Agronomy Research Biosystem Engineering Special Issue 1, 269-276, 2012

Influence of different pretreatment methods on bioethanol production from wheat straw

M. Tutt, T. Kikas and J. Olt

Institute of Technology, Estonian University of Life Sciences, Kreutzwaldi 56, EE51014, Tartu, Estonia; e-mail: marti.tutt@emu.ee

Abstract. Article investigates the influence of different pretreatment methods on sugar conversion and bioethanol production. Different dilute acid and alkaline pretreatment methods are compared to determine the best pretreatment method to give the highest glucose and ethanol yields under the mild operating conditions. Wheat straw is used as a raw material as it is the most widely grown cereal in Europe. Dilute sulfuric acid, hydrochloric acid, nitric acid and potassium hydroxide solutions are used for pretreatment in combination with enzymatic hydrolysis. Results indicate that the highest cellulose-to-glucose conversion rate of 316.7 g kg^{-1} of biomass is achieved by the pretreatment with nitric acid. The lowest glucose concentration of 221.3 g kg^{-1} is achieved by hydrochloric acid. In the wheat straw samples pretreated with sulfuric acid and KOH, two different approaches are used. Solid phase of half the samples is rinsed with water before adding enzymes, and the rest of the samples are not. The rinsed samples pretreated with KOH solution give the highest ethanol yield of 104.3 g kg^{-1}, while the lowest ethanol yield is 67.7 g kg^{-1} from samples pretreated with HCl solution. Unrinsed samples and rinsed samples pretreated with sulfuric acid give an ethanol yield of 78.7 g kg^{-1} and 92.0 g kg^{-1}, respectively. These results indicate that rinsing the solid phase of the samples with distilled water before hydrolysis removes most of the inhibitory compounds formed during the pretreatment with dilute acid and increases fermentation efficiency by approximately 12%.

Key words: wheat straw, glucose, dilute acid pretreatment, cellulose

Introduction

Rising energy dependency on fossil fuels, increasing emissions of greenhouse gases and risks associated with the price fluctuations on the international energy markets has led to a move towards the research and production of alternative, renewable, efficient and cost-effective energy sources with lesser emissions (Dwivedi et al., 2009). Among many renewable energy alternatives for transportation fuels, four different energy sources are considered the most sustainable in the foreseeable future. These are biofuels, hydrogen, solar energy and syngas. At the moment, biofuels are considered the most favorable choice among these, because biofuels are renewable, biodegradable and cost-effective compared to using hydrogen or solar energy as transportation fuel (Nigam & Singh, 2011). Biofuels are classified as primary and secondary biofuels. The primary biofuels are natural and unprocessed biomass such as fuel-wood, wood chips and pellets. These are used by direct combustion for heating, cooking or power production. The secondary biofuels are produced by processing of biomass. For example, ethanol, biodiesel, methanol, etc. The secondary biofuels are further divided into first, second and third generation biofuels on the basis of raw material and technology used for their production (Larson, 2008). Second–generation liquid biofuels are produced from lignocellulosic biomass, such as agricultural residues, grass and wood. It involves biological or thermochemical processing of the material to break the lignin structure and disrupt the crystalline structure of cellulose. The most widely produced second generation biofuel in the world is cellulosic ethanol (http://www.globalrfa.org/pr_021111.php).

Liquid biofuels are being researched mainly to replace conventional liquid fuels, such as diesel and petrol. The advantage of the second generation biofuels is the fact that it does not compete directly with the food market. It is possible to use entire above-ground biomass of a plant, thus enabling better efficiency and land use. Downside of the second generation biofuel production is the need for large investments and sophisticated processing equipment, compared to first generation (Stevens et al., 2004). In the future, the production of ethanol is expected to include both, traditional grain/sugar crops and lignocellulosic materials (Demirbas, 2011). Production of ethanol from lignocellulosic raw material and utilizing it as a substitute for petrol could help promote rural development, reduce greenhouse gases, and achieve independence from outside energy providers (Demirbas, 2005).

From different available raw materials wheat straw was chosen because it is the most widely grown cereal in Europe. For example, in 2004 the annual wheat straw production in Europe was approximately 132 million tons. Only a small portion of wheat straw is used for animal feed and bedding or for industrial use, and although the industrial use has been growing in the recent years, most of the wheat straw is still left on the fields or disposed of as waste (Sarkar, et al., 2012). Promoting the use of wheat straw as a raw material for bioethanol production could help increase the cellulosic ethanol production in Europe and reduce the quantity of biomass that goes to waste.

