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Preparation of nanocellulose by hydrolysis with ionic liquids and two-step hydrolysis with ionic liquids and enzymes

MARTA BABICKA[1], MAGDALENA WOŹNIAK[1], KINGA SZENTNER[1], SŁAWOMIR BORYSIAK[2], KRZYSZTOF DWIECKI[3], IZABELA RATAJCZAK[1]

[1]Department of Chemistry, Faculty of Forestry and Wood Technology, Poznań University of Life Sciences, Poland
[2]Institute of Chemical Technology and Engineering, Poznan University of Technology, Poland
[3]Department of Food Biochemistry and Analysis, Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Poland

Abstract*: The aim of this study was to compare parameters of nanocellulose obtained by two different procedures: hydrolysis with ionic liquids (1-allyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium acetate) and hydrolysis with ionic liquids in combination with hydrolysis using a cellulolytic enzyme from Trichoderma reesei. Avicel cellulose was treated with two ionic liquids: 1-allyl-3-methylimidazolium chloride (AmimCl) and 1-ethyl-3-methylimidazolium acetate (EmimOAc). In the two-step hydrolysis cellulose after treatment with ionic liquids was additionally hydrolyzed with a solution of enzymes. In order to characterize the obtained material, the following analyses were used: infrared spectroscopy, X-ray diffraction and dynamic light scattering. The results indicated that cellulose obtained by two-step nanocellulose production methods (first hydrolysis with ionic liquids and then with enzymes) showed similar parameters (particle size, XRD patterns and degree of crystallinity) as the material after the one-step process, i.e. hydrolysis with ionic liquids.*

Keywords: nanocellulose, ionic liquids, enzymes

INTRODUCTION

Nanocellulose is a very popular material, as evidenced by numerous patents and scientific articles related to this topic [Charreau et al. 2020; Ribeiro et al. 2019]. This material is widely used in many areas: food technology, medicine, pharmacy, cosmetics, electronics, furniture and environmental protection or in the search for new composite materials [Moohan et al. 2020; Trache et al. 2020; Zinge and Kandasubramanian 2020]. A wide range of potential applications for nanosized cellulose results from its unique properties, such as light weight, large surface area, low density, outstanding strength properties, biodegradability and biocompatibility [Heise et al. 2021; Phanthong et al. 2018].

Cellulose of nanometric dimensions can be obtained by various methods, e.g. mechanical techniques, such as grinding, grating or using high-power lasers, and chemical methods, where acids and bases are used [George et al. 2015; Kalia et al. 2011; Lee et al. 2014]. In the production of nanocellulose acid hydrolysis or hydrolysis in combination with other methods, most often mechanical, is commonly used. In this process, acids such as hydrochloric, hydrobromic or sulfuric acid are typically used. In the case of obtaining nanocellulose as a result of acid hydrolysis, significant amounts of the acids are needed, which makes this method harmful to the environment [De Aguiar et al. 2020; Man et al. 2011; Trache et al. 2020]. Another disadvantage of this method is connected with the low efficiency of this process and the resulting product showing reduced thermal stability of obtained nanocellulose [Clought et al. 2014; Du and Qian 2011]. Alternative, environmentally safe methods of nanocellulose production are hydrolysis processes with the use of ionic liquids or enzymes. Ionic liquids (IL) are compounds that are attractive thanks to such properties as melting point below 100°C, chemical and thermal stability, recyclability, low vapor pressure, non-volatility and non-flammability [Prudêncio et al. 2020; Tan et al. 2015]. Imidazolium ionic liquids such as 1-butyl-3-methylimidazolium bisulfate, 1-ethyl-3-methylimidazole chloride or 1-butyl-3-

methylimidazolium chloride are most often used to obtain nanocellulose [Grząbka-Zasadzińska et al. 2019; Mao et al. 2017; Tan et al. 2015]. The method of producing nanocellulose using ionic liquids has both advantages (using small amounts of solvents, working with an odorless and relatively safe solvent at atmospheric pressure), but unfortunately also disadvantages (unsatisfactory efficiency) [Brodeur et al. 2011; Karimian et al. 2019; Noor et al. 2020]. To obtain nanocellulose, enzymes (cellulases) are also used. These enzymes are produced, among others, by some species of fungi, bacteria and insects (*Trichoderma*, *Aspergillus*, *Clostridium* or *Zophobas*) [Michelin et al. 2020; Szentner et al. 2019; Zielińska et al. 2021]. The type of cellulolytic enzymes determines the efficiency of nanocellulose production [Zielińska et al. 2021]. In order to find the most optimal manner to obtain nanocellulose various methods are combined, e.g. a combination of hydrolysis using ionic liquids with enzymatic hydrolysis [Babicka et al. 2021; Siqueira et al. 2019; Tibolla et al. 2019; Yassin et al. 2019].

