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Influence of harvesting time on biochemical composition and glucose yield from hemp

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Abstract. This article investigates the influence of different harvesting times of hemp samples on their biochemical composition and glucose conversion yield. Samples were harvested from experimental fields of the Estonian University of Life Sciences from July to September in 2011. Dilute sulfuric acid solution was used for pretreatment in combination with enzymatic hydrolysis. Results indicate that the highest glucose conversion rate of 204.1 g kg^{-1} of dry matter of biomass was achieved by samples harvested on the 18th of August. The lowest glucose yield of 170.3 g kg^{-1} was achieved by samples harvested on 25th of August, which also had a very low hydrolysis efficiency of 46.9%. Biochemical composition and glucose conversion efficiencies of samples vary in time. Samples harvested in September have higher cellulose and lignin content than samples harvested in July. However, glucose conversion efficiencies decrease significantly in later samples. Average hydrolysis efficiency was 51.4%.

Key words: *glucose, dilute acid pretreatment, cellulose, hemp.*

INTRODUCTION

The increasing industrialisation and motorisation of the world has led to a steep rise in the demand for petroleum-based fuels (Agrawal, 2007). Today fossil fuels take up to 80% of the primary energy consumed in the world, of which 58% is consumed by the transportation sector alone (Escobar et al., 2009). The transportation sector is almost fully dependent on liquid fuels such as petrol and diesel. Continuously increasing oil prices have raised more support for the use of renewable energies. The key advantage of the utilisation of renewable sources for the production of biofuels is the use of natural bio-resources (that are geographically more evenly distributed than fossil fuels) to provide an independent and secure energy supply. Among biofuels, ethanol is one of the most appealing choices, because it can be blended with petrol or used in its pure form in modified engines (Hahn-Hagerdal et al., 2006; Tan et al., 2008). The dominating substrates used for ethanol production today are either pure sugars (sucrose from sugarcane) or easily degradable carbohydrates (starch from cereals or corn). A shift to lignocellulosic plant material is making the utilisation of other crops possible and will enable the production of transportation fuel from herbaceous biomass, corn stalks and straw (Kreuger et al., 2011). Utilising agricultural residual and waste substrates as raw materials for fuel ethanol production will also minimise the potential conflict between food and fuel.

Cellulosic ethanol production is a complex process compared to the first generation grain or sugarcane ethanol production. Firstly, it is necessary to break the lignin seal and hemicellulose sheathing over cellulose, and disrupt the crystalline structure of cellulose. Only then is it possible to degrade the cellulose in the biomass to sugar monomers. This disruption is achieved by the pretreatment process that is usually followed by enzymatic hydrolysis (Dwivedi et al., 2009; Kim et al., 2011).

The energy crop investigated in this study was industrial hemp. Hemp has several features that make it a suitable feedstock for cellulosic ethanol production. Firstly, it yields high biomass per hectare and adapts easily to different climate conditions. Secondly, the cellulose content of hemp is usually between 35–55%. Hemp fibres have many industrial applications, for example it is used in composite materials, in textile or pulp and paper production (Gonzales-Garcia et al., 2012). Hemp has also been reported as a good solid fuel for combustion. The high cellulose content and above ground biomass yield per hectare makes hemp a suitable crop for bioethanol production (Sipos et al., 2010). Since hemp also has quite a high content of lignin, it is necessary to use hydrothermal pretreatment before enzymatic hydrolysis to make cellulose fibres more easily accessible for enzymes.

Pretreatment with a dilute acid was used in the current research. Dilute acid pretreatment has been the most widely used method for pretreatment of the lignocellulosic material. This method uses cheap chemicals, mild operating conditions and is simple to perform. The downside of the dilute acid pretreatment method is a low conversion rate and formation of byproducts that are inhibitory for the following fermentation process. For the pretreatment with dilute acid, 0.5–1.5% acid solution is added to the biomass to hydrolyse hemicellulose during 5–60 minutes at 130–200 °C. Higher temperatures require a shorter time of pretreatment (Dien et al. 2006; Yang et al., 2009).

The pretreatment process is usually followed by enzymatic hydrolysis to convert the cellulose fibres and hemicellulose to fermentable sugars and fermentation to convert sugars to ethanol. Hydrolysis is carried out by different cellulases which are produced by lignocellulose degrading bacteria or fungi, for example *Trichoderma reesei*. The main factors that affect the hydrolysis rate of cellulose are accessibility of cellulose fibres to enzymes, crystallinity of cellulose and hemicellulose, and lignin content (Sun et al., 2002; Kim et al., 2011). Presence of lignin and hemicellulose makes the access of enzymes to cellulose fibres difficult. Therefore, removal of lignin and hemicellulose as well as increase in porosity during the pretreatment process increases the hydrolysis rate significantly (Dwivedi et al., 2009). At the same time, presence of dissolved lignin can also inhibit the hydrolysis, so that not all of the accessible cellulose is converted to sugars. Enzymatic hydrolysis can be carried out with total solid loadings up to 20%. If solid loading is higher than that, the constant stirring and equal distribution of enzymes in the mixture becomes difficult to achieve.

