

## Comparison of different pretreatment methods for straw for lignocellulosic bioethanol production

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**Abstract.** In order to reduce carbon dioxide emissions from the combustion of fossil fuels, the production of biofuels from lignocellulosic agricultural residues is the focus of industrial and scientific interest. The feedstocks of the second generation used for bioethanol production are lignocellulose-containing raw materials like different types of straw, or other plants like *miscanthus x giganteus*. In all these plants, the cellulose in the lignocellulose is not accessible to enzymes. Therefore, lignin and/or hemicelluloses have to be removed by a specific pretreatment in order to make enzymatic degradation of cellulose possible. We examined and compared the pretreatment of wheat straw by means of steam treatment and steam explosion treatment. After hydrolysis, glucose concentrations up to 300 g kg<sup>-1</sup> were reached both for steam-pretreated straw and steam-exploded straw. After fermentation, ethanol concentrations ranging from 120–140 g kg<sup>-1</sup> were achieved. Results suggest that the explosion process slightly favors the solubilisation of sugars and, therefore, enhances ethanol production. Only at higher temperature and longer incubation time does the explosion process not seem to be necessary. In addition to this, we examined most of the lignocellulosic residuals in Austria available for bioethanol production. As a result, we can show that even in a country not focused on agricultural production all the bioethanol needed for E10 can easily be provided.

**Keywords:** straw, steam explosion, biomass potential, E10.

### INTRODUCTION

The combustion of fossil fuels is responsible for 73% of carbon dioxide emissions into the atmosphere as reported by the US environmental protection agency (EPA 2010). Therefore it contributes significantly to global warming. Interest in the development of methods to reduce greenhouse gases has increased enormously. In order to reduce such emissions, the production of biofuels from lignocellulosic agricultural residues is the focus of industrial and scientific interest. The proposal for the new directive of the European parliament on the promotion of the use of energy from renewable sources also favors the use of ‘advanced biofuels’ with high greenhouse gas savings EU (2012).

The feedstocks of second generation currently used for bioethanol production are lignocellulose-containing raw materials like the straw from wheat (*Triticum vulgare*), rye (*Secale cereale*), oat (*Avena sativa*) and corn (*Zea mays*) as well as chinese silver grass (*Miscanthus x giganteus*) or reed (*arundo donax*). In all these plants, the cellulose in the lignocellulose is not accessible to enzymes. Therefore, lignin and/or hemicelluloses have to be removed by specific pretreatment in order to make enzymatic degradation of cellulose possible. Later, the sugars are fermented with yeast

to ethanol. The solution is then distilled, rectified and can be used as a chemical or blended with gasoline to give E 5, E 10 or E 85.

The pretreatment methods can be classified into three different types: thermophysical methods, acid-based methods and alkaline methods (Talebnia et al., 2010). Thermophysical methods like steam pretreatment, steam explosion or hydrothermolysis open the structure, make most of the celluloses accessible to enzymes and solubilize the hemicelluloses.

Among all pretreatment methods, steam explosion seems to be the state of the art technology for most of the commercial or pre-commercial pilot and demonstration plants for second generation bioethanol. So far, it is unknown whether the ‘explosion’ is really needed in the pretreatment (Brownell et al., 1986; Li et al, 2005; Schütt et al., 2012).

We examined and compared the pretreatment of wheat straw by means of steam treatment and steam explosion treatment (Zabihi et al., 2010). There are just few analysis about the necessity of pressure release (explosion). So the aim of this paper is the comparison of the steam explosion pretreatment and the steam pretreatment.

For this purpose, wheat straw was pretreated in two different ways. In both ways, wheat straw was heated up to 160–200 °C and incubated for 10 to 20 minutes. Then the pressure (and the temperature) was released suddenly (steam explosion) or the temperature (and also the pressure) was decreased slowly. The different pretreated wheat straws were dried and analyzed by means of scanning electron microscopy and / or hydrolyzed and fermented to determine the sugar-concentration (glucose and xylose) and, after fermentation, the ethanol-concentration.

