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Dependence of the hydrolysis efficiency on the lignin content in lignocellulosic material

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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 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 yields and hydrolysis efficiency were used to evaluate the suitability of different energy crops for bioethanol production. The results show that 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 7–9 g 100 g^{-1}. The lower hydrolysis efficiency also resulted in a lower ethanol yield in the next step of the bioethanol production process.

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Introduction

Due to the increasing energy demand, depletion of fossil fuels and global warming a great deal of attention has been paid to finding new alternative and sustainable energy sources [1,2]. One of the more attractive options to replace liquid fossil fuels is the production of alternative renewable fuels like bioethanol. Global production of bioethanol reached 88.8 million m^3 in 2013 [3]. Most of the bioethanol however, is produced from corn and sugarcane although, first commercial-scale cellulosic ethanol plants have also started operating. Bioethanol production from corn and sugarcane has raised many concerns due to the increased use of agricultural land, which has led to a rise in prices of food and other agricultural commodities [4,5]. Since demand for biofuels has been increasing together with demand for food, a lot of attention in recent years has been directed to the utilization of lignocellulosic biomass [6,7].

Lignocellulosic biomass, which is non-edible residues of food crop production or non-edible whole plant biomass, is the most promising feedstock for the bioethanol production considering their great availability, low cost and sustainable supply [8]. Such ligno-cellulosic feedstock materials include by-products (cereal straw, sugar cane bagasse, forest residues), wastes (organic components of municipal solid wastes), and dedicated feedstocks (purpose-grown vegetative grasses, short rotation forests and other energy crops) [9,10]. In addition, low-cost crop and forest residues, wood process wastes,

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and the organic fraction of municipal solid wastes can all be used as lignocellulosic feedstocks [2,9].

Plant biomass is primarily composed of plant cell walls of which about 75% consists of polysaccharides, cellulose and hemicellulose [10], which could be used for ethanol production. The cellulose consists of a linear chain of D-glucose linked by β-(1,4)-glycosidic bonds to each other [8,10,11] while the hemicelluloses are a group of heterogeneous branched polysaccharides [10] built of pentoses, hexoses and sugar acids [8,10,11]. The lignocellulosic biomass is traditionally transformed to ethanol using three step process – pretreatment of biomass to open the biomass structure for further treatment, acid or enzymatic hydrolysis to degrade the cellulose polymers to sugar monomers and fermentation of sugars to ethanol [12,13].

However, since the three main structural units of lignocellulosic biomass – cellulose, hemicellulose and lignin, are organized into a complex structure [8], the plant cell walls are very recalcitrant to chemical and/or biological degradation [14,15]. To overcome this, various pretreatment methods have been proposed. A pretreatment is necessary to break down the crystalline structure of any type of plant biomass [16] and this is targeted to improving the conversion steps by separating the biomass components and/or by providing easier access to cellulose to improve the efficiency of conversion steps [17,18].

Since different types of feedstock have varying cellulose, hemicelluloses and lignin contents, suitable pre-treatment method must be found for particular biomass [10,19,20]. Furthermore, it is important to select the biomass with the composition suitable for bioethanol production. From the previous work it is known that high cellulose content of the biomass enables to gain high glucose and ethanol yields [21,22] while hemicellulose and lignin decrease the bioethanol production potential by acting as a physical barrier, preventing the cellulose in the biomass to be hydrolysed [1].

The paper gives overview of research conducted to investigate the dependence of hydrolysis efficiency on the lignin content in lignocellulosic material. Traditional three step bioethanol production process with acid pretreatment was used study the conversion of different types of biomasses to bioethanol. Hydrolysis efficiency and glucose and ethanol yields were considered to estimate the effect of biomass composition to bioethanol production process.

Materials and methods

Biomass

Various alternative crops with different lignin, cellulose and hemicellulose contents were chosen to investigate their suitability for bioethanol production. 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, reed and additionally sample of silage was used as a biomass. The more complete description of biomass growth and sampling conditions are described in previous paper [23].

The fibre analysis to determine the percentage of lignin, Acid Detergent Fibre (ADF), and Neutral Detergent Fibre (NDF) in the dry mass (DM) of all plant samples was conducted in the Plant Biochemical Laboratory of Estonian University of Life Sciences (Tecator ASN 3430) [24]. All samples were ground with Cutting Mill ZM 200 (Retsch GmbH).

Bioethanol production

Dilute acid pretreatment followed by enzymatic hydrolysis was used for degradation of cellulose into glucose. 750 ml of 1% H_2SO_4 solution was added to 75 g of dried and milled biomass (moisture content < 5%) for the pretreatment of biomass. Biomass samples in acid solution were heated for 30 min at a temperature of 150 ± 3 °C and at a pressure of 5 bar. In order to avoid enzyme inactivation at pH ranges of pH < 4 and pH > 6, the samples were cooled below 50 °C and pH of the mixture was neutralized with $Ca(OH)_2$ to achieve pH between 4.5 and 5.

Pretreatment was followed by enzymatic hydrolysis using enzyme complex Accellerase 1500 which was added to a sample in a proportion of 0.2 ml per g of biomass. Hydrolysis lasted 48 h under constant stirring and at temperature of 50 °C.

Fermentation with dry yeast *Saccharomyces cerevisiae* was used in order to convert the glucose to ethanol. 2.5 g of dry yeast was added to all samples and fermentation process was carried out at room temperature under low oxygen conditions in 1000 ml glass bottles, sealed with a fermentation tube. Fermentation lasted for 7 days after which, the ethanol concentration was measured.

Glucose and ethanol concentrations were measured reflectometrically using RQflex 10 reflectometer and Reflectoquant alcohol and Reflectoquant glucose & fructose tests by Merck Inc.

Results and discussion

Biomass analysis

Biomass can be characterised according to its biochemical composition – cellulose, hemicellulose and lignin content. For this fibre analysis was conducted and the results are presented in Table 1. If bioethanol production is the goal, the cellulose content is the most important parameter to consider

Table 1 – Percentage of cellulose (CEL), hemicellulose (HC) and lignin in biomass samples of different energy crops.

Energy crop	CEL (%)	HC (%)	Lignin (%)
Hemp	53.86	10.60	8.76
Reed	49.40	31.50	8.74
Rye	42.83	27.86	6.51
Amur silver-grass.	42.00	30.15	7.00
Silage	39.27	25.96	9.02
Energy grass cv Szarvasi-1	37.85	27.33	9.65
Sunflower	34.06	5.18	7.72
Jerusalem artichoke (Oct)	25.99	4.50	5.70
Jerusalem artichoke (Sep)	20.95	5.48	5.05

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