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Influence of Mechanical Pre–treatment on Fermentable Sugar Production from Lignocellulosic Biomass

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Abstract. Mechanical pre-treatment of lignocellulosic biomass has been extensively applied in biofuel production despite its high energy requirements. To balance the consumed energy with the energy produced, careful selection and evaluation of pre-treatment parameters, equipment and desired outcome is needed. The study aims to determine optimal hay and barley straw biomass particle size in view of sugar yields, energy consumption and treatment time. The results show that there is no significant difference ($p > 0.05$) in sugar yields from hay biomass with particle sizes 0.25 mm, 1 mm and 10 mm. Energy requirements for the production of 1 kg of sugar from hay range from 1.8–10.7 MJ. At the same time barley straw proved to be inappropriate for sugar extraction due to low sugar yields (below 40 mg g^{-1} dry mass) and high energy consumption (18.5–76.2 MJ to produce 1 kg sugar). Thus, after the careful selection of biomass, mechanical pre-treatment followed by enzymatic hydrolysis can be an effective technique in biofuel production from biomass.

Key words: lignocellulosic biomass, pre-treatment, fermentable sugars, milling.

INTRODUCTION

Lignocellulosic biomass is regarded as a sustainable renewable resource to produce biofuels, e.g., bioethanol or biobutanol. A major portion of this biomass contains cellulose, hemicellulose and lignin. Cellulose and hemicellulose, in turn, can be converted to fermentable sugars. At the same time, lignin forms a protective covering and impedes the hydrolysis of cellulose and hemicellulose. Therefore, biomass pre-treatment is required for the depolymerisation of lignin prior to the conversion of cellulose and hemicellulose into fermentable sugars (Chaturvedi & Verma, 2013). Many combinations of various technologies have been proposed for the pre-treatment of lignocellulosic materials to produce biofuels. These methods can be divided into chemical (acid, alkaline), biological, oxidative and physical (mechanical) methods (Kumar et al., 2009). So that these technologies could be used outside laboratory and research environments, they must be effective as well as simple, user-friendly and economically sustainable. One of the simplest approaches is mechanical size reduction that has many advantages, like facilitating an increase in total accessible surface area, reduction in cellulose crystallinity (Barakat et al., 2014) and no formation of toxic by-products (Agbor et al., 2011). Cutting or crushing, coarse milling, chipping, shredding and grinding are among the most often reported size reduction approaches (Barakat et al., 2014).

The selection of the most appropriate mechanical pre-treatment method is associated with the economical parameters of the equipment, since it has been reported that pre-treatment is generally the most energy consuming step in biofuel production (Mosier et al., 2005). Sometimes energy consumption is even higher than the theoretical energy content available in the biomass (Kumar et al., 2009), thus making some techniques inefficient. Nevertheless, a large number of studies have been performed over the years to find the most appropriate mechanical pre-treatment conditions and equipment (Shi et al., 2009; Barakat et al., 2014) as there is a need for technological simplicity that must have an overall effect on production yields. It has been demonstrated that size reduction increases sugar (Pedersen & Meyer, 2009; Mezule et al., 2015) or biofuel (Menind & Normak, 2010) yields and is closely linked with energy requirements (Cadoche & López, 1989). At the same time, data on linking sugar yields with energy requirements is limited or the connection has not been demonstrated for biomass available at temperate climates (Da Silva et al., 2010) where inedible lignocellulosic biomass can be found in vast amounts. Recently, landowners have been even required to remove biomass cultures from the fields to receive financial support (Zalite et al., 2014; Rural Support Service of the Republic of Latvia, 2015), thus making the resource an unwanted waste.

The aim of this study is to determine optimal hay and barley straw biomass particle size in view of sugar yields, energy consumption and treatment time. For the evaluation biomass was milled in a commercial cutting mill with exchangeable sieves that was selected owing to its ability to produce variable biomass fractions and the potential for large-scale application. Detailed fractionation of the biomass was not performed due to the low probability of fractionation by the potential end user—the industry.

