

Fast-growing tree species as sugars sources

N. Yavorov*, I. Valchev

Department of Pulp, Paper and Printing Arts, University of Chemical Technology and Metallurgy, 8 St. Kliment Ohridski Blvd., 1756 Sofia, Bulgaria

Received: September 13, 2019; Accepted: October 02, 2019

The use of lignocellulosic biomass for the production of bio-based products is a worldwide tendency. The present study examines steam explosion pretreatment and subsequent enzymatic hydrolysis of different fast-growing tree species (poplar, willow, black locust, paulownia and ailanthus) to obtain sugars that can be substrate in various fermentation processes. Experimental results showed that the pretreatment of species with low wood density can be carried out under milder conditions compared to those with higher density. It was established that the efficiency of hydrolysis by a commercial cellulase complex also depends on the wood density, and the highest yield of glucose is reached from paulownia. The topochemical kinetic mechanism of cellulase hydrolysis of fast-growing tree species was established, and it was found that the activation energy and the pre-exponential factor increase with wood density decrease. The determinant for the process rate is the pre-exponential factor.

Keywords: Fast-growing tree, Lignocellulosic biomass, Steam explosion, Enzymatic hydrolysis, Fermentable sugars

INTRODUCTION

The rapid increase in energy demand and the unsustainable use of natural resources are among the major global concerns. The excessive dependency on petroleum fuel leads to various concerns regarding economics, environment and energy security. In this context, use of plant biomass is the favourable substitute to the conventional sources of energy production [1]. Lignocellulosic biomass is the most abundant source of renewable biopolymers on Earth and a promising cheap raw material for the production of fuels, chemicals and materials [2–4]. The major polymeric components in lignocellulosic biomass are: cellulose, hemicelluloses and lignin. Compositions vary for different types of biomass [5]. The conversion of lignocellulosic materials into bioproducts enables to avoid competition between their manufacturing and food production [6]. Lignocellulosic biomass broadly includes agricultural residues, forest-based plants, food wastes, agricultural by-products and purpose-grown energy crops, such as switch grass, fast-growing tree species, etc. [7]. The production of biomass from fast-growing trees for energy purposes has become a new line of agricultural production, which is defined as the agro power industry [8]. The wood biomass from plantations of fast-growing tree species, such as paulownia, poplar, black locust, eucalyptus and willow, can be an attractive alternative for bioethanol production. Fast-growing tree species grown in short-rotation plantations are a promising source of lignocellulosic biomass because of high

yield, opportunities for use on lower-quality lands and biodiversity support at the landscape level [9, 10]. Biochemical conversion of lignocellulosic biomass involves a pretreatment step to depolymerise its lignin-carbohydrates complex and subsequent enzymatic hydrolysis for releasing fermentable sugars [11]. The effective pretreatment plays a key role, since it critically influences the subsequent enzymatic hydrolysis [12]. A large number of pretreatment technologies have been developed on a wide variety of lignocellulosic materials, since individual raw materials have different physicochemical characteristics. The pretreatment methods can be classified into physical, chemical, physicochemical, biological methods and by some combinations of these processes [4, 13]. Among these methods, steam explosion (SE) is one of the most commonly used physicochemical methods for pretreatment of wood-based raw materials relying on recovering partially-degraded hemicelluloses from wood tissue by an attractive hydrolysing medium that enables a wide variety of reactions without catalyst [12, 14]. SE is a combination of physical and chemical effects, and involves exposing lignocellulosic biomass to high-pressure saturated steam at temperatures typically between 160 and 260°C for several seconds to a few minutes and then quickly reducing pressure to atmospheric condition [15–17]. This leads to disruption of the lignocellulosic complex, removal of hemicelluloses, partial removal and/or redistribution of lignin and considerable enhancement of the accessibility of the enzymes to the cellulose fibrils [18].

* To whom all correspondence should be sent:
E-mail: nyavorof@gmail.com

Several wood-based raw materials have shown positive effects on pretreatment with SE, such as poplar wood [17, 19–21], eucalyptus wood [22, 23] and spruce wood [24]. The present study aims to evaluate the processes and the potential for obtaining sugars from fast-growing tree species (poplar, willow, black locust, paulownia and ailanthus) by SE pretreatment and enzymatic hydrolysis. In order to clarify the influence of the pretreatment on enzymatic hydrolysis, the relationship between structural features and sugars yield was investigated. Additionally, the dependence of the kinetic parameters of cellulase hydrolysis on wood density of the species concerned is reported.

