.001	15.0 ± 1.50	139	0.17	312
					0.5	515
					3.0	751
					5.0	1020
					24.0	1139



G/MCCh + 15% Lignin pH 4	2.00 ± 0.04	0.022 ± 0.003	9.00 ± 1.53	132	0.17	242
					0.5	355
					3.0	586
					5.0	957
					24.0	963
G/MCCh + 5% Lignin pH 8	2.00 ± 0.08	0.029 ± 0.002	5.66 ± 1.24	128	0.17	221
					0.5	319
					3.0	500
					5.0	576
					24.0	756
G/MCCh + 10% Lignin pH 8	1.98 ± 0.15	0.032 ± 0.003	4.48 ± 0.40	129	0.17	178
					0.5	383
					3.0	491
					5.0	545
					24.0	705
G/MCCh + 15% Lignin pH 8	2.06 ± 0.05	0.24 ± 0.000	8.52 ± 1.36	139	0.17	234
					0.5	361
					3.0	477
					5.0	592
					24.0	733

Based on the results shown in (Table 5), it was found that the addition of HW lignin fraction at 5, 10 and 15% by weight significantly affect of the flexibility samples and reduced of tensile strength of sponges, relative to the starting material without the addition of lignin. It was found that the addition of lignin fractions also caused a decrease WRV.

**Table 6.** Physical and mechanical properties of MCCh composites in the form of sponges with SW (softwood) lignin fraction addition

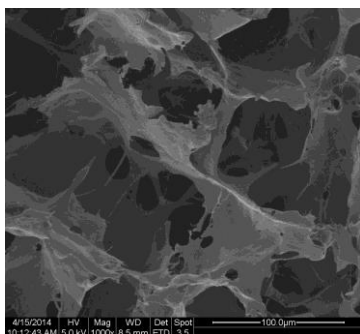
Symbol of sample	Thickness, mm	Tensile strenght MPa	Elongation at max. tension %	WRV, %	Sorption time, h	Sorption index Ws %
G/MCCh	2.06 ± 0.02	0.034 ± 0.001	7.88 ± 2.1	181	0.17	179
					0.5	318
					3.0	410
					5.0	460
					24.0	508
G/MCCh +	2.15 ± 0.01	0.044 ±	5.28 ± 0.91	167	0.17	161

5% Lignin pH 2		0.003			0.5	253
					3.0	362
					5.0	459
					24.0	495
G/MCCh + 10% Lignin pH 2	2.28 ± 0.03	0.055 ± 0.003	5.17 ± 0.89	163	0.17	169
					0.5	287
					3.0	375
					5.0	465
G/MCCh+ 15% Lignin pH 2	2.28 ± 0.07	0.035 ± 0.003	9.30 ± 1.20	158	0.17	247
					0.5	322
					3.0	444
					5.0	508
G/MCCh + 5% Lignin pH 4	2.02 ± 0.03	0.025 ± 0.002	10.50 ± 0.90	198	0.17	239
					0.5	355
					3.0	488
					5.0	538
G/MCCh + 10% Lignin pH 4	2.04 ± 0.02	0.028 ± 0.001	14.00 ± 1.4	205	0.17	183
					0.5	302
					3.0	424
					5.0	545
G/MCCh + 15% Lignin pH 4	2.21 ± 0.02	0.030 ± 0.003	7.82 ± 1.56	210	0.17	158
					0.5	269
					3.0	427
					5.0	506
G/MCCh + 5% Lignin pH 8	2.03 ± 0.09	0.030 ± 0.002	9.78 ± 1.19	175	0.17	173
					0.5	284
					3.0	371
					5.0	411
					24.0	583

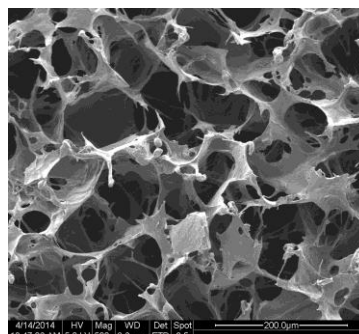
G/MCCh + 10% Lignin pH 8	2.18 ± 0.08	0.024 ± 0.004	11.70 ± 1.50	181	0.17	123
					0.5	221
					3.0	410
					5.0	518
					24.0	560
G/MCCh + 15% Lignin pH 8	1.92 ± 0.07	0.027 ± 0.001	9.84 ± 1.34	202	0.17	213
					0.5	292
					3.0	470
					5.0	478
					24.0	582

Based on the results shown in (Table 6), the addition of SW lignin fraction at 5, 10 and 15% by weight relative to the chitosan, influenced on flexibility of sponges (elongation at maximum tension), without influencing the strenght. Increasing of WRV with increasing of lignin participate in the composite was observed (Table. 6). For MCCh/Lignin biocomposite (with lignin pH 4 in an amount of 5, 10 and 15% by weight), WRV reached about 9-16% higher than the value of WRV for sponge produced from the chitosan.

The results in (Tables 5 and 6) show, that with the increase of time sponge being kept in demineralized water is increases absorptive capacity of all tested sponges. In the SEM photo of the surface of MCCh/Lignin composites in the form of sponges was observed expanded inner surface (Figure 3).



Starting dressing material (SEM 500x)



MCCh/15% Lignin composites (SEM 500x)

**Figure 3.** SEM photo of the surface of MCCh/Lignin composites in the form of sponges

The obtained results show that lignin fractions extracted from black liquid (hardwood and softwood), can be used for construction biocomposites in the form of a sponge, which can be used as dressings and hygienic materials.

#### **4. Conclusions**

1. Lignin fractions obtained from black liquor were characterized by different chemical structure and purity, depending on the method of isolation.
2. The participation of lignin in a composite of MCCh/Lignins in amounts of 5, 10 and 15% wt relative to the chitosan significantly affected the flexibility of the sponge dressing obtained (elongation at maximum stress) and sorption properties, with small decreasing of strength for HW lignin fraction.
3. The result showed that with increasing retention time of sponge being kept in demineralised water increased absorptive capacity for all tested forms of sponge.
4. Prepared biopolymer MCCh/Lignin composite in the form of sponge meets the basic criteria of mechanical and sorption, which makes it possible to use it as a dressing and hygienic material

#### List of abbreviations:

MCCh – microcrystalline chitosan

MCCh/Lignin – biopolymer microcrystalline chitosan and lignin composite

DD – deacetylation degree

WRV – water retention of value

SW – lignin from softwood black liquor

HW – lignin from hardwood black liquor

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