MATERIALS AND METHODS

Feedstock preparation

Hay (Dry weight (DW) 92.8 ± 1.3%) and barley straw (DW 94.2 ± 0.7%) harvested in 2015 in Latvia were used as the test material. Previously reported chemical compositions of the biomass were adopted for this study (35–45% cellulose, 30–50% hemicellulose and 8–20% lignin for barley straw (Chen et al., 2007) and 25–40% cellulose, 35–50% hemicellulose and 10–30% lignin for hay (Kumar et al., 2009). The material was milled at a mechanical cutting mill (Retsch SM100, Haan, Germany) with 1.5 kW drive and parallel section rotor with a peripheral speed of 9.4–11.4 ms^{-1}. For all individual tests 400 g of biomass was used. Particle size was controlled with the help of sieve size and type (0.25 mm and 1 mm trapezoid holes, 10 mm square holes). After each milling the sieves were carefully cleaned to exclude any transfer of biomass. Practical electrical energy consumption (E_p, kWh) was measured with a 3–phase indicator (Orno OR–WE–505, Mikolow, Poland). Theoretical energy (E_t, kWh) was calculated according to Eq. 1:

$$E_t = P \times t \quad (1)$$

where: P – drive power (kW); t – milling time, h.

After the milling all samples were collected to separate containers, carefully closed to avoid moisture and stored for further processing. All experiments were prepared in triplicate.

Dry weight content was analysed with the moisture analyser Kern DBS (Kern & Sohn GmbH, Germany).

Enzymatic hydrolysis

Prior hydrolysis 3% w/v of the collected milled biomass was diluted in 0.05 M sodium citrate buffer (mono-sodium citrate pure, AppliChem, Germany) and boiled for 5 min (1 atm) to eliminate any indigenous microorganisms. After cooling to room temperature a laboratory-prepared enzyme (0.2 FPU ml^{-1}, Mezule et. al, 2015) was added to the diluted substrates and incubated on an orbital at 30 °C. Samples for sugar analyses were collected after dilution with buffer, prior enzyme addition and after 24 and 48 h of incubation. At least 2 samples from each test were collected for reducing sugar measurements.

Reducing sugar analysis

Total reducing sugar concentration was measured with the dinitrosalicylic acid (DNS) method (Ghose, 1987). In brief, all samples were centrifuged at 6,600 g for 5 min (MiniSpin Plus, Eppendorf). Then 0.1 ml of the supernatant was mixed with 0.1 ml of 0.05 M sodium citrate buffer and 0.6 ml of DNS (3,5-dinitrosalicylic acid, Sigma, Germany). For blank control, distilled water was used instead of the sample. Then all samples were boiled for 5 min and transferred to cold water. Further 4 ml of distilled water was added. Absorption was measured with spectrophotometer at 540 nm (Camspec M501, UK). To obtain absolute concentrations, a standard curve against glucose was prepared.

Data analysis

MS Excel 2007 t-test (two tailed distribution) and ANOVA single parameter tool (significance level ≤ 0.05) were used for the analysis of variance of data from various sample setups.

RESULTS AND DISCUSSION

The mechanical pre-treatment of biomass to reduce its size and facilitate hydrolysis has been widely used despite its high energy requirements (Barakat et al., 2014). To create a balance between sugar yields and energy consumption various factors like size and equipment must be evaluated. In this research biomass pre-treatment was performed with a commercial cutting mill with exchangeable sieves. Particles were milled by passing them through a sieve where they were reduced by the high-speed mechanical impacts and shearing inside the grinding chamber (Silva et al., 2012).

The obtained fractions were below 0.25 mm, 1 mm and 10 mm (Fig. 1, samples 1–3 respectively) in size. Milling time decreased with the increasing sieve size and the same amount of biomass was obtained twice the faster ($p < 0.05$) with the 10 mm sieve compared to 0.25 or 1 mm, irrespective of biomass source. At the same time, the processing of barley straw required a 18–44% longer milling time than hay. Regular blocking of the mill was observed for barley straw irrespective of the sieve size.

Processing difficulties connected to barley straw could be attributed to different initial density and moisture content, which afterwards decreased the material's flowability (Tumuluru et al., 2014). Nevertheless, visual observations showed that samples milled with 10 mm sieve contained distinct biomass pieces that could explain fractions producing low sugar yields (Mezule et al., 2015). Both 0.25 mm and 1 mm size samples had homogeneous biomass fractions without any distinct particles. Samples of 0.25 mm fractions were almost powder-like and created dust easily (during milling 10–50% of the material was lost). Thus, dust masks were required for operators and the powder was a potential health hazard. Fractions of 1 mm did not show such properties.

