The Analysis of the distribution of available mesopores in cellulosic pulp, using Inverse Size Exclusion Chromatography-ISEC

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Abstract: *The Analysis of the distribution of available mesopores in cellulosic pulp, using Inverse Size Exclusion.* Chromatography-ISEC The aim of the presented research was to determine available mesopores in cellulose masses obtained by industrial methods. Cellulose masses obtained from fast-growing poplar species (Populus sp.) can be used in the production of biofuels of the second generation. One of the factors influencing the effectiveness of hydrolysis is the availability of pores in the material for acids and enzymes used during this process. For this purpose the reverse spatial exclusion chromatography (ISEC) with the use of dextran standards and selected sugars was applied to verify the distribution of available mesopores in cellulose masses. The analysis showed the material with the highest share of available pores, the selection of which will allow to increase the efficiency and profitability of biofuel production from lignocellulosic materials.

Keywords: mesopores, cellulosic pulp, HPLC, ISEC

INTRODUCTION

The development of a low-carbon economy is associated with the development of new industries such as green technology-based energy. It should include plantations of energy crops such as energy willow, fast-growing poplars, including genetic modification to optimise biomass harvesting for fuel purposes, which could be used in other industries if needed (Szechyńska-Hebda et al. 2016). Such wood could be used additionally in the wood and paper industry.

The production of second-generation biofuels from lignocellulosic materials such as wood requires the breaking down of lignocellulosic complexes (LCC) in order to increase production efficiency by making the enzymes and acids used in biofuel production more easily accessible (Fatehi 2013, Mota et al. 2017).

One of the methods of breaking down LCC complexes found in wood biomass is alkaline treatment used to produce pulp. Cellulose masses thanks to the breaking down of the wood structure and LCC complexes are attractive material for the production of biofuels of the second and third generation (Miazek et al. 2017, Przybysz, Buzała et al. 2019). During the production of cellulose masses, the material is subject to partial chemical delignification of sulphate, allowing the recovery of chemicals used in the process. This makes it possible to reduce the environmental impact of the process (Anastas and Warner. 1998). During the production of pulp there is also a loss of extractive and structural substances such as hemicellulose and low-polymerization cellulose. As a result of the process, the cellulose itself is subject to a change in its structure which makes it more accessible to enzymes (Przybysz Buzała et al. 2019).

One of the important aspects influencing the process of hydrolysis of enzymatic lignocellulose biomass is the availability of cellulose fibres for enzymes. This parameter is dependent on the porosity of the material. The most commonly used porosity measurement method is nitrogen adsorption, mercury porosimetry, and nuclear magnetic resonance spectroscopy (NMR) Bayer et al. 2010). Due to IUPAC recommendations concerning the necessity to take into account the type and application of the examined material, an interesting

analytical method is ISEC - Inverse Size Exclusion Chromatography. This method is based on the application of reverse spatial exclusion chromatography to determine the available pore area for standards and pore distribution in the analyzed material (Striegel et al. 2009, Zawadzki et al. 2016). An additional advantage of this method is the testing of the material in the wet state. This prevents the collapse of pores with the smallest diameters.

MATERIALS

Cellulose masses were obtained as a result of cooperation with the Institute of Papermaking and Printing in Łódź from californian and japanese poplar wood. The masses were made of 2,5-year-old poplar wood. Before dissolving, the wood was chipped into 8 × 16 × 25 mm chips. The sulfate method was used under conditions: 1 000
- the share of active alkalis (NaOH and Na2S) was 19 and 26% respectively,
- the method was 30% sulfide,
- liquid module 4,
- the maximum temperature of the process was 160°C which was obtained within 2 hours from the start of the process and maintained for another 2 hours,
- the cooling time was less than 20 minutes at 40°C,
- cooling time was less than 30 minutes at 25°C.

The wood material was washed diffusively for at least 12 h after alkaline solution. The material prepared in this way was defibered at a head speed of 8000 rpm-1. The pulp thus obtained was separated to remove the non-fibrous elements and was washed to remove alkalis from the obtained material which could cause hydrolysis of the obtained material. An aqueous solution of lithium chloride was used to protect the material from microbial growth and stored at approximately 6°C.

