Effect of lignin content of lignocellulosic material on hydrolysis efficiency

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Abstract. Lignocellulosic material is the most promising feedstock for bioethanol production; however, due to the varying physicochemical characteristics of different biomasses, it is necessary to select a biomass with a composition suitable for bioethanol production. For this purpose several different alternative non-food energy crops were chosen to investigate their suitability for bioethanol production, considering their cellulose, hemicellulose and lignin content. The traditional three-step bioethanol production process was used, where dilute acid was applied for biomass pre-treatment. Glucose and ethanol concentrations were measured during the process. Glucose and ethanol yields and hydrolysis efficiency were used to evaluate the suitability of different energy crops for bioethanol production. The results show that, with most biomass types, the glucose yield increases as the cellulose content in the biomass rises. However, a sharp decrease in hydrolysis efficiency was noted in the lignin content range of 7 to 9 g 100 g^{-1}. The lower hydrolysis efficiency also resulted in a lower ethanol yield in the next step of the bioethanol production process for these samples.

Key words: bioethanol, lignocellulosic biomass, lignin, biofuel, acid pre-treatment.

INTRODUCTION

Lignocellulosic biomass is considered an attractive feedstock for the production of a second-generation biofuel – bioethanol. Traditional lignocellulosic feedstocks include mainly energy crops (purpose-grown vegetative grasses, short rotation forests etc.) and agricultural residues (e.g. corn stover, wheat straw, sugar cane bagasse) (Tutt et al., 2012; Tutt et al., 2013; Cybulska et al., 2014) but also different kinds of waste (e.g. organic components of municipal solid waste) (Sims et al., 2010; Raud et al. 2014; Tutt et al., 2014). The main advantage of the production of second-generation biofuels from non-edible feedstock is that it limits the direct food versus fuel competition associated with first-generation biofuels (Naik et al., 2010; Nigam & Singh, 2011; Haghighi Mood et al., 2013).

Lignocellulosic materials are the most promising feedstock for bioethanol production, considering their great availability, low cost, and sustainable supply (Agbor et al., 2011). Plant biomass is primarily composed of plant cell walls, of which about 75% consists of polysaccharides (Phitsuwan et al., 2013), which could be used for ethanol production. The conversion of lignocellulosic biomass to alcohol traditionally requires a three-step process – the pre-treatment of biomass, acid or enzymatic hydrolysis, and fermentation/distillation (Mosier et al., 2005; Naik et al., 2010;

Demirbas, 2011). However, the complicated structure of lignocellulosic material makes it recalcitrant to chemical and biochemical reactions (Min et al., 2013; Xu et al., 2013). Due to many physical and chemical factors, such as the presence of lignin, the crystallinity of cellulose, and the presence of covalent cross-linkages between lignin and hemicelluloses in the plant cell wall, lignocellulosic biomass is naturally resistant to microbial attack (Li et al., 2011).

Different biomass pre-treatment methods have been reported to overcome the recalcitrance of lignocellulosic biomass. The goal of the pre-treatment is to enhance the conversion steps by separating the biomass components and/or by providing easier access to cellulose (Chiaramonti et al., 2012; Conde-Mejia et al., 2012). The main techniques for the selective fractionation of hemicelluloses from the biomass include the use of acids, water (liquid or steam), organic solvents and alkaline agents (Girio et al., 2010; Cybulska et al., 2014). Different pre-treatment methods have different targets. For example, acid treatment breaks down the glycosidic bonds of polysaccharides, freeing individual monosaccharides and thereby removing hemicellulose. Alkaline pre-treatment, on the other hand, alters and removes the lignin structure (Min et al., 2013; Barakat et al., 2014). Physicochemical pre-treatment methods, like steam-explosion and AFEX pre-treatment, alter the biomass structure by separating the individual fibres in combination with partial hemicellulose hydrolysis and solubilization (Alvira et al., 2010).

However, during pre-treatment, various enzyme inhibitors/deactivators and toxic substances are produced, which may also inhibit the later fermentation processes, leading to lower ethanol yields and productivities (Girio et al., 2010; Cybulska et al., 2014). These compounds are principally generated from lignocellulosic components during pre-treatment (Phitsuwan et al., 2013) and, depending on the operational conditions, degradation products are formed both from sugars (furan derivatives and organic acids) and, to a lesser extent, from lignin (phenolic compounds) (Girio et al., 2010; Cybulska et al., 2014). The type and amount of the generated toxic compounds depend on the raw material and the harshness of the pre-treatment conditions (Alvira et al., 2010). In addition, several other factors have been reported to affect the hydrolysis of cellulose, including the porosity of the biomass, cellulose fibre crystallinity, and lignin and hemicellulose content. The presence of lignin and hemicellulose makes it difficult for cellulase enzymes to access the cellulose fibres and thus reduces the efficiency of the hydrolysis process (Sun & Cheng, 2002).