The aim of this study was to compare parameters of nanocellulose obtained by two different procedures: hydrolysis with ionic liquids (1-allyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium acetate) and hydrolysis with ionic liquids in combination with hydrolysis using a cellulolytic enzyme from *Trichoderma reesei*.

MATERIALS AND METHODS

Hydrolysis with ionic liquids

The Avicel PH-101cellulose material (Sigma Aldrich Chemie, Darmstadt, Germany) was mixed with ionic liquids (1-allyl-3-methylimidazolium chloride (AmimCl) (≥97.0%) and 1-ethyl-3-methylimidazolium acetate (EmimOAc) (≥95.0%)) at a weight ratio of 1:5. Both ionic liquids were purchased from Sigma Aldrich Chemie, Darmstadt, Germany. The reactions were run for ~ 15 min (until the mixture becomes homogeneous) at the temperature of 80°C using a heating mantle with intense magnetic stirring (ChemLand, Stargard, Poland). The hydrolysis reactions were stopped by adding 15 ml of a mixture of water and acetone (1:1) to the reaction solutions. The obtained products were washed with a mixture of water and acetone, next filtered and dried initially at room temperature and finally over P_2O_5 (Sigma Aldrich Chemie, Darmstadt, Germany).

Hydrolysis with the enzyme

The next step of the study was to run enzymatic hydrolysis of the cellulosic material after treatment with ionic liquids. The Avicel cellulose after hydrolysis with EmimOAc and AmimCl was added to citrate buffer (50 mM, pH = 4.8) at a ratio of 50 : 1 (mg/ml) and incubated for 30 min at 50°C with a shaking speed of 150 rpm using an incubated shaker (Lab Companion, JeioTech, Korea). Then the cellulolytic enzyme (from the microscopic fungus *Trichoderma reesei* ATCC 26921 with an activity of 700 units/g, Sigma Aldrich Chemie, Darmstadt, Germany) diluted in citrate buffer (1:50 by volume) was added to the cellulose at a ratio of 1: 2 by volume. The mixture was incubated at 50°C for 30 min. After this time the reaction was stopped by boiling the sample for 5 min. The samples were centrifuged (Universal 320, Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany), washed with deionized water and dried in a laboratory dryer (Pol-Eko-Aparatura, Wodzisław Śląski, Poland).

As a result of the experiment using the raw cellulose material (Avicel) four different samples were obtained, the denotations of which are presented in Table 1.

Table 1. Description of symbols of obtained samples

Samples	Hydrolysis steps	
	Treatment with ionic liquids	Treatment with enzymes
A	AmimCl	-
E	EmimOAc	-
AT	AmimCl	*Trichoderma reesei*
ET	EmimOAc	*Trichoderma reesei*

FTIR spectroscopy. Cellulose samples were mixed with KBr (Sigma Aldrich Chemie, Darmstadt, Germany) and analyzed using a Nicolet iS5 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). XRD analysis. The supermolecular structures of cellulose samples were analyzed using the X-ray diffraction (XRD) analysis (TUR M-62 X-ray diffractometer, Carl Zeiss AG, Jena, Germany). DLS analysis. The particle sizes expressed as the hydrodynamic diameter of cellulose samples were determined using the DLS method (Zetasizer Nano ZS-90 instrument, Malvern, UK).

RESULTS AND DISCUSSION

The FTIR spectra of Avicel cellulose, cellulose after hydrolysis with ionic liquids and the material after hydrolysis with IL and enzymes are presented in Figs. 1 and 2.

