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Suitability of various plant species for bioethanol production

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Abstract. The aim of this research was to investigate glucose yield from different sorts of biomass and their suitability for bioethanol production. The amount of glucose obtained from different samples was also compared with their cellulose, hemicellulose, lignin content and in some cases with harvesting time. Dilute acid pretreatment at temperature of 150°C was used together with enzymatic hydrolysis. Herbaceous biomass from 7 different species was investigated: hemp, sunflower, energy grass, reed, silage, Jerusalem artichoke (*Helianthus tuberosus*) and *Miscanthus sacchariflori*s. Hemp had the highest cellulose content of 53.86%, while Jerusalem artichokes contained only 21–26% of cellulose. The highest lignin content was found in energy grass and silage, 9.65% and 9.02%, respectively. The most important properties of herbaceous material for bioethanol production and high glucose yields are high cellulose content and availability of biomass. In compliance with the cellulose concentrations, the best glucose yield of 312.7g kg^{-1} of biomass was obtained from hemp samples and the lowest results of 122.7g kg^{-1} of biomass from sunflower.

Keywords: Renewable energy, biomass, glucose, dilute acid pretreatment, cellulose, lignin.

INTRODUCTION

Out of many renewable energy resources, biomass is given high priority as it can be directly utilized for the production of various alternative transportation fuels, especially ethanol (Dwivedi et al., 2009). Ethanol is an attractive alternative fuel because it is a renewable resource and it is oxygenated, providing thus potential for reducing emissions in engines.

In many areas of Estonian agriculture practical utilization of agricultural by-products and biowaste is lacking. Moreover, biomass from the management of nature reserves is largely unused. Production of ethanol from cellulosic feedstock and its utilization as a substitute for gasoline could help in promoting rural development, reducing greenhouse gases, and achieving better energy independence (Demirbas & Bioethanol, 2005).

The aim of this research was to investigate glucose yield from different sorts of biomass and their suitability for bioethanol production. Biomass species were chosen according to the principle that they should grow in Estonia, but should not compete with the food market.

Cellulose

Cellulose is a linear polysaccharide which may consist of a few hundred to tens of thousands D–glucose monomers. It is found mostly in plant cell wall constructions. Cellulose is a water insoluble compound that is difficult to biodegrade. In nature, cellulose is degraded by bacteria and fungi which possess corresponding enzymes (Guenet & Cellulose, 2008). Microorganisms degrade cellulose primarily in to stages as follows:

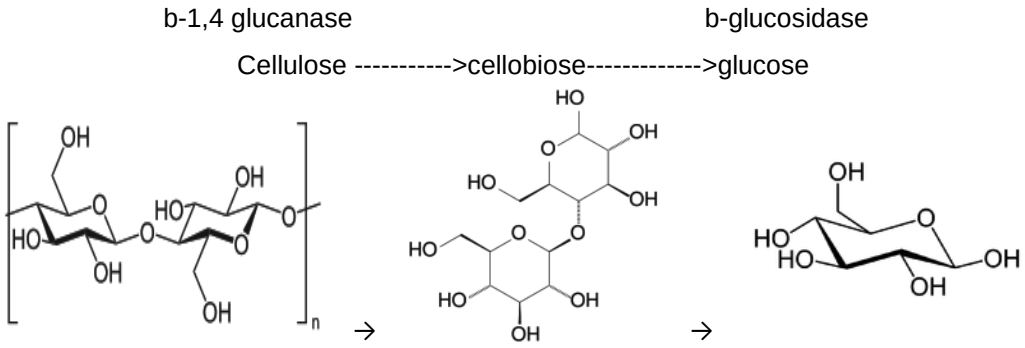

Figure 1. Degradation of cellulose into glucose (Wang, 2009).

First, as seen in Fig. 1, cellulose is degraded into cellobiose which consists of two glucose monomers, and then it is further degraded into glucose.