EXPERIMENTAL

Materials

The following fast-growing tree species were investigated: paulownia (*Paulownia elongata* S.Y. Hu (PE) and *Paulownia tomentosa* Thunb. (PT)), poplar (*Populus alba* L. (PA) and *Populus ×euramericana* (Dode) Guinier (PEA), cultivars (cvs.) 'I 214' and 'I 45 51'), willow (*Salix viminalis rubra* L. cv. 'X/3' (SVR)), black locust (*Robinia pseudoacacia* L. (RPA), cvs. 'Jáskiséri', 'Pordim' and 'Tsarevets') and ailanthus (*Ailanthus altissima* (Mill.) Swingle (AA)). The tree species were harvested and collected from the Experimental Station for Fast-growing Forest Tree Species, Svishtov, Bulgaria (43°37'N 25°21'E). Initially, they were chipped to a particle size of 20–25 mm. Chemical analyses were based on the following methods: for cellulose according to Kürschner and Hoffer [25], pentosans (TAPPI standard T 223 cm-10), lignin (TAPPI standard T 222 om-11), hot water solubility (TAPPI standard T 207 cm-08) and ash (TAPPI standard T 211 om-12). The results are reported as percentages of oven-dry weight. The wood density was determined according to DIN EN 52182 standard.

Methods

Steam explosion pretreatment. The SE pretreatment of wood chips was carried out in a 2 L stainless steel laboratory-scale system at a solid-to-liquid ratio of 1:10 (w/v). The raw materials were steamed at the desired temperature (190 and 200°C, respectively) under pressure for 10 min. Defibration of the pretreated materials was achieved by a rapid decompression. After the pretreatment, the solid and liquid fractions were separated in a cyclone. The solid fraction was washed with distilled water to remove the dissolved components. The separated

prehydrolysates were analysed for sugar composition. The effects of temperature and time on the pretreatment process were calculated based on the severity factor (Eq. 1) defined by Overend *et al.* [26].

$$\log(R_0) = \log(t \cdot \exp^{(T-100)/14.75}) \quad (1)$$

where R_0 is the severity parameter, T is the reaction temperature (°C), 100 is a reference temperature (°C), t is time (min) and 14.75 is an empirical parameter related to temperature and activation energy.

Enzymatic hydrolysis. Pretreated solids of various severity factors were enzymatically hydrolysed by the commercial complex Cellic CTec2 (produced by *Trichoderma reesei*) containing 125 FPU/g of cellulase (filter paper activity). All hydrolysis runs were carried out in polyethylene bags in a water bath at 10% solids loading (g of dry solids/volume of the hydrolysis mixture) and enzyme loading – 5% of Cellic CTec2 per g of dry pretreated substrate (approximately 6.3 FPU/g). The process was conducted at a temperature of 50°C, pH of 5.0 (according to the manufacturer's recommendations) and reaction time of 2880 min. The treatment was stopped by heating the samples at 100°C for 10 min.

HPLC analysis. The sugars (glucose, xylose and cellobiose) and furan compounds (5-hydroxymethylfurfural (5-HMF) and furfural) contents were measured on a Dionex HPLC system (Dionex Inc., CA, USA) equipped with a Shodex RI-101 RI detector (Showa Denko KK, Kawasaki, Japan), according to the National Renewable Energy Laboratory (NREL) analytical methods for biomass [27]. The separation was performed in a Hi-Plex H column, 7.7 mm × 300 mm (Agilent Technologies, USA) at 65°C with ultrapure water produced by a Simplicity® water purification system (Merck KGaA, Darmstadt, Germany) as eluent at a flow rate of 0.5 mL/min. Injected volume: 20 µL. The results were evaluated by the Chromeleon 6.80 software. The amount of sugars was calculated as a percentage (%) on a dry matter basis.

RESULTS AND DISCUSSION

Chemical composition. The broad-leaved tree species studied differ considerably in their density (Table 1). The results obtained show that the black locusts are characterized with the highest density, it being 773 kg/m³ in the case of the wood of RPA cv. 'Pordim'. Lowest is the density of the wood of PT – 240 kg/m³.

