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Explosive decompression pretreatment: nitrogen vs. compressed air

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Abstract: Lignocellulosic material is the most promising feedstock for the bioethanol production however, due to complicated physico-chemical characteristics of biomasses, it is necessary to pretreat the biomass before the bioethanol production. The goal of the pretreatment is to open the biomass structure for enzymatic hydrolysis to gain higher sugar and ethanol yields in further processes. In this paper a novel explosive decompression pretreatment is studied where two gases – nitrogen and compressed air are utilized for pressure generation. For this, traditional three-step bioethanol production process was used, where explosive decompression pretreatment with N_2 gas or compressed air was applied for biomass pretreatment. Glucose and ethanol concentrations were measured during the process. Glucose and ethanol yields and process efficiencies were used to evaluate the effect of explosive decompression pretreatment and its suitability for biomass pretreatment in bioethanol production process. Results show that the highest glucose yield was gained when nitrogen gas was used, while difference in glucose yield compared to that of autohydrolysis was negligible when compressed air was applied.

Key words: lignocellulose, explosive decompression, bioethanol, pretreatment.

INTRODUCTION

Lignocellulosic material is widely used source of biomass for energy production. This kind of biomass is usually burned directly for heat and energy production or converted to other types of fuel. In addition, due to the depletion of conventional fossil fuels and EU 2020 targets (Commission) the conversion of biomass to liquid fuels, like ethanol, has attracted particular attention. Lignocellulosic biomass is promising feedstock for the ethanol production considering its great availability, low cost, and sustainable supply (Agbor et al., 2011; Min et al., 2013; Phitsuwan et al., 2013). Plant biomass is primarily composed of plant cell walls of which about 75% are polysaccharides (Phitsuwan et al., 2013) that could be used for ethanol production. Therefore, it has become a major focus of intensive research and development (Agbor et al., 2011; Tutt et al., 2013; Raud et al., 2014; Tutt et al., 2014).

The bioethanol production includes three step process – pretreatment of biomass, hydrolysis of cellulose to sugars and fermentation of sugars to ethanol (Tutt et al., 2012; Raud et al., 2015). The conversion of cellulose to ethanol is difficult since the cellulose fibers in the biomass are tightly packed with hemicellulose and lignin cover. The pretreatment is necessary in order to break down the biomass structure to gain access to the sugars from cellulose and hemicellulose. The goal of the pretreatment is to improve the further conversion steps (Conde-Mejía et al., 2012) and therefore, various pretreatment methods have been proposed to enhance bioethanol production process (Alvira et al., 2010; Demirbas, 2011; Chiaramonti et al., 2012).

The pretreatment methods can be divided into biological, chemical, – physical, and combined chemical-physical methods. However, since various types of biomasses have different characteristics, there is no ideal pretreatment method, but the suitable pretreatment method must be adopted to each specific lignocellulosic biomass (Alvira et al., 2010). The biological pretreatment methods apply various types of fungi to degrade hemicellulose and lignin however, the method is ineffective due to low hydrolysis rate (Alvira et al., 2010). The physical methods on the other hand are mainly targeted to increasing the surface area and reduction of particle size of biomass and they are used usually in combination with other pretreatment methods (Haghighi Mood et al., 2013). Chemical pretreatment methods use chemicals such as acids, alkali, organic solvents, and ionic liquids, which have a significant effect on the native structure of biomass (Agbor et al., 2011; Tutt et al., 2012). However, the use of chemicals makes the production process too expensive due to the high cost of chemicals and the production of toxic materials during the process (Shirkavand et al., 2016).

More effective methods of enhancing the biomass digestibility are combinational pretreatments, where physical parameters like temperature and pressure are combined with other pretreatment methods. Amongst them steam-explosion, ammonia fibre explosion (AFEX), CO_2 or SO_2 explosion are the most used methods (Agbor et al., 2011). These methods include pretreatment of biomass with pressurized steam, ammonia, CO_2 or SO_2, respectively, which is followed by rapid decompression to an atmospheric pressure (Tutt et al., 2014). The decompression causes destruction of lignin and hemicellulose, which enables enzymes to gain better access to cellulose in further treatment. Additionally, the CO_2 forms carbonic acid when dissolved in water, which increases the hydrolysis rate (Shirkavand et al., 2016). However, the disadvantage of these methods is the formation of inhibitory compounds and they are more effective on low-lignin content biomass (Chiaramonti et al., 2012).