The aim of this research was to assess the potential of hemp as a feedstock for bioethanol production and investigate how different harvesting times influence it.

MATERIALS AND METHODS

Biomass

Industrial hemp was chosen as a raw material in this work, because it has a high biomass yield and high cellulose content, which makes it suitable for ethanol production. Furthermore, hemp does not compete directly with the food market. Hemp samples were harvested from July to September 2012, from the experimental fields of the Estonian University of Life Sciences. Hemicellulose, cellulose and lignin contents of aboveground biomass samples were determined in the Laboratory of Plant Biochemistry of the Estonian University of Life Sciences (see table 1). Standard methods of the Association of Official Analytical Chemists (AOAC 973.18) and methods by Tecator Company (fibre determination using the Fibertec M&I systems) were used in the analysis. Samples were milled to a particle size of 1–3 mm and dried. Dry matter content was 90 ± 3%.

Table 1. Hemicellulose, cellulose and lignin contents in dry mass of hemp samples harvested at different times

Harvesting time	Hemicellulose %	Cellulose %	Lignin %
28th July	10.54	33.06	6.48
4th August	14.34	32.98	6.29
11th August	10.90	36.76	7.37
18th August	9.36	39.31	7.60
25th August	9.20	36.34	7.98
1st September	10.04	37.84	8.16
8th September	8.81	43.30	8.15

Methods

Pretreatment with dilute acid, followed by enzymatic hydrolysis, was used in this work. Size of the samples was 100 g of dried (DM 90 ± 3%) and milled hemp to which 1,000 mL of 1% sulfuric acid solution was added. All samples were heated for t = 60 minutes at a temperature T = 130 ± 3ºC and a pressure of p = 3 bar. As enzymes are inactivated when temperature is T > 70 °C or 4 > pH > 7, the sample was cooled to a temperature below 50 °C and K_2CO_3 was added to neutralise the pH. Pretreatment was followed by enzymatic hydrolysis with the enzyme complex Accellerase 1,500. Enzyme mixture was added to the sample at a ratio of 0.3 mL per g of biomass. Hydrolysis lasted for t = 24 hours under constant stirring and at a temperature T = 50 °C. After the hydrolysis process, glucose concentration in all of the samples was measured reflectometrically using RQflex 10 reflectometer and Reflectoquant glucose & fructose test. D–glucose and D–fructose are converted into D–glucose–6–phosphate. This is oxidised by NAD under the catalytic effect of glucose–6–phosphate dehydrogenase to gluconate–6–phosphate. In the presence of diaphorase, the NADH formed in the process reduces a tetrazolium salt to a blue formazan that is then determined reflectometrically.

At least three parallel samples were analysed from each harvesting time. Averaged results are used in figures and deviations are shown by vertical lines. Data was processed with programs Microsoft Excel and GraphPad Prism 5.

RESULTS AND DISCUSSION

Results confirm that the cellulose and lignin content of the hemp biomass rises with maturity (Fig. 1). Samples harvested at the end of July and beginning of August have the lowest cellulose and lignin content of 32.98 and 6.29%, while the latest harvest, cut on September 8th, has one of the highest cellulose and lignin contents of

43.30 and 8.15%, respectively. As hemp matures, the cellulose and lignin contents in plant cells increase, which causes the stalk to harden. This corresponds well with other research done on cellulose and lignin content in herbaceous crops like corn stover and wheat straw (Buranov and Mazza, 2008). However, the hydrolysis efficiency decreases in samples of later harvests (Figs 1, 2). This can be explained by the increasing lignin content of the samples. The higher the lignin content, the more difficult it is for enzymes to reach cellulose fibres and convert them to glucose monomers. The highest hydrolysis efficiency of 59.1% was achieved in the hemp sample harvested on August 4th, which also had the lowest lignin content of 6.29%. The lowest hydrolysis efficiency of 46.4% was achieved in the hemp sample harvested on September 8th. Although it had the highest cellulose content, it also had one of the highest lignin contents. In order to achieve better hydrolysis efficiencies from late harvest hemp samples, longer pretreatment times or higher temperatures should be used. Furthermore, the pretreatment with a dilute base could be used to break the lignin seal of the fibres as the dilute acid pretreatment affects mainly hemicellulose sheathing. Average hydrolysis efficiency for all samples was 51.4%.

