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Examination of changes in the morphology of lignocellulosic fibers treated with e-beam irradiation

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HIGHLIGHTS

- E-beam irradiation was investigated as pretreatment method for the biofuel production.
- We examine changes in the morphology of lignocellulosic materials.
- E-beam irradiation induced increase of surface area and average pore diameter.

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ABSTRACT

Ionizing radiation was applied as a substrate pretreatment method in the process of bioethanol production. The aim of the presented work was to determine the changes in the morphology of willow plant fibers caused by the interaction of a high energy electron beam with lignocellulosic biomass. The microstructure was examined with a scanning electron microscope and X-ray computer microtomography. Additionally, sorption analysis was carried out in order to determine specific surface area and porosity. The analysis carried out after the treatment of lignocellulose with an electron beam indicated destruction of cell walls, observed as a decrease in the smoothness and an increase in the roughness of the surface of the fibers. The changes in surface texture and fiber integrity affected the specific surface area and porosity of the tested samples. The specific surface area, the total volume of pores and the average pore diameter were calculated based on the isotherms of nitrogen sorption. The increase in the specific surface area was observed to occur simultaneously with the increase in the average diameter of pores.

1. Introduction

The method of producing second generation biofuels has become the subject of investigation as a technology for developing environmentally friendly energy sources. Lignocellulose is one of the most widespread materials in the world, but its application is limited due to high pretreatment costs. It is a highly organized, complex plant material composed mostly of semi-crystalline cellulose, amorphous hemicellulose and lignin. Plant cell walls are composed of cellulose chains packed into microfibrils covered by hemicellulose and lignin, creating macrofibrils (Verardi et al., 2012). In the biofuel production process, lignocellulose undergoes pretreatment in order to increase its susceptibility to the enzymes hydrolyzing cellulose, which consists in removing lignin and hemicellulose, reducing the crystallinity of cellulose, and increasing porosity (Kumar et al., 2009).

Lignocellulosic biomass can be modified with the use of physical or chemical methods in order to increase the yield of the saccharification process (Kumar et al., 2009). One of the physical methods that can be applied in the pretreatment of lignocellulose is irradiation with gamma rays or an electron beam, which causes changes in its chemical and physical properties. The effect of irradiation of different lignocellulosic materials, such as wheat straw, rice straw, sugarcane bagasse, sawdust, and poplar bark, on the effectiveness of the hydrolysis process has already been reported on (Bak et al., 2009; Chung et al., 2012; Duarte et al., 2012; Kumakura and Kaetsu, 1978, 1983; Yang et al., 2008).

An effective pretreatment method must increase the accessibility of cellulose to enzymes and facilitate mass transfer of the hydrolysis products by eroding the surface and exposing the inner part of the cell wall. Particle size and specific surface area have been indicated as factors which influence the effectiveness of the

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saccharification process with enzymes. As reported by Zeng et al. (2007), before pretreatment the smaller particles are more susceptible to hydrolysis than the bigger ones. After pretreatment, pores appear in the lignocellulose, the surface area of the large particles increases, making the cellulose more exposed and accessible to the cellulase enzyme (Zeng et al., 2007). Grethlein has confirmed the impact of lignocellulose porosity on the yield of hydrolysis with cellulase from *Trichoderma reesei*. According to the results there is a linear relationship between the initial yield and pore size up to a diameter of 51 Å, which represents the molecular size of cellulase (Grethlein, 1985). The impact of electron beam irradiation on surface smoothness and pore structure of lignocellulose has been tested on henequen fibers. Han and Choi (2010) observed an increase in surface roughness for low doses of up to 30 kGy, and an increase in the total surface area for doses of up to 100 kGy.

The aim of the presented work was to determine the changes in the morphology of willow plant fibers caused by the interaction of a high energy electron beam with lignocellulosic biomass. The willow plant was selected for the experiments because of its high level of biomass production in a short time and adaptability to different environmental conditions. In the presented work, the microstructure of lignocellulose was examined with a scanning electron microscope (SEM) and X-ray micro computer tomography (micro-CT). The changes in porosity and specific surface area were estimated using sorption analysis.