Several different pretreatment methods for wheat straw have been studied in the past, but no method has yet emerged as being efficient, but also simple and cost effective. Methods using moderate pretreatment conditions are cost effective, but usually have low sugar and ethanol yields. Pretreatment methods using high temperatures and harsh conditions have much better sugar and ethanol conversion yields, but they need expensive chemicals and equipment, thus making them economically not viable (Kim, et al., 2011).

Aim of this research was to investigate how different pretreatment methods with moderate conditions differ in hydrolysis and fermentation efficiencies. The influence of rinsing the solid phase of wheat straw samples on the sugar and ethanol conversion yields was also investigated.

Materials and methods

Biomass

Wheat straw was chosen as a raw material in this work, because it is the most widely grown cereal in Europe and much of the wheat straw is going to waste. Wheat is also grown in Estonia, and since straw does not compete directly with the food market, it makes wheat straw a good choice for bioethanol production.

Wheat straw samples were harvested in August, 2011, from the experimental fields of Estonian University of Life Sciences. Ash, hemicellulose, cellulose and lignin contents of straw samples were determined in the Laboratory of Plant Biochemistry of Estonian University of Life Sciences (see table 1).Standard methods of Association of Official Analytical Chemists (AOAC 973.18) and methods by company of Tecator (fibre determination using the Fibertec M&I systems) were used in the analysis. Samples were milled to a particle size of 1–3 mm and dried to a moisture content of less than 10%.

Table 1. Ash, hemicellulose, cellulose and lignin contents in dry mass of wheat straw samples

Sample	Ash %	Hemicellulose %	Cellulose %	Lignin %
Wheat straw	3.57	31.01	46.47	7.94

Pretreatment of a biomass

Cellulosic ethanol production is a complex process compared to first generation grain or sugarcane ethanol production. As the first step, it is necessary to break the lignin seal and hemicellulose sheathing over cellulose, and disrupt the crystalline structure of cellulose. Only then it is possible to degrade the cellulose in the biomass to sugar monomers. This disruption is achieved by the pretreatment process which is usually followed by enzymatic hydrolysis (Dwivedi et al., 2009; Kim et al., 2011).

Pretreatment with dilute acid

Pretreatment with dilute acid has been the most widely used method for pretreatment of the lignocellulosic material. This method 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 byproducts that are inhibitory for the following fermentation process. In the pretreatment with dilute acid, 0.5–1.5% sulfuric 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; Dien et al., 2006). Besides sulfuric acid, nitric acid also has shown good results in cellulose-to-sugars conversion yields, but nitric acids higher price makes it less cost effective.

Pretreatment with alkali

Pretreatment with alkali removes lignin and part of the hemicellulose, thus increasing the accessibility of enzymes to cellulose in later phases of hydrolysis. All of the cellulose and most of the hemicellulose is left in an insoluble polymeric form. This process uses alkali such as NaOH, KOH and $Ca(OH)_2$ and temperatures of 120–180°C. Pretreatment with alkali has been reported to give better ethanol yields than pretreatment with dilute acid. This is due to better fermentation efficiency, because formation of inhibitory byproducts is avoided. Downside of the method is a slightly lower sugar conversion rate. Pretreatment with alkali is best used for biomass with high lignin content (Gupta, 2008; Hamelinck et al., 2005; Mosier et al., 2005).

Enzymatic hydrolysis

The pretreatment is usually followed by enzymatic hydrolysis to convert the cellulose fibres and hemicellulose to fermentable sugars. Hydrolysis is carried out by different cellulase enzymes which are usually produced by lignocellulose degrading bacteria or fungi, for example *Trichoderma reesei*. The main factors that affect the hydrolysis rate of cellulose are accessibilty of cellulose fibers to enzymes, crystallinity of cellulose and hemicellulose, and lignin content (Sun & Cheng, 2002). Presence of lignin and hemicellulose makes the access of enzymes to cellulose fibres difficult. Therefore, the removal of lignin and hemicellulose as well as the increase of porosity during the pretreatment process increases the hydrolysis rate significantly (Dwivedi et al., 2009). At the same time, the presence of dissolved lignin can also inhibit the hydrolysis, so that not all of accessible cellulose is converted to sugars. Enzymatic hydrolysis can be carried out with total solid loadings up to 20%. If solid loading is higher than that, the constant stirring and equal distribution of enzymes in the mixture becomes difficult to achieve.