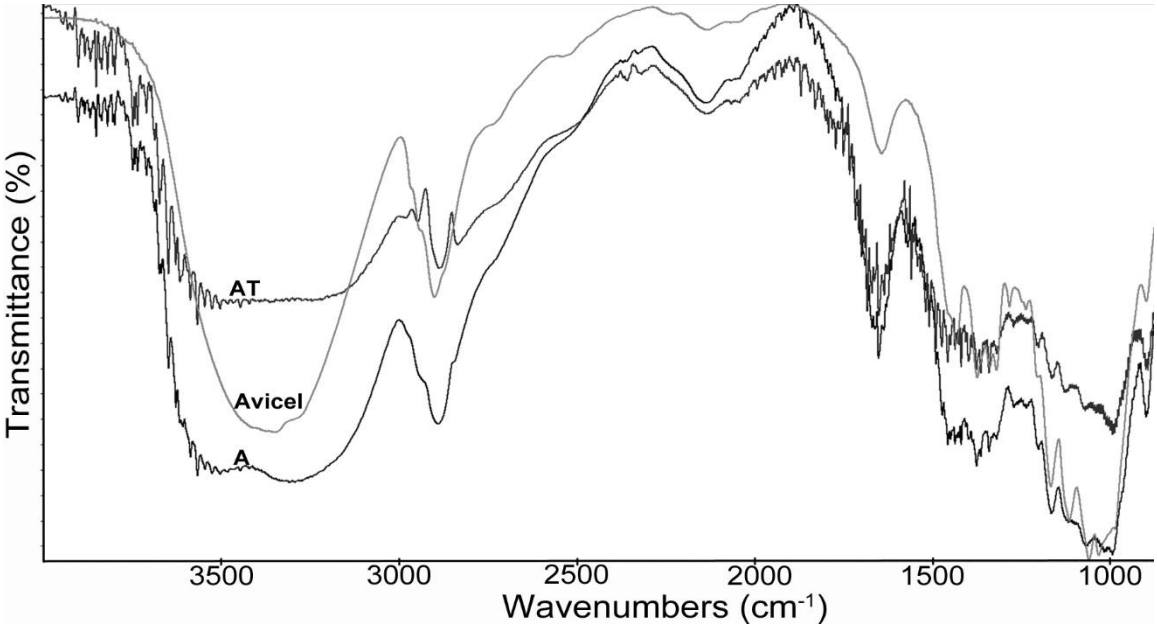

Figure 1. FTIR spectra of Avicel, (A) Avicel cellulose treated with AmimCl, (AT) Avicel cellulose treated with AmimCl and the enzyme.

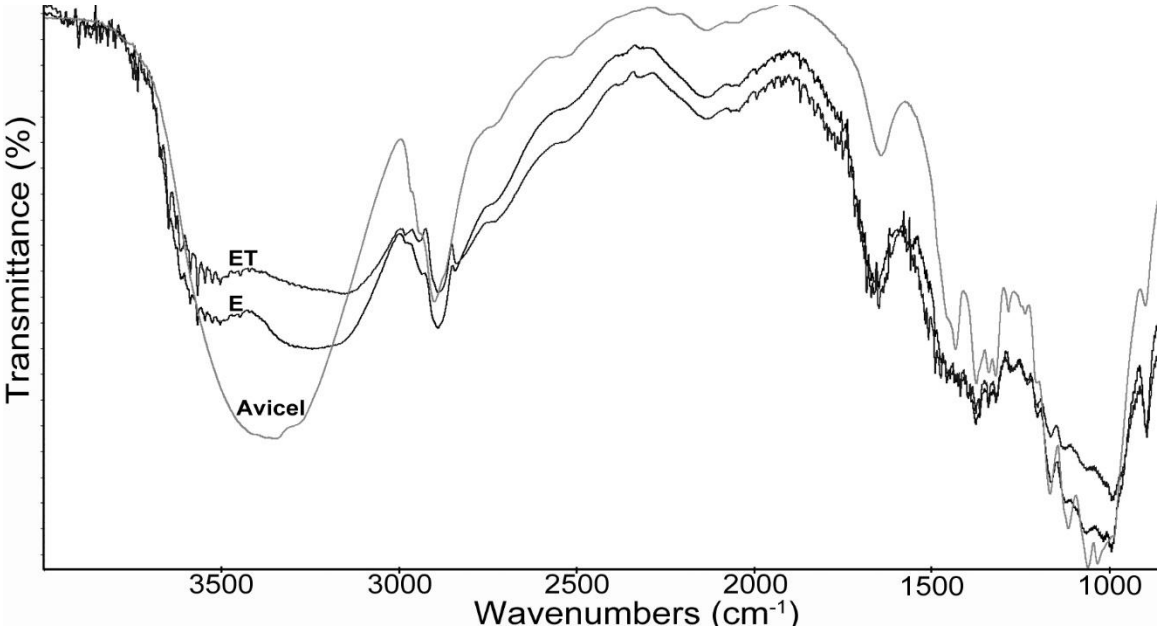

Figure 2. FTIR spectra of Avicel, (E) Avicel cellulose treated with EmimOAc, (ET) Avicel cellulose treated with EmimOAc and the enzyme.

