COMPARISON OF DIFFERENT PRETREATMENT METHODS ON DEGRADATION OF RYE STRAW

Marti Tutt, Timo Kikas, Juri Olt
Estonian University of Life Sciences
marti.tutt@emu.ee

Abstract. This article investigates the influence of different pretreatment methods on sugar conversion and ethanol production of rye straw. Different dilute acid and alkaline pretreatment methods were compared to each other with the aim of getting the best glucose and ethanol yields under mild operating conditions using rye straw as a raw material. From different available feedstocks rye straw was chosen because it has high biomass growth and rye is a widely grown cereal in Northern Europe. Dilute sulfuric acid, nitric acid and potassium hydroxide solutions were used for pretreatment in combination with enzymatic hydrolysis. The results indicate that the highest cellulose to glucose conversion rate of 324.0 $g \cdot kg^{-1}$ of biomass was achieved by nitric acid pretreatment of rye straw. It also had the highest ethanol yield of 96.9 $g \cdot kg^{-1}$. The lowest glucose concentrations were achieved by KOH pretreatment. In wheat and rye straw samples pretreated with sulfuric acid and KOH, two different approaches were used. The solid part of half the samples was rinsed with water before adding enzymes and the rest of the samples were unrinsed before enzymatic hydrolysis. The results indicate that approximately 8 % of cellulose is converted to sugars during acid pretreatment and is dissolved in the liquid phase.

Key words: rye straw, glucose, dilute acid pretreatment, cellulose, biomass.

Introduction

Liquid biofuels are being researched mainly to replace the conventional liquid fuels, such as diesel and petrol. The advantage of the second generation biofuels is the fact that they do 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 the first generation [1]. In the future, the production of ethanol is expected to include both, traditional grain/sugar crops and lignocellulosic materials [2]. 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 [3].

Cellulosic ethanol production is a complex process compared to the 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 [4; 5]. Several different pretreatment methods for straw have been studied in the past, but no method has yet emerged as being efficient, but also simple and cost effective. The methods using moderate pretreatment conditions are cheap and simple, 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 [5]. In hydrolysis conversion, cellulose and hemicellulose present in biomass is broken down to sugar molecules and then fermented to produce ethanol. Lignin is removed during the pretreatment process and utilized. Hydrolysis processes are often combined with the thermochemical pretreatment phase in temperatures 120 – 200 ºC followed by enzymatic hydrolysis [4].

From different available raw materials rye straw was chosen because it has high above ground biomass growth and it is a widely grown cereal in Northern Europe. Only a small portion of 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 straw is still left on the fields or disposed of as waste [6]. Promoting the use of rye straw as a raw material for bioethanol production could help increase the cellulosic ethanol production in Estonia and in Europe and reduce the quantity of biomass that goes to waste.

The aim of the 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 rye straw samples on the sugar and ethanol conversion yields was also investigated.

Materials and methods

Biomass

Rye straw was chosen as a raw material in this work, because it is a widely grown cereal in Northern Europe and much of the rye straw is going to waste. Rye is also grown in Estonia, and since straw does not compete directly with the food market, it makes rye straw a good choice for bioethanol production. The rye straw samples were harvested in August, 2011, from the experimental fields of the Estonian University of Life Sciences. Ash, hemicellulose, cellulose and lignin contents of straw samples were determined in the Laboratory of Plant Biochemistry of the 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. The 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 rye straw samples

Sample	Ash, %	Hemicellulose, %	Cellulose, %	Lignin, %
Rye straw	5.21	27.86.	42.83	6.51

Pretreatment

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 – 200 ºC. Higher temperatures require shorter time of pretreatment [7; 8]. Besides, sulfuric acid, nitric acid has shown good results in cellulose-to-sugars conversion yields.

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 ºC. 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 [9 – 11].

The pretreatment process 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 accessibility of cellulose fibers to enzymes, crystallinity of cellulose and hemicellulose, and lignin content [12]. Presence of lignin and hemicellulose makes the access of enzymes to cellulose fibers difficult. Therefore, the removal of lignin and hemicellulose as well as the increase of porosity during the pretreatment process increases the hydrolysis rate significantly [4]. At the same time, the presence of dissolved lignin can also inhibit 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, nitric acid and potassium hydroxide solution were used for pretreatment. The size of samples was 100 g of dried (moisture content <10 %) and milled rye straw to which 1000 mL of 1 % acid or alkaline solution was added. All samples were heated for t = 60 minutes at a temperature T = 130 ± 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 50 ºC and K_2CO_3 or HCl was added to neutralize the pH. Pretreatment was followed by enzymatic hydrolysis with the enzyme complex Accellerase 1500. In rye straw samples pretreated with sulfuric acid and KOH, two different approaches were used. The solid part of half the samples was rinsed with water before adding enzymes and the rest of the samples were unrinsed before enzymatic hydrolysis. 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 the RQflex 10 reflectometer and the 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 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. The fermentation process was carried out for 7 days under low oxygen conditions in 1000 mL glass bottles, sealed with a fermentation tube. No glucose was detected in the samples after fermentation. The ethanol concentration in fermented samples was measured by gas chromatography. At least 3 parallel samples were analyzed with each pretreatment method. Averaged results are used in figures and deviations are shown by vertical lines. The data were processed with programs Microsoft Excel and GraphPad Prism 5.