Standards used for porosity analysis of material by HPLC-ISEC(Inverse Size Exclusion Chromatography) liquid chromatography. The following standards were used to determine the distribution of pores available in the analysed material (Table 1): a set of 10 dextran standards (D), part d.a. Fluoride, maltose, glucose, ethylene glycol, triethylene glycol, methanol.

Table 1 General characteristics of standards for the determination of pore distribution available in the analysed material (Radomski 2015)

Marking at work	M_p	M_n	M_w	Radius r_n	Marking at work	M_p	M_n	M_w	Radius r_n
	/(kg·mol^{-1})			/nm		/(kg·mol^{-1})			/nm
Dx1k	1,08	1,01	1,27	0,86	ethylene glycol	0,062	0,062	0,062	0,30
Dx5k	4,44	3,26	5,22	1,72	POEn2	0,106	0,106	0,106	0,37
Dx50k	43,5	35,6	48,6	5,35	POEn4	0,194	0,194	0,194	0,47
Dx80k	66,7	55,5	80,9	6,56	POEn9	0,430	0,397	0,434	0,63
Dx150k	123,6	100,3	147,6	8,95	glucose	0,180	0,180	0,180	0,48
Dx270k	196,3	164,2	273,0	11,21	maltose	0,342	0,342	0,342	0,59
Dx410k	276,5	236,3	409,8	13,31	methanol	0,032	0,032	0,032	0,24
Dx670k	401,3	332,8	667,8	15,94					

Redistilled water was used as an eluent. Before HPLC analysis, the eluate was each time de-aerated with an ultrasonic scrubber for a minimum of 3 h. Methodology of pore structure analysis with ISEC is based mainly on the works (Szadkowski et al. 2015, Radomski 2015, Zawadzki et al. 2016).

Porosity determination by ISEC method was carried out using Shimadzu HPLC liquid chromatograph. The basic composition of HPLC analytical equipment included; CTO-10A furnace in which there is space for two 20 µl dosing loops and two columns, two LC-20AD pumps, DGU-20A degasser, RID-10A refractometer detector, CBM-10A control module enabling control and detection from the level of LC Solution v.1.21 SP1 software.
Two blank Macherey-Nagel steel columns with a length of 250 mm and an internal diameter of 4.0 mm were used for testing. The columns were filled with reference lignocellulose material and pre-treated material .according to the work (Zawadzki et al.2016).

After loading the fraction of analyzed material (japanese and californian poplar wood at the age of three and five years as well as cellulose pulp) the columns were equipped with metal filters 1/4 4.6 mm 5 µm on both sides of the column before closing. The purpose of the filters was to prevent mechanical soiling of the RID-10A detector used for porosity analysis. The double-sided application of the filters allowed the column to be turned over to better stabilize the deposit. For the analysis, standards were made, the specifications of which are presented in chapter 4.1. In the case of dextrane standards Dx 1k and Dx 5k, about 1 mg of the standard was dissolved in 1 cm^3 of redistilled water. The standards Dx 12 to Dx 670 k were made in solution so that approx. 0.5 mg was dissolved in 1 cm^3 of redistilled water. The methanol standard was made so that about 10 mg of methanol was dissolved in 1 cm^3 of redistilled water. The remaining standards were prepared at a ratio of about 2 mg per 1 cm^3 of redistilled water.

The analysis was carried out on a column stabilized in the flow of eluent in the form of redistilled water. The initial stabilization flow was 0.0200 ml/min for 12 h, then the flow was increased by 0.0200 ml/min every 0.5 h. When the flow rate reached 0.5 ml/min, the RID-10a detector was activated. A flush of the cell was performed on the detector. After stabilization of the obtained signals from the detector, dextran standards from Dx 670k to Dx 80k. at a flow rate of 0.5ml/min were introduced to the column. For standards Dx 50k to Dx 12k, the eluent flow was 0.7 ml/min. For standards below Dx 12k, the eluent flow was 1 ml/min. The change in the eluent flow was associated with an improvement in the separation of the standards according to size during analysis.