In the spectra of all cellulose samples a wide band in the range of 3350–3480 cm−1 corresponding to the O-H bond and a band at 2900 cm-1 corresponding to the C-H stretching limitation are observed [Tan 2015]. For both methods of nanocellulose preparation (hydrolysis with ionic liquids and two-step hydrolysis with ionic liquids and enzymes), a material with a similar chemical structure was obtained, which confirms the presence of bands at the same wavenumber values. Moreover, the addition of enzymatic hydrolysis after the hydrolysis with ionic liquid did not affect the cellulose structure, which confirms the presence of the same bands in the spectra of the material for both methods of nanocellulose preparation. The diffraction profiles of Avicel cellulose after hydrolysis with ionic liquids and cellulose after hydrolysis with ionic liquids and enzymes are shown in Fig. 3.

Cellulose diffractograms after treatment with the AmimCl and EmimOAc ionic liquids showed peaks at 2θ = 15°, 17° and 22.7° corresponding to the planes (1-10), (110) and (200), respectively [French 2014], which is characteristic to cellulose I, although the intensity is much less than that of raw Avicel. Moreover, peak was observed at the diffraction angles of 20°, corresponding to planes (110). This indicates that cellulose I is partially converted to cellulose II by the action of ionic liquids. It is worth emphasizing that the polymorphic transformation of cellulose occurred to a greater extent in the case of hydrolysis with the use of EmimOAc ionic liquid. The same effect (conversion from cellulose I to cellulose II) was observed in our earlier work, where cellulose in the form of Avicel and Whatman after enzyme pretreatment was hydrolyzed with AmimCl and EmimOAc [Babicka et al. 2021]. These results are also in line with the work described by Cheng et al. (2011), where hydrolysis of various lignocellulosic materials (Avicel, switchgrass, pine and eucalyptus) with EmimOAc resulted in the formation of cellulose II.