In this paper a novel combinational biomass pretreatment method – explosive decompression pretreatment is evaluated. In case of explosive pretreatment, the biomass is mixed with water and additionally N_2 gas is added to the reactor to elevate the pressure (Raud et al., 2016). As an economical alternative to N_2 gas, the compressed air could be used since air consists in approximately 78% of nitrogen. Nitrogen molecules are smaller than water molecules and gas molecules used in other pretreatment methods (like CO_2 and SO_2) and under high pressure and temperature can more effectively penetrate the cell walls of biomass. When the pressure is released in an explosive manner, the dissolved nitrogen gas expands and thereby, opens the biomass structure and increases its surface area for following enzymatic hydrolysis. The explosive decompression pretreatment method is economically attractive since no catalysts or chemicals are added in these processes, which makes the pretreatment process cheaper.

The purpose of this work was to assess the effect of explosive decompression pretreatment method using nitrogen and, as an alternative, compressed air. Experiments were conducted using several different temperature and pressure combinations. Resulting pretreated biomass was used in the standard three-step lignocellulosic bioethanol production process. Glucose and ethanol concentrations of the samples were measured after hydrolysis and fermentation processes, respectively. Results were analysed in order to find the most suitable pretreatment conditions and to study the efficiency of the pretreatment.

MATERIALS AND METHODS

Biomass

Barley straw was used as a biomass in all experiments. The biomass was grown in Tartu area (Estonia), on loamy soil and harvested in August. The samples were dried to a moisture content less than 10% and ground with Cutting Mill ZM 200 (Retsch GmbH) to a particle-size 1 mm or less.

Pretreatment

The explosive decompression pretreatment method was used to break the cell structure of the biomass and expose the cellulose to further enzymatic treatment. The experimental setup with the pressure reactor and heater is presented in Fig. 1. 100 g of dry biomass was weighed, placed into a pressure vessel and mixed with distilled water until watery biomass paste with a volume of 1 litre was gained. The reactor was closed and pressurised with compressed air or nitrogen gas to a pressure of 1 to 60 bars. The pressurized samples were then heated in the reactor vessel with ceramic heater to temperatures of 25–175 °C. The temperature in the pressure vessel was measured with a thermocouple and controlled using electronic controller unit. When the intended temperature was reached, the reactor was cooled down to at least 80 °C, if necessary, and pressure was released through a valve in an explosive manner. The biomass was kept in the reactor for 3 hours from the start of the heating until the explosive decompression. After the pre-treatment the samples were cooled to a temperature below 50 °C for enzymatic hydrolysis.

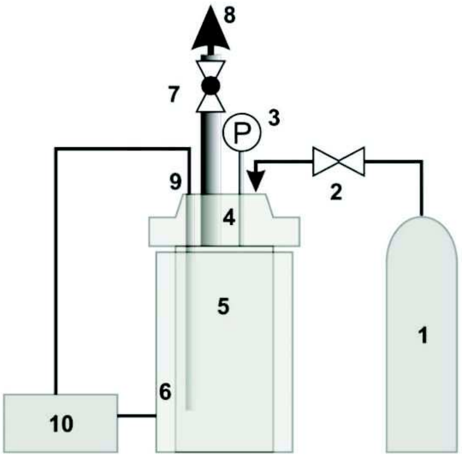

Figure 1. Schematic of the explosive decompression pretreatment system: 1 – nitrogen tank; 2 – pressure control valve; 3 – manometer; 4 – modified reactor vessel cap; 5 – reactor vessel; 6 – ceramic contact heater; 7 – pressure release valve; 8 – into ventilation system; 9 – thermocouple; 10 – controller unit.