2. Experimental

The material studied were lignocellulosic fibers from willow plants (*Salix viminalis* L.). The plants were cut down after 2 years of cultivation. The cut-off branches of the plants were air-dried to 10% moisture content and ground in a laboratory mill to particles 2–10 mm in length. Samples of the material were exposed to electron beam irradiation with doses from 25 to 300 kGy using the 10 MeV–10 kW linear electron accelerator "Elektronika" at the Institute of Nuclear Chemistry and Technology in Warsaw. The irradiation was carried out in air atmosphere in multiple stages, delivering a dose of 25 kGy in one pass, thus minimizing the temperature effect of high-dose irradiation. The microstructure of the fibers and integrity of the cell walls were studied with a scanning electron microscope and x-ray micro computer tomography.

SEM images of the lignocellulosic material were obtained using a Leo 1530 Gemini scanning electron microscope with a low accelerating voltage of 2 kV. The samples for SEM examination were prepared according to a standard procedure, fixed with conductive glue and coated with a thin layer of gold. The samples were examined at magnifications of 500, 5000, and 25,000 times.

The internal 3D structure of the fibers was examined with the use of the XRADIA Micro XCT-400 X-ray micro computer tomography system. Calculations of the changes in the total porosity of the samples as well as in the total surface area and cell wall thickness were performed.

The specific surface area of the samples was determined based on the isotherms of nitrogen physical sorption measured experimentally using Quadrasorb-SI equipment (Quantachrome Co). Before the measurements, the samples were degassed at a temperature of 100 °C for 48 h. The adsorption and desorption of nitrogen on the surface of lignocellulose were measured at a temperature of −195.8 °C, within the entire range of relative pressure. The specific surface area was determined using the Brunauer–Emmett–Teller method and the BET equation, which permit determination of the specific surface area within the range of relative pressure (P/P_0) from 0.05 to 0.35. The total volume of the pores present in the lignocellulose samples was determined by the Barret–Joyner–Halenda method and using the BJH equation based on the isotherms of the physical desorption of nitrogen. The method is based on the assumption that during capillary condensation at a relative pressure below 0.4, the only effect which occurs with a further increase in the pressure is the thickening of the mono-layer of the absorbate formed on the surface of the pore walls. The distributions of the pore sizes determined by the BJH method were used to estimate the average pore diameter.

3. Results

3.1. Scanning electron microscopy

The SEM method was applied to examine the changes caused by ionizing radiation on the surface of the lignocellulosic material. The images at 500× magnification, presented in Fig. 1, show typical structures of wood tissue with longitudinal fibers and vessels partially destroyed during mechanical pretreatment. This magnification was not sufficient to observe the influence of irradiation on the microstructure of wood tissue.

To observe the influence of irradiation on the cell walls of lignocellulose, a magnification of 5000× was used. The images are shown in Fig. 2. SEM images of a lignocellulosic material before treatment show a flat, smooth surface. Irradiation of the samples leads to morphological changes in the material's structure. After irradiation, some cleavage of the fibers in the external layers can be observed. These effects of irradiation are already visible in the sample irradiated at 25 kGy. The increase in surface roughness and porosity is observed with increasing dose up to 300 kGy. After pretreatment with 300 kGy, the destruction of the smooth structure of the surface and breaking of the cell walls can be observed. Higher magnification (25000×) revealed an increase in surface roughness of the lignocellulosic material, but it did not make it possible to characterize the changes in the porosity of the surface.

3.2. X-ray computed microtomography

X-ray computer microtomography is a non-destructive method for the characterization of materials, allowing to obtain a flat or spatial image of the material. In contrast to classical tomography applicable to medical diagnostics, microtomography allows to obtain images at very high resolutions, typically in the range of