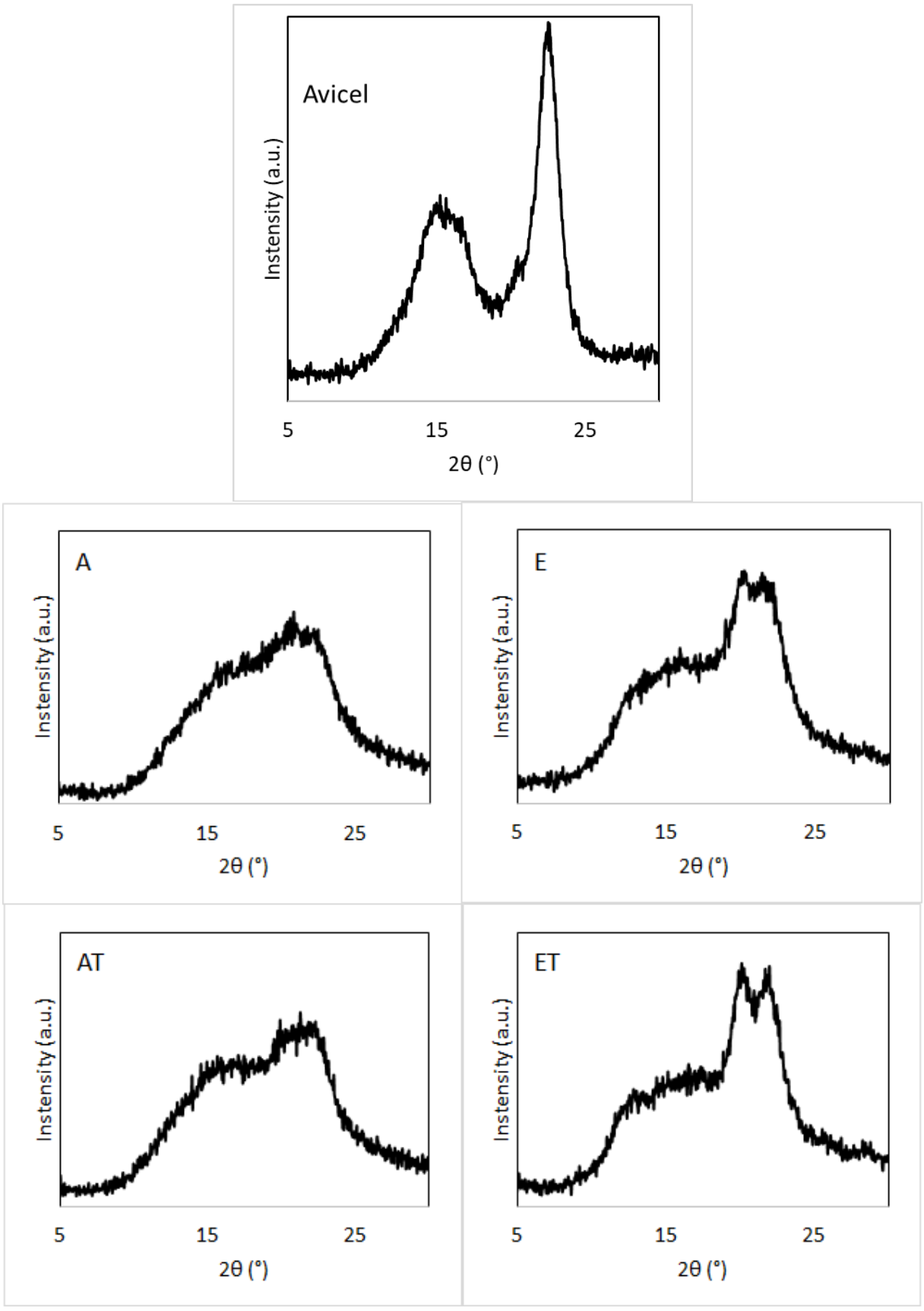

Figure 3. XRD patterns of Avicel, (A) Avicel cellulose treated with AmimCl, (E) Avicel cellulose treated with EmimOAc, (AT) Avicel cellulose treated with AmimCl and the enzyme, (ET) Avicel cellulose treated with EmimOAc and the enzyme.

Table 2. The degree of crystallinity of cellulose samples

Samples	Crystallinity index (%)
Avicel	61
A	14
AT	16
E	28
ET	30

The values of the crystallinity index indicated that hydrolysis with ionic liquids influenced crystallinity of the raw cellulose samples. The crystallinity index for Avicel cellulose was 61%, for the material treated with AmimCl it was 14% and for that treated with EmimOAc it was 28%. These observations are consistent with our previous work [Babicka et al. 2021], where it was also observed that the AmimCl ionic liquid is responsible for the reduction of the degree of crystallinity in cellulose samples. Analysis of these results shows that the enzymatic treatment of the samples after hydrolysis with ionic liquids did not significantly affect the value of crystallinity. When comparing the obtained results with our previous work, it can be seen that the degree of crystallinity of cellulose obtained in the case of pretreatment with ionic liquids is lower than that of the material with enzymatic hydrolysis used as a pretreatment [Babicka et al. 2021].

The particle size of the obtained cellulose material was measured using the dynamic light scattering method, the results obtained are shown in Fig. 4.