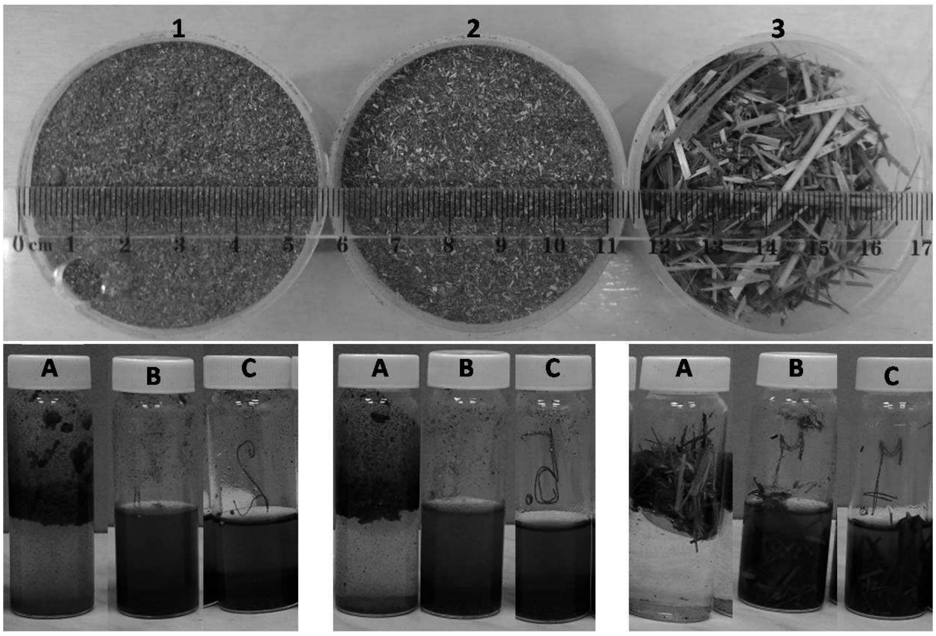

Figure 1. Hay biomass fractions after milling with 0.25 mm (1), 1 mm (2) and 10 mm (3) sieves (upper picture) and subsequent biomass sample after wetting (A), after 5 min of boiling (B) and after 24 h of hydrolysis (C).

After wetting (addition of 3% w/v buffer), 10 mm samples did not show any colouration of the buffer (Fig. 1., 3A). Slight coloration was observed only after boiling. However, it did not reach the level of samples milled with 0.25 and 1 mm sieves, which showed good wetting properties and distinct coloration after adding buffer. They also adopted a more brownish colour after boiling that could be explained with the more extensive formation of water soluble lignocellulose substances.

Analyses of fermentable sugar concentration during the production process did not show any significant differences ($p > 0.05$) between the hay samples milled with different sieves (Fig. 2). About 20 mg g^{-1} sugar was released already after milling, (including in the samples with 10 mm fractions), an additional 24–29% were released after boiling, indicating that boiling, which was initially used only for the neutralisation of indigenous microflora (Mezule et al., 2012), also supports the degradation of complex structures and sugar release. A slight variation in hay-sample sugar content was observed only after 48 h of hydrolysis when the sugar content in 0.25 mm size samples was 8–11% higher than in 1 and 10 mm samples ($p > 0.05$). Since longer hydrolysis time did not produce a significant increase in sugar concentration ($p > 0.05$), only 24 h hydrolysis data were used for further evaluation (low economic benefit in prolonged incubation).

