



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



Mariusz J. Stolarski<sup>a,\*</sup>, Michał Krzyżaniak<sup>a</sup>, Michał Łuczyński<sup>b</sup>, Dariusz Załuski<sup>a</sup>, Stefan Szczukowski<sup>a</sup>, Józef Tworkowski<sup>a</sup>, Janusz Gołaszewski<sup>a</sup>

<sup>a</sup> 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

<sup>b</sup> University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, Department of Chemistry, Plac Łódzki 4, 10-957 Olsztyn, Poland

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## 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%.

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## 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](mailto:mariusz.stolarski@uwm.edu.pl) (M.J. Stolarski).

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<sup>-1</sup> year<sup>-1</sup>, when cultivated on good soils and under favorable weather conditions (Adegbi 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<sup>-1</sup> year<sup>-1</sup>) (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 Łęźany (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 “Zubr”. 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<sup>-1</sup>. Live mycorrhizal mycelium as a liquid suspension at 30–35 cm<sup>3</sup> was applied to each plant. Mineral fertilization with NPK was applied at 13, 50 and 90 kg ha<sup>-1</sup>, 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

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