It has been observed that since different lignocellulosic materials have different physicochemical characteristics, it is necessary to adopt suitable pre-treatment technologies based on the biochemical composition of the lignocellulosic biomass (Alvira et al., 2010). It has been shown that a biomass with a high cellulose content helps to gain high glucose and ethanol yields (Kikas et al., 2014) while the other major components of biomass decrease the bioethanol production potential by acting as a physical barrier, preventing the cellulose in the biomass to be hydrolysed by enzymes (Alvira et al., 2010).

The purpose of this study was to investigate the effect of biomass composition on the bioethanol production potential. Different non-food biomass types were collected and used in the traditional three-step bioethanol production process, where dilute acid was used for biomass pre-treatment. Glucose and ethanol concentrations were measured

during the process. The bioethanol production potential was evaluated on the basis of glucose and ethanol yields and hydrolysis efficiency.

MATERIALS AND METHODS

Description of the biomass

Different alternative crops were chosen to investigate their suitability for bioethanol production, considering their lignin content. The species selected were: Jerusalem artichoke (*Helianthus tuberosus L.*), fibre hemp (*Cannabis sativa L.*) cv USO-31, energy sunflower (*Helianthus annuus L.*) cv Wielkopolski, Amur silver-grass (*Miscanthus sacchariflorus*), energy grass cv Szarvasi-1, rye and reed. In addition, a sample of silage was used as a biomass. A more detailed description of the growth parameters and sampling conditions of the biomass is given in a previous paper (Kikas et al., 2014).

The percentage of lignin, Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) in the dry mass (DM) of all the plant samples was determined in the Laboratory of Plant Biochemistry at the Estonian University of Life Sciences (Tecator ASN 3430). Standard methods of the Association of Official Analytical Chemists (AOAC 973.18) and the methods of the company Tecator (fibre determination using the Fibertec M&I systems) were applied in the analysis. All samples were ground up with Cutting Mill SM 100 comfort (Retsch GmbH) and then with Cutting Mill ZM 200 (Retsch GmbH).

Bioethanol production

A dilute acid pre-treatment followed by enzymatic hydrolysis was used for the degradation of cellulose into glucose. For the pre-treatment, 750 mL of a 1% (m m^{-1}) H_2SO_4 solution was added to 75 g of dried and milled biomass (moisture content < 5%, m m^{-1}). All the samples were heated for 30 minutes at a temperature of 150 ± 3 °C and at a pressure of 5 bar. The samples were cooled to below 50 °C and the pH of the mixture was neutralized with $Ca(OH)_2$ to achieve a pH between 4.5–5.5 in order to avoid enzyme inactivation at pH ranges of pH < 4 and pH > 6.

The pre-treatment was followed by enzymatic hydrolysis using the enzyme complex Accellerase 1500. The enzymes were added to the sample in a proportion of 0.2 mL per g of biomass. The hydrolysis lasted for 48 hours under constant stirring and at a temperature of 50 °C. As a result most of the biomass was dissolved and the mixture turned into a brown liquid. After the hydrolysis, the glucose concentration in all the samples was measured reflectometrically (RQflex 10 reflectometer, Reflectoquant glucose & fructose test).

Fermentation with the yeast *Saccharomyces cerevisiae* was used in order to convert the glucose to ethanol. 2.5 g of dry yeast *Saccharomyces cerevisiae* was added to all the samples and the fermentation process was carried out at room temperature in 1,000 mL glass bottles sealed with a fermentation tube and a water lock that provided low-oxygen conditions. The fermentation lasted for 168 h, after which the ethanol concentration was measured. The concentration was measured reflectometrically using the RQflex 10 reflectometer and the Reflectoquant alcohol test by Merck Inc.

RESULTS AND DISCUSSION

Biomass analysis

In this study biomass was characterized on the basis of its relative proportion of cellulose, hemicellulose, lignin, and ash as outlined in Table 1. The potential glucose and ethanol yields are directly proportional to the cellulose content of the biomass (Kikas et al., 2014); therefore, a biomass with higher cellulose content is usually preferred for ethanol production. From Table 1 it can be seen that reed and hemp exhibited the highest cellulose content of 49.40 and 53.86 g 100g^{-1}, respectively, while Jerusalem artichoke had by far the lowest cellulose content regardless of the harvesting time. Reed together with Amur silver-grass also had high hemicellulose content. The highest lignin content was measured in energy grass and silage, 9.65 and 9.02 g 100 g^{-1}, respectively. The lowest hemicellulose and lignin content was also found in samples of Jerusalem artichoke. Furthermore, its biochemical composition depended on the harvesting time of the biomass since the biochemical composition of plants changes during the vegetation period (Tutt et al., 2013; Kikas et al., 2014). The highest ash content of 9.78 g 100 g^{-1} was found in sunflower samples.