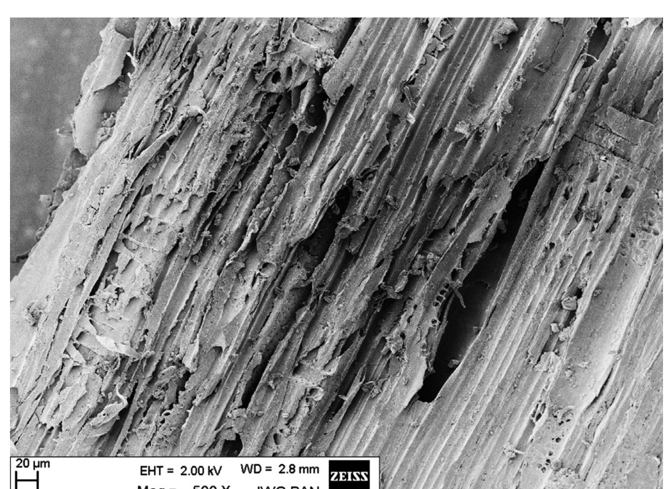

Fig. 1. SEM image of the surface of willow plant fibers – control sample at 500× magnification.

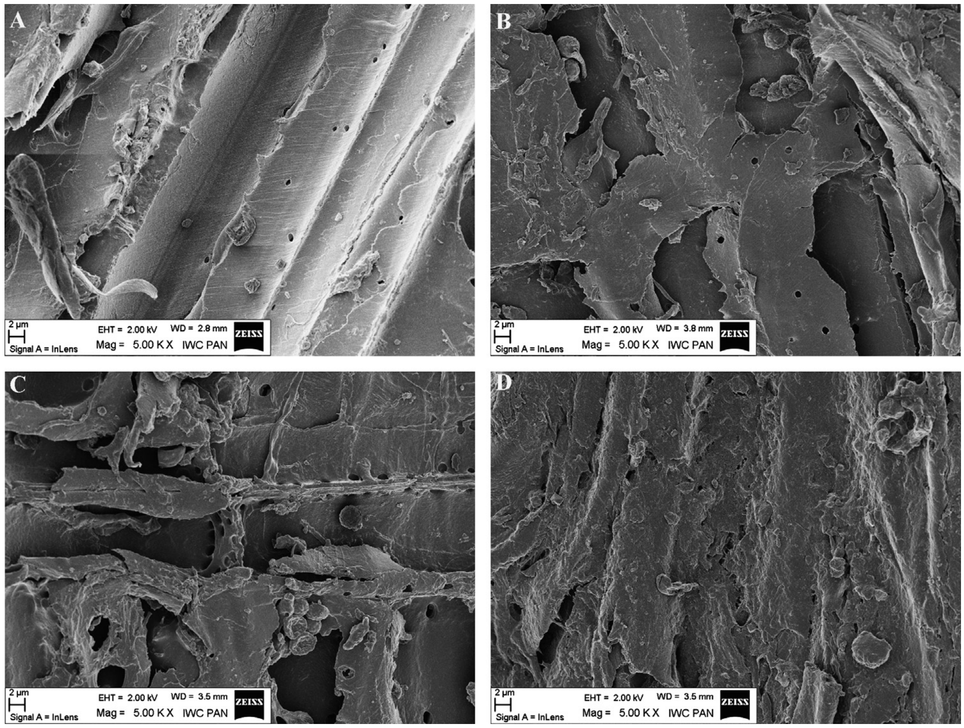

Fig. 2. SEM images of the surface of willow plant fibers at 5000 × magnification: A–control, B–25 kGy, C–100 kGy, D–300 kGy.

100 nm. While scanning, the sample was rotated in predetermined angular steps. In each of the set angular positions the transmission image (projection) of an object was recorded. Then, based on a whole series of transmission images, a computer reconstruction of the internal structure of the test object was performed. Examples of the transmission images and computer simulated structures obtained for the lignocellulosic samples are shown in Fig. 3.

Wood is classified into two major categories: softwood and hardwood. According to Mayo et al. (2010), hardwood can be characterized by longitudinal fibers of the order of 1 mm in length and 10 μm in average diameter as structural elements, and by large vessels as the elements used for transporting water, evident in the images as pores. According to the morphology of the tissue observed using micro-CT (Fig. 3), the willow plant can be classified as hardwood. Comparing the cross-sections of samples before and after irradiation, it can be concluded that under the influence of radiation there occurs partial tearing of the fibers and destruction of the cell walls.

