Industrial Crops and Products 75 (2015) 66–75

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Lignocellulosic biomass from short rotation woody crops as a feedstock for second-generation bioethanol production

Mariusz J. Stolarski[a,*], Michał Krzyżaniak[a], Michał Łuczyński[b], Dariusz Załuski[a], Stefan Szczukowski[a], Józef Tworkowski[a], Janusz Gołaszewski[a]

[a] University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, Department of Plant Breeding and Seed Production, Plac Łódzki 3, 10-724 Olsztyn, Poland

[b] University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, Department of Chemistry, Plac Łódzki 4, 10-957 Olsztyn, Poland

ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 9 April 2015
Accepted 10 April 2015
Available online 6 May 2015

Keywords:
Willow
Poplar
Black locust
Chemical composition
Bioethanol

ABSTRACT

Lignocellulosic biomass can be used as a substrate in an integrated biorefinery, including in the production of second-generation biofuels. Therefore, this study analyzed the chemical composition of biomass of willow, poplar and black locust, depending on the method of soil enrichment (lignin, mineral fertilization, mycorrhiza inoculation and control - no enrichment), and harvest cycle (three- and four-year), as potential feedstock in the production of second-generation bioethanol. The highest content of cellulose in the experiment was found in willow biomass obtained from the control plot in the 3-year harvest cycle. Although the content of cellulose in poplar biomass was similar regardless of its harvest cycle, it was lower than in willow biomass by an average of 5% points. Furthermore, the average content of cellulose in biomass of black locust harvested in a 3-year cycle was the lowest. Of the species under study, the highest content of lignin was found in biomass of poplar, both in the 3-year and 4-year harvest cycle. The study found that although the choice of SRWC species used as a source of polysaccharides must take into account the percentage content in biomass, species and soil enrichment methods must also be chosen to ensure high biomass yield per unit area because differences in the potential yield of individual polysaccharides per 1 ha in some cases exceeded 1000%.

1. Introduction

Biomass is one of the most easily available feedstock and sources of energy in the world. Depending on its properties, it is used in different branches of the food, forestry, construction, energy, medicine and chemical industries. Ethanol, used in production of transport fuels, is one of products obtained from biomass (Dalla Marta et al., 2014; Johnson and Silveira, 2014). The ethanol production output in 2013 amounted to 88.69 billion liters. Brazil and the USA are the two major ethanol producing countries (over 80% market share), where it is produced from sugarcane (Brazil) or starch, mainly from corn (USA) (Gupta and Verma, 2015). However, studies have shown that biofuels obtained in this manner frequently do not contribute to greenhouse gas reduction and they require a large amount of energy for their production (Fargione et al., 2008). However, efforts are being made to use corn kernel, bagasse and sugarcane-trash to produce cellulosic ethanol. This could be a way of reducing GHG emissions from maize and sugar cane plantations (Alonso Pippo et al., 2011; Shrestha et al., 2012). Moreover, they compete with food production directly, by the biorefineries using their products (crops) and because they are grown on good quality soils where food crops could be grown. This results in food price increases. This can be shown in an example of a drought in the USA – a country in which 40% of corn production output is used to produce bioethanol. Due to the drought and a considerable crop yield drop in 2012/2013, the average farm price for corn was $6.89 per bushel compared with $6.22 in 2011/2012 (USDA, 2014). Moreover, according to the Action Aid report, the price of corn in developed countries increased by $6.6 billion over six years because a large portion of it was used to produce ethanol (Action Aid International USA, 2012).

Therefore, attempts have been made to produce second-generation ethanol from cellulose and hemicellulose obtained from non-food crops or residues from food crops. Such feedstock can be obtained from both forestry and agricultural resources. (Kim and Dale, 2004) reported that 442 billion liters of second generation ethanol can be produced from lignocellulosic biomass and

* Corresponding author. Tel.: +48 895234838.
E-mail address: mariusz.stolarski@uwm.edu.pl (M.J. Stolarski).

http://dx.doi.org/10.1016/j.indcrop.2015.04.025
0926-6690/

that total crop residues and wasted crops can produce 491 billion liters of ethanol per year. de Vries et al., (2014) found that second-generation biofuels from miscanthus and black locust perform substantially better than first generation systems based on rapeseed and sugarbeet. They contribute much more to GHG emission reduction, have much higher net energy yields and better resource use efficiencies (soil erosion and N leaching were also lower). However, the sustainability of second-generation biofuels may also be disputable if producers follow the path of manufacturers of first generation fuels (e.g., deforestation, soil organic carbon content, water and nutrients use, intensification, long transport distance, etc.) (Mohr and Raman, 2013).

This is the reason why the development of lignocellulosic biorefineries has been attracting increasing attention in many countries around the world, mainly in Brazil, the USA, Canada, Japan, India, China and in Europe (Mussatto et al., 2010). The development of ethanol production technology is still limited by the cost of its production, but the progress in enzyme engineering, modern methods of pre-treatment and studies of the fermentation process have brought the production of second-generation ethanol closer to implementation and made it more feasible (Gonzalez-Garcia et al., 2012). Work on developing new, more cost-effective solutions and full-scale production technology of second-generation ethanol is under way. According to a survey conducted by the National Renewable Energy Laboratory (NREL), the majority of installations of this type in the USA are still in the demonstrative or concept phase (Schwab et al., 2015). It is the same in Europe, where much further studies are needed to improve the technology of industrial conversion of lignocellulosic raw materials into ethanol.

A biorefinery uses the concept of separation and utilization of all the organic fractions obtained from lignocellulose. The production process of ethanol from lignocellulose usually consists of pre-treatment, enzymatic hydrolysis of polysaccharides, ethanol fermentation in a monosaccharide solution, distillation, rectification and dehydration of the obtained alcohol (Margeot et al., 2009; Viikari et al., 2012; Kelbert et al., 2015; Chiaramonti et al., 2012). Processing the fractions which are not used in the ethanol production process helps to improve the economic balance of the installation, while providing a broad range of potential products of a biorefinery, depending on the market demand. These processes can be categorized as alternative (which use the same feedstock as in the process of alcohol production) or those based on fractions of lignocellulosic feedstock which are not used in the process. Examples of a competitive process in terms of feedstock for the ethanol production process include other fermentation pathways (which lead to compounds such as butanol, isopropanol or acetone) as well as chemical methods of conversion, for example, of cellulose to levulinic acid or obtaining furfural by catalytic transformation of hemicelluloses. It is noteworthy that cellulose, especially as nanocellulose, is itself a valuable product (Mathew et al., 2014).

Agricultural lignocelluloses, which can potentially be used as a substrate in an integrated biorefinery, including in the production of second-generation biofuels, can be obtained from agricultural residues, i.e. corn stover, sugarcane bagasse, cereal straw, rice husk, etc. or dedicated perennial crops, such as miscanthus, poplar, willow (García et al., 2014; Gonçalves et al., 2013; Karlsson et al., 2014; Krzyżaniak et al., 2014). Among perennial species, short rotation woody crops (SRWC) provide a stable yield and there is a well-developed technology for their cultivation. The yield of willow and poplar can reach as much as 30 Mg of d.m. ha^{-1} $year^{-1}$, when cultivated on good soils and under favorable weather conditions (Adegbidi et al., 2001; Johansson and Karačić, 2011; Stolarski et al., 2011b). However, it is lower in agricultural practise (5–15 Mg of d.m. ha^{-1} $year^{-1}$) (Wilkinson et al., 2007; Mola-Yudego, 2011; Stolarski et al., 2011a, 2015). The lower yield in agriculture is caused by different factors, including the cultivation of such plants on land of lower quality, which is less usable for the cultivation of edible crops. The choice is frequently dictated by economic factors. Farmers concentrate high-yield production of edible crops on high quality soils, and defective or marginal soils are left for the production of less-demanding plants. Therefore, different methods of enriching soil and improving its quality are being sought (Labrecque et al., 1997; Rooney et al., 2009; Aronsson et al., 2014; Stolarski et al., 2014a). Soil enrichment aims to obtain higher biomass yield, thereby resulting in larger amounts of cellulose and hemicellulose produced at an SWRC plantation. Moreover, the plant species, cultivar, age, soil and the conditions of plant growth all contribute to the chemical composition of biomass obtained from an SRWC plantation (López et al., 2008; Guidi et al., 2009; Wróblewska et al., 2009; Stolarski et al., 2011b; Serapiglia et al., 2013; Carmona et al., 2015). Therefore, the aim of this study was to assess the biomass chemical composition of 3 SRWC species, depending on the method of soil enrichment and harvest cycle, as potential feedstock in the production of second-generation bioethanol.