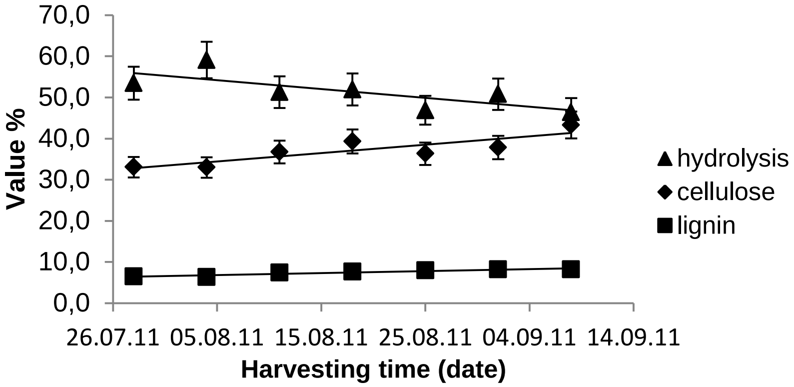

Figure 1. Correlation of cellulose/lignin contents and hydrolysis efficiencies of hemp samples with harvesting times.

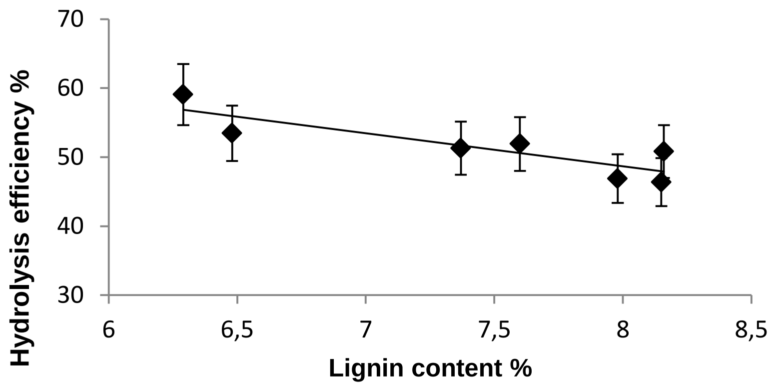

Figure 2. Dependence of hydrolysis efficiencies from the lignin content of the hemp samples with different harvesting times.

Glucose yield from the enzymatic hydrolysis was analysed for all hemp samples and the results were correlated with the harvesting time and cellulose content to assess the potential of hemp as a feedstock for bioethanol production. The corresponding results are shown in figure 3. Linear correlation was observed between glucose yield and cellulose content, as only cellulose fibres in lignocellulosic material are converted to glucose monomers. However, the highest glucose yield of 204.1 g per kg of dry matter was achieved from hemp samples harvested on August 18th and not from samples harvested on September 8th which had the highest cellulose content. This can be explained by the increasing lignin content and correspondingly decreasing hydrolysis efficiency of glucose conversion in hemp samples. From the point of glucose conversion and bioethanol production, the best harvesting time of hemp is in the middle of August when the plant has almost reached its maximum growth, but has not yet fully matured.

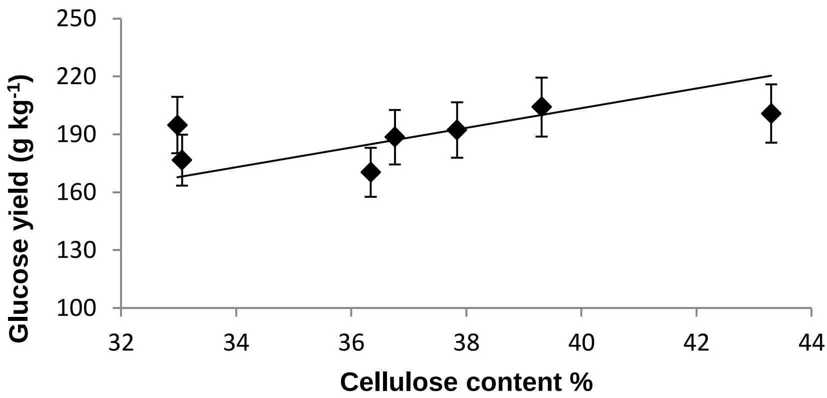

Figure 3. Dependence of glucose yield from cellulose content.

CONCLUSIONS

Aim of this research was to assess the potential of hemp as a feedstock for bioethanol production and investigate how different harvesting times influence it. Results confirm that cellulose and lignin content of a sample rises in time. Samples harvested at the end of July and beginning of August have the lowest cellulose and lignin content of 32.98 and 6.29%, while the latest harvest, cut on September 8th, have one of the highest cellulose and lignin contents of 43.30 and 8.15%, respectively. The hydrolysis efficiency decreases in samples of later harvests. Average efficiency for all samples was 51.4%. The highest glucose yield of 204.1 g per kg of dry matter was given by hemp samples harvested on August 18th and not by samples from later harvests which had higher cellulose contents. In conclusion, hemp is a good feedstock for bioethanol production, because of its high glucose yield and high above ground biomass yield. The best harvesting time of hemp for bioethanol production is in the middle of August when the plant has almost reached its maximum growth, but has not yet fully matured.

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