Table 1. Hemicellulose (HC), cellulose (CEL), lignin and ash content (g 100 g^{-1}) in the biomass samples of different energy crops

Energy crop	HC	CEL	Lignin	Ash
Amur silver-grass	30.15	42.00	7.00	5.37
Energy grass cv Szarvasi-1	27.33	37.85	9.65	7.01
Hemp	10.60	53.86	8.76	5.25
Sunflower	5.18	34.06	7.72	9.78
Rye	27.86	42.83	6.51	5.21
Reed	31.50	49.40	8.74	n/a
Silage	25.96	39.27	9.02	n/a
Jerusalem artichoke (Oct)	4.50	25.99	5.70	4.56
Jerusalem artichoke (Sep)	5.48	20.95	5.05	5.15

Glucose production from biomass

Fig. 1 illustrates how the glucose yield from the selected energy crops related to the cellulose content of different types of biomass. As expected, based on previous research, the results show that the glucose yield is proportional to the cellulose content in biomass. Since cellulose is a polymer that consists of glucose monomers, glucose is the main product formed during the enzymatic hydrolysis of cellulose. As a result, when the cellulose content in the biomass rises, the glucose yield from this biomass should also increase. However, two types of biomass – sunflower and reed – showed considerably lower glucose yields than what could be expected based on the cellulose content of the biomass. The glucose yield from reed was 24% lower than expected, while the glucose yield from sunflower was nearly two times lower than what could be expected based on the cellulose content of the respective biomass.

In order to explain this deviation from the expected glucose yields, other parameters, like hemicellulose and lignin content of the biomass, were analyzed. While no correlation was found between hemicellulose content and glucose yield, there was a distinct relationship between the lignin content of the biomass and hydrolysis efficiency. Fig. 2 illustrates the change in hydrolysis efficiency of various types of biomass

depending on their lignin content. A hydrolysis efficiency of over 60% was achieved with many different biomass types that had low or high lignin content. However, a sharp decrease in hydrolysis efficiency can be seen in the lignin content range of 7–9 g 100 g^{-1}, where the lowest efficiencies were seen with sunflower and reed. These results suggest that in case of acid pre-treatment where hemicellulose is hydrolysed during the pre-treatment, the absolute value of lignin content in the biomass is less important than the range within which it lies. This points to a possibility that the efficiency of enzymatic hydrolysis is dependent not only on the presence of lignin in the biomass but also on the way the cellulose macrofibrils are wrapped in the lignin sheath. Too little or too much lignin does not provide the cellulose fibres with effective coverage and thus makes them more accessible for the enzymes after the removal of hemicellulose, which in turn results in a more efficient hydrolysis of cellulose.
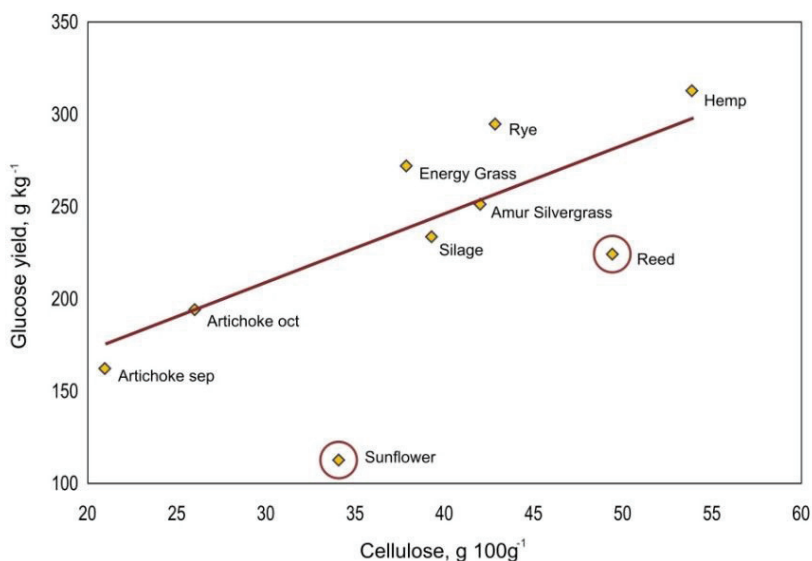

Figure 1. The dependence of glucose yield on the cellulose content of different biomass types. The red line indicates a linear dependence of the glucose yield on the cellulose content; outliers from the main linear trendline are circled.