2. Materials and methods

2.1. Field experiment

The study was based on a three-factorial field experiment, set up in late April 2010 at the Didactic and Research Station in Łężany (53°59′N, 21°04′E), owned by the University of Warmia and Mazury in Olsztyn. It was located on soil of low usability for traditional agricultural production for food or fodder crops. The experiment was set up in a split-plot design. Three plant species (factor A) and eight methods of soil enrichment (factor B) were randomly assigned to 72 plots in three replications with 20 plants per plot. Subsequently, single plants were randomly harvested from each plot in 2012 (after 3 years of vegetation) and in 2013 (after four years of vegetation) (factor C). However, due to the high costs of chemical analyses and limited budget, a reduced composition of factors in the experiment were used to assess the biomass chemical composition. Since several levels of factor B were excluded from the assessment, three effects of factor A were assessed (black locust, willow, poplar) as well as four effects of factor B (control, lignin, mineral fertilization, mycorrhiza inoculation) and two effects of factor C (three- and four-year harvest cycle) and their interactions (AB, AC and ABC).

Willow of the *Salix viminalis*, species, poplar *Populus nigra* × *P. Maximowiczii* Henry cv. Max-5 and black locust (*Robinia pseudoacacia*) were used in field trials. A willow cultivar UWM 006 grown in UWM, Olsztyn, was used in the experiment. The cultivar is registered with the Polish Center for Crop Studies under the name of "Żubr". Poplar was provided by a farm in northern Austria and black locust by a forest nursery in Poland. The crops were planted at the density of 11.11 thousand per ha. Willow and poplar were manually planted from cuttings and black locust from seedlings. Lignin (a waste product in paper production) was applied at the dose of 13.3 Mg ha^{-1}. Live mycorrhizal mycelium as a liquid suspension at 30–35 cm^3 was applied to each plant. Mineral fertilization with NPK was applied at 13, 50 and 90 kg ha^{-1}, respectively.

2.2. Obtaining biomass for laboratory analyses

Appropriate three- and four-year old willow, poplar and black locust trees from different combinations of soil enrichment and the control plot were felled in December 2012 and 2013. Plants were cut down with a DCS520 (Makita) chain saw 5–10 cm above the ground level. Subsequently, the whole shoots with branches were cut up into chips on site with a Junkkari HJ 10 G (Junkkari) chipper, working together with a tractor (New Holland) with the power of 130HP. While chipping shoots, representative samples of biomass (approx. 5 kg) for the methods of soil enrichment under study were taken from each plot for laboratory analyses. The samples were packed in foil bags and sent to the laboratory.

2.3. Laboratory tests and determination of the yield of selected polysaccharides

Biomass samples were delivered to the laboratory and laboratory samples were then isolated for chemical analyses. The chemical composition was determined in biomass obtained from whole shoots with bark. In order to prepare the samples for further analyses, moisture content was determined by drying and weighing. To this end, biomass was dried at the temperature of 105 ± 2 °C in a Premed KBC G-65/250 drier (PN 80/G-04511) until a constant weight was achieved.

The wood for chemical analyses was prepared following the PN-92/P-50092 standard. Samples were ground using a sieve with 1.0 mm square screens (mill Fritsch type 15). The material was passed through brass sieves to separate the 0.5–1.0 mm fraction. Standard methods were applied for the chemical composition of biomass. Before determination of the cellulose, lignin and holocellulose content, extraction in 96% ethyl alcohol was performed in a Soxhlet apparatus with TAPPI T 204 cm-07 (Baeza and Freer, 2000; Fengel and Wegener, 1989). The material was then dried under laboratory conditions and the extracted substances (lipids, waxes, resins and others) were dried at 103 ± 2 °C and the contents of cellulose (with the Seifert method, according to PN-92/P-50092), lignin (according to Tappi T 222 om-06), using 72% H_2SO_4, pentosans (with Tollen's method – TAPPI 223 cm-01), holocellulose (using sodium chlorite, with the PN-75/50092 standard) (Rowell, 2005) and base-soluble substances (a 1% aqueous solution of NaOH, which provides information about the amount of hemicelluloses in wood, according to TAPPI T 212 om-07). Apart from incomplete extraction of hemicelluloses, the main drawback of this method is that some of the lignin hydrolyzes and undergoes partial condensation. Therefore, in order to provide a more precise content, the content of hemicellulose was also calculated from the difference between the holocellulose and cellulose contents. The content of substances soluble in cold and hot water were determined according to TAPPI T 204 cm-07. In one of the analyses, the content of substances soluble in cold water was first determined followed by the content of substances soluble in hot water. However, the content of substances soluble in ethanol and the content of substances soluble in 1% NaOH were determined in the other two separate analyses. A pH analysis was conducted according to PN-Z-15011-1. All of the analyses were done in three replications.

The potential yield of cellulose, hemicellulose and holocellulose (Mg of d.m. ha^{-1} $year^{-1}$) was calculated by multiplying the dry weight yield of the SRWC species calculated in different combinations (Stolarski et al., 2014a,b) by the percentage content of individual polysaccharides in dry biomass.

2.4. Statistical analysis

Because of the limitation discussed here and because only some plots were chosen for assessment, the chemical composition of biomass was analyzed statistically in a completely randomized design (CRD).

A three-way analysis of variance was carried out to determine the effects of species (factor A), soil enrichment procedure (factor B), harvest cycle (factor C) and all interactions (AB, AC, BC, ABC). Homogeneous groups for the all examined characteristics were determined by means of a Tukey (HSD) multiple test with the significance level set at $P < 0.05$.

PCA (Principal component analysis) was applied to evaluate the chemical features of the biomass. The number of components was selected based on Kaiser's criterion using the method of eigen values (λ_i) larger than one (>1). The diagram of the Component Scores for the first two PCs was presented in the form of a biplot. The results of the tests were analyzed statistically using STATISTICA PL software.

3. Results

All parameters of the chemical composition of the biomass under study were significantly different across all of the main experiment factors and between them. Significant differences were not found only in the content of substances soluble in ethanol and cellulose content across the harvest cycles and for the pentosane content across the methods of soil enrichment (Table 1).

The content of substances soluble in cold water was the highest in biomass of black locust, both in the 3- and 4-year harvest cycle, by an average of 8.90% of d.m. and 7.91% of d.m., respectively (Table 2). A significantly lower value of this attribute was observed in poplar biomass and the significantly lowest was in willow biomass. The highest content of substances soluble in cold water was found in plots in which black locust was fertilized with mineral fertilizers in a 3- or 4-year harvest cycle - the homogeneous group a and b, respectively. Similar relationships between the species under study were observed for the content of substances soluble in hot water.

The average content of substances soluble in ethanol was the highest in poplar biomass, in both the 3- and 4-year harvest cycles (8.24% of d.m. and 8.60% of d.m., respectively) (Table 3). The value

Table 1
The ANOVA F-test statistics.

Source of variation	df	Substances soluble in cold water	Substances soluble in hot water	Substances soluble in ethanol	Substances soluble in 1% NaOH	Cellulose	Holocellulose	Hemicellulose	Lignin	Pentosans	pH
Species (A)	2	1470.4**	878.7**	263.8**	797.9**	1346.9**	273.2**	112.2**	1041.5**	16.5**	102.3**
Soil enrichment procedure (B)	3	42.9**	7.4**	7.6**	16.7**	24.8**	19.8**	3.8*	28.7**	2.7NS	5.5**
Harvest cycle (C)	1	64.6**	6.5*	1.6NS	409.2**	2.0NS	661.4**	403.4**	39.1**	76.0**	657.8**
AB	6	84.9**	20.7**	35.9**	50.2**	11.4**	12.2**	3.8**	42.6**	3.7**	38.1**
AC	2	83.3**	40.2**	21.5**	64.7**	58.2**	18.3**	14.9**	50.9**	23.4**	124.5**
BC	3	234.0**	75.6**	79.1**	43.0**	4.9**	14.0**	5.0**	15.0**	4.6**	20.0**
ABC	6	139.9**	74.3**	45.3**	25.1**	17.8**	20.8**	9.4**	16.8**	4.0**	77.4**
Error	48										
Total	71										

df = number degrees of freedom.
NS = not significant.
* $P < 0.05$.
** $P < 0.01$.

Table 2
The content of substances soluble in cold and hot water in SRWC biomass.