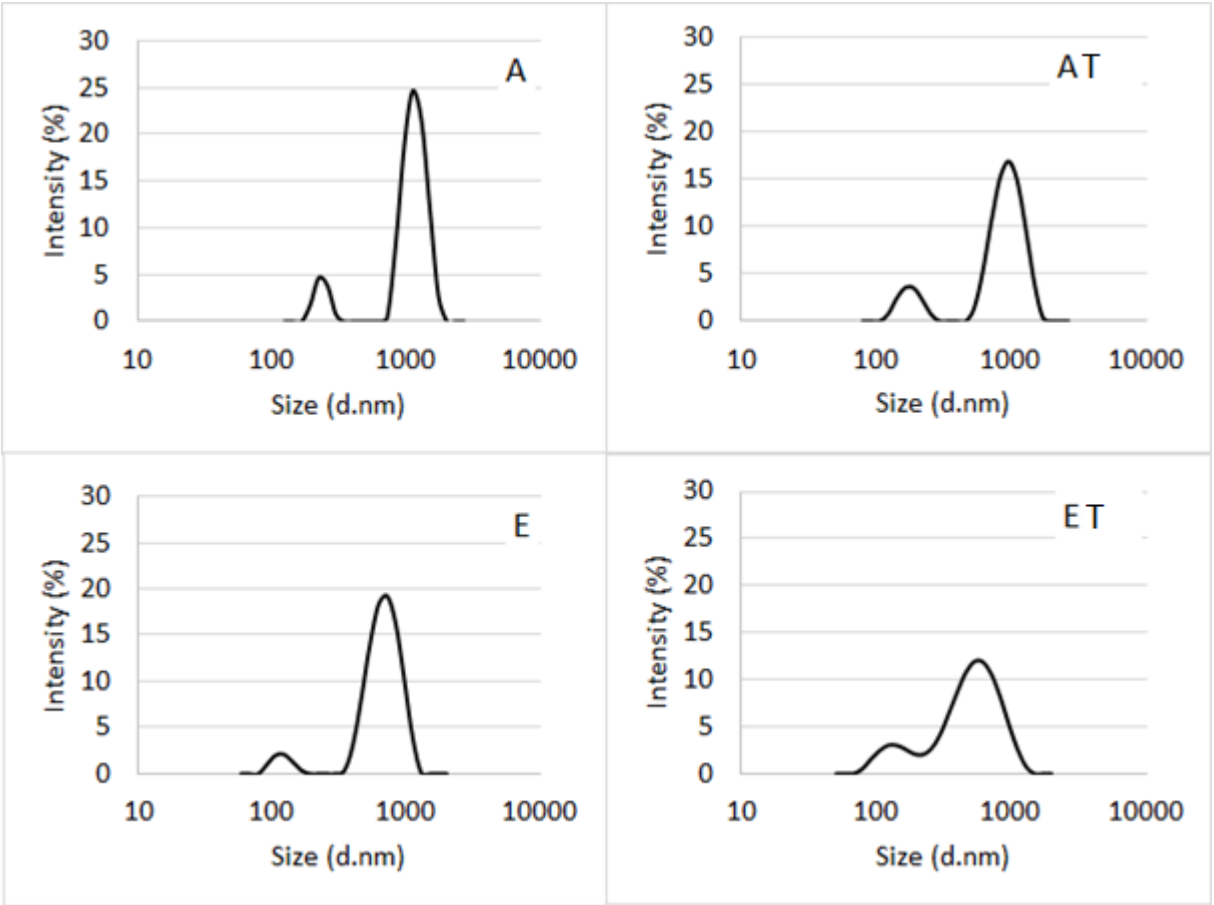

Figure 4. DLS patterns of (A) Avicel cellulose treated with AmimCl, (E) Avicel cellulose treated with EmimOAc, (AT) Avicel cellulose treated with AmimCl and the enzyme, (ET) Avicel cellulose treated with EmimOAc and the enzyme.

The DLS results show that each cellulose material has two fractions with different particle size. The particle size of approx. 1000 nm was recorded for all the cellulose materials (only ET had the particle size below 1000 nm). The raw Avicel cellulose particle size was in the range of 1300-4800 nm, which indicates that hydrolysis did not take place in all of the cellulose volume. A smaller particle fraction was also recorded for each cellulose sample. For cellulose treated with AmimCl and for cellulose treated with AmimCl and the enzyme the peak of approx. 200 nm was observed. For the sample treated with EmimOAc the particle size was about 130 nm for both treatment variants (with and without enzymatic hydrolysis). Slight differences in particle size can be seen when using different ionic liquids; however, the enzymatic hydrolysis process as the second step in nanocellulose production has no significant effect on the size of cellulose particles. A smaller particle size and lower polydispersity of cellulose were reported in our previous work [Babicka et al. 2021], where the order of the nanocellulose preparation process was reversed: enzymatic pre-treatment was applied, followed by hydrolysis with ionic liquids. After enzymatic pretreatment Avicel cellulose had a particle size of approx. 200 nm and after additional hydrolysis with ionic liquids the particle size was below 100 nm [Babicka et al. 2021]. This indicates that the use of enzymatic hydrolysis first as a pretreatment of cellulose material followed by hydrolysis with ionic liquids results in a smaller particle size and a lower polydispersity of the cellulosic material compared to the two-step process, where hydrolysis with IL was applied as the first step.

CONCLUSIONS

In this study two methods of obtaining nanocellulose were compared. The first method was a one-step hydrolysis with ionic liquids, in which two different ionic liquids, AmimCl and EmimOAc, were used. The second method was the two-step hydrolysis of cellulose, where first hydrolysis of cellulose took place with ionic liquids as a pretreatment method followed by the use of enzymes. The results showed that cellulose obtained in the two-step nanocellulose production methods (first hydrolysis with IL and then with enzymes) shows similar parameters (particle size, XRD patterns and degree of crystallinity) to those of the material after the single step process (hydrolysis with IL).