Table 1. Chemical composition and wood density

Tree species	ρ_0 (kg/m ³)	Lignin (%)	Cellulose (%)	Pentosans (%)	Ash (%)	Hot water solubility (%)
PE	311	22.03	46.42	22.47	0.42	5.86
PT	240	20.73	47.78	22.29	0.84	8.35
AA	592	20.85	48.07	25.75	0.83	5.21
SVR	420	25.38	45.05	22.25	1.38	6.15
PEA cv. 'I 214'	418	24.81	49.85	23.23	0.53	2.18
PEA cv. 'I 45 51'	400	23.93	49.24	22.03	0.57	2.39
PA	325	21.23	48.79	24.62	0.65	2.56
RPA cv. 'Pordim'	773	26.56	48.25	21.92	0.35	6.51
RPA cv. 'Jászkiséri'	720	23.41	45.78	21.90	0.52	6.85
RPA cv. 'Tsarevets'	661	23.05	48.89	22.73	0.41	4.03

The density is relatively low in the case of poplars and willows, whereas the wood of ailanthus takes an intermediate position with respect to this indicator. PEA cvs. 'I 214' and 'I 45 51', which also have the lowest percentage of substances soluble in hot water, are characterized with the highest cellulose content (49.85% and 49.24%, respectively). The presence of these substances is most often connected with the content of some low-molecular, extractive and pectic substances capable of being hydrolysed and dissolved at a temperature of up to 100°C. From Table 1 it is seen that their amount predominates in PT (8.35%). Similar results have been also reported by Balatinecz and Kretschmann [28], where holocellulose amounts in poplar wood were found to be approximately 80%, made up of 50% cellulose and 30% hemicelluloses. The total content of cellulose and pentosans in case of the fast-growing tree species studied is within 67.30% – 73.82%, it being highest in the case of ailanthus and poplars, and lowest in the case of willow that is characterised with high lignin content. The wood with highest density of RPA cv. 'Pordim' contains the highest lignin amount, and that with lowest density of PT is characterised with very low lignin content. Other visible dependences between the wood chemical composition and density cannot be found.

It is known that the chemical composition of the wood of the individual tree species varies considerably. With respect to one tree species only, there is a difference in the chemical composition which is related to a number of factors, some of which are: time of harvesting, growth conditions, tree part from which the sample for analysis is taken, etc. [29, 30].

It can be noted that the chemical composition of the wheat straw, which is the main raw material in

the production of sugars for bioethanol, is characterised with a similar cellulose (44%) and lignin (24.3%) content whereas the content of pentosans (33.5%), ash (4.6%) and substances soluble in hot water (20.7%) is considerably higher [31].

Steam explosion pretreatment. To facilitate the enzyme action into the complex lignocellulosic structure of wood biomass, a SE pretreatment was carried out. The effect of structural differences between the tree species was evaluated by applying the same pretreatment conditions. Different steam pretreatment severities ($\log R_0 = 3.65$ and 3.94 , respectively) were applied to compare the degree of hemicelluloses dissolution and recovery in a monomeric form with limited degradation of solubilised sugars to furan derivatives.

The SE pretreatment of wood chips of broad-leaved fast-growing tree species showed that the wood density has a determining effect on the pretreatment conditions. It was established that in the case of the tree species with density below 350 kg/m³, the process may be carried out at $\log R_0 = 3.65$ or lower. In the case of the tree species with higher density it is necessary to apply higher severity in order to defibre the wood in the explosion process.

The amount of the dissolved wood components in the SE pretreatment carried out at $\log R_0 = 3.94$ is lowest in the case of black locusts that are also characterized with the highest density.

The analysis of the prehydrolysates generated during the SE pretreatment showed that the monosaccharides content increases along with concomitant increase in severity of the pretreatment (Table 2).

Table 2. Yield and amount of dissolved substances after SE pretreatment

Tree species	Severity factor (R_0)	Solid recovery (%)	Glucose (%)	Xylose (%)	Cellobiose (%)	5-HMF (%)	Furfural (%)
PE	3.65	67.51	1.08	2.19	0.56	0.12	0.22
	3.94	63.02	1.95	2.86	1.02	0.29	0.35
PT	3.65	67.12	1.12	2.0	0.89	0.11	0.26
	3.94	62.74	1.98	2.81	1.16	0.33	0.37
PA	3.65	67.72	1.62	2.98	1.12	0.22	0.35
	3.94	62.31	1.87	3.65	1.31	0.36	0.49
SVR	3.65	68.81	0.71	0.97	0.13	0.09	0.17
	3.94	62.34	0.91	1.55	0.43	0.11	0.19
PEA cv. 'I 214'	3.94	63.27	1.04	2.68	0.68	0.19	0.34
PEA cv. 'I 45 51'	3.94	63.71	0.62	1.32	0.70	0.15	0.35
AA	3.94	63.33	0.93	1.85	0.66	0.08	0.18
RPA cv. 'Pordim'	3.94	66.81	1.14	2.28	0.98	0.03	0.24
RPA cv. 'Jászkiséri'	3.94	66.76	1.26	2.09	1.56	0.11	0.18
RPA cv. 'Tsarevets'	3.94	65.14	1.13	2.11	0.68	0.03	0.17