Species (A)	Soil enrichment procedure (B)	Harvest cycle (C)			
		Three-year	Four-year	Three-year	Four-year
		Content of substances soluble in cold water (% d.m.)		Content of substances soluble in hot water (% d.m.)	
Black locust	Control	8.93 ± 0.22 b	7.47 ± 0.07 d	9.41 ± 0.30 cd	10.02 ± 0.10 bc
	Lignin	7.84 ± 0.12 cd	7.80 ± 0.13 cd	9.72 ± 0.27 c	9.59 ± 0.11 cd
	Mineral fertilization	10.52 ± 0.24 a	8.91 ± 0.19 b	11.02 ± 0.31 a	10.03 ± 0.21 bc
	Mycorrhiza	8.29 ± 0.17 bc	7.45 ± 0.05 d	8.36 ± 0.37 ef	9.53 ± 0.28 cd
Mean		8.90 ± 1.07 A	7.91 ± 0.63 B	9.63 ± 1.02 A	9.79 ± 0.30 A
Poplar	Control	6.21 ± 0.16 ef	5.70 ± 0.27 f	8.57 ± 0.44 e	7.83 ± 0.36 f
	Lignin	5.96 ± 0.13 f	8.39 ± 0.03 bc	8.84 ± 0.31 de	8.17 ± 0.27 ef
	Mineral fertilization	5.18 ± 0.22 g	8.03 ± 0.01 c	6.56 ± 0.16 gh	10.38 ± 0.23 b
	Mycorrhiza	8.05 ± 0.19 c	4.62 ± 0.01 gh	9.19 ± 0.12 d	7.86 ± 0.19 f
Mean		6.35 ± 1.11 D	6.69 ± 1.65 C	8.29 ± 1.10 B	8.56 ± 1.13 B
Willow	Control	5.88 ± 0.06 f	5.48 ± 0.07 fg	7.74 ± 0.08 fg	5.88 ± 0.02 h
	Lignin	6.34 ± 0.28 e	5.29 ± 0.18 g	7.20 ± 0.39 g	6.44 ± 0.15 gh
	Mineral fertilization	4.59 ± 0.16 h	6.12 ± 0.04 ef	5.98 ± 0.02 h	7.15 ± 0.22 g
	Mycorrhiza	6.66 ± 0.29 e	5.15 ± 0.34 g	8.00 ± 0.05 f	6.00 ± 0.10 h
Mean		5.87 ± 0.85 E	5.51 ± 0.42 F	7.23 ± 0.83 C	6.37 ± 0.53 D

± Standard deviation A,B,C... homogenous groups interaction factors AC; a,b,c... homogenous groups interaction ABC.

Table 3
The content of substances soluble in ethanol and 1% NaOH in SRWC biomass.

Species (A)	Soil enrichment procedure (B)	Harvest cycle (C)			
		Three-year	Four-year	Three-year	Four-year
		Content of substances soluble in ethanol (% d.m.)		Content of substances soluble in 1% NaOH (% d.m.)	
Black locust	Control	8.01 ± 0.12 cd	6.59 ± 0.17 gh	29.40 ± 0.51 bc	24.61 ± 0.18 g
	Lignin	6.09 ± 0.37 hi	6.76 ± 0.33 g	28.42 ± 0.28 c	22.10 ± 0.36 i
	Mineral fertilization	8.83 ± 0.40 bc	7.66 ± 0.13 de	31.60 ± 0.12 a	27.66 ± 0.12 cd
	Mycorrhiza	6.85 ± 0.27 fg	6.33 ± 0.48 h	27.01 ± 0.03 de	27.38 ± 0.16 d
Mean		7.45 ± 1.13 C	6.84 ± 0.59 D	29.11 ± 1.76 C	25.44 ± 2.38 E
Poplar	Control	7.78 ± 0.26 d	8.37 ± 0.31 c	32.09 ± 0.78 a	29.41 ± 0.47 bc
	Lignin	8.07 ± 0.11 cd	9.34 ± 0.22 b	30.92 ± 0.42 ab	30.02 ± 0.31 b
	Mineral fertilization	6.89 ± 0.16 fg	9.32 ± 0.34 b	30.04 ± 0.06 b	31.07 ± 0.29 ab
	Mycorrhiza	10.21 ± 0.19 a	7.38 ± 0.15 ef	31.49 ± 0.16 a	31.16 ± 1.47 ab
Mean		8.24 ± 1.28 B	8.60 ± 0.87 A	31.13 ± 0.88 A	30.41 ± 1.03 B
Willow	Control	6.87 ± 0.41 fg	6.18 ± 0.06 hi	27.35 ± 0.50 d	23.56 ± 0.09 h
	Lignin	7.01 ± 0.17 f	6.62 ± 0.15 gh	27.48 ± 0.47 d	26.17 ± 0.19 ef
	Mineral fertilization	5.64 ± 0.13 i	7.61 ± 0.33 de	25.70 ± 0.24 f	25.09 ± 0.25 fg
	Mycorrhiza	7.53 ± 0.11 e	6.71 ± 0.27 g	26.40 ± 0.46 e	23.91 ± 0.29 gh
Mean		6.76 ± 0.75 D	6.78 ± 0.58 D	26.73 ± 0.84 D	24.68 ± 1.09 F

± Standard deviation A,B,C... homogenous groups interaction factors AC; a,b,c... homogenous groups interaction ABC.

of this attribute ranged from 5.64% of d.m. to 10.21% of d.m. in the whole experiment, in both mineral-fertilized willow and in poplar, under which mycorrhiza was applied in a three-year harvest cycle, respectively. The biomass of poplar, obtained in 3- and 4-year harvest cycles, contained the largest amounts of substances soluble in 1% NaOH, 31.13% of d.m. and 30.41% of d.m., respectively. The lowest value of this attribute was observed in willow biomass obtained in the 4-year harvest cycle (24.68% d.m.).

Of the species under study, the significantly highest content of lignin was found in the poplar biomass, in both the 3-year and 4-year harvest cycles (27.43% of d.m. and 25.98% of d.m., respectively) (Table 4). The content of lignin in willow and black locust biomass was lower compared with its content in poplar by 3–5% points, on average. A higher content of lignin in poplar was found in plots where poplar was cultivated in a combination with lignin added in order to enrich the soil, and a higher content of lignin in black locust was found in control plots. It is difficult at this stage to clearly identify the reason why the soil enrichment methods applied in the experiment resulted in a decrease in lignin content in black locust biomass compared with the control plot. This may have been caused by some species-specific physiological factors.

Willow biomass obtained in the 4-year harvest cycle contained the largest amounts of pentosanes (22.10% of d.m.). The same homogeneous group "A" included black locust biomass obtained in the same harvest cycle. On the other hand, black locust biomass obtained in a 3-year harvest cycle contained the significantly smallest amount of pentosanes. The pH value of the species biomass ranged from 4.90 to 6.13 for poplar fertilized with mineral fertilizers and harvested in a 4-year cycle and willow on a control plot and mycorrhiza in a 3-year harvest cycle, respectively (Table 4).

The contents of holocellulose and hemicellulose were significantly different across all the main experiment factors and interactions between them (Table 5). It must be emphasized that willow biomass obtained in the 4-year harvest cycle contained the largest amounts of holocellulose (77.80% of d.m., on average). The value of this attribute in willow in the plots where soil was enriched with lignin, mineral fertilization and mycorrhiza was 78% of d.m. The second homogeneous group in terms of mean content of holocellulose included poplar biomass harvested in a 4-year cycle and the third was willow biomass harvested in a 3-year cycle.

Willow biomass obtained in the 3- and 4-year harvest cycle contained the largest amounts of cellulose, 43.82% of d.m. and 42.92% of d.m., respectively (Table 5). The highest content of cellulose in the experiment was found in willow biomass obtained from the control plot in the 3-year harvest cycle. The methods of soil enrichment applied in this experiment caused a decrease in cellulose content in willow biomass. The methods of soil enrichment applied in the experiment tended to decrease cellulose content in willow biomass

Table 4
The content of lignin, pentosans in SRWC biomass and pH.

Species (A)	Soil enrichment procedure (B)	Harvest cycle (C)					
		Three-year Lignin (% d.m.)	Four-year	Three-year Pentosans (% d.m.)	Four-year	Three-year pH	Four-year
Black locust	Control	23.69 ± 0.27 e	24.89 ± 0.49 d	21.10 ± 0.27 e	22.36 ± 0.13 ab	5.11 ± 0.11 fg	5.39 ± 0.04 ef
	Lignin	22.86 ± 0.20 f	22.69 ± 0.03 fg	19.84 ± 0.10 g	22.16 ± 0.07 b	6.04 ± 0.05 ab	4.92 ± 0.08 h
	Mineral fertilization	22.07 ± 0.24 g	21.54 ± 0.23 g	21.05 ± 0.18 e	22.14 ± 0.37 b	5.38 ± 0.07 ef	5.38 ± 0.07 ef
	Mycorrhiza	22.84 ± 0.24 f	23.47 ± 0.06 ef	20.70 ± 0.47 f	21.65 ± 0.21 d	5.14 ± 0.07 fg	5.25 ± 0.10 f
Mean		22.86 ± 0.63 E	23.15 ± 1.29 D	20.67 ± 0.58 C	22.08 ± 0.33 A	5.42 ± 0.40 B	5.24 ± 0.21 C
Poplar	Control	27.91 ± 0.23 ab	25.98 ± 0.10 c	20.89 ± 0.44 f	21.46 ± 0.12 d	5.26 ± 0.03 f	5.14 ± 0.03 fg
	Lignin	28.62 ± 0.11 a	26.51 ± 0.37 bc	21.82 ± 0.98 cd	21.42 ± 0.40 d	5.35 ± 0.04 ef	5.21 ± 0.04 f
	Mineral fertilization	27.32 ± 0.7 b	25.94 ± 0.31 c	21.49 ± 0.23 d	21.22 ± 0.27 de	5.45 ± 0.03 e	4.90 ± 0.04 h
	Mycorrhiza	25.86 ± 0.28 c	25.51 ± 0.18 cd	20.92 ± 0.05 ef	21.48 ± 0.22 d	5.73 ± 0.04 c	5.35 ± 0.03 ef
Mean		27.43 ± 1.11 A	25.98 ± 0.43 B	21.28 ± 0.62 B	21.40 ± 0.25 B	5.45 ± 0.19 B	5.15 ± 0.17 C
Willow	Control	22.94 ± 0.29 f	23.07 ± 0.24 ef	21.44 ± 0.24 d	22.56 ± 0.11 a	6.13 ± 0.07 a	5.37 ± 0.06 ef
	Lignin	23.11 ± 0.10 ef	24.44 ± 0.38 de	21.24 ± 0.28 de	21.97±0.10 bc	5.57 ± 0.13 d	5.27 ± 0.07 f
	Mineral fertilization	24.52 ± 0.22 de	22.62 ± 0.35 fg	21.98±0.34 bc	21.87±0.22 c	5.95 ± 0.05 b	5.02 ± 0.06 gh
	Mycorrhiza	23.70 ± 0.08 e	23.35 ± 0.59 ef	21.61 ± 0.34 d	22.01±0.44 bc	6.13 ± 0.13 a	5.04 ± 0.08 g
Mean		23.57 ± 0.67 C	23.37 ± 0.78 CD	21.57 ± 0.39 B	22.10 ± 0.35 A	5.95 ± 0.25 A	5.17 ± 0.17 C