Hydrolysis

Enzymatic hydrolysis with enzyme complex Accellerase 1500 was used to convert cellulose in the biomass to glucose. Enzyme mixture was added to samples pretreatment at a ratio of 0.3 ml per g of biomass and the flask was filled with distilled water to 1,000 ml volume. Hydrolysis lasted 24 hours at a temperature of 50 °C under constant stirring in rotating shaker/incubator (Unimax 1010, Heidolph Instruments GmbH & Co.KG). After the hydrolysis, the glucose concentration in all the samples was measured.

Fermentation

Dry yeast *Saccharomyces cerevisiae* in ratio of 25 g of yeast per kg of biomass was added to all samples to start the fermentation process. Fermentation process was carried out at room temperature under low oxygen conditions in 1,000 mL glass bottles, sealed with a fermentation tube. Fermentation lasted for 7 days after which, the ethanol concentration in the mixture was measured.

Chemical analysis

Dry matter content was analyzed with a moisture analyzer Ohaus MB 45. The percentage of lignin, Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) in the dry mass (DM) of the biomass samples was determined at the Plant Biochemistry Laboratory of Estonian University of Life Sciences (Tecator ASN 3430) (AOAC, 1990; Van Soest et al., 1991). The glucose and ethanol concentrations in the mixture were determined using Analox GL6 analyzer (Analox instruments Ltd.).

Averaged results of at least three parallel measurements are used in figures and corresponding standard deviations are shown by vertical lines.

RESULTS AND DISCUSSION

Biomass analysis

Barley straw was used as a sample biomass to investigate the effect of the explosive decompression pretreatment method on the bioethanol production process. A biomass can be characterized on the basis of its relative proportion of cellulose, hemicellulose, and lignin (Table 1). Previous research has shown that energy crops for ethanol production should be selected based on their cellulose content since ethanol yield per kg of biomass was directly proportional to the cellulose content in the energy crop (Kikas et al., 2016). As seen in Table 1, the barley straw used in these experiments as a sample biomass contained 45.73% of cellulose. Relatively high cellulose content makes it a suitable biomass for bioethanol production.

Table 1. Results of the biomass analysis (n = 3)

Component	Content (%) [1]
Hemicellulose	32.61 ± 0.53
Cellulose	45.73 ± 0.21
Lignin	5.25 ± 0.00
Ash	3.86 ± 0.06
Dry matter	n.a.

[1] Determined at the Plant Biochemistry Laboratory of Estonian University of Life Sciences.

Hydrolysis of biomass

The Fig. 2 illustrates the change in glucose yield at different temperatures when N_2 gas or compressed air was used to increase the pretreatment pressure. The glucose yield was the lowest when no additional pressure was used in pretreatment and only increase in temperature affected the biomass structure. Approximately the same glucose yields were gained when the compressed air was used to increase pressure in the pressure reactor. Using compressed air for pretreatment, a 68% increase in glucose yield can be seen in Fig. 2 when the temperature was increased from 100 °C to 150 °C. The increase in glucose yield was gained due to the autohydrolysis of biomass caused by the elevated temperature. The autohydrolysis leads to the hydrolysis of hemicelluloses and the lignin disruption, resulting in increase in cellulose reactivity with cellulose in the following enzymatic hydrolysis process (Samuel et al., 2013). The explosive pretreatment with compressed air had little to no effect on the glucose yield, which was approximately the same as with autohydrolysis.