During chromatographic analysis, the column with the tested samples was placed in an oven set to 35°C. The analysis was performed using wood shavings of japanese and californian poplar in moist state, after draining. In the case of porosity analysis of cellulose masses due to problems with placing them in chromatographic columns it was necessary to apply the process of changing the solvent from water to non-polar solvent. The solvent exchange procedure was the same as for the determination of pores by nitrogen adsorption. After loading the columns with a sample dried in a vacuum dryer, the reverse process was applied (moistening the material with increasingly polar eluent in order to rinse out the residual solvent which could limit the bed penetration of standards dissolved in redistilled water. After the analysis, the samples were dried to constant weight.

RESULTS

As a result of the experiment, different pore distribution was observed depending on the species of poplar from which they were obtained and the content of alkali used to obtain them.
Japanese poplar obtained with the addition of 26% alkali shows the largest pore area available for the standards. It shows the largest available pore area for standards with hydrodynamic radii up to about 1,5 nm and above 8 nm.

A similar relationship is drawn for california poplar. However, the pore area available for the standards for california poplar is lower (about 1,9 cm^3/g) than for maximum poplar (about 2,9 cm^3/g) for the lowest hydrodynamic rays of the standards.

The highest sum of available areas of the relevant pores is shown by the cellulose masses obtained from california poplar with 19% alkali. It shows a large share of pores from the range 1÷5 nm. It shows the second content of pores from the range above 5 nm and from 0.5÷1 nm and equal to the available pore surface area with cellulose mass obtained from poplar japanese with addition of 19% of alkali.
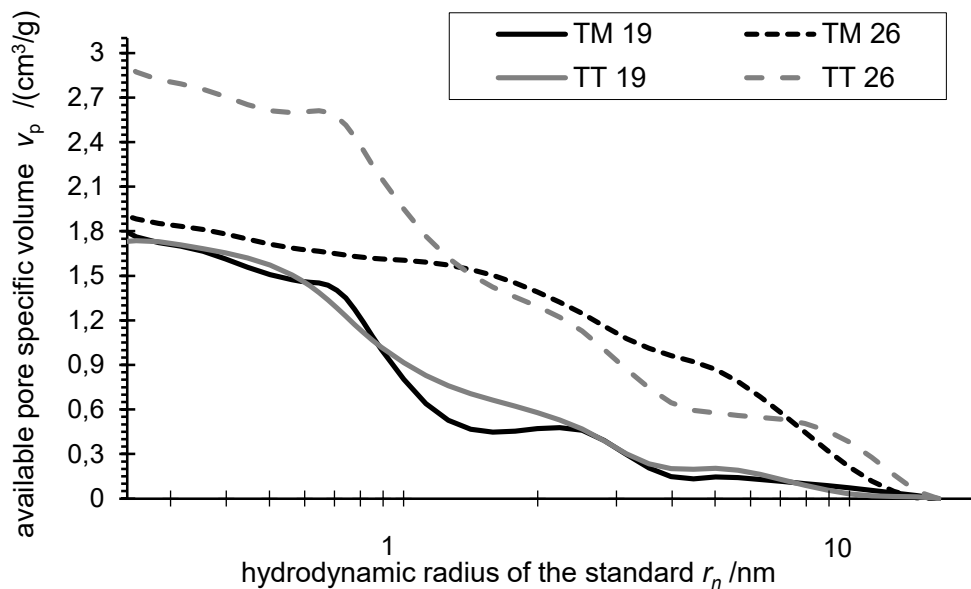
Figure 1. The available specific pore volume for the hydrodynamic radius of the standard (TM 19 - poplar japanese with addition 1% alkali, TM 26- poplar japanese with addition 26% alkali, TT 19- poplar california with addition 19% alkali, TT 26 - poplar california with addition 26% alkali)
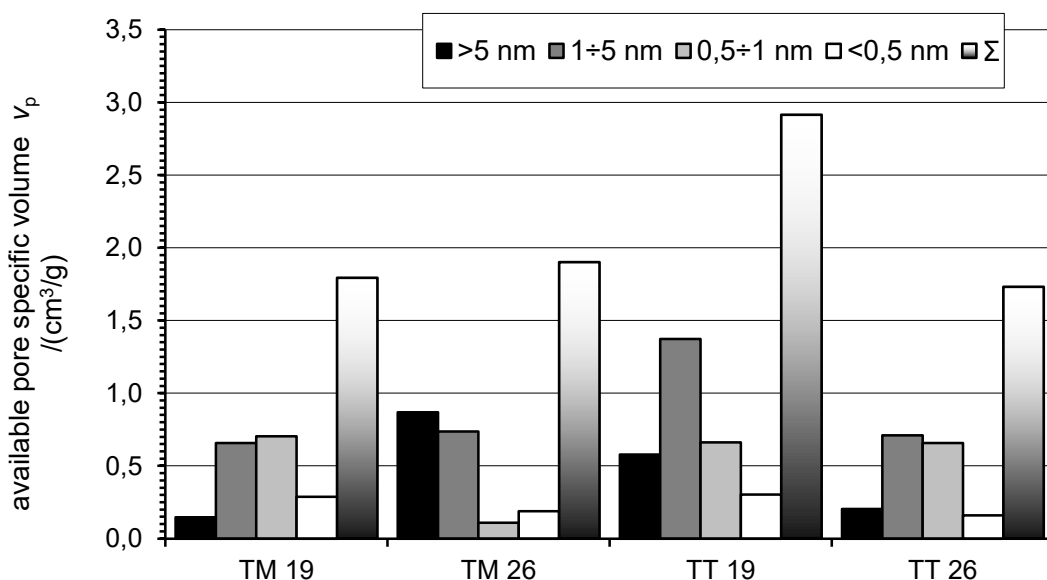
Figure 2. Pore size distribution depending on the poplar species and alkali concentration (TM 19 - poplar japanese with addition 19% alkali, TM 26- poplar japanese with addition 26% alkali, TT 19 - poplar california with addition 19% alkali, TT 26- poplar california with addition 26% alkali)