± Standard deviation A,B,C... homogenous groups interaction factors AC; a,b,c... homogenous groups interaction ABC.

Table 5
The content of holocellulose, cellulose and hemicellulose in SRWC biomass.*

Species (A)	Soil enrichment procedure (B)	Harvest cycle (C)					
		Three-year Holocellulose (% d.m.)	Four-year	Three-year Cellulose (% d.m.)	Four-year	Three-year Hemicellulose (% d.m.)	Four-year
Black locust	Control	71.87 ± 0.33 de	76.18 ± 1.54 b	37.45 ± 0.39 e	40.47 ± 0.37 c	34.42 ± 0.67 e	35.71 ± 1.89 cd
	Lignin	69.21 ± 0.74 f	75.51 ± 0.12 bc	37.24 ± 0.22 ef	38.44±0.54 de	31.97±0.70 gh	37.08±0.51 bc
	Mineral fertilization	68.00 ± 0.37 g	72.86 ± 0.91 cd	35.49±1.08 f	38.21±0.35 de	32.51±0.72 fg	34.64±1.12 de
	Mycorrhiza	72.77 ± 0.44 cd	73.33 ± 0.35 cd	37.95±0.41 e	37.26±0.29 ef	34.82±0.79 d	36.07±0.27 c
Mean		70.46 ± 2.06 F	74.47 ± 1.66 D	37.03±1.10 D	38.60±1.27 C	33.43±1.41 D	35.88±1.33 B
Poplar	Control	72.98 ± 1.14 cd	77.32 ± 0.41 ab	39.47±0.35 cd	38.40±0.35 de	33.52±0.97 f	38.93±0.42 ab
	Lignin	72.19 ± 0.35 d	77.24 ± 0.08 ab	37.56±0.38 e	37.34±0.17 e	34.63±0.72 de	39.89±0.10 a
	Mineral fertilization	73.65 ± 0.21 c	75.71 ± 0.40 bc	38.39±0.16 de	38.40±0.26 de	35.26±0.32 cd	37.32±0.56 b
	Mycorrhiza	70.99 ± 0.77 e	77.68 ± 0.16 ab	38.33±0.48 de	38.59±0.70 d	32.66±0.34 fg	39.09±0.81 ab
Mean		72.46 ± 1.20 E	76.99 ± 0.83 B	38.44±0.77 C	38.18±0.63 C	34.02±1.18 CD	38.81±1.08 A
Willow	Control	76.51 ± 0.56 b	76.57 ± 0.61 b	44.51±0.18 a	42.33±0.26 bc	32.00±0.50 gh	34.24±0.41 ef
	Lignin	73.99 ± 0.70 c	78.26 ± 0.40 a	42.56 ± 0.31 bc	43.10 ± 0.45 b	31.43 ± 1.01 h	35.16 ± 0.52 cd
	Mineral fertilization	74.24 ± 0.83 c	78.19 ± 0.17 a	43.99 ± 0.34 ab	42.68 ± 0.11 bc	30.25 ± 1.17 i	35.51 ± 0.06 cd
	Mycorrhiza	76.48 ± 0.25 b	78.17 ± 0.15 a	44.23 ± 0.26 ab	43.57 ± 0.25 b	32.25 ± 0.50 g	34.61 ± 0.26 de
Mean		75.31 ± 1.36 C	77.80 ± 0.81 A	43.82 ± 0.82 A	42.92 ± 0.54 B	31.48 ± 1.08 E	34.88 ± 0.60 C

± Standard deviation A,B,C... homogenous groups interaction factors AC; a,b,c... homogenous groups interaction ABC.
* The compounds cannot be added up as 100%, but they should be understood as the content in biomass.

and the others compared with control plots. This may be attributed to physiological factors associated with quicker growth, biomass increase and higher yield from the plots in which different methods of soil enrichment were used than in the control plots. The content of cellulose in poplar biomass was similar regardless of its harvest cycle. However, it was significantly lower than in willow biomass (by an average of 5% points). Furthermore, the content of cellulose in black locust biomass harvested in a 4-year cycle was in the same homogeneous group as poplar. On the other hand, the cellulose content in black locust biomass harvested in a 3-year cycle was the lowest (significantly) and it was only 35.49% d.m. in a plot where mineral fertilization was applied. The largest amount of hemicellulose was found in biomass of poplar harvested in a 4-year cycle and the smallest was in biomass of willow harvested in a 3-year cycle.

The principal component analysis (PCA) yielded three principal components (F1, F2, F3) which, when combined, explained 81% of the variability of the plots under study (Table 6). The first principal component (F1) after Varimax rotation explained 35% of the variability between the plots under study based on the substances soluble in hot and cold water and the content of cellulose and holocellulose. Furthermore, component F3 explained 23.72% of the variance based on substances soluble in 1% NaOH, content of lignin and substances soluble in ethanol. The third dimension – component F2–was based on the content of hemicellulose and pH.

A biplot is an enhanced form which explains the variability of the plots under study (Fig. 1). The plot shows the chemical parameters as vectors whose coordinates are based on the size of factorial loads of the components which explain the variability to the greatest extent (F1 = 34.68% and F3 = 23.72%). A biplot also includes a projection of the plots under study on the plane made by components F1 and F3.

A biplot analysis is based on the positions of vectors and points of the plots relative to the vertical axis determined by dimension F1 (which divides the diagram into right and left sides) and a horizontal axis F3 (which divides the diagram into its upper and lower part). The vector's meaning is consistent with its sign (plus or minus) and its length is consistent with the values shown in Table 6. The main information that can be gained from the plot is the fact that the biomasses of the species under study differed enough to create 3 separate sets (groups of points). Willow biomass (W) was characterized by the fact that, due to a high content of cellulose and holocellulose, it contained little lignin or substances soluble in ethanol or in 1% NaOH. In general, black locust (BL) contained the greatest amount of substances soluble in hot and cold water, and

Table 6
Original (raw) and rotated (Varmiax rotation) factorial loadings.

Feature	Original			Varimax rotation		
	F1	F2	F3	F1	F2	F3
Cold water	−0.78	−0.18	−0.53	*0.95*	0.04	−0.08
Hot water	−0.82	−0.07	−0.37	*0.89*	0.08	0.1
Ethanol	−0.67	0.36	0.19	0.42	0.28	*0.61*
1% NaOH	−0.75	0.12	0.59	0.34	-0.09	*0.89*
Celullose	0.88	−0.22	0.14	*−0.78*	−0.27	−0.4
Holocellose	0.72	0.58	−0.01	*−0.73*	0.53	−0.2
Hemicellose	−0.13	0.91	−0.17	0.01	*0.91*	0.21
Lignin	−0.19	0.5	0.74	−0.29	0.2	*0.85*
Pentosans	0.65	0.37	−0.32	−0.48	0.45	−0.48
pH	0.15	−0.76	0.42	−0.17	*−0.86*	0.03
Eigenvalue (λ_i)	4.06	2.35	1.69	3.47	2.27	2.37
Percentage of explained variance	40.64	23.54	16.89	34.68	22.66	23.72

Italics indicate significant coefficients.
Significant at $P < 0.05$.