The results of HPLC also indicated the presence of a significant content of soluble oligomeric sugars. 5-HMF and furfural derived from glucose and xylose degradation, respectively, were also detected in the prehydrolysates.

The data obtained during the SE pretreatment of the wood showed that the low wood density favours the process and allows its implementation under milder conditions. In general, pretreatments at high severity $R_0 \geq 4$) showed a produced higher xylose recovery but also higher sugars degradation and inhibitors production [20].

Enzymatic hydrolysis. The effect of the pretreatment in the case of the examined fast-growing tree species on the efficiency of the subsequent enzymatic hydrolysis was studied. The generated hydrolysates were analysed by means of HPLC analysis to establish their composition. The obtained results about the yield of monosaccharides – glucose and xylose, are summarized and presented in Table 3. The results showed that the highest yield of glucose is reached after 2880 min in the case of PT that is characterized with the lowest density. Relatively good results were also obtained in the

Table 3. Yield of sugars after enzymatic hydrolysis of SE pretreated wood

Tree species	Severity factor (R_0)	Glucose (%)	Xylose (%)
PE	3.65	38.98	2.57
	3.94	39.31	2.99
PT	3.65	44.13	2.61
	3.94	44.63	2.86
PA	3.65	35.92	2.34
	3.94	36.94	2.42
SVR	3.65	26.45	2.84
	3.94	30.06	3.19
PEA cv. 'I 214'	3.94	33.78	1.92
PEA cv. 'I 45 51'	3.94	33.16	2.92
AA	3.94	33.52	3.26
RPA cv. 'Pordim'	3.94	27.51	3.66
RPA cv. 'Jászkiséri'	3.94	32.34	3.29
RPA cv. 'Tsarevets'	3.94	34.91	3.08

case of the tree species PE and PA and RPA cv. 'Tsarevets'. The glucose yield in the case of these tree species is comparable with and higher than that after enzymatic hydrolysis of wheat straw [32]. It was established that the efficiency of the enzymatic hydrolysis is lower in RPA cv. 'Pordim' that is characterized with the highest density and lignin content. The remaining tree species take an intermediate position, not yielding considerably to wheat straw. The xylose yield after the enzymatic hydrolysis is within 1.92% to 3.66% and practically does not depend on the density of the tree species. It was also established that the severity of the SE pretreatment does not exercise considerable effect on the efficiency of the enzymatic hydrolysis in the case of the tree species with low density Paulownia and PA, which is yet another confirmation of the possibility for implementation of the process under milder conditions. The results obtained are an indicator of the high potential of the fast-growing tree species with low density as a raw material for production of sugars.

It was established that the kinetics of the cellulosic action in all fast-growing tree species is most exactly described by the Prout-Tompkins topochemical equation [33]. The applicability of a topochemical kinetic mechanism is an indicator of the zonal course of the process that is characterized with opening of new active centres in case of diffusion limitations. The fibrous structure of the wood biomass is determining for the applicability of the topochemical kinetic mechanism observed. Unlike wood, the kinetics of the cellulosic action in wheat straw is most exactly described by the exponential kinetic equation [31]. The predominant content of non-fibrous structures in wheat straw is probably determining for the observed difference and for the applicability of the exponential kinetic model valid for processes taking place at heterogeneous surfaces [34]. During the study of the effect of the properties of the fast-growing tree species on the kinetics of the enzymatic action, well expressed linear dependences (Eqs. 2 and 3) between the wood density, on the one hand, and the activation energy and the pre-exponential factor, on the other hand, were established (Fig. 1).

$$E = E^0 - m \cdot \rho \quad (2)$$

$$\ln \lambda = \ln \lambda^0 - n \cdot \rho \quad (3)$$

where: E^0 , m , $\ln \lambda^0$ and n are empirical constants.