Analysis

Dilute sulfuric acid, hydrochloric acid, nitric acid and potassium hydroxide solution were used for pretreatment. The size of samples were 100 g of dried (moisture content <10%) and milled wheat straw to which 1,000 mL of 1% acid or alkaline solution was added. All samples were heated for $t = 60$ minutes at a temperature $T = 130 \pm 3$ºC and a pressure of $p = 3$ bar. As enzymes are inactivated when temperature $T > 70$ºC or $4 > pH > 7$, the sample was cooled to a temperature below 50ºC and K_2CO_3 or HCl was added to neutralize the pH. Pretreatment was followed by enzymatic hydrolysis with the enzyme complex Accellerase 1,500. Enzyme mixture was added to the sample at a ratio of 0.3 mL per g of biomass. Hydrolysis lasted for $t = 24$ hours under constant stirring and at a temperature $T = 50$ºC. After the hydrolysis process, glucose concentration in all of the samples was measured reflectometrically using RQflex 10 reflectometer and Reflectoquant glucose & fructose test. D–glucose and D–fructose are converted into D–glucose–6–phosphate. This is oxidized by NAD under the catalytic effect of glucose–6–phosphate dehydrogenase to gluconate–6–phosphate. In the presence of diaphorase, the NADH formed in the process reduces a tetrazolium salt to a blue formazan that is then determined reflectometrically.

In order to start the fermentation process, 2.5 g of dry yeast *Saccharomyces cerevisiae* was added to all of the samples. Fermentation process was carried out for 7 days under low oxygen conditions in 1,000 mL glass bottles, sealed with a fermentation tube. No glucose was detected in the samples after fermentation. Ethanol concentration was measured reflectometrically using RQflex 10 reflectometer and Reflectoquant alcohol test by Merck Inc. Under the catalytic effect of alcohol dehydrogenase, alcohol is oxidized by NAD to acetaldehyde. In the presence of an electron transmitter, the NADH formed in the process reduces a tetrazolium salt to a blue formazan that is determined reflectometrically.

At least 3 parallel samples were analyzed with each pretreatment method. Averaged results are used in figures and deviations are shown by vertical lines. Data was processed with programs Microsoft Excel and GraphPad Prism 5.

Results and discussion

The influence of different pretreatment methods on glucose and ethanol yields from wheat straw was investigated to determine the most efficient method for bioethanol production under moderate pretreatment conditions. Influence of washing the solid phase of wheat straw samples on the sugar and ethanol conversion yield was also investigated.

Results show that the highest cellulose to glucose conversion rate of 316.7 g kg^{-1} of biomass was achieved with the pretreatment by nitric acid (results shown in fig. 1). This indicates that nitric acid removes most of the hemicellulose from the sample and leaves the cellulose fibres easily accessible for enzymes. By far the lowest glucose yield of 221.3 g kg^{-1} was achieved by hydrochloric acid. Although the same acid concentrations were used, nitric acid pretreatment gave 30.1% higher glucose yield than the pretreatment with hydrochloric acid. This shows that 1% HCl acid solution is not strong enough to remove hemicellulose from the samples. Higher acid concentrations or longer pretreatment times could be used to overcome low glucose yield, but it would make the pretreatment with hydrochloric acid unfeasible compared to that with nitric acid or sulfuric acid.

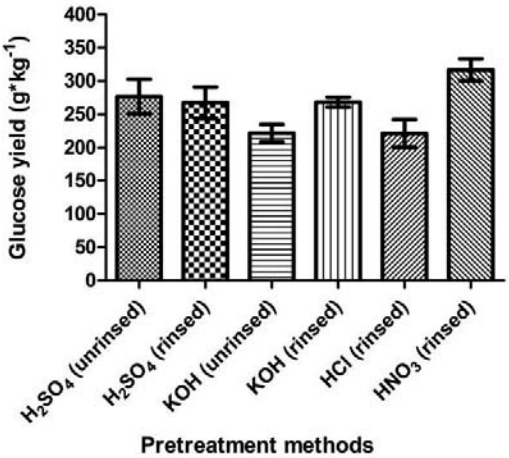

Fig. 1. The influence of different pretreatment methods on the glucose yield from wheat straw samples.

In wheat straw samples pretreated with sulfuric acid, two different approaches were used. Solid phase of half of the samples was rinsed with water before adding enzymes, and the rest of the samples were not. The results indicated that the unrinsed samples pretreated with sulfuric acid gave a glucose yield of 276.7 g kg^{-1}while samples that were rinsed before hydrolysis gave a glucose yield of 267.3 g kg^{-1}. Approximately 3.5% of cellulose is converted to sugars during the pretreatment with acid and is dissolved in the liquid phase. In case of pretreatment with diluted KOH, the unrinsed samples gave a glucose yield of 221.7 g kg^{-1}while samples that were rinsed before hydrolysis gave a glucose yield of 267.5 g kg^{-1}. This can be explained by the different thickness of rinsed and unrinsed alkaline pretreated samples, presence of dissolved lignin and short hydrolysis time. Unrinsed samples were very thick and difficult to stir, and dissolved lignin in solution is a known inhibitor to enzyme activity (Berlin et al., 2006).

