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Using steam explosion pretreatment method for bioethanol production from floodplain meadow hay

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Abstract. This article investigates influence of the steam explosion pretreatment method at different temperatures on sugar conversion rates and bioethanol production efficiencies from floodplain meadow hay. Floodplain meadow hay is used as a raw material, because these semi-natural grasslands need regular maintenance to preserve their high biodiversity. So far, this biomass has been largely unused, but it could provide a good feedstock for bioethanol production.
In this work, steam explosion pretreatment is used in combination with enzymatic hydrolysis. Effects of steam explosion pretreatment on the fibre content and cell wall structure are also studied. Results from fibre analysis show, that the floodplain meadow hay has very high lignin content of 24.16%, but relatively low cellulose content of 27.19%. Highest cellulose to glucose conversion rate of 234.6 g kg^{-1} and ethanol yield of 115.7 g kg^{-1} of biomass were achieved with the steam explosion pretreatment at 200°C. Scanning electron microscope (SEM) images show that pretreatment at 150°C does very little damage to plant cells, while steam explosion at 200°C disintegrates most of the plant cell walls and exposes cellulose fibres.

Key words: floodplain meadow hay, glucose, bioethanol, steam explosion.

INTRODUCTION

Biofuels are considered one of the most sustainable options in the foreseeable future for replacing fossil fuels in transportation sector (Nigam & Singh, 2011). The most widely produced biofuel in the world is bioethanol (Global Renewable Fuels Alliance, 2011). Most of the bioethanol is produced from corn or sugar cane, but the share of cellulosic bioethanol is rapidly increasing. The advantage of the cellulosic bioethanol, compared to traditional grain/sugar ethanol, is the fact that it is possible to use entire above-ground biomass of a plant for bioethanol production, thus enabling better efficiency and land use. Downside of the cellulosic bioethanol production is the need for large investments and sophisticated processing equipment (Stevens et al., 2004). In the future, the production of bioethanol is expected to include both, traditional grain/sugar crops and lignocellulosic materials (Demirbas, 2011).

Lignocellulosic raw materials represent the most abundant global resource for production of liquid biofuels (Lin & Tanaka, 2006; Talebnia et al., 2010). Since demand for biofuels has been increasing together with demand for food, a lot of attention has been recently directed to the utilization of biomass from grasslands (McKendry, 2002; Heinsoo et al., 2010).

In Estonia, there is nearly 20,000 hectares of floodplain meadows with high biodiversity, that need regular maintenance (Kukk & Sammul, 2006). If these meadows are not maintained, they will quickly overgrow and lose much of their biodiversity. Floodplain meadows produce every year over 100,000 tons of biomass, which until now has found little use. In order to promote the management of semi-natural meadows, alternative uses for the biomass are required without changing the traditional management principals like harvesting time, avoidance of fertilizers and use of heavy equipment (Heinsoo et al., 2010). One alternative possibility is to use natural meadow hay as feedstock for bioethanol production and utilize it as a substitute for petrol. This would also help to promote rural development, reduce greenhouse gases and decrease the dependence from energy import (Demirbas, 2005).

Several different pretreatment methods for lignocellulosic biomass have been studied in the past. Among those, steam explosion and dilute acid pretreatment are the most widely used. In steam explosion pretreatment, lignocellulosic biomass is heated at elevated temperatures of 150–250°C with high pressure steam. After a few minutes of incubation time, the heated biomass is subjected to explosive decompression thereby, physically and chemically modifying the biomass (Cantarella et al., 2004). When biomass is exposed to high temperatures: hemicellulose is degraded, part of lignin is solubilized and cellulose binding is reduced. Under instantaneous decompression, superheated water flashes into steam and steam volume expands explosively. The impact force generated by flashing and volume expansion destroys cell structure. This tears materials into small pieces, cellulose fibre-bundles are separated from one another and their structures loosened thereby, re-distributing lignin and fully exposing cellulose (Chen & Zhang, 2012).