Results and discussion

The influence of different pretreatment methods on glucose and ethanol yields from rye straw were investigated to determine the most efficient method for bioethanol production under moderate pretreatment conditions. The results show that the highest cellulose to glucose conversion rate of 324.0 $g \cdot kg^{-1}$ of biomass was achieved with the pretreatment by nitric acid (results shown in Figure 1).

This indicates that nitric acid removes most of the hemicellulose from the sample and leaves the cellulose fibres easily accessible for enzymes. In the rye straw samples pretreated with sulfuric acid, two different approaches were used. The 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 294.7 $g \cdot kg^{-1}$ while the samples that were rinsed before hydrolysis gave a glucose yield of 270.6 $g \cdot kg^{-1}$. Approximately 8 % of cellulose is converted to sugars during the pretreatment with acid and is dissolved in the liquid phase. If the sample is rinsed after pretreatment then we lose that 8 % sugar, but we also remove some of the inhibitory byproducts and most of acid residues, so use of chemicals for pH adjustment is significantly reduced. In case of pretreatment with diluted KOH, the unrinsed samples gave a glucose yield of 219.0 $g \cdot kg^{-1}$ which is by far the lowest result. In contrast to sulfuric acid pretreatment, the samples that were rinsed before hydrolysis gave a glucose yield of 245.3 $g \cdot kg^{-1}$, which is approximately 10.7 % higher than the unrinsed results. This can be explained by the different thickness of the washed and unwashed alkaline pretreated samples, presence of dissolved lignin and short hydrolysis time. The unwashed samples were very thick and difficult to stir, and dissolved lignin in solution is a known inhibitor to enzyme activity [13]. Lower glucose yield of alkaline pretreated samples compared to acid pretreatment could be as well the result of biochemical composition of samples. Rye straw has quite low lignin content, but high hemicellulose content and alkaline pretreatment removes only the lignin part from the sample leaving the hemicellulose structure unhindered.

The rinsed samples pretreated with nitric acid gave the best ethanol yield of 96.6 $g \cdot kg^{-1}$ (results in Figure 2) which was expected because these samples had the highest glucose yield. On the other hand, the rye straw samples pretreated with KOH (rinsed) gave an ethanol yield of 80.9 $g \cdot kg^{-1}$ regardless of their relatively low glucose yield of 245.3 $g \cdot kg^{-1}$. The samples pretreated with sulfuric acid (rinsed) had quite a high glucose yield but lower ethanol yield than the KOH (rinsed) pretreated samples. This can be explained by the formation of byproducts during the acid pretreatment process which later inhibit the fermentation process [14]. Since these byproducts are not formed during the 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.

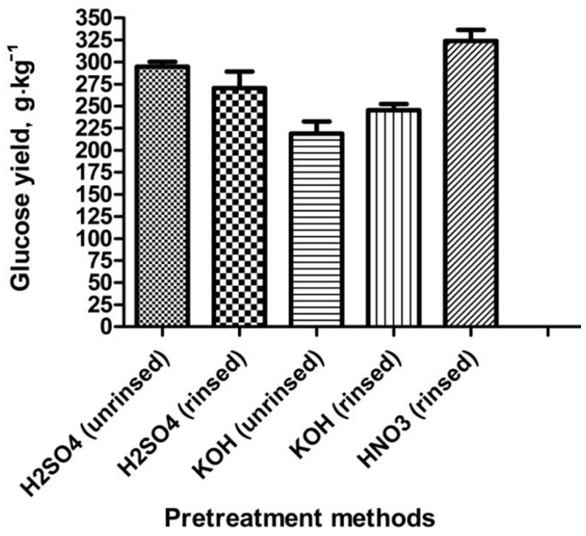

Fig. 1. **Influence of different pretreatment methods on glucose yield from rye straw samples**

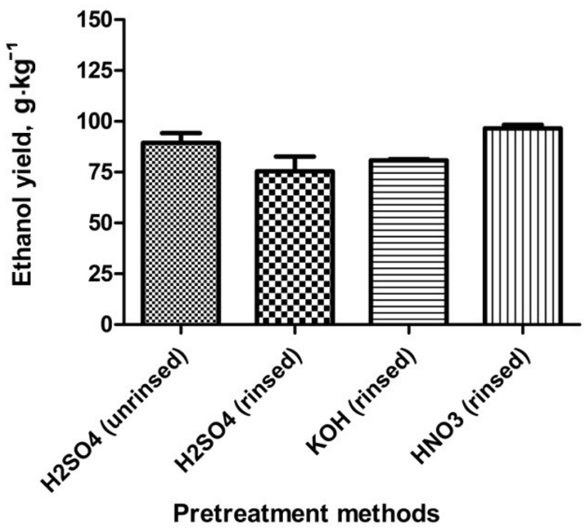

Fig. 2. **Influence of different pretreatment methods on ethanol yield from rye straw samples**

The results showed (Table 2) that the samples pretreated with nitric acid (rinsed) had the best hydrolysis efficiency of 75.6 %, but mediocre fermentation efficiency of 58.6 %. In contrast, the samples pretreated with sulfuric acid (rinsed) had the hydrolysis efficiency of 63.2 % and the fermentation efficiency of 54.4 %.