In addition to this, we examined most of the lignocellulosic residuals in Austria available for bioethanol production e.g. straw from wheat (*Triticum vulgare*), rye (*Secale cereale*), oat (*Avena sativa*) and corn (*Zea mays*) as well as Chinese silver grass (*Miscanthus x giganteus*). The bioethanol yield of each specific plant was determined by standard experimental design: pretreatment by means of steam explosion, enzymatic hydrolysis using cellulases and fermentation with yeast. On this basis and on the basis of the quantity of feedstock available, we calculated the total bioethanol production capacity for Austria. As a result, we can show that even in a country not focused on agricultural production all the bioethanol needed for E 10 can easily be provided.

## MATERIALS AND METHODS

### Raw material

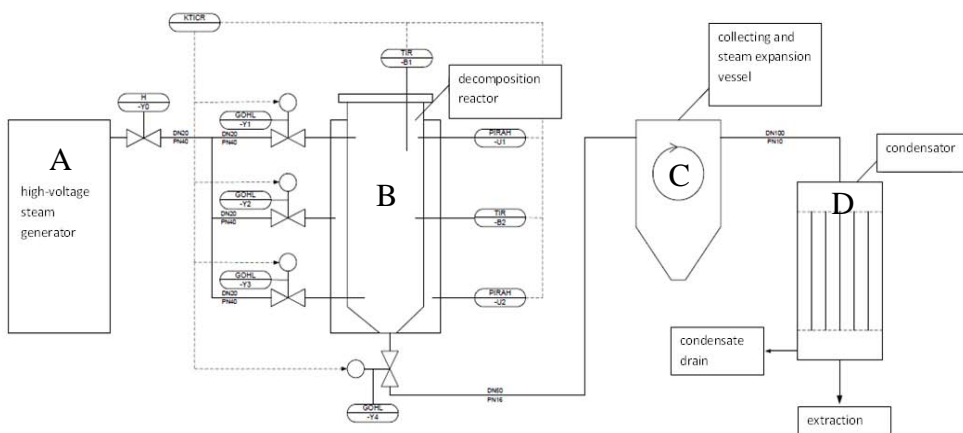
Wheat straw (*Triticum vulgare*), oat straw (*Avena sativa*), rye straw (*Secale cereal*) and corn straw (*Zea mays*) were obtained from local producers in Austria. The air-dried straw was chopped up by a garden shredder (Viking GE 260, Kufstein, Austria) to a length of 2–3 cm. The straw was stored at room temperature. The moisture content was 9%.

### Pretreatment

Straw was pre-soaked with distilled water for 15 minutes with a mass ratio of one part distilled water to one part wheat straw. Both pretreatment methods (steam treatment and steam explosion treatment) had the following pretreatment conditions:

incubation times 10 and 20 minutes and pretreatment temperature from 160 °C to 200 °C (Horn et al., 2011).

The steam explosion pretreatment was performed in a laboratory scale steam explosion unit (VAM GmbH & Co KG, Linz, Austria). The 15 L decomposition reactor was loaded with 1,800 g presoaked straw. Saturated steam from a high-voltage steam generator was injected, when wheat straw was heated under pressure by a jacket heating up to the pretreatment temperatures for 10 or 20 minutes. By opening a ball valve the pressure was released suddenly, so the wheat straw was transferred to the collecting and steam expansion vessel where the pretreated straw was withdrawn.



**Figure 1.** Laboratory scale steam explosion unit. Main equipment: (A) high-voltage steam generator, (B) decomposition reactor, (C) collecting and steam expansion vessel and (D) condenser.

For steam pretreatment the laboratory scale steam explosion unit was also used. The decomposition reactor was filled with 1,040 g pre-soaked wheat straw. Instead of opening the ball valve to the collecting and steam expansion vessel, the pressure was released by an additional valve very slowly, so that the pressure decreased at 45 bar h<sup>-1</sup>. The steam treated straw was removed by means of a stainless steel basket from the decomposition reactor. Therefore the quantity of straw had to be decreased to 1,040 g.