Dilute acid pretreatment uses cheap chemicals, mild operating conditions and is simple to perform. Downside of the dilute acid pretreatment method is a low conversion rate and formation of by-products that are inhibitory for the following fermentation process (Tutt et al., 2012; Tutt et al., 2013). Furthermore, most of the lignin remains intact. In the pretreatment with dilute acid, 0.5–1.5% sulphuric acid solution is added to the biomass to hydrolyse hemicellulose during 5–60 minutes at 130–200ºC. Higher temperatures require shorter time of pretreatment (Yang et al., 2009; Kim et al., 2011).

Aim of this research was to investigate bioethanol production from floodplain meadow hay and to compare at different conditions the influence of steam explosion pretreatment to the glucose and ethanol yield. Results from steam explosion pretreatment were also compared to the results of dilute acid pretreatment of floodplain meadow hay

MATERIALS AND METHODS

Biomass

Meadow hay samples were harvested in July, 2012, from the floodplains of Emajõgi. Samples were milled to a particle size of 1–3 mm and stored at a room temperature. Dry matter content of samples was 90.4%.

Pretreatment

Steam explosion pretreatment was used in this work. Sample size was 900 g of pre-dried and milled hay, which was soaked in 900 g of distilled water. Pretreatment was performed in a laboratory scale steam explosion system, seen in Fig. 1, at the University of Applied Sciences Upper–Austria. Steam explosion was carried out at temperatures T = 150–200°C and incubation times of 10–30 minutes. Pretreated material was then dried at temperature T = 40°C to a dry matter content of 95%.

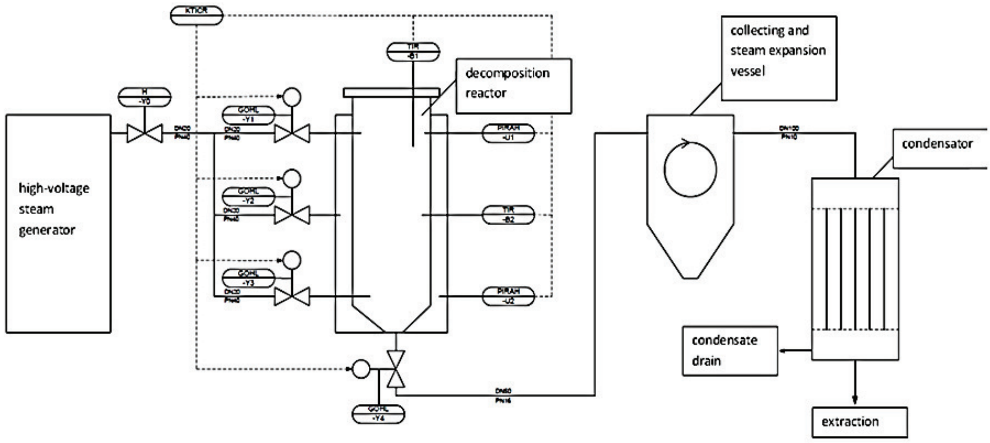

Figure 1. Laboratory scale steam explosion unit (Eisenhuber et al., 2013).

Pretreatment with dilute acid, followed by enzymatic hydrolysis, was used for the comparison tests. Size of the samples was 100 g of dried (DM 90.4%) and milled meadow hay to which 1,000 mL of 1% sulphuric 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 (Tutt et al., 2012; Tutt et al., 2013).

Hydrolysis and fermentation

Pretreatment phase was followed by enzymatic hydrolysis with enzyme complex Accellerase 1500. Enzyme mixture was added to the sample at a ratio of 0.3 mL per g of biomass. Hydrolysis of the pretreated material was carried out at 10% dry matter content in citrate buffer, c = 50 mmol L^{-1} and at pH = 5 (adjusted with NaOH). The samples were incubated for 72 h at 50°C in a shaking incubator at rotational speed of 2.5 s^{-1}.

Fermentation of glucose into ethanol was executed using yeast *Saccharomyces cerevisiae* in 1 litre bottles that were sealed with fermentation tubes. Volume of fermentation medium was 500 mL. Fermentation medium had a pH = 4.6 which was adjusted with H_2SO_4. Fermentation medium contained 100 mL hydrolysate, 2 mL $CaCl_2 \cdot 2H_2O$, 2 mL KH_2PO_4, 2 mL $MgSO_4 \cdot 7H_2O$, 0.44 g $(NH_4)_2HPO_4$. Yeast suspension (2 mL) was added to the solution and fermentation was carried out at 30°C for 120 hours.