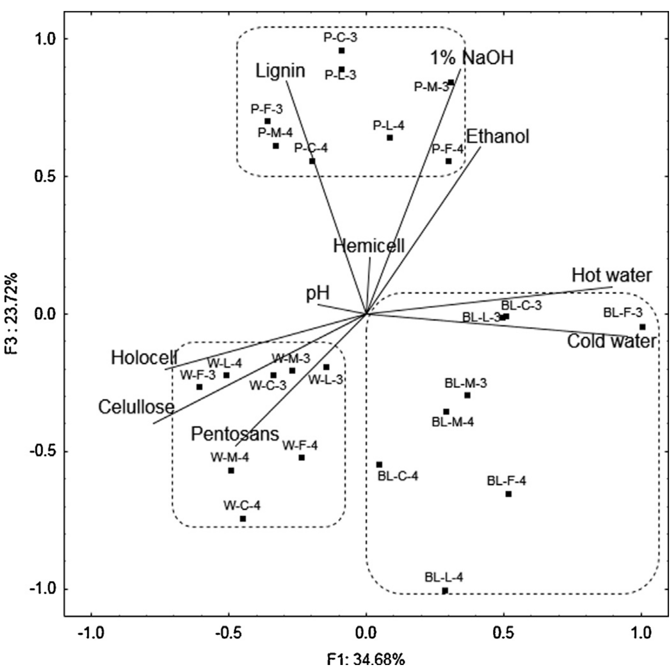

Fig. 1. Biplot. BL-Black Locust, P-Poplar W-willow, C-control, L-lignin, F-mineral fertilization, M-mycorrhiza; 3-year harvest cycle, 4-year harvest cycle.

the biomass of poplar (P) contained the largest amount of lignin and the highest content of substances soluble in 1% NaOH and ethanol.

The chemical composition of biomass is one of the elements used to assess its practical usability. However, in a broader perspective, since one should bear in mind the practicality of obtaining the substrate, it is important to assess the production output potential for cellulose, hemicellulose or holocellulose from a unit area of an SRWC species plantation. It has been shown in this study that a willow plantation can yield 3.6–4.2 Mg of dry cellulose ha^{-1} $year^{-1}$ when lignin and mineral fertilization are used to enrich soil in a 3- and 4-year harvest cycle (Fig. 2). A similar yield of cellulose, although lower by about 11%, was obtained in the cultivation of poplar and also when lignin and mineral fertilization were used to enrich soil in 3- and 4-year harvest cycles. The second homogeneous group in terms of cellulose yield per unit area included willow and poplar, harvested on control plots and with mycorrhiza used to enrich the soil. Furthermore, the potential yield of cellulose in the cultivation of black locust was the lowest and it was in the third homogeneous group, regardless of the method of soil enrichment and the plant harvest cycle. The data presented in Fig. 2 show that the yield of cellulose was differentiated mainly by plant species and partly by the method of soil enrichment, whereas the harvest cycle was statistically insignificant.

Obviously, the potential yield of holocellulose was higher than that of cellulose. The significantly highest holocellulose yield was obtained in willow cultivation on soil enriched with lignin with a 4-year harvest rotation (7.3 Mg ha^{-1} $year^{-1}$ d.m., Fig. 3). A similar yield of holocellulose was obtained in the cultivation of willow and poplar on soil enriched with mineral fertilizers in a 3- and 4-year harvest cycle and poplar cultivated on soil enriched with lignin in a 4-year harvest cycle. Meanwhile, the highest potential hemicellulose yield (3.7 Mg of dry matter ha^{-1} $year^{-1}$) was obtained from poplar cultivated on soil enriched with lignin in a 4-year harvest cycle (Fig. 4). The yield of holocellulose and hemicellulose was differentiated mainly by plant species and partly by the methods of soil enrichment and the harvest cycle. In general, a higher yield of those polysaccharides was obtained in a 4-year harvest cycle compared with the 3-year cycle, but the differences were only significant in some cases.

It must be emphasized that the yield of different polysaccharides in the biomass of the SRWC under study, cultivated with different methods of soil enrichment and harvested in different cycles, depended greatly on the biomass yield from 1 ha. Therefore, for example, cellulose content in biomass of willow fertilized with lignin and harvested in a 4-year cycle was higher by about 5.5% points than the cellulose content in black locust biomass in a control plot or when fertilized with mineral fertilizers and harvested in a 3-year harvest cycle (Tab. 5), whereas the yield of willow cellulose was more than 10 times higher than from black locust (Fig. 2).

4. Discussion

The study described in this paper has shown that the chemical composition of biomass was significantly differentiated by the SRWC species, the method of soil enrichment and by the harvest cycle. Of the species under study, willow was characterized by the highest content of cellulose and holocellulose. It was also found that the mean contents of cellulose and holocellulose in the biomasses of black locust, poplar and willow in 3-year and 4-year harvest cycles were higher compared with the biomasses obtained in a 2-year harvest cycle (Stolarski et al., 2013b). The conditions of plant growth, which were different with respect to lignin, fertilizers or mycorrhize added to soil, and in the control plot, caused the content of individual polysaccharides to differ. However, at this stage it is difficult to identify a clear trend in different species, because a high yield of polysaccharides, e.g., cellulose, was also obtained from control plots. There have also been literature reports suggesting that

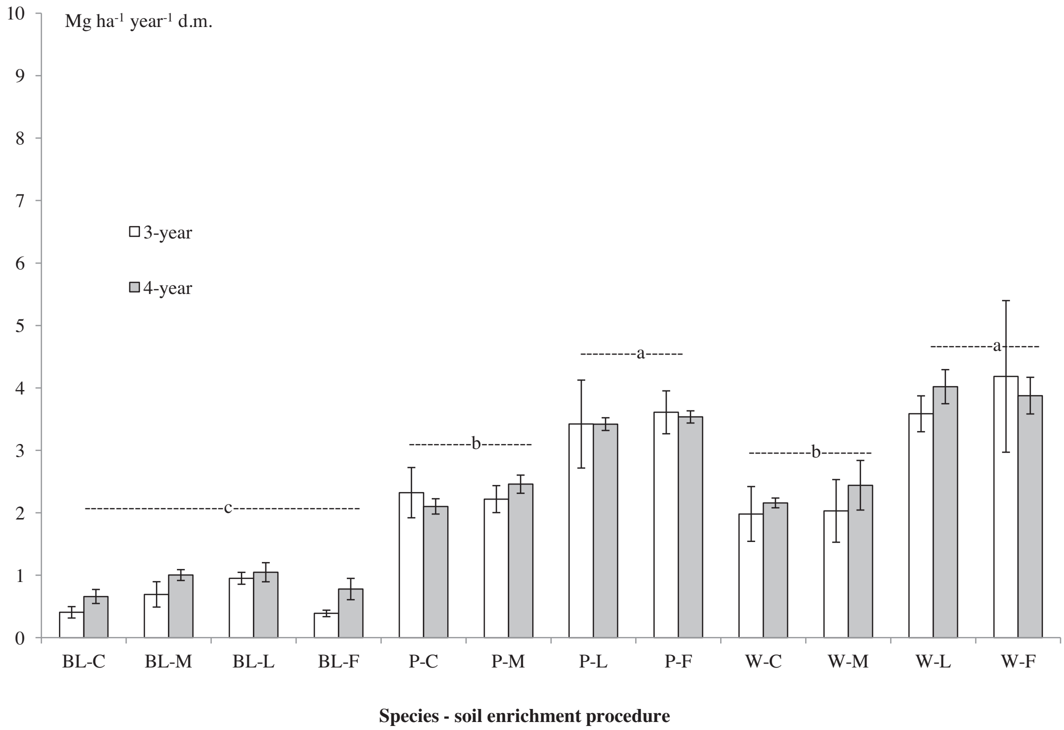

Fig. 2. The yield of cellulose harvested with the SRWC biomass (the error bars show the standard deviation; a, b, c,. .. homogeneous groups; BL-black locust, P-poplar W-willow; C-control, L-lignin, F-mineral fertilization, M-mycorrhiza).