For further experiments the pretreated wheat straws were dried at 40 °C to a moisture content of 5%.

The severity factor (1) describes the intensity of the reaction conditions, temperature and incubation time during pretreatment. It allows comparison of different variations of these variables. They were calculated according to Overend & Chornet (1987) as:

$$S = \log_{10} [t * \exp [(T-100) 14.75^{-1}]] \quad (1)$$

where  $S$  = severity factor,  $t$  = incubation time [min],  $T$  = temperature [°C], 14.75 = activation energy value.

### Enzymatic hydrolysis

For cellulose conversion into C6-sugars, the enzyme mixture Accellerase 1500 from Genencor® was used. The enzyme concentration was 30 FPU g<sup>-1</sup> cellulose. The enzymatic hydrolysis of the pre-treated straw was carried out at 10% dry matter content in citrate buffer, c = 50 mmol L<sup>-1</sup> (pH 5: adjusted with NaOH, c = 4 mol L<sup>-1</sup>). The incubation was done for 72 h at 50 °C in a shaking incubator at rotational speed of 2.5 s<sup>-1</sup>.

### Fermentation

The fermentation of glucose into ethanol was done by yeast *Saccharomyces cerevisia*. The fermentation medium (pH 4.6: adjusted with H<sub>2</sub>SO<sub>4</sub>, c = 4 mol L<sup>-1</sup>) contained 100 mL hydrolyzate, 2 mL CaCl<sub>2</sub>\*2H<sub>2</sub>O ( $\gamma$  = 150 g L<sup>-1</sup>), 2 mL KH<sub>2</sub>PO<sub>4</sub> ( $\gamma$  = 143 g L<sup>-1</sup>), 2 mL MgSO<sub>4</sub>\*7H<sub>2</sub>O ( $\gamma$  = 75 g L<sup>-1</sup>) and 0.44 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>. 2 mL of a yeast suspension (with a cell count of 1.77 x 10<sup>9</sup> mL<sup>-1</sup>) were added and then the fermentation was done at 30 °C for 168 hours (Kahr et al., 2012). All experiments are done in triplicates. Arithmetic mean and standard deviation have been calculated.

### Chemical analyses

The dry substance content was analysed with a moisture analyzer (Ohaus MB 45, Parsippany, USA). The quantitative determination of fibre content (cellulose, hemicellulose and lignin) from wheat straw was analysed according to the fibre detergent analysis method of van Soest (1991) and VDLUFA (2004).

Saccharides, organic acids, ethanol and furans from liquids were quantified 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<sub>2</sub>SO<sub>4</sub> (c = 5 mmol L<sup>-1</sup>) eluent and an isocratic flow rate of 0.8 mL min<sup>-1</sup> was used. The signals were acquired with a refractive index (RI) and a UV-detector at 210 nm wavelength.

### SEM-images

To compare the morphological structure of the different pretreated straw (pretreatment condition: 200 °C, 10 min and 200 °C, 20 min), scanning electron microscope (SEM) images were taken with a scanning electron microscope VEGA 2 LMU from Tescan.

### Availability of straw and calculation of bioethanol potential in Austria

The availability of straw in Austria was calculated on the basis of the area under cultivation of the respective plants, the total harvest and the ratio of crop / straw as shown in Table 1. The data were obtained from Statistics Austria

**Table 1.** Crop yield and straw in Austria 2011

Substrate	Area [ha]	Yield [t]	Ratio crop/straw	Straw yield [t]
Rye	45,945	202,002	1:0.9	181,802
Oat	25,029	109,807	1:1.1	120,788
Corn	217,100	2,453,133	1:1	2,453,133
Wheat	304,334	1,781,837	1:0.8	1,425,470

## RESULTS AND DISCUSSION

### Severity