REFERENCES

1. BABICKA M., WOŹNIAK M., SZENTNER K., BARTKOWIAK M., PEPLIŃSKA B., DWIECKI K., BORYSIAK S., RATAJCZAK I., 2021: Nanocellulose production using ionic liquids with enzymatic pretreatment, Materials, 14, 12, 3264
2. BRODEUR G., YAU E., BADAL K., COLLIER J., RAMACHANDRAN K.B., RAMAKRISHNAN S., 2011: Chemical and physicochemical pretreatment of lignocellulosic biomass: A review, Enzyme Research 2011
3. CHARREAU H., CAVALLO E., FORESTI M.L., 2020: Patents involving nanocellulose: Analysis of their evolution since 2010, Carbohydrate Polymers, 237, 116039
4. CHENG G., VARANASI P., LI C., LIU H., MELNICHENKO Y.B., SIMMONS B.A., KENT M.S., SINGH S., 2011: Transition of cellulose crystalline structure and surface morphology of biomass as a function of ionic liquid pretreatment and its relation to enzymatic hydrolysis, Biomacromolecules, 12, 933–941
5. CLOUGH M.T., GEYEY K., HUNT P.A., SON S., VAGT U., WELTON T., 2014: Ionic liquids: Not always innocent solvents for cellulose, Green Chemistry, 17, 231-243
6. DE AGUIAR J., BONDANCIA T.J., CLARO P.I.C., MATTOSO L.H.C., FARINAS C.S., MARCONCINI J.M., 2020: Enzymatic deconstruction of

sugarcane bagasse and straw to obtain cellulose nanomaterials, ACS Sustainable Chemistry & Engineering, 8, 2287–2299
7. DU H., QIAN X., 2011: The effects of acetate anion on cellulose dissolution and reaction in imidazolium ionic liquids, Carbohydrate Research, 346, 1985–1990
8. FRENCH A.D., 2014: Idealized powder diffraction patterns for cellulose polymorphs, Cellulose, 21, 885–896
9. GEORGE J.,SABAPATHI S.N., 2015: Cellulose nanocrystals: Synthesis, functional properties, and applications, Nanotechnology, Science and Applications, 8, 45–54
10. GRZĄBKA-ZASADZIŃSKA A., SKRZYPCZAK A., BORYSIAK S., 2019: The influence of the cation type of ionic liquid on the production of nanocrystalline cellulose and mechanical properties of chitosan-based biocomposites, Cellulose, 26, 4827–4840
11. HEISE K., KONTTURI E., ALLAHVERDIYEVA Y., TAMMELIN T., LINDER M.B., NONAPPA, IKKALA O., 2021: Nanocellulose: Recent fundamental advances and emerging biological and biomimicking applications, Advanced Materials, 33
12. KALIA S., DUFRESNE A., CHERIAN B.M., KAITH B.S., AVÉROUS L., NJUGUNA J., NASSIOPOULOS E., 2011: Cellulose-based bio- and nanocomposites: A review, International Journal of Polymer Science, 2011
13. KARIMIAN A., PARSIAN H., MAJIDINIA M., RAHIMI M., MIR S.M., SAMADI KAFIL H., SHAFIEI-IRANNEJAD V., KHEYROLLAH M., OSTADI H., YOUSEFI B., 2019: Nanocrystalline cellulose: Preparation, physicochemical properties, and applications in drug delivery systems, International of Biological Macromolecules, 133, 850–859
14. LEE H.V., HAMID S.B.A., ZAIN S.K., 2014: Conversion of lignocellulosic biomass to nanocellulose: Structure and chemical process, The Scientific World Journal, 2014
15. MAN Z., MUHAMMAD N., SARWONO A., BUSTAM M.A., KUMAR M.V., RAFIQ S., 2011: Preparation of cellulose nanocrystals using an ionic liquid, Journal of Polymers and the Environment, 19, 726–731
16. MAO J., ABUSHAMMALA H., HETTEGGER H., ROSENAU T., LABORIE M.P., 2017: Imidazole, a new tunable reagent for producing nanocellulose, part I: Xylan-coated CNCs and CNFs, Polymers, 9, 473
17. MICHELIN M., GOMES D.G., ROMANÍ A., POLIZELI M., TEIXEIRA J.A., 2020: Nanocellulose Production: Exploring the Enzymatic Route and Residues of Pulp and Paper Industry, Molecules, 25, 3411
18. MOOHAN J., STEWART S.A., ESPINOSA E., ROSAL A., RODRÍGUEZ A., LARRAÑETA E., DONNELLY R.F., DOMÍNGUEZ-ROBLES J., 2020: Cellulose nanofibers and other biopolymers for biomedical applications. A review, Applied Sciences, 10, 65
19. NOOR S.M., ANUAR A.N., TAMUNAIDU P., GOTO M., SHAMELI K., HALIM M., 2020: Nanocellulose production from natural and recyclable sources: A review, IOP Conference Series: Earth and Environmental Science, 479
20. PHANTHONG P., REUBROYCHAROEN P., HAO X., XU G., ABUDULA A., GUAN G., 2018: Nanocellulose: Extraction and application, Carbon Resources Conversion, 1, 32–43
21. PRUDÊNCIO C., VIEIRA M., VAN DER AUWERAER S., FERRAZ R., 2020: Recycling old antibiotics with ionic liquids, *Antibiotics, 9*, 578