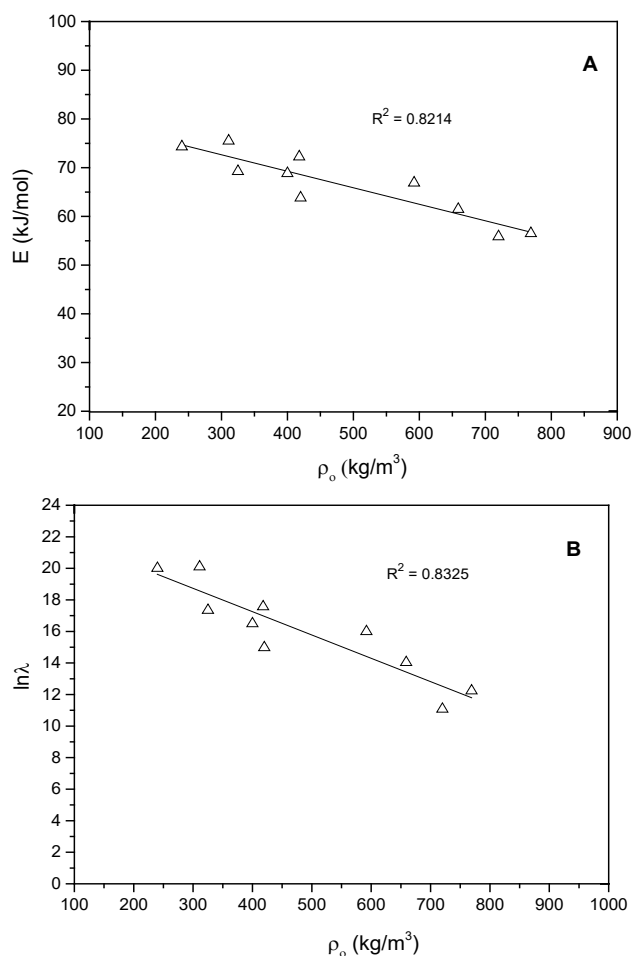


Fig. 1. Dependence of A: activation energy, B: pre-exponential factor on the wood density.

Both kinetic parameters increase with the decrease of the wood density, but determining is the effect of the pre-exponential factor. The combination of chemical interaction and diffusion processes affects the rate of the process of enzymatic hydrolysis of the examined fast-growing tree species with various density. The process of hydrolysis is limited by the diffusion of the large enzyme molecules in the capillary system of the fibrous matrix, which also determines the observed regularity of rate decrease with the increase of the steric difficulties with the increase of the wood density.

CONCLUSIONS

When studying the composition of the fast-growing tree species, no expressed dependences between the chemical composition and the density were observed, but it was established that the wood with the highest density is characterized with the highest lignin content, and that with the lowest density has the lowest lignin content. It was established that the low wood density favours the implementation of the SE pretreatment at lower

severity and ensures the necessary efficiency of the enzymatic hydrolysis. The results of the enzymatic hydrolysis showed that the highest glucose yield is obtained in the case of PT that is also characterized with the lowest density. The efficiency of the enzymatic hydrolysis of the tree species with low density is comparable and higher than that of enzymatic hydrolysis of wheat straw. A common feature of the kinetics of enzymatic hydrolysis of the pretreated fast-growing tree species is the topochemical mechanism that is characterized with a zonal course of the process with diffusion limitations. It was established that by the decrease of the wood density, the pre-exponential factor, that has a determining effect on the process rate, increases.