The rinsed samples pretreated with KOH gave the best ethanol yield of 104.3 g kg^{-1} results in fig. 2. On the other hand, wheat straw samples pretreated with HNO_3 gave an ethanol yield of only 95.0 g kg^{-1}regardless of the highest glucose yield of 316.7 g kg^{-1}. This can be explained by the formation of byproducts during acid pretreatment process which later inhibit fermentation process (Helle et al., 2003). Since these byproducts are not formed during alkaline pretreatment phase, the fermentation is more effective and more sugars are used for ethanol production rather than for the formation of organic acids and other unwanted byproducts.

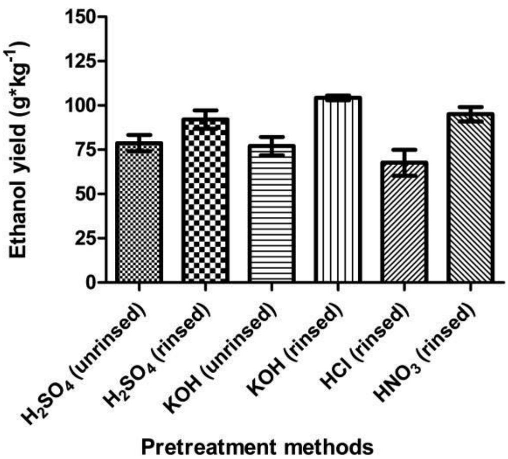

Fig. 2. The influence of different pretreatment methods on the ethanol yield from wheat straw samples.

The effect of inhibitory compounds was seen in the wheat straw samples pretreated with sulfuric acid as well. The rinsed wheat straw samples pretreated with sulfuric acid gave approximately 14.5% higher ethanol yield than the samples that were not rinsed (ethanol yields of 92.0 and 78.7 g kg^{-1}, respectively). These results indicate that although washing removes 3.5% of sugars from a pretreated sample, it also removes a quantity of compounds that later inhibit the fermentation, thus resulting in a higher ethanol yield.

Results showed (table 2) that the samples pretreated with nitric acid (rinsed) had the best hydrolysis efficiency of 68.1%, but mediocre fermentation efficiency of 59.2%. In contrast, the samples pretreated with sulfuric acid (rinsed) had a hydrolysis efficiency of 57.5% and fermentation efficiency of 68.0%. This shows that nitric acid fractionates cellulose fibres and removes hemicellulose better than sulfuric acid, but byproducts of the pretreatment with nitric acid are more difficult to remove with rinsing and thus have a bigger negative impact on fermentation.

Table 2. Hydrolysis and fermentation efficiencies of different pretreatment methods

Pretreatment method	Glucose yield (g kg^{-1})	Ethanol yield (g kg^{-1})	Hydrolysis efficiency (%)	Fermentation efficiency (%)
H_2SO_4 (unrinsed)	276.7	78.7	59.5	56.1
H_2SO_4 (rinsed)	267.3	92.0	57.5	68.0
KOH (unrinsed)	221.7	77.0	47.7	68.3
KOH (rinsed)	268.2	104.3	57.7	76.3
HCl (rinsed)	221.3	67.7	47.6	59.9
HNO_3 (rinsed)	316.7	95.0	68.1	59.2

The highest fermentation efficiency of 76.3% was given by the samples pretreated with KOH (rinsed). This indicates that byproducts that impede fermentation are not formed during pretreatment with alkali. The downside of alkaline pretreatment method is its slightly lower hydrolysis efficiency compared to the dilute acid pretreatment methods. The alkaline pretreatment process removes lignin from samples, but leaves most of the hemicellulose intact which makes access of enzymes to cellulose fibres difficult. Dissolved lignin is also a known inhibitor of enzyme activity.

Conclusions

The aim of this research was to investigate the different pretreatment methods of wheat straw to find the most efficient and cost effective method using moderate pretreatment conditions. The influence of rinsing the solid phase of wheat straw samples after the pretreatment phase on the sugar and ethanol conversion yields was also investigated.