Using the transmission images, 3D models of structures were generated. In the models, the partial destruction of lignocellulose fibers can be observed. Computer calculations performed for the models show changes in the internal structure determining features such as porosity, particle and pore sizes and their statistical distributions. Before irradiation, the measured average pore diameter was 6.12 μm (standard deviation SD = 1.32 μm), and the average wall thickness was 4.27 μm (SD = 0.7 μm). Ionizing radiation caused a decrease in the average pore diameter and an increase in the mean wall thickness as well as increase in porosity of about 4.5%. The total wall area based on the total sample volume from 0.85 1/μm to 0.95 1/μm increases after irradiation with 300 kGy.

The SEM and micro-CT methods revealed the morphological changes occurring during radiation pretreatment of lignocellulose. As suggested by Zeng et al. (2007), the intermicrofibrillar cavities formed during pretreatment cannot be observed with SEM. To obtain detailed information on porosity and specific surface area, sorption analysis was performed.

3.3. *Analysis of porosity and surface area*

The specific surface area of the lignocellulose samples and their open porosity were measured based on the isotherms of the physical sorption of nitrogen determined experimentally. The results, including the specific surface area and the average pore diameter, are presented in Figs. 4 and 5. It can be seen that the specific surface area of the lignocellulose samples irradiated with different doses increases with the dose and exceeds 0.9 m^2/g for 300 kGy (Fig. 4). However, the total pore volume of the willow samples fluctuates around 0.001 cm^3/g and no unequivocal trend can be observed, even for such a high radiation dose as 300 kGy. The average pore diameter of the samples treated with different radiation doses increases with increasing dose and exceeds 140 Å in the sample treated with 300 kGy (Fig. 5). The obtained results indicate that the total volume of the pores does not increase in spite of the fact that the surface area and pore diameter increase. This may be attributed to a progressive expansion of pores during radiation treatment, destruction of walls, and simultaneous agglomeration of small pores into larger structures.

4. Conclusions

Lignocellulosic materials are complex polymer structures whose morphology can be observed on a micro scale with scanning electron microscopy or computer tomography. Scanning electron microscopy is useful for the characterization of surface changes. After irradiation, a partially disordered surface and a decrease in the smoothness and integrity of fiber walls were observed. Micro computer tomography enables characterization of the morphology of the internal parts of the samples. The transmission images and the calculations performed showed that irradiation of lignocellulose induced an increase in porosity accompanied by the destruction of cell wall integrity, which was observed as an increase in wall thickness and a decrease in average pore diameter. The measurements based on the isotherms of