Hydrolysis of biomass was done by using a specific enzyme complex called Accellerase 1500, produced by Danisco U.S. Inc. Accellerase 1500 contains multiple enzyme activities, mainly exoglucanase, endoglucanase, hemi–cellulase and beta–glucosidase. It is produced by genetically modified strain of *Trichoderma reesei. Trichoderma reesei* is a mesofilic fungus that is found in tropical soils and produces different cellulose degrading enzymes (Vitikainen et al., 2010).

Lignin

Lignin is a complex chemical compound that is an integral part of the secondary cell wall of the plants. Lignin is one of the most slowly decomposing components of dead vegetation, contributing a major fraction of the material that becomes humus as it decomposes. Lignin is a cross-linked macromolecule, structure seen in fig. 2, with molecular masses over 10,000, being relatively hydrofobic and aromatic. Because of its cross-linking with the other cell wall components, it minimizes the accessibility of cellulose and hemicellulose to microbial enzymes (Sjöström, 1993).

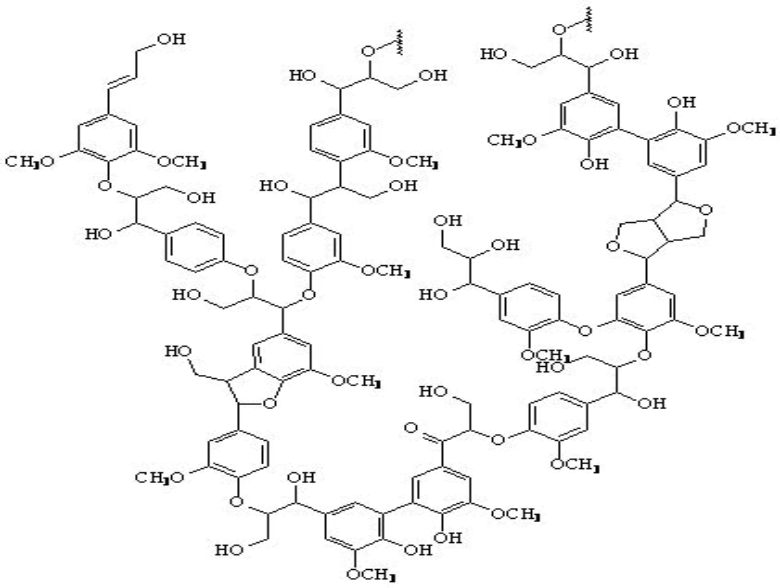

Figure 2. Structure of lignin (http://www.research.uky.edu/odyssey/images/lignin.jpg, 2011).

MATERIALS AND METHODS

Biomass

Different plant species which grow in Estonia, but do not compete directly with the food market or animal feed, were chosen for research. Herbaceous biomass from 7 different species was investigated: hemp, sunflower, energy grass, reed, silage, Jerusalem artichoke (*Helianthus tuberosus*), and *Miscanthus sacchariflori*.

Table 1. Content of ash, hemicellulose, cellulose and lignin in biomass samples.

Sample	Ash %	Hemicellulose %	Cellulose %	Lignin %
En.grass	7.01	27.33	37.85	9.65
Misc.sacchariflori.	5.37	30.15	42.00	7.00
Sunfower	9.78	5.18	34.06	7.72
Jerusalem artichoke (Aug.)	5.15	5.48	20.95	5.05
Jerusalem artichoke (Oct.)	4.56	4.50	25.99	5.70
Hemp	5.25	10.60	53.86	8.76
Silage	-	25.96	39.27	9.02
Reed	-	31.50	49.40	8.74

All samples were harvested between August and October in 2010 from the experimental fields of Estonian University of Life Sciences, except for reed which was cut in October from Lake Kuremaa. Ash, hemicellulose, cellulose and lignin content of biomass samples was determined in Laboratory of Plant Biochemistry of Estonian University of Life Sciences as seen in Table 1. Standard methods of Association of Official Analytical Chemists (AOAC 973-18) and methods by the company Tecator (Fibre determination using the Fibertec M&I systems) were used for analysis. Jerusalem artichoke had two harvesting periods and both samples were analysed. Silage was pressed and then dried press cake was used for analysis. All samples were cut down to particle size of 3–5 cm and dried to a moisture content of less than 5%.