Analysis

Dry matter content was analysed with a moisture analyser Ohaus MB 45. The fibre analysis (cellulose, hemicellulose and lignin) was performed according to the methods of Association of Official Analytical Chemists (AOAC 973.18) and methods by Tecator company (fibre determination using Tecator, Part No. 1000 1217, Serial No. 1706, U = 200 - 240 V, f = 50/60 Hz, P = 1,000 W). Fibre analysis results were also checked with acid hydrolysis. Acid hydrolysis was done according to the methods of National Renewable Energy Laboratory of USA for determination of structural carbohydrates and lignin in biomass (NREL, 2012).

Saccharides, organic acids, ethanol and furans in sample solutions were measured by high performance liquid chromatography (HPLC). The HPLC system Agilent Technologies 1200 Series with a Varian Metacarb 87 H column (300·7.8 mm) at 65°C, H_2SO_4 ($c = 5$ mmol L^{-1}) eluent and an isocratic flow rate of 0.8 mL min^{-1} was used. The signals were acquired with a refractive index (RI) and a UV–detector at 210 nm wavelength.

In order to compare the morphological structure of untreated and steam exploded raw material, scanning electron microscope (SEM) images were taken with a scanning electron microscope VEGA 2 LMU from Tescan.

Averaged results are used in figures and standard deviations are shown by vertical lines. Data was processed with programs Microsoft Excel and GraphPad Prism 5.

RESULTS AND DISCUSSION

Bioethanol production from natural meadow hay using steam explosion pretreatment was studied in this work. Glucose and ethanol results were compared with those from dilute acid pretreatment method that had been used previously for meadow hay pretreatment.

Table 1. Cellulose, hemicellulose, lignin and dry matter content of meadow hay samples (HS150– steam exploded hay at 150°C)

	Cellulose (%)	STD_2 (%)	Hemicellulo se (%)	STD_2 (%)	Lignin (%)	STD_2 (%)	Dry matter (%)
Hay (untreated)	27.19	0.69	29.15	0.48	24.16	0.29	90.43
HS150	32.66	0.23	26.51	0.15	26.49	0.31	93.46
HS170	33.42	1.11	25.98	0.38	28.82	0.12	93.96
HS180	33.87	0.36	24.63	1.02	29.05	0.09	95.20
HS200	35.07	0.36	15.27	0.48	33.71	0.17	95.83

Results from fibre analysis show that natural meadow hay, which was cut in the middle of July, has fully matured and therefore, has very high lignin content of 24.16%, but relatively low cellulose content of 27.19% in the untreated sample, see Table 1. Meadow hay also has relatively high hemicellulose content of 29.15%, which makes it difficult for enzyme molecules to reach cellulose fibres and degrade these into glucose without using adequate pretreatment conditions. High content of lignin in plant fibres leads to a creation of protective barrier that prevents plant cell destruction by fungi and bacteria. For the conversion of biomass to biofuel, the cellulose and hemicellulose must be broken down into their corresponding monomers (Kumar et al., 2009).

Steam explosion pretreatment disrupts the structure of plant cell walls and removes hemicellulose, but it dissolves only a fraction of lignin. Approximately 95% of lignin remains in an insoluble form. Since no chemicals are used in the pretreatment phase, the steam explosion method requires high temperatures to effectively dissolve hemicellulose, as seen in Fig. 2. Effectiveness of steam explosion increases rapidly at temperatures over 180°C. Steam explosion at 200°C lowers hemicellulose content of meadow hay sample to 15.27%, compared to the 29.15% in the untreated sample.

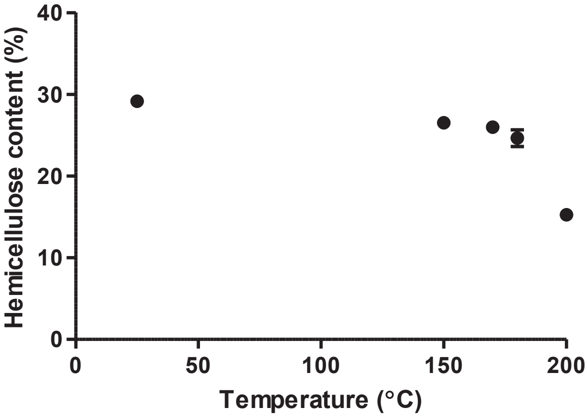

Figure 2. The correlation of hemicellulose content in meadow hay samples, from steam explosion pretreatment temperatures.

Scanning electron microscope images also confirm that steam explosion at 200°C is very effective in disrupting the plant cell wall structure, while temperatures under 180°C clearly seem to be inadequate, see Fig. 3. SEM images show that steam explosion at 150°C has done very little damage to cell walls compared to the untreated sample, while steam explosion at 200°C has destroyed most of the cell walls and exposed cellulose fibres.

Hydrolysis results show that the highest cellulose to glucose conversion rate of 234.6 g kg^{-1} of biomass was achieved with the steam explosion pretreatment at 200°C (results shown in Fig. 4). This shows that steam explosion at 200°C removes most of the hemicellulose from the sample and leaves the cellulose fibres easily accessible for enzymes. By far the lowest glucose yield of 83.8 g kg^{-1} was achieved by steam explosion pretreatment at 150°C. Although the pretreatment temperature was only 50 degrees lower, the glucose yield was 2.8 times smaller than the results achieved with pretreatment at 200°C temperatures. This shows that steam explosion conditions at 150°C are not effective enough to remove hemicellulose from the samples.

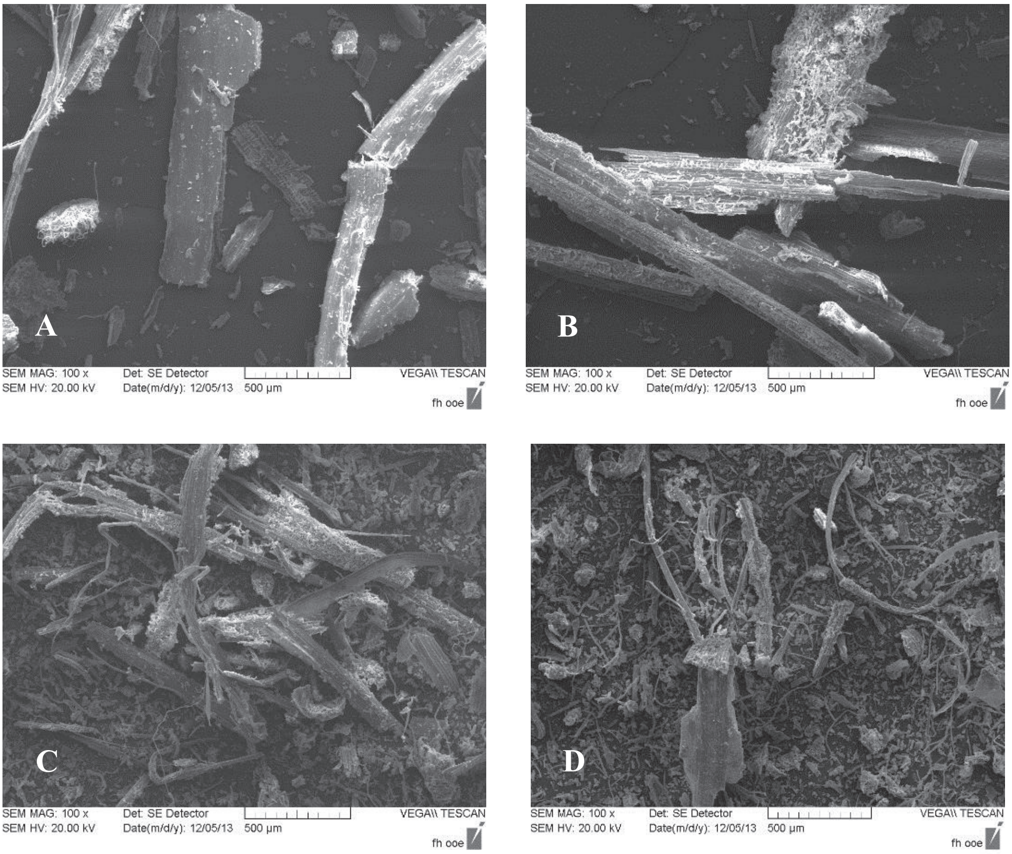

Figure 3. Comparison of scanning electron microscope (SEM) images of meadow hay samples – untreated (A) and steam exploded at different temperatures (B – 150°C, C – 170°C, and D – 200°C).