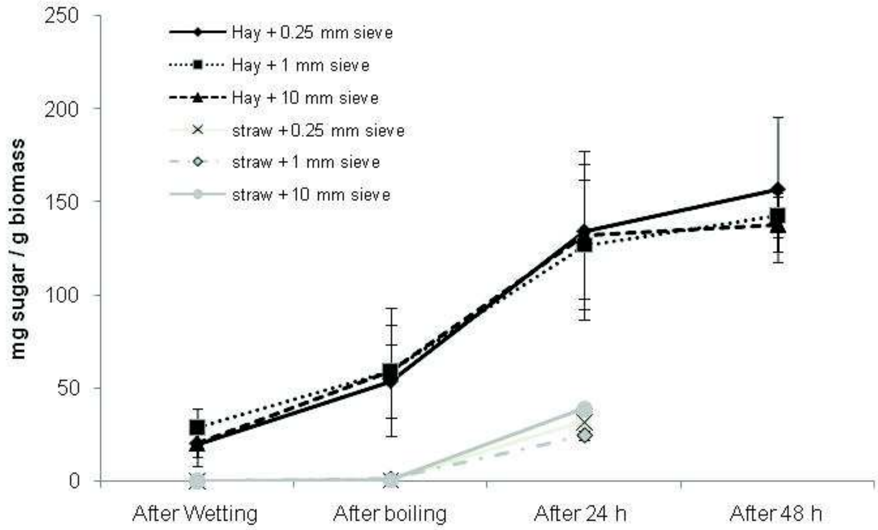

Figure 2. The amount of fermentable sugars produced from hay and barley straw biomass milled with 0.25, 1 and 10 mm sieves after wetting, boiling, 24 and 48 h of hydrolysis. Each standard deviation represents an average from at least 3 individual sample measurements.

Higher sugar yields were obtained from hay compared to barley straw. The amount extracted from barley straw after 24 h was 70% lower than from hay and was statistically different ($p < 0.05$) for the various sizes (Fig. 2). Nevertheless, these differences were attributed to deviations from the 24–39 mg g^{-1} sugar content and did not correlate with biomass size, and this contradicts with other studies on the processing of straw (Pedersen & Meyer, 2009). Furthermore, no or minor release of sugars was observed after sample wetting and boiling. The low release of sugars could be explained not only with the high crystallinity and low hydrolysability of barley straw (Vandenbossche et al., 2014) but also with the higher amount of *p*-hydroxycinnamates (abundant in monocots—barley straw), which have been shown to have a negative correlation with enzymatic digestibility if no chemical pre-treatment is applied (Li et al., 2012). Thus, barley straw might be inappropriate for biofuel production with the pre-treatment/hydrolysis method used in this study.

Analyses of energy requirements for the production of fermentable sugars showed that generally much more energy is required to process straw (Table 1) compared to hay. To produce 1 kg of sugar from hay, milling required 1.8–10.7 MJ depending on biomass size, however, for barley straw it required 18.5–76.2 MJ, thus further supporting the observations on the inapplicability of this resource for energy production.

Table 1. The amount of energy consumed and fermentable sugar produced per kg of hay or barley straw biomass (24 h of hydrolysis)

Sample	Sieve size, mm	Energy consumed, MJ kg^{-1}	Sugar produced, kg kg^{-1}
Hay	0.25	1.44	0.13
	1	0.49	0.13
	10	0.24	0.13
Barley straw	0.25	2.43	0.03
	1	0.99	0.02
	10	0.72	0.04

Research on mechanical pre-treatment generally involves the preparation of very fine particles (Barakat et al., 2014) and, subsequently, high consumption of energy (Da Silva et al., 2010). However, this research showed that hay fractions below 10 mm produce reasonable quantities of reducing sugars and there was no need to prepare powder-like fractions below the size of 0.25 mm, thus widening the variety of equipment that may be used for biomass pre-treatment. Energy consumption was not only influenced by particle size but also by biomass type. Seemingly similar biomass materials required significantly different ($p < 0.05$) amounts of energy, creating a problem when all-year, all-type biomass conversion technologies are planned. Similarly, pre-processing biomass to eliminate unproductive material would produce additional costs.

Nevertheless, the selected equipment still required a lower amount of energy compared to theoretical or reported values (Da Silva et al., 2010), allowing the application of even harder materials, e.g., hardwood, in biofuel production (Cadoche & López, 1989).

CONCLUSIONS

The results showed that particle size has no significant influence ($p > 0.05$) on sugar yields when fractions with the size of 0.25 mm, 1 mm and 10 mm are used. Nevertheless, the general observation that smaller biomass size generates more sugar proved to be correct in this study. Barley straw cannot be used as a biofuel substrate if mechanical pre-treatment is combined with enzymatic hydrolysis for sugar production because the potential yields were low and energy needed only for pre-treatment ranged from 18.5–76.2 MJ kg^{-1} of the sugar produced. At the same time it was possible to produce 1 kg of sugar from hay by using only 1.8 MJ for milling. In order to use barley straw as a renewable resource for energy production other pre-treatment/hydrolysis methods should be evaluated.

This study demonstrated that mechanical milling can be an effective, simple and sustainable approach, if the most appropriate treatment conditions, equipment and biomass type are used. However, the biomass type used must be thoroughly considered before production.

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