Pretreatment and hydrolysis

Different methods have been used for biomass pretreatment. Very high conversion rates of cellulose to sugars of 70-90% are usually reported with AFEX (Ammonia Fiber Expansion) and steam explosion pretreatment methods. Both methods require quite severe operating conditions, temperature of 70-200ºC and pressure of 5-30 bar with AFEX, and temperature of 180-240ºC and pressure of 10-40 bar with steam explosion. For degradation of cellulose into glucose in this research, dilute acid pretreatment followed by enzymatic hydrolysis was used. This method is simple and uses cheap chemicals and mild operating conditions. The downside of the method is lower conversion rate and a possibility of inhibitory byproducts formation. Pretreatment is achieved by breaking the lignin seal and hemicellulose sheathing over cellulose and by disrupting the crystalline structure of cellulose (Dien et al., 2006; Yang et al, 2009).

The size of the samples was 75g of dried and milled biomass (moisture content < 5%) to which 750ml of 1% H_2SO_4 solution was added. All samples were heated for t = 30 minutes at the temperature T = 150 ± 3ºC and the pressure p = 5 bar. After the sample had cooled under 50ºC, $Ca(OH)_2$ was added in order to regulate the pH = 4,5-5 because enzymes are inactivated when pH < 4 or if pH > 6. Pretreatment was followed by enzymatic hydrolysis with enzyme complex Accellerase 1500. Enzymes were added to the sample with a concentration of 0,2ml g^{-1} of biomass. Hydrolysis lasted for t = 48 hours under constant stirring and at the temperature T = 50ºC. As a result most of the biomass (TS = 10%) was dissolved and turned into brown liquid. After the hydrolysis process, glucose concentration in all samples was measured reflectometrically by RQflex 10 reflectometer and Reflectoquant glucose & fructose test. D-glucose and D-fructose are converted into D-glucose-6-phosphate. This is oxidized 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 blue formazan which is determined reflectometrically.

At least 3 parallel samples were analyzed from every different biomass sort. Averaged results are used in figures and deviation is showed by vertical lines. Data was processed with programs Microsoft Excel and Graph Pad Prism 5.

RESULTS AND DISCUSSION

Glucose yield from different sorts of biomass and their suitability for bioethanol production was studied in this work. Results varied greatly between samples from different plant species.

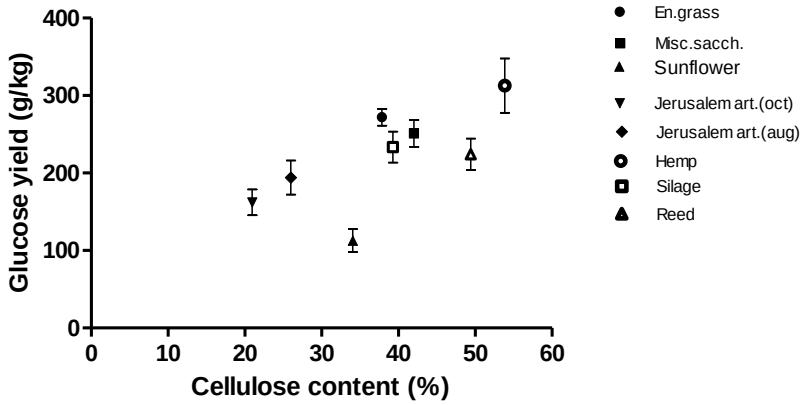

Figure 3. Dependence of glucose yield on cellulose content.