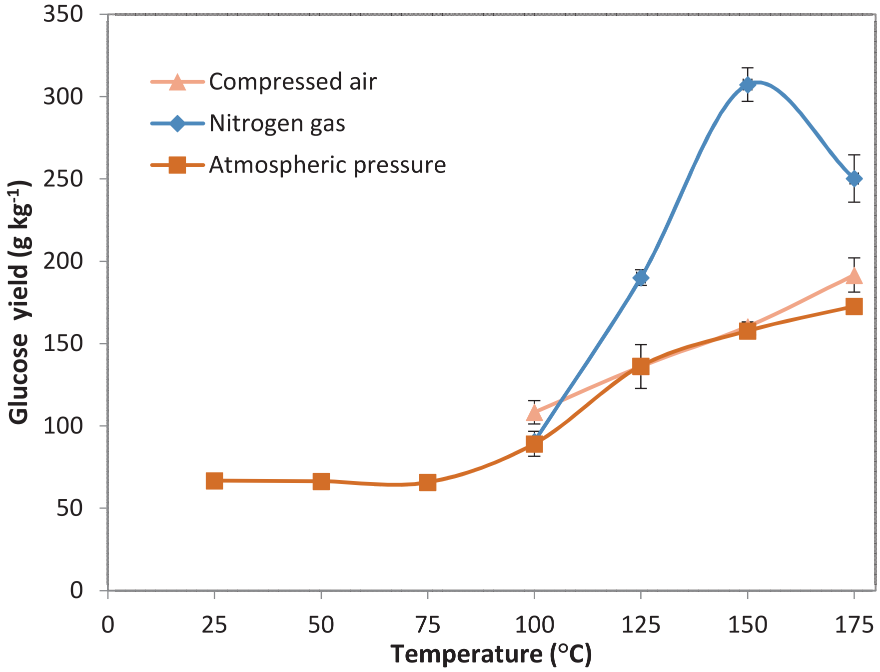

Figure 2. Comparison of glucose yields gained at pressure of 30 bars with different pretreatment methods when the pretreatment temperature was gradually increased.

However, in case of explosive decompression with N_2 gas, a sharp – 218% increase in glucose yield was noted when the pretreatment temperature was increased from 100 °C to 150 °C. At 150 °C explosive decompression with N_2 gas enabled to gain 95% higher glucose yield than when using explosive decompression with compressed air or autohydrolysis at higher temperatures.

During the pretreatment the small N_2 molecules penetrate more efficiently into the biomass fibres and cells than gases found in compressed air. When the pressure is suddenly decreased, the dissolved gas expands and opens the cellulosic structure of biomass and thereby, increases the accessible surface area of biomass for further enzymatic hydrolysis. Although the pressurized air contains 78% of N_2, it does not effectively penetrate into the biomass cells and therefore, it is not as effective for use in explosive pretreatment of biomass as nitrogen gas.

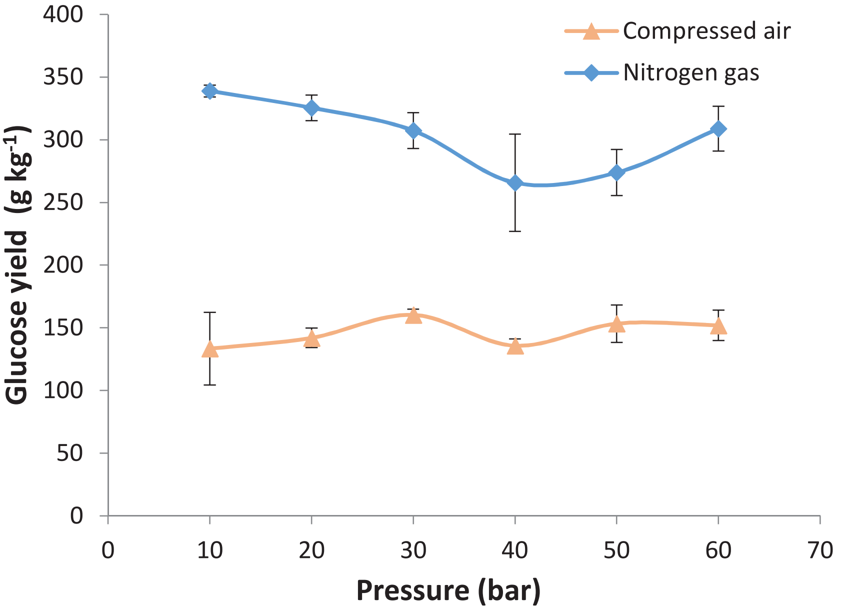

Figure 3. Comparison of glucose yields gained with different pretreatment methods at 150 °C when the pretreatment pressure was gradually increased.