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The highest available specific pore surface area from the range above 5 nm is shown by the cellulose mass obtained from japanese with 26% alkali. The smallest sum of available specific pore area is shown by the california poplar with 26% alkali.

CONCLUSION

1. The method of measurement of pore distribution by inverse spatial exclusion chromatography shows the difference in available pore areas depending on the content of alkali used and the species of wood from which they were obtained.
2. The available specific pore area in the pulp pulp is not related to the amount of alkali used and the species of wood from which it was obtained.
3. The choice of alkali content for the production of pulp for biofuel purposes should be chosen individually depending on the species and type of biomass used.

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Streszczenie: *Analiza rozkładu dostępnych mezoporów w masach celulozowych za pomocą odwrotnej chromatografii wykluczenia przestrzennego (ISEC).* Celem przedstawionych badań było oznaczenie dostępnych mezoporów w masach celulozowych uzyskanych metodami przemysłowymi. Masy celulozowe pozyskane z szybkorosnących gatunków topoli (*Populus* sp.) mogą znaleźć zastosowanie przy produkcji biopaliw II generacji. Jednym z czynników wpływających na skuteczność hydroliz jest dostępność porów w materiale dla kwasów i enzymów stosowanych podczas tego procesu. W tym celu weryfikacji rozkładu dostępnych mezoporów w masach celulozowych zastosowano odwrotną chromatografię wykluczenia przestrzennego (ISEC) z zastosowanie wzorców dekstranowych oraz wybranych cukrów. Przeprowadzona analiza wskazała materiał o największym udziale dostępnych porów, którego wybór pozwoli na zwiększenie wydajność i opłacalność produkcji biopaliw z materiałów lignocelulozowych.

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