Samples pretreated with KOH (rinsed) gave the best ethanol yield of 104.3 g kg^{-1} regardless of the glucose yields inferior to those of nitric acid and unrinsed sulfuric acid. The wheat straw samples pretreated with HNO_3 gave the highest glucose yield of 316.7 g kg^{-1}, but an ethanol yield of 95.0 g kg^{-1} which was less than expected. This can be explained by the formation of byproducts during acid pretreatment process that later inhibit the fermentation process. Since byproducts that inhibit fermentation are not formed during alkaline pretreatment phase, the fermentation is much more effective and more sugars are used for ethanol production rather than for the formation of organic acids and other unwanted byproducts.

The samples pretreated with nitric acid (rinsed) had the best hydrolysis efficiency of 68.1%, but poor fermentation efficiency of 59.2%. It is in contrast to the results from the samples pretreated with sulfuric acid (rinsed) which had a hydrolysis efficiency of 57.5% and fermentation efficiency of 68.0%. This can be due to the fact that nitric acid fractionates cellulose fibres and removes hemicellulose better than sulfuric acid, but compounds that are formed during nitric acid pretreatment are more difficult to remove with washing and thus have a bigger negative impact on fermentation. Best fermentation efficiency of 76.3% was achieved by samples pretreated with KOH (rinsed). The downside of alkaline pretreatment method is its lower hydrolysis efficiency compared to sulfuric and nitric acid pretreatment methods.

In the light of these results we can conclude that from the point of ethanol production process under mild pretreatment conditions, the most effective method is KOH pretreatment process combined with rinsing the samples before the hydrolysis.

References

Dwivedi, P., Alavalapati, J.R.R., Lal, P., 2009. Cellulosic ethanol production in the United States: Conversion technologies, current production status, economics and emerging developments, *Energy for Sustainable Development*, **13**, 174–182.

Nigam, P.S., Singh, A., 2011. Production of liquid biofuels from renewable resources, *Progress in Energy and Combustion Science*, 37, 52–68.

Larson, E.D., 2008. Biofuel production technologies: status prospects and implications for trade and development, United Nations Conference on Trade and Development, New York and Geneva.

Global Renewable Fuels Alliance, 11.02.2011. World Ethanol Fuel Production data from F.O.Licht, http://www.globalrfa.org/pr_021111.php.

Stevens, D.J., Worgetten, M., Saddler, J., 2004. Biofuels for transportation: an examination of policy and technical issues. IEA Bioenergy Task **39**, Liquid Biofuels Final Report 2001–2003, Canada.

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

Demirbas, A., 2005. Bioethanol from cellulosic materials: a renewable motor fuel from biomass, *Energy Sources*, **27**, 327–337.

Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., 2012. Bioethanol production from agricultural wastes: An overview, *Renewable Energy*, **37**, 19–27.

Kim, J-W., Kim, K.S., Lee, J-S., Park, S.M., Cho, H-Y., 2011. Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid, Bioresource Technology, **102**, 8992–8999.

Yang, Y., Sharma–Shivappa, R., Burns, J.C., Cheng, J.J., 2009. Dilute Acid Pretreatment of Oven-dried SwitchgrassGermplasms for Bioethanol Production, *Energy & Fuels*, **23**, 3759–3766.

Dien, B., Jung, H., Vogel, K., Casler, M., Lamb, J., Iten, L., Mitchell, R., Sarath, G., 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfaalfa, reed canarygrass and switchgrass, *Biomass Bioenergy*, **30**, 880–891.

Gupta, R., 2008. Alkaline pretreatment of biomass for ethanol production and understanding the factors influencing the cellulose hyrdolysis, A Dissertation, Auburn University, Alabama, USA.

Hamelinck, C.N., Hooijdonk, G.V., Faaji, A., Ethanol from lignocellulosic biomass: technoeconomicperfomance in short-, middle- and long-term, Biomass&Bioenergy, 2005, **28**, 384–410.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y., Holtzapple, Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresource Technology, 2005, **96(6),** 673–686.

Sun, Y., Cheng, J., Hydrolysis of lignocellulosic material for ethanol production: a review, Bioresource Technology, 2002, **83**, 1–11.

Berlin, A., Balakshin, M., Gilkes, N., Kadla, J., Maximenko, V., Kubo, S., Saddler, J., Inhibition of cellulase, xylanase and glucosidase activities by softwood lignin preparations, Journal of Biotechnology, 2006, 198–209.

Helle, S., Cameron, D., Lam, J., White, B., Duff, S., Effect of inhibitory compounds found in biomass hyrdosylates of growth and xylose fermentation by a genetically engineered strain of *S. cerevisiae*, Enzyme and Microbial Technology, 2003, **33**, 786–792.