Fig. 3 illustrates the effect of pretreatment pressure to the glucose yield of biomass at 150 °C. As can be seen, the pretreatment pressure has little effect on the glucose yield of biomass. When compressed air was used to generate pressure, the glucose yield changed only to a small degree. On the other hand, pretreatment with nitrogen gas resulted in higher results but the glucose yield had a U-shaped dependence where the minimum glucose yield was gained at the pressure of 40 to 50 bars. In addition, depending on the temperature, the pretreatment with N_2 gas enabled to gain 90–150% higher glucose yield than when compressed air was used for pretreatment. This shows that even though the presence of pressure and its explosive release has effect on the biomass, the extent of pressure has negligible effect on the pretreatment process. However, choosing the suitable gas for pressure elevation and temperature combination enables to gain considerably higher glucose yields.

Fermentation

After hydrolysis, the fermentation with yeast *Saccharomyces cerevisiae* was used to convert the glucose into ethanol. Compressed air or nitrogen gas were used to reach pressure of 30 bars inside the pressure reactor. Comparison experiments were conducted at near atmospheric pressure with no gas-added pressure. Based on gained results the ethanol yield was calculated and used to estimate the effect of different pretreatment conditions to ethanol production process (Fig. 4).

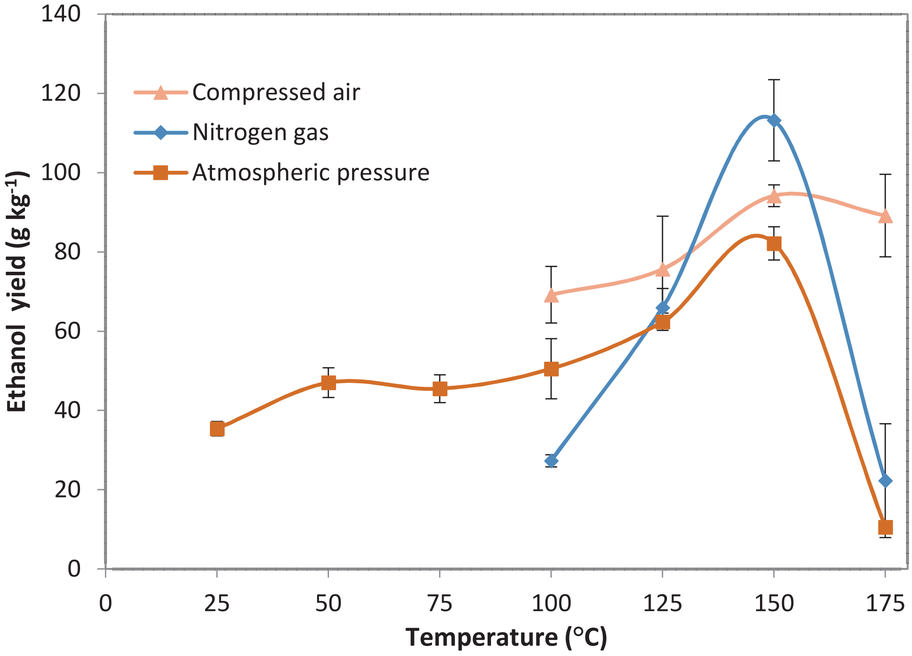

Figure 4. Comparison of ethanol yields gained with different pretreatment methods at 30 bars and at near atmospheric pressure when the pretreatment temperature was gradually increased.

The ethanol yields increased as the pretreatment temperature was increased. Lower ethanol yields were gained at temperatures below 125 °C and at 175 °C. The highest ethanol yields were gained with all pretreatment methods at 150 °C. At these conditions, 82 g ethanol per kg of biomass was gained when no pressure was applied during pretreatment. However, when compressed air was used to elevate the pressure to 30 bars during pretreatment a 14.2% higher ethanol yield was gained. Even higher ethanol yield – 113 g per kg of biomass was gained when explosive decompression pretreatment was used with nitrogen gas.