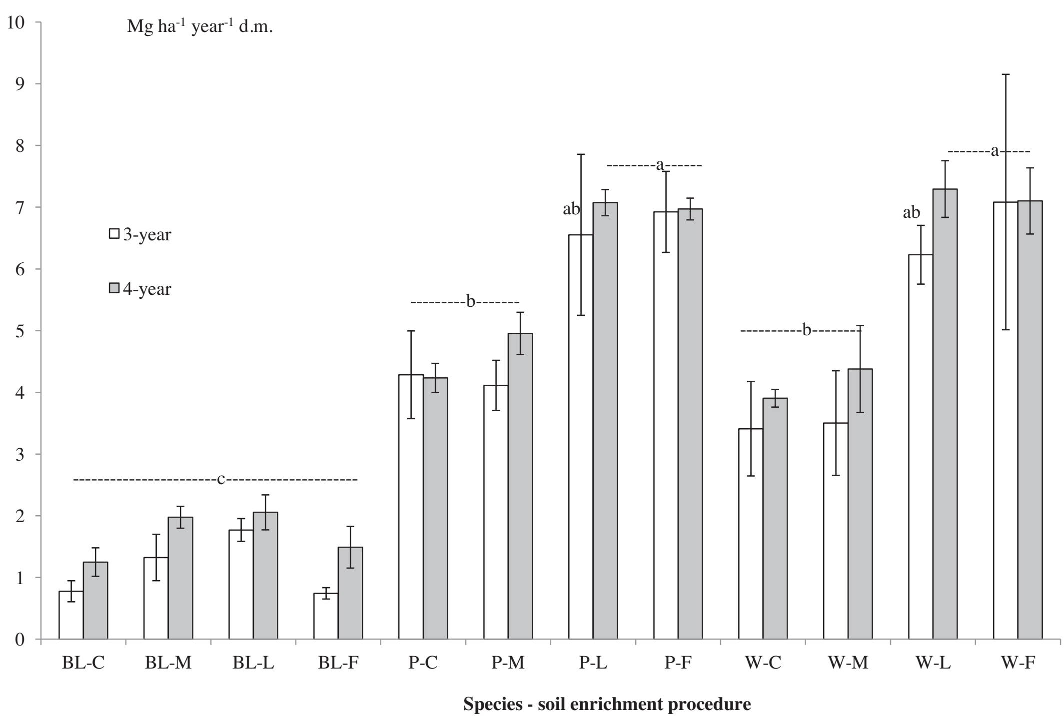

Fig. 3. The yield of holocellulose harvested with the biomass of SRWC (the same legend as for Fig. 2).

the chemical composition of wood biomass depends not only on the species and cultivar, but also on such factors as a plant age, the plant part taken for analysis, time of harvest and the conditions of plant growth (Baeza and Freer, 2000; Rowell et al., 1997; Guidi et al., 2009; Komorowicz et al., 2009; Wróblewska et al., 2009; Stolarski et al., 2011b, 2013a; Serapiglia et al., 2013; Carmona et al., 2015).

In general, biomass obtained in shorter harvest rotations contains more extractable substances which are soluble in water and alkalis and less cellulose compared with biomass obtained in longer harvest rotations. For example, the content of hot-water-soluble substances in five willow cultivars decreased from 8.4 to 5.2% d.m., and the content of cellulose increased from 40.2 to 44.6% d.m., in a

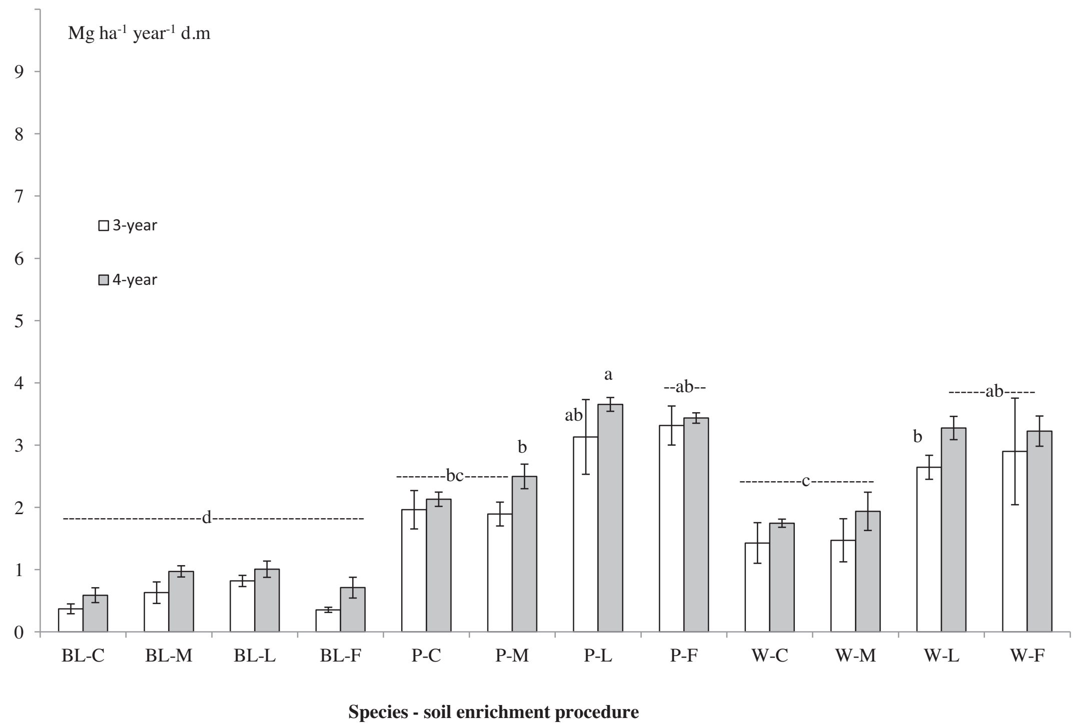

Fig. 4. The yield of hemicellulose (theoretical value) harvested with the biomass of SRWC (the same legend as for Fig. 2).

one-year and three-year harvest cycle, respectively (Stolarski et al., 2011b). On the other hand, the content of lignin reported in these studies was 24.4 and 21.8% d.m., respectively. The content of cellulose in willow biomass reported in another study increased from 39.3 to 45.6% d.m., and the content of lignin decreased from 26.0 to 20.0% d.m., in a one-year and a four-year harvest cycle, respectively (Komorowicz et al., 2009). Furthermore, the content of cellulose in a 4-year harvest cycle in four willow cultivars ranged from 42.4 to 45.3% d.m., and that of lignin ranged from 19.9 to 21.1% d.m. (Stolarski et al., 2013a). In addition, poplar wood obtained in a two-year harvest cycle contained less cellulose (42.5% d.m.) and more lignin (22% d.m.) than wood obtained in a four-year rotation – 51.6% d.m. and 19% d.m., respectively (Guidi et al., 2009). Significant differences have been shown in other studies to exist in cellulose content (42.38–48.70% d.m.), in holocellulose content (83.32–88.29% d.m.) and in lignin content (17.69–24.84% d.m.) between 19 poplar clones, when biomass was obtained in a two-year harvest cycle (Carmona et al., 2015). Moreover, (Serapiglia et al., 2013) found significant differences in regard to the content of cellulose and lignin between 18 poplar clones grown in two different types of soil conditions in a three-year harvest cycle. Cellulose content in biomass obtained on silt loam soil across all the clones averaged 42.3% and was significantly greater than cellulose content in biomass obtained on gravelly loam soil (average 41.0% dm.). The lignin content was 22.0% d.m. and 22.3% d.m., respectively. It was found throughout the experiment that across both sites and genotypes, cellulose content ranged from 38.7% d.m. to 44.5% d.m., whereas lignin ranged from 20.9% d.m. to 24.1% d.m. On the other hand, (González-Garćia et al., 2010) reported that the cellulose content in biomass obtained in a five-year harvest rotation was 43.2% d.m., and that of lignin was 21.3% d.m. Furthermore, the content of cellulose in biomass obtained from commercial poplar clones NM6 and D105 after 13 years of cultivation was 39% d.m., that of hemicellulose was 21% d.m, and lignin was 27% d.m. Zamora 2013. The content of cellulose and lignin in biomass of black locust was found to be 46.5 and 27.1% d.m., respectively (Pinto et al., 2005).

An analysis of these findings shows that the potential yield of cellulose, hemicellulose and holocellulose from 1 ha varied significantly across SRWC species and, for willow and poplar, partly across methods of soil enrichment and harvest cycle. Therefore, it must be emphasized that the choice of SRWC species used as a source of specific polysaccharides must take into account not only the percentage content in biomass, but, most of all, the species and soil enrichment methods must be chosen to ensure high biomass yield per unit area. Because although differences between species and methods of soil enrichment were significant, they ranged from about a dozen to just over 20%, whereas differences in the potential yield of different polysaccharides from 1 ha have been shown to exceed 1000% in some cases (e.g., a comparison of cellulose yield in willow fertilized with lignin in a 4-year harvest rotation and black locust in a 3-year harvest rotation in a control plot or in one fertilized with mineral fertilizers). Therefore, if differences in yield are considerable, species and methods of soil enrichment should be chosen which ensure higher yield, which potentially reduces the cost of production, harvest and transport of biomass and reduces the cost of the carbohydrate in manufacturing a specific product such as second-generation bioethanol or another bioproduct.