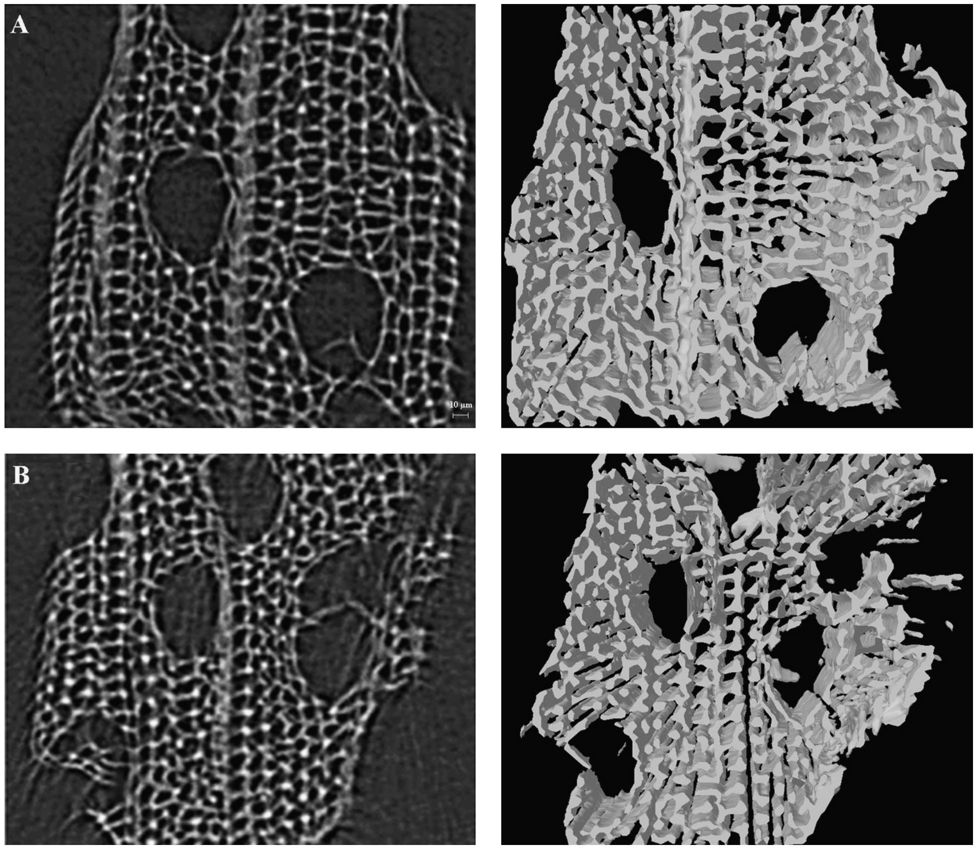

Fig. 3. Transmission images and computer simulations of the willow plant structure: A- control sample, B- sample irradiated with 300 kGy.

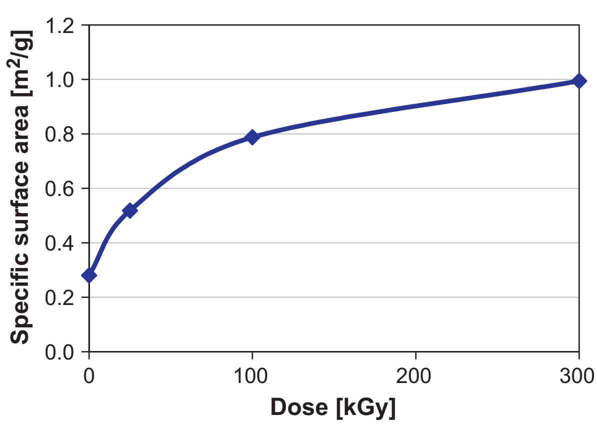

Fig. 4. Surface area of the willow samples treated with different radiation doses determined using the BET method.

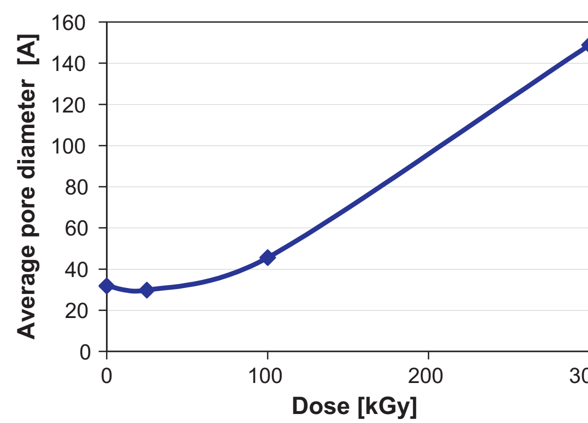

Fig. 5. Average pore diameter of the willow samples treated with different radiation doses estimated using the BJH method.

nitrogen physical sorption confirmed that irradiation of lignocellulose effected an increase in surface area and average pore diameter. There were no significant changes in total pore volume, which can be connected with the destruction of small pores and aggregation into larger ones. The changes observed during the study indicated a positive effect of ionizing radiation on the morphology of lignocellulose from the point of view of the biofuel production process. The greater surface area, porosity and pore diameter should increase the reactivity of the material and facilitate mass transfer, thus increasing the efficacy of the process. Irradiation with an electron beam is a widely applied method of polymer degradation and modification. The effect of the interaction of ionizing radiation with lignocellulosic biomass depends on many factors, such as phenolic compounds content and moisture content, as well as how the process is carried out. Irradiation with an electron beam is a multistage process which allows to reduce the temperature effect of high-dose irradiation so that the observed changes can be attributed to irradiation.

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