The increased ethanol yields gained with nitrogen gas as operative gas in pretreatment was expected since the glucose yields at these conditions were more than two times higher. On the other hand, while the glucose yields of pretreatment with compressed air as operative gas and near atmospheric pressure pretreatment were very similar, ethanol yields gained with compressed air pretreatment were considerably higher than those gained with autohydrolysis.

The ethanol yields, in case of nitrogen explosion pretreatment and autohydrolysis methods, decreased significantly when temperature above 150 °C was used while in case of compressed air explosion pretreatment the decrease was insignificant. The sharp decrease in ethanol yield can be attributed to formation of inhibiting compounds at higher pretreatment temperatures. The pretreatment temperatures above 150 °C probably result in formation of compounds that inhibit the fermentation process, which leads to lower ethanol yields. The explosive decompression pretreatment with decompressed air enabled to gain higher ethanol yield at 175° than with nitrogen gas of pretreating at atmospheric pressure at this temperature, however it still remained lower than pretreating with nitrogen gas at 150 °C. In addition, it is not reasonable to use too high temperatures due to high energy cost associated with this.

Furthermore, Fig. 5 shows that in case of autohydrolysis and compressed air explosion pretreatments the fermentation efficiency exceeds 100% at majority of pretreatment temperatures. This suggests that these pretreatments were not efficient enough in opening the biomass structure and thus, the enzymatic hydrolysis continued during the fermentation period. Extremely low fermentation efficiencies at temperatures above 150 °C confirm that at higher temperatures compounds are formed that inhibit the yeast.

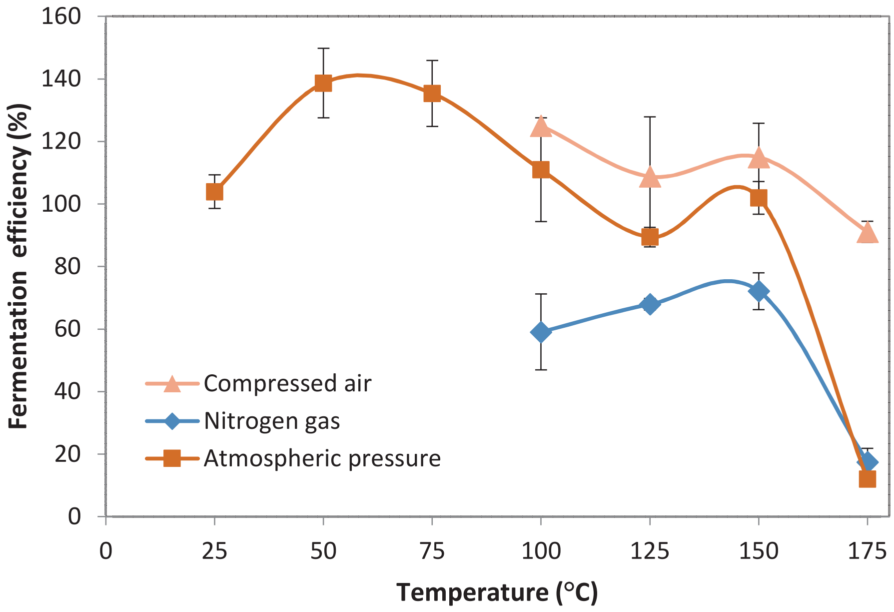

Figure 5. Comparison of fermentation efficiencies gained with different pretreatment methods at 30 bars or at atmospheric pressure when the pretreatment temperature was gradually increased.

CONCLUSIONS

In order to evaluate the effect of explosive decompression pretreatment method, two different gases were used as operative gas in explosion at different temperature and pressure combinations and the results were compared to those of autohydrolysis using barley hay as a sample biomass. Resulting pretreated biomass was used in the standard three step lignocellulosic bioethanol production process, where the glucose and ethanol concentrations of the samples were measured in order to quantify the effect of different pretreatment methods.