It must be emphasized that from the point of view of the second-generation bioethanol production process, the content of cellulose and hemicellulose (the main sources of fermentable sugar) is of the greatest importance. Therefore, SRWC species and technologies should be sought to ensure the greatest yield of these polysaccharides from 1 ha. Lignocellulosic biomass of forest origin (fir, pine), classified as softwood, contains more mannose and glucose than willow, poplar or black locust wood (classified as hardwood), which usually contains more pentoses, such as xylose. Furthermore, hemicellulose in hardwood is acetylated to a greater extent than softwood. The branched structure of hemicellulose makes it more susceptible to hydrolysis than cellulose (Palmqvist and Hahn-Hägerdal, 2000). The yield of the second-generation bioethanol production process will also be affected by the content and type of lignin in biomass. This plays an important role in the initial stages of conversion which involve hydrolysis of lignocellulosic mass and making cellulose and hemicellulose polymers available for further decomposition. A high content of lignin hinders the release of monosaccharides by hydrolysis (Zamora et al., 2013). Compared with softwood plants, wood of SRWC contains less lignin, which is a beneficial characteristic from the point of view of the process. Moreover, a relatively low ash content in SRWC biomass (1–2% d.m.) can have a beneficial effect on the process outcome, because (Valentas et al., 2009) have shown that a high content of ash in biomass has a negative impact on the ethanol production process and, consequently, on the final product quantity. Moreover, the production process of second-generation ethanol may be affected by pH of lignocellulosic material. Pre-treatment is one of the key stages of the process. Depending on the type of pre-treatment (physical, chemical, a combination of both), the biomass pH may have a positive (acidic pre-treatment and pH below 7, alkaline pre-treatment and pH above 7), or a negative effect when these relationships are reversed. The other steps of the production process do not depend on the material pH, as it is necessary to interfere with the pH of pre-treated material in order to bring the pH of the environment of enzymatic hydrolysis to a set value, appropriate for a specific enzymatic cocktail. On the other hand, it must be emphasized that the choice of parameters of the process of conversion and treatment of material is of key importance for the quality of second-generation bioethanol production. Depending on non-specific process conditions, it can yield a whole range of products which inhibit enzymatic hydrolysis and fermentation. These compounds include organic acids, furans and phenolic compounds (Palmqvist and Hahn-Hägerdal, 2000), which can also be present among extractable and soluble substances in small amounts. These compounds are known to disturb the metabolism of yeast in an independent and synergistic manner, thereby impairing fermentation and consistently limiting ethanol production when their concentration is too high (Jing et al., 2009).

Moreover, lignin, which is not desirable for the second-generation bioethanol production process, may be the most important component of lignocellulosic biomass for the entire integrated biorefinery. The extremely rich matrix of this organic biopolymer has interesting mechanical and thermoplastic properties and provides great opportunities to use lignin in plastic production (protein-lignin blends, starch-lignin blends, PHA polyhydroxyalkanoates [PHA], polylactides and polyglycolides, epoxy resin blends and many others) (Doherty et al., 2011). Lignin is a raw material which, when isolated, can be hydrolyzed and yields mixtures of compounds with small molecular weights. When these are separated, they yield a wide range of substrates for further transformations – both into polymers and compounds with potential medical and cosmetic applications. Despite the large volumes of lignin available on the market (50 million tonnes in 2010 only 1 million tonnes were commercially used for low-value products, the rest was burnt as a low-value fuel. Only kraft, organosolv and high-grade lignin may have high value application. Their global annual production in 2011 was estimated at ca. 61,000 tonnes (Smolarski, 2012). Therefore, low purity lignin may also find application in soil improvement to enhance the plant development conditions and their yield.

The aim of this outline is to emphasize how important it is to know the characteristics of feedstock and its potential yield from a unit area in the biorefinery design process. The content of individual organic fractions in feedstock will affect process design, in both its technological and economic aspects García et al., 2014. The opportunity to plan and control, to a certain extent, the composition of biomass of SRWC and the amount of feedstock to be obtained allows for the economical design of production processes.

5. Conclusion

Due to its chemical composition and a polysaccharide-yielding potential, biomass of short rotation woody crops can be used as feedstock for second-generation bioethanol and bioproduct production. However, the chemical composition of biomass and polysaccharide-yielding potential has been shown to vary between the species, harvest cycle and methods of soil enrichment under study. Compared with the other species, willow biomass was characterized by a high content of cellulose and holocellulose and a lower content of lignin, substances soluble in ethanol and in 1% NaOH. On the other hand, black locust contained the greatest amount of substances soluble in hot and cold water, whereas poplar biomass contained the largest amount of lignin and it dominated as the species with the highest content of substances soluble in 1% NaOH and ethanol.

It has been shown that a willow plantation can yield 3.6–4.2 Mg of dry cellulose ha^{-1} year^{-1} when lignin and mineral fertilization are used to enrich soil. A similar yield of cellulose, although lower by about 11%, was obtained in the cultivation of poplar under the same cultivation conditions as for willow. Furthermore, the potential yield of cellulose in black locust cultivation was significantly lower, regardless of the method of soil enrichment or the plant harvest cycle.

This study has shown that the choice of SRWC species used as a source of polysaccharides must take into account the percentage content in biomass and, crucially, the species and soil enrichment methods must be carefully selected to ensure a high biomass yield per unit area, because differences in the potential yield of individual polysaccharides per 1 ha exceeded 1000% in some cases. The study presented in this paper needs to be continued to balance the polysaccharide yields with the soil enrichment options to improve the sustainability of the processes. This will be the subject of a different publication, which will consider costs, energy intensity and GHG emissions in the biomass production of the SRWC species under study with different methods of soil enrichment.

Acknowledgements

This work has been financed by the Strategic Program of the National (Polish) Centre for Research and Development (NCBiR): "Advanced Technologies for Energy Generation. Task 4: Elaboration of Integrated Technologies for the Production of Fuels and Energy from Biomass, Agricultural Waste and other Waste Materials".

References

Action Aid International USA, 2012. Fueling the food crisis: The cost of developing countries of US corn ethanol expansion, Washington, p. 30.
Adegbidi, H.G., Volk, T.A., White, E.H., Abrahamson, L.P., Briggs, R.D., Bickelhaupt, D.H., 2001. Biomass and nutrient removal by willow clones in experimental bioenergy plantations in New York State. Biomass Bioenergy 20, 399–411.
Alonso Pippo, W., Luengo, C.A., Alonsoamador Morales Alberteris, L., Garzone, P., Cornacchia, G., 2011. Energy recovery from sugarcane-trash in the light of 2nd generation biofuels part 1: current situation and environmental aspects. Waste Biomass Valor. 2, 1–16.
Aronsson, P., Rosenqvist, H., Dimitriou, I., 2014. Impact of nitrogen fertilization to short-rotation willow coppice plantations grown in Sweden on yield and economy. Bioenergy Res. 7, 993–1001.
Baeza, J., Freer, J., 2000. Chemical characterization of wood and its components. In: Hon, D.N.-S., Shiraishi, N. (Eds.), Wood and Cellulosic Chemistry. , second ed. Marcel Dekker Inc., New York, pp. 275–369.
Carmona, R., Nunez, T., Alonso, M.F., 2015. Biomass yield and quality of an energy dedicated crop of poplar (*Populus* spp.) clones in the Mediterranean zone of Chile. Biomass Bioenergy 74, 96–102.
Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P., Cherchi, F., 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass Bioenergy 46, 25–35.
Dalla Marta, A., Mancini, M., Orlando, F., Natali, F., Capecchi, L., Orlandini, S., 2014. Sweet sorghum for bioethanol production: c rop responses to different water stress levels. Biomass Bioenergy 64, 211–219.

de Vries, S.C., van de Ven, G.W.J., van Ittersum, M.K., 2014. First or second generation biofuel crops in Brandenburg, Germany? A model-based comparison of their production-ecological sustainability. Eur. J. Agron. 52 (Part B), 166–179.

Doherty, W.O.S., Mousavioun, P., Fellows, C.M., 2011. Value-adding to cellulosicethanol Lignin polymers. Ind. Crop. Prod. 33, 259–276.

Fargione, J., Hill, J., Tilman, D., Polasky, S., Hawthorne, P., 2008. Land clearing and the biofuel carbon debt. Science 319, 1235–1238.

Fengel, D., Wegener, G., 1989. Wood. Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin-New York.

García, A., González Alriols, M., Labidi, J., 2014. Evaluation of different lignocellulosic raw materials as potential alternative feedstocks in biorefinery processes. Ind. Crop. Prod. 53, 102–110.

Gonçalves, F.A., Sanjinez-Argandona, E.J., Fonseca, G.G., 2013. Cellulosic ethanol and its co-products from different substrates, pretreatments, microorganisms and bioprocesses: a review. Nat. Sci. 5, 624–630.

González-Garćia, S., Gasol, C.M., Gabarrell, X., Rieradevall, J., Teresa Moreira, M., Feijoo, G., 2010. Environmental profile of ethanol from poplar biomass as transport fuel in Southern Europe. Renewable Energy 35, 1014–1023.

Gonzalez-Garcia, S., Moreira, M.T., Feijoo, G., Murphy, R.J., 2012. Comparative life cycle assessment of ethanol production from fast-growing wood crops (Black locust, eucalyptus and poplar). Biomass Bioenergy 39, 378–388.

Guidi, W., Tozzini, C., Bonari, E., 2009. Estimation of chemical traits in poplar short-rotation coppice at stand level. Biomass Bioenergy 33 (12), 1703–1709.