Table 2

Hydrolysis and fermentation efficiencies of different pretreatment methods

Pretreatment method	Glucose yield, $g \cdot kg^{-1}$	Ethanol yield, $g \cdot kg^{-1}$	Hydrolysis efficiency, %	Fermentation efficiency, %
H_2SO_4 (unrinsed)	294.7	89.4	68.8	59.7
H_2SO_4 (rinsed)	270.7	75.4	63.2	54.4
KOH (unrinsed)	219.0	–	51.1	–
KOH (rinsed)	245.3	80.9	57.3	64.8
HNO_3 (rinsed)	324.0	96.6	75.6	58.6

This shows that nitric acid fractionates cellulose fibres and removes hemicellulose better than sulfuric acid, and also sugars are more effectively used for ethanol production.

The highest fermentation efficiency of 64.8 % was given by the samples pretreated with KOH (rinsed). This indicates that much less byproducts which impede fermentation are formed during pretreatment with alkali. The downside of alkaline pretreatment method is its lower hydrolysis efficiency compared to the dilute acid pretreatment methods. The alkaline pretreatment process removes lignin from the samples, but leaves most of the hemicellulose intact, which makes access of enzymes to cellulose fibres difficult if samples have high hemicellulose content. Dissolved lignin is also a known inhibitor of enzyme activity.

Conclusions

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

The samples pretreated with sulfuric acid (rinsed) gave the best glucose and ethanol yields of 324.0 $g\cdot kg^{-1}$ and 99.6 $g\cdot kg^{-1}$, respectively. The alkaline pretreated samples (rinsed) had the best fermentation efficiency of 64.8 %, but lower glucose yield meant that overall ethanol production was smaller than, for example, when using nitric acid pretreatment. This can be explained by the formation of byproducts during the acid pretreatment process that later inhibit the fermentation process. Since byproducts that inhibit fermentation are not formed during the 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. On the other hand, because hemicellulose is not dissolved during the alkaline pretreatment, access to cellulose fibers is hindered, thus resulting in lower sugar yields. Acid pretreatment should be preferred in case of high hemicellulose content and alkaline pretreatment in case of high lignin content of biomass.

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 the nitric acid pretreatment process combined with rinsing the samples before hydrolysis. Sulfuric acid pretreatment gives slightly lower sugar and ethanol yields, but since sulfuric acid is cheaper than nitric acid, it is more preferred in large scale industrial processes.

References

1. Stevens, D.J., Worgetten, M., Saddler, J., Biofuels for transportation: an examination of policy and technical issues. IEA Bioenergy Task 39, Liquid Biofuels Final Report 2001-2003, Canada, 2004.
2. Demirbas, A., Competitive liquid biofuels from biomass, *Applied Energy*, 2011, 88, 17-28.
3. Demirbas, A., Bioethanol from cellulosic materials: a renewable motor fuel from biomass, *Energy Sources*, 2005, 27, 327-337.
4. Dwivedi, P., Alavalapati, J.R.R., Lal, P., Cellulosic ethanol production in the United States: Conversion technologies, current production status, economics and emerging developments, *Energy for Sustainable Development*, 2009, 13, 174-182.
5. Kim, J-W., Kim, K.S., Lee, J-S., Park, S.M., Cho, H-Y., Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid, Bioresource Technology, 2011, 102, 8992-8999.
6. Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., Bioethanol production from agricultural wastes: An overview, *Renewable Energy*, 2012, 37, 19-27.
7. Yang, Y., Sharma–Shivappa, R., Burns, J.C., Cheng, J.J., Dilute Acid Pretreatment of Oven-dried Switchgrass Germplasms for Bioethanol Production, *Energy & Fuels*, 2009, 23, 3759-3766.
8. Dien, B., Jung, H., Vogel, K., Casler, M., Lamb, J., Iten, L., Mitchell, R., Sarath, G., Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfaalfa, reed canarygrass and switchgrass, *Biomass Bioenergy*, 2006, 30, 880-891.
9. Gupta, R., Alkaline pretreatment of biomass for ethanol production and understanding the factors influencing the cellulose hyrdolysis, A Dissertation, Auburn University, Alabama, USA, 2008.
10. Hamelinck, C.N., Hooijdonk, G.V., Faaji, A., Ethanol from lignocellulosic biomass: technoeconomic perfomance in short-, middle- and long-term, Biomass&Bioenergy, 2005, 28, 384-410.
11. 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.
12. Sun, Y., Cheng, J., Hydrolysis of lignocellulosic material for ethanol production: a review, Bioresource Technology, 2002, 83, 1-11.
13. 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.
14. 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.