During the conversion of biomass to ethanol it is essential to minimize sugar losses to reduce the production cost of ethanol (Alvira et al., 2010). Therefore, for an efficient bioethanol production process it is vital to achieve a high glucose yield. This could be achieved by choosing a biomass with a lignin content of below or above 7–9 g 100 g^{-1}, where the lignin coverage of the cellulose fibrils is not optimal, in combination with choosing the most suitable pre-treatment method.
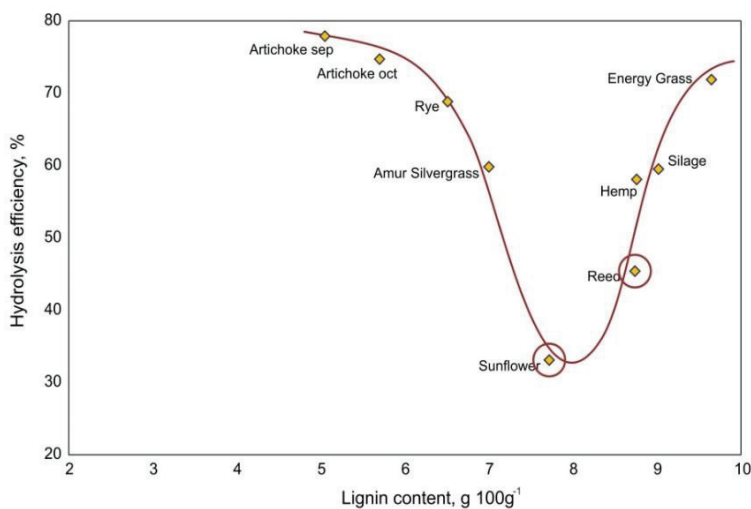
Figure 2. The dependence of the hydrolysis efficiency of different types of biomass on the lignin content. The red line indicates s dependence of hydrolysis efficiency on the lignin content; two outliers from the cellulose-glucose linear trendline are circled.

Ethanol production

As ethanol is the final product of this process, the ethanol yields from different energy crops were also analyzed. Fig. 3 describes the dependence of ethanol yield on the cellulose content of different types of biomass. The results show that ethanol yield is proportional to the cellulose content of the biomass – more ethanol was gained from biomasses with higher cellulose content. This is expected as the ethanol yield is directly proportional to the amount of glucose available for fermentation and the glucose yield was shown to be proportional to the cellulose content of the biomass.
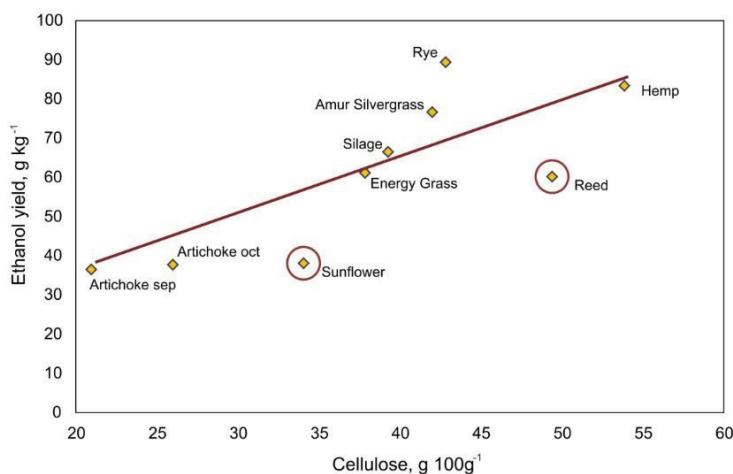

Figure 3. The dependence of ethanol yield on the cellulose contents of different types of biomass. The red line indicates a linear dependence of ethanol yield on the cellulose content; two outliers from the cellulose-glucose linear trendline are circled.

Fig. 3 also shows that the rye sample has a higher glucose yield, while sunflower and reed have lower glucose yields than assumed from the general trendline. The lower ethanol yield of reed and sunflower is expected since the glucose yield (Fig. 1) and hydrolysis efficiency (Fig. 2) of these energy crops were also lower than those of other samples with similar cellulose contents.

CONCLUSIONS

Different types of non-food biomass were collected and used in the traditional three-step bioethanol production process, where dilute acid was used for biomass pre-treatment to study the effect of biomass composition on the bioethanol production potential. In this study, glucose and ethanol yields together with hydrolysis efficiency were used to evaluate the suitability of different energy crops for bioethanol production. The results show that although glucose and ethanol yields rise when the cellulose content of biomass increases it is not the only parameter that influences the glucose and ethanol yields. Considerably lower glucose and ethanol yields were gained when the lignin content of the biomass was between 7–9 g 100 g^{-1}, which leads to lower ethanol yields. This suggests that the efficiency of enzymatic hydrolysis is dependent not only on the pre-treatment conditions and on the presence of lignin in the biomass, but also on the way the cellulose fibrils are wrapped in the lignin sheath.

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