Gupta, A., Verma, J.P., 2015. Sustainable bio-ethanol production from agro-residues: a review. Renew.Sustain. Energy Rev. 41, 550–567.

Jing, X., Zhang, X., Bao, J., 2009. Inhibition performance of lignocellulose degradation products on industrial cellulase enzymes during cellulose hydrolysis. Appl. Biochem. Biotechnol. 159, 696–707.

Johansson, T., Karačić, A., 2011. Increment and biomass in hybrid poplar and some practical implications. Biomass Bioenergy 35, 1925–1934.

Johnson, F.X., Silveira, S., 2014. Pioneer countries in the transition to alternative transport fuels: Comparison of ethanol programmes and policies in Brazil, Malawi and Sweden. Environ. Innov. Soc. Transit. 11, 1–24.

Karlsson, H., Börjesson, P., Hansson, P.-A., Ahlgren, S., 2014. Ethanol production in biorefineries using lignocellulosic feedstock –GHG performance, energy balance and implications of life cycle calculation methodology. J. Clean. Prod. 83, 420–427.

Kelbert, M., Romaní, A., Coelho, E., Pereira, F.B., Teixeira, J.A., Domingues, L., 2015. Lignocellulosic bioethanol production with revalorization of low-costagroindustrial by-products as nutritional supplements. Ind. Crop. Prod. 64, 16–24.

Kim, S., Dale, B.E., 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy 26, 361–375.

Komorowicz, M., Wróblewska, H., Pawłowski, J., 2009. Chemical composition and energetic properties of biomass from selected renewable energy sources (in Polish). Ochrona Środowiska i Zasobów Naturalnych 40, 402–410.

Krzyżaniak, M., Stolarski, M.J., Waliszewska, B., Szczukowski, S., Tworkowski, J., Załuski, D., Śnieg, M., 2014. Willow biomass as feedstock for an integrated multi-product biorefinery. Ind. Crop. Prod. 58, 230–237.

Labrecque, M., Teodorescu, T.I., Daigle, S., 1997. Biomass productivity and wood energy of Salix species after 2 years growth in SRIC fertilized with wastewater sludge. Biomass Bioenergy 12 (6), 409–417.

López, F., García, M.M., Yánez, R., Tapias, R., Fernández, M., Díaz, M.J., 2008. Leucaena species valoration for biomass and paper production in 1 and 2 year harvest. Bioresour. Technol. 99, 4846–4853.

Margeot, A., Hahn-Hagerdal, B., Edlund, M., Slade, R., Monot, F., 2009. New improvements for lignocellulosic ethanol. Curr. Opin. Biotechnol. 20, 372–380.

Mathew, A.P., Oksman, K., Karim, Z., Liu, P., Ahmed Khan, S., Naseri, N., 2014. Process scale up and characterization of wood cellulose nanocrystalshydrolysed using bioethanol pilot plant. Ind. Crop. Prod. 58, 212–219.

Mohr, A., Raman, S., 2013. Lessons from first generation biofuels and implications for the sustainability appraisal of second generation biofuels. Energy Policy 63, 114–122.

Mola-Yudego, B., 2011. Trends and productivity improvements from commercial willow plantations in Sweden during the period 1986–2000. Biomass Bioenergy 35, 446–453.

Mussatto, S.I., Dragone, G., Guimaraes, P.M.R., Silva, J.P.A., Carneiro, L.M., Roberto, I.C., Vincente, A., Domingues, L., Teixeira, J.A., 2010. Technological trends, global market, and challenges of bio-ethanol production. Biotechnol. Adv. 28, 817–830.

Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates: II: inhibitors and mechanisms of inhibition. Bioresour. Technol. 74, 25–33.

Pinto, P.C., Evtuguin, D.V., Neto, C.P., 2005. Chemical composition and structural features of the macromolecular components of plantation *Acaciat mangium* wood. J. Agric. Food Chem. 53, 7856–7862.

Rooney, D.C., Killham, K., Bending, G.D., Baggs, E., Weih, M., Hodge, A., 2009. Mycorrhizas and biomass crops: opportunities for future sustainable development. Trends Plant Sci. 14, 542–549.

Rowell, R.M., Han, J.S., Bisen, S.S., 1997. Changes in fiber properties during the growing season. In: Rowell, R.M., Young, R.A., Rowell, J.K. (Eds.), Paper and Composite from Agro-based Resources. Lewis Publishers, Boca Raton, New York, London, Tokyo, pp. 23–37.

Rowell, R., 2005. Wood Chemistry and Wood Composites. Taylor and Francis Group, Boca Raton-London-New York-Singapore.

Schwab, A., Geiger, J., Lewis, J. 2015. 2013 Survey of Non-Starch Ethanol and Renewable Hydrocarbon Biofuels Producers Technical Report NREL/TP-6A10-63389. (</www.nrel.gov/publications>).

Serapiglia, M., Cameron, K., Stipanovic, A., Abrahamson, L., Volk, T., Smart, L., 2013. Yield and woody biomass traits of novel shrub willow hybrids at two contrasting sites. Bioenergy Res. 6, 533–546.

Shrestha, P., Pometto III, A., Khanal, S., van Leeuwen, J., 2012. Second-generation biofuel production from corn-ethanol industry residues. In: Gopalakrishnan, K., van Leeuwen, J., Brown, R.C. (Eds.), Sustainable Bioenergy and Bioproducts. Springer, London, pp. 71–87.

Stolarski, M.J., Szczukowski, S., Tworkowski, J., Klasa, A., 2011a. Willow biomass production under conditions of low-input agriculture on marginal soils. Forest Ecol. Manag. 262, 1558–1566.

Stolarski, M.J., Szczukowski, S., Tworkowski, J., Wroblewska, H., Krzyzaniak, M., 2011b. Short rotation willow coppice biomass as an industrial and energy feedstock. Ind. Crop Prod. 33, 217–223.

Smolarski, N., 2012. High-Value Opportunities for Lignin: Unlocking its Potential. Frost & Sullivan. <http://www.greenmaterials.fr/wp-content/uploads/2013/01/High-value-Opportunities-for-Lignin-Unlocking-its-Potential-Market-Insights.pdf/> (accessed 06.03.15).

Stolarski, M.J., Krzyżaniak, M., Waliszewska, B., Szczukowski, S., Tworkowski, J., Zborowska, M., 2013a. Lignocellulosic biomass derived from agricultural land as industrial and energy feedstock. Drewno 56 (189), 5–23.

Stolarski, M.J., Szczukowski, S., Tworkowski, J., Klasa, A., 2013b. Yield: energy parameters and chemical composition of short-rotation willow biomass. Ind. Crop. Prod. 46, 60–65.

Stolarski, M.J., Krzyżaniak, M., Szczukowski, S., Tworkowski, J., Bieniek, A., 2014a. Short rotation woody crops grown on marginal soil for biomass energy. Pol. J. Environ. Stud. 23 (5), 1727–1739.

Stolarski, M.J., Krzyżaniak, M., Szczukowski, S., Tworkowski, J., Załuski, D., Bieniek, A., Gołaszewski, J., 2014b. Effect of increased soil fertility on the yield and energy value of short-rotation woody crops. Bioenergy Res., http://dx.doi.org/10.1007/s12155-014-9567-9.

Stolarski, M.J., Krzyżaniak, M., Szczukowski, S., Tworkowski, J., Grygutis, J., 2015. Changes of the quality of willow biomass as renewable energy feedstock harvested with biobaler. J. Elem. 20 (3), http://dx.doi.org/10.5601/jelem.2014.19.3.769.

USDA, 2014. Feed Grains: Yearbook Tables, <http://www.ers.usda.gov/data-products/feed-grains-database/feed-grains-yearbook-tables.aspx#.VCpvsfl_uaU/> (accessed 26.12.14).

Valentas, K.J., Gauto, V., Gillitzer, P., Keitz, M.C., Lehman, C., Taff, S., et al., 2009. ; 2009. p. In: Biofuel feasibility study of Chisago, Isanti and Pine counties in Minnesota. St. Paul (MN). The University of Minnesota, pp. 92.

Viikari, L., Vehmaanperä, J., Koivula, A., 2012. Lignocellulosic ethanol: from science to industry. Biomass Bioenergy 46, 13–24.

Wilkinson, J.M., Evans, E.J., Bilsborrow, P.E., Wright, C., Hewison, W.O., Pilbeam, D.J., 2007. Yield of willow cultivars at different planting densities in a commercial short rotation coppice in the north of England. Biomass Bioenergy 31, 469–474.

Wróblewska, H., Komorowicz, M., Pawłowski, J., Cichy, W., 2009. Chemical and energetical properties of selected lignocellulosic raw material. Folia Forestalia Polonica Series B 40, 67–78.

Zamora, D.S., Wyatt, G.J., Apostol, K.G., Tschirner, U., 2013. Biomass yield, energy values, and chemical composition of hybrid poplars in short rotation woody crop production and native perennial grasses in Minnesota, USA. Biomass Bioenergy 49, 222–230.