REFERENCES

1. S. Nanda, A. K. Dalai, J. A. Kozinski, *J-FOR*, **3**, 15 (2013).
2. F. H. Isikgor, C. R. Becer, *Polym. Chem.*, **6**, 4497 (2015).
3. M. Bilal, M. Asgher, H. M. N. Iqbal, H. Hu, X. Zhang, *Int. J. Biol. Macromol.*, **98**, 447 (2017).
4. A. K. Kumar, S. Sharma, *Bioresour. Bioprocess.*, **4**, 7 (2017).
5. M. Zhou, J. Yang, H. Wang, T. Jin, D. J. Hassett, T. Gu, in: *Bioenergy Research: Advances and Applications*, V. K. Gupta, M. G. Tuohy, C. P. Kubicek, J. Saddler, F. Xu (eds.), Elsevier BV, Oxford, 2014, p. 131.
6. K. Buzala, P. Przybysz, J. Rosicka-Kaczmarek, H. Kalinowska, *Cellulose*, **22**, 663 (2015).
7. K. Ullah, V. K. Sharma, S. Dhingra, G. Braccio, M. Ahmad, S. Sofia, *Renew. Sust. Energ. Rev.*, **51**, 682 (2015).
8. A. Szostak, G. Bidzinska, E. Ratajczak, M. Herbec, *Drewno*, **56**, 85 (2013).
9. S. González-García, M. T. Moreira, G. Feijoo, R. J. Murphy, *Biomass Bioenergy*, **39**, 378 (2012).
10. A. Procentese, F. Raganati, G. Olivieri, M. E. Russo, M. de la Feld, A. Marzocchella, *N. Biotechnol.*, **39**, 135 (2017).
11. J. Xu, Z. Wang, J. J. Cheng, *Bioresour. Technol.*, **102**, 7613 (2011).
12. E. Domínguez, A. Romani, L. Domingues, G. Garrote, *Appl. Energy*, **187**, 777 (2017).
13. P. Alvira, E. Tomás-Pejó, M. Ballesteros, M. J. Negro, *Bioresour. Technol.*, **101**, 4851 (2010).
14. F. Cotana, C. Buratti, M. Barbanera, E. Lascaro, *Bioresour. Technol.*, **198**, 470 (2015).
15. Y. Sun, J. Cheng, *Bioresour. Technol.*, **83**, 1 (2002).
16. H. Rabemanolontsoa, S. Saka, *Bioresour. Technol.*, **199**, 83 (2016).
17. T. Auxenfans, D. Crônier, B. Chabbert, G. Paës, *Biotechnol. Biofuels*, **10**, 36 (2017).
18. N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple, M. Ladisch, *Bioresour. Technol.*, **96**, 673 (2005).
19. W. R. Grous, A. O. Converse, H. E. Grethlein, *Enzyme Microb. Technol.*, **8**, 274 (1986).
20. M. Cantarella, L. Cantarella, A. Gallifuoco, A. Spera, F. Alfani, *Biotechnol. Prog.*, **20**, 200 (2004).
21. J. S. Martín-Davison, M. Ballesteros, P. Manzanares, X. P. B. Sepúlveda, A. Vergara-Fernández, *Int. J. Green Energy*, **12**, 832 (2015).
22. A. P. Nunes, J. Pourquie, *Bioresour. Technol.*, **57**, 107 (1996).
23. R. Martín-Sampedro, M. E. Eugenio, J. C. García, F. Lopez, J. C. Villar, M. J. Diaz, *Biomass Bioenergy*, **42**, 97 (2012).
24. M. Muzamal, K. Jedvert, H. Theliander, A. Rasmuson, *Holzforchung*, **69**, 61 (2015).
25. K. Kürschner, A. Hoffer, *Techn. Chem. Papier und Zellstoff Fabr.*, **26**, 125 (1929).
26. R. P. Overend, E. Chornet, J.A. Gascoigne, *Phil. Trans. R. Soc. Lond. A*, **321**, 523 (1987).
27. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, NREL, Golden, 2008.
28. J. J. Balatinez, D. E. Kretschmann, in: *Poplar Culture in North America*, D.I. Dickmann, J. G. Isebrands, J. E. Eckenwalder, J. Richardson (eds.), NRC Research Press, Ottawa, 2001, p. 227.
29. F. Pitre, F. Lafarguette, B. Boyle, N. Pavy, S. Caron, N. Dallaire, P. Poulin, M. Ouellet, M. Morency, N. Wiebe, E. Lim, A. Urbain, G. Mouille, J. Cooke, J. Mackay, *Tree Physiol.*, **30**, 1273 (2010).
30. M. Stolarski, M. Krzyżaniak, M. Łuczyński, D. Załuski, S. Szczukowski, J. Tworkowski, J. Gołaszewski, *Ind. Crops Prod.*, **75**, 66 (2015).
31. G. Radeva, I. Valchev, S. Petrin, E. Valcheva, P. Tsekova, *Cellulose Chem. Technol.*, **46**, 61 (2012).
32. I. Valchev, S. Petrin, in: *R&D on Processes for Solid, Liquid and Gaseous Fuels from Biomass* (Proc. 21st EU BC and E, Copenhagen, 2013), A. Eldrup, D. Baxter, A. Grassi, P. Helm (eds.), ETA-Florence Renewable Energies, Italy, 2013.
33. I. Valchev, N. Yavorov, S. Petrin, *Holzforchung*, **70**, 1147 (2016).
34. S. Singh, D. Dutt, C. H. Tyagi, *BioResources*, **6**, 154 (2010).