22. RIBEIRO R., POHLMANN B.C., CALADO V., BOJORGE N., PEREIRA N., 2019: Production of nanocellulose by enzymatic hydrolysis: Trends and challenges. Engineering in Life Sciences, 19, 279–291
23. SIQUEIRA G.A., DIAS I., ARANTES V., 2019: Exploring the action of endoglucanases on bleached eucalyptus kraft pulp as potential catalyst for isolation of cellulose nanocrystals, International of Biological Macromolecules, 133, 1249–1259
24. SZENTNER K., WAŚKIEWICZ A., KAŹMIERCZAK S., WOJCIECHOWICZ T., GOLIŃSKI P., LEWANDOWSKA E., WASIELEWSKI O., 2019: Enzymatic hydrolysis of cellulose using extracts from insects, Carbohydrate Research, 485,107811
25. TAN X.Y., ABD HAMID S.B., LAI C.W., 2015: Preparation of high crystallinity cellulose nanocrystals (CNCs) by ionic liquid solvolysis, *Biomass Bioenergy, 81*, 584–591
26. TIBOLLA H., PELISSARI F.M., MARTINS J.T., LANZONI E.M., VICENTE A.A., MENEGALLI F.C., CUNHA R.L., 2019: Banana starch nanocomposite with cellulose nanofibers isolated from banana peel by enzymatic treatment: In vitro cytotoxicity assessment, Carbohydrate Polymers, 207, 169–179
27. TRACHE D., TARCHOUN A.F., DERRADJI M., HAMIDON T.S., MASRUCHIN N., BROSSE N., HUSSIN M.H., 2020: Nanocellulose: From fundamentals to advanced applications, Frontiers in Chemistry, 8
28. YASSIN M.A., GAD A., GHANEM A.F., ABDEL REHIM M.H., 2019: Green synthesis of cellulose nanofibers using immobilized cellulase, Carbohydrate Polymers, 205, 255–260
29. ZIELIŃSKA D., SZENTNER K., WAŚKIEWICZ A., BORYSIAK S., 2021: Production of nanocellulose by enzymatic treatment for application in polymer composites, Materials, 14, 2124
30. ZINGE C., KANDASUBRAMANIAN B., 2020: Nanocellulose based biodegradable polymers, European Polymer Journal, 133, 109758

Streszczenie: *Otrzymywanie nanocelulozy poprzez hydrolizę cieczami jonowymi oraz hydrolizę dwuetapową cieczami jonowymi i enzymami*. Celem pracy było porównanie parametrów nanocelulozy otrzymanej dwoma różnymi metodami: na drodze hydrolizy cieczami jonowymi (chlorek 1-allilo-3-metyloimidazoliowy i octan 1-etylo-3-metyloimidazoliowy) z hydrolizą cieczami jonowymi w połączeniu z hydrolizą enzymatyczną, przy użyciu enzymów celulolitycznych z *Trichoderma reesei*. Celuloza Avicel została potraktowana dwoma cieczami jonowymi: chlorkiem 1-allilo-3-metyloimidazoliowym (AmimCl) i octanem 1-etylo-3-metyloimidazoliowym (EmimOAc). W hydrolizie dwuetapowej, celuloza po obróbce cieczami jonowymi była dodatkowo poddana hydrolizie roztworem enzymów. W celu scharakteryzowania otrzymanego materiału zastosowano następujące analizy: spektroskopię w podczerwieni, dyfrakcję rentgenowską oraz dynamiczne rozpraszanie światła. Wyniki wykazały, że celuloza otrzymana w wyniku dwuetapowego procesu produkcji nanocelulozy (w pierwszej kolejności hydroliza cieczami jonowymi, a następnie enzymami) wykazuje podobne parametry (wielkość cząstek, strukturę nadcząsteczkową i stopień krystaliczności) jak materiał po jednoetapowym procesie - hydrolizie cieczami jonowymi.

Słowa kluczowe: nanoceluloza, ciecze jonowe, enzymy

Corresponding author:

Izabela Ratajczak
Department of Chemistry, Faculty of Forestry and Wood Technology
Poznań University of Life Sciences,
75 Wojska Polskiego St., 60-625 Poznań, Poland
e-mail: izabela.ratajczak@up.poznan.pl