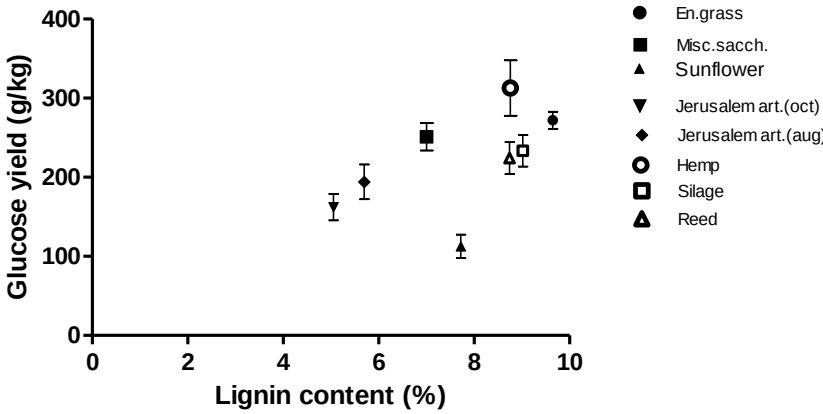

Figure 4. Dependence of glucose yield on lignin content.

The results indicate that glucose yield depends directly on the cellulose content of the sample, as shown in Fig. 3. The higher the cellulose content, the higher is the glucose yield with two exceptions of sunflower and reed. Sunflower and Jerusalem artichoke (Oct.) gave the lowest glucose yield of 122.7g kg^{-1} and 162.2g kg^{-1} of biomass, the cellulose content of which was 34.06% and 20.95%, respectively. Jerusalem artichoke (Aug.) gave a glucose yield of 194.1g kg^{-1}, but it had a cellulose

content of 25.99%. Cellulose and lignin content of a plant varies in time. A plant culture harvested in summer tends to have higher cellulose content and give better glucose yields than the same culture that is harvested in autumn (Bals et al., 2010). Sunflower has low cellulose content but quite high lignin and ash levels. Lignin minimizes the accessibility of cellulose to enzymes, resulting in lower glucose yield. Reed has high cellulose content but also high lignin and hemicellulose content, as shown in Fig. 4 and Table 1. This indicates that pretreatment has not removed hemicellulose completely and some of the remaining hemicellulose together with lignin is impeding enzymes from reaching the cellulose. The best glucose yield of 312.7g kg^{-1} was obtained from hemp samples that had the highest cellulose concentration of 53.86%.

Table 2. Glucose yield from theoretical value.

Sample	Glucose yield %
En.grass	71.86
Misc.saccharifl.	59.80
Sunfower	33.08
Jerusalem artichoke (Aug.)	74.70
Jerusalem artichoke (Oct.)	77.88
Hemp	58.06
Silage	59.49
Reed	46.42

The percentages of glucose yield from possible maximum values can be seen in Table 2. Jerusalem artichokes gave the highest yield of 77.42% and 74.70%, regardless of the harvesting time. This culture had the lowest cellulose content, but also the lowest lignin, hemicellulose and ash content. With few inhibiting factors, cellulose was easily accessible for degradation. The lowest yield percentage could be observed in sunflower and reed with 33.08% and 46.42%, respectively. Both cultures have high lignin content, but reed has also very high hemicellulose and cellulose content. In this case, the low yield percentage is possibly caused by inadequate pretreatment process. Dilute acid pretreatment at 150ºC for 30 minutes is not sufficient to remove high concentration of hemicellulose and lignin from the sample.

CONCLUSIONS

Accellerase The aim of this research was to investigate the glucose yield from different sorts of biomass and their suitability for bioethanol production. The results indicate that glucose yield depends on the cellulose content of the sample. The higher the cellulose content, the higher is the glucose yield with two exceptions: sunflower and reed. No direct correlation was found between glucose yield and lignin content, because samples with high lignin had also high cellulose concentrations, such as hemp.

Samples with low lignin had also low cellulose content and gave small glucose yield, such as Jerusalem artichokes. The best glucose yield of 312.7g kg^{-1} was obtained from hemp samples that had the highest cellulose concentration of 53.86%. The best yield percentage of 77.42% and 74.70% could be observed in Jerusalem artichokes, but since its cellulose content was low, it only released 162.2 and 194.1 grams of glucose per kg of biomass. Sunflower gave the lowest glucose yield of 122.7g kg^{-1}. So we can conclude that in the light of these results, the most suitable culture for bioethanol production is hemp and the least suitable is sunflower.

Studies with fermentation and bioethanol production from the above mentioned plant species are still ongoing to see if the results of ethanol yield concur with the results from glucose yield research.

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