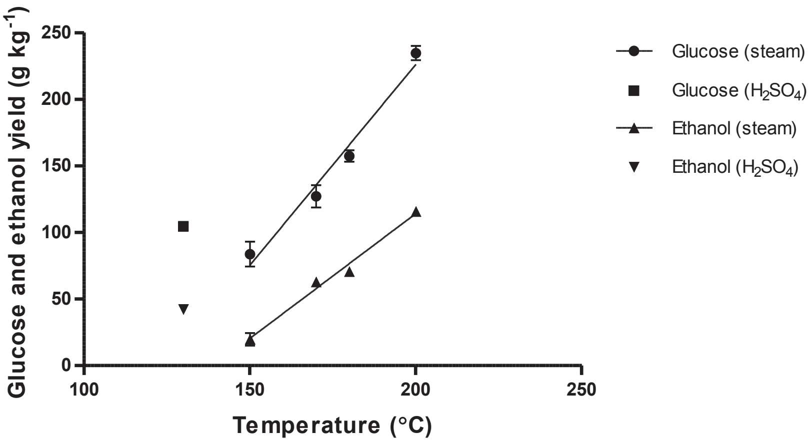

Figure 4. Influence of different pretreatment conditions on the glucose and ethanol yields from floodplain meadow hay samples.

The same correlation was also seen between steam explosion temperature and ethanol yield. The highest ethanol yield of 115.7 g kg^{-1} was achieved by pretreatment at 200°C and the lowest ethanol yield of 19.8 g kg^{-1} was achieved by pretreatment at 150°C.

Table 2. Hydrolysis and fermentation efficiencies at different pretreatment conditions (HS- steam explosion pretreatment at temperatures 150–200°C; HD- dilute acid pretreatment at 130°C)

Pretreatment method	Glucose yield g kg^{-1}	Ethanol yield g kg^{-1}	Hydrolysis efficiency %	Fermentation efficiency %
HS150	83.8	19.8	30.8	46.2
HS170	127.1	62.8	46.7	96.9
HS180	157.4	70.7	57.9	88.1
HS200	234.6	115.7	86.3	96.7
HD130	115.2	41.1	42.4	69.8

The floodplain meadow hay pretreated with dilute acid gave glucose and ethanol yields of 115.2 g kg^{-1} and 41.1 g kg^{-1}, respectively. This shows that pretreatment of meadow hay with dilute acid is more effective than steam explosion at 150°C, but less effective than steam explosion pretreatment at temperatures over 170°C. Hydrolysis efficiencies at different pretreatment conditions are given in Table 2. The highest hydrolysis efficiency of 86.3% and one of the highest fermentation efficiencies of 96.7% was achieved by steam explosion at 200°C.

CONCLUSIONS

Results from fibre analysis show, that natural meadow hay has very high lignin content of 24.16%, but relatively low cellulose content of 27.19%. This means that it is difficult for enzyme molecules to reach cellulose fibres and degrade these into glucose without using high temperature pretreatment conditions.

Highest cellulose to glucose conversion rate of 234.6 g kg^{-1} and ethanol yield of 115.7 g kg^{-1} of biomass were achieved with the steam explosion pretreatment at 200°C. The lowest glucose yield of 83.6 g kg^{-1} and ethanol yield of 19.8 g kg^{-1} were given by samples pretreated with steam explosion at 150°C. These results were confirmed by scanning electron microscope images which show that pretreatment at 150°C does very little damage to plant cell walls. Fully matured meadow hay is quite durable to steam explosion pretreatment thus, higher temperatures and harsher conditions, preferably 200°C have to be used.

Although floodplain meadow hay has relatively low cellulose content and high lignin content, it is suitable raw material for bioethanol production. Floodplain meadows produce over 100,000 tons of biomass per year and if steam explosion pretreatment at 200°C, followed by enzymatic hydrolysis, is used, then it would be possible to produce approximately 11,570 tons of bioethanol from this biomass.

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