The results show that nitrogen is most suitable operative gas for explosive decompression pretreatment giving the highest glucose yield at 150 °C and at pressure of 30 bars. Although major component in compressed air is also nitrogen, considerably lower glucose yields were gained using explosive decompression pretreatment with compressed air as operative gas. The results with compressed air were almost the same as those gained in autohydrolysis pretreatment, where no added pressure was used in addition to elevated temperature. This shows that compressed air, although consisting for the most part of nitrogen, is not as effective in explosive decompression pretreatment as nitrogen. As the explosive decompression pretreatment with nitrogen as operative gas enabled to gain the highest glucose yields, the highest ethanol yields where gained with this pretreatment method in the next stage of the process as well.

REFERENCES

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A. & Levin, D.B. 2011. Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances* **29**, 675–685.

Alvira, P., Tomás-Pejó, E., Ballesteros, M. & Negro, M.J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology* **101**, 4851–4861.

AOAC (1990). 'Official methods of analysis of the Association of Official Analytical Chemists'.

Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P. & Cherchi, F. 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. *Biomass and Bioenergy* **46**, 25–35.

Commission, E. Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC.

Conde-Mejía, C., Jiménez-Gutiérrez, A. & El-Halwagi, M. 2012. A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. *Process Safety and Environmental Protection* **90**, 189–202.

Demirbas, A. 2011. Competitive liquid biofuels from biomass. *Applied Energy* **88**, 17–28.

Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jouzani, G., Najafi, G.H., Gholami, M. & Ardjmand, M. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews* **27**, 77–93.

Kikas, T., Tutt, M., Raud, M., Alaru, M., Lauk, R. & Olt, J. 2016. Basis of Energy Crop Selection for Biofuel Production: Cellulose vs. Lignin. *International Journal of Green Energy* **13**, 49–54.

Min, D., Yang, C., Shi, R., Jameel, H., Chiang, V. & Chang, H. 2013. The elucidation of the lignin structure effect on the cellulase-mediated saccharification by genetic engineering poplars (Populus nigra L. × Populus maximowiczii A.). *Biomass and Bioenergy* **58**, 52–57.

Phitsuwan, P., Sakka, K. & Ratanakhanokchai, K. 2013. Improvement of lignocellulosic biomass in planta: A review of feedstocks, biomass recalcitrance, and strategic manipulation of ideal plants designed for ethanol production and processability. *Biomass and Bioenergy* **58**, 390–405.

Raud, M., Kesperi, R., Oja, T., Olt, J. & Kikas, T. 2014. Utilization of urban waste in bioethanol production: Potential and technical solutions. *Agronomy Research* **12**, 397–406.

Raud, M., Olt, J. & Kikas, T. 2016. N2 explosive decompression pretreatment of biomass for lignocellulosic ethanol production. *Biomass and Bioenergy* **90**, 1–6.

Raud, M., Tutt, M., Olt, J. & Kikas, T. 2015. Effect of lignin content of lignocellulosic material on hydrolysis efficiency. *Agronomy Research* **13**, 405–412.

Samuel, R., Cao, S., Das, B.K., Hu, F., Pu, Y. & Ragauskas, A.J. 2013. Investigation of the fate of poplar lignin during autohydrolysis pretreatment to understand the biomass recalcitrance. *RSC Advances* **3**, 5305–5309.

Tutt, M., Kikas, T. & Olt, J. 2012. Influence of different pretreatment methods on bioethanol production from wheat straw. *Agronomy Research* **10**, 269–276.

Tutt, M., Kikas, T. & Olt, J. 2013. Influence of harvesting time on biochemical composition and glucose yield from hemp. *Agronomy Research* **11**, 215–220.

Tutt, M., Kikas, T., Kahr, H., Pointner, M., Kuttner, P. & Olt, J. 2014. Using steam explosion pretreatment method for bioethanol production from floodplain meadow hay. *Agronomy Research*, **12**(2), 417–424.

Shirkavand, E., Baroutian, S., Gapes, D.J. & Young, B.R. 2016. Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment – A review. *Renewable and Sustainable Energy Reviews* **54**, 217–234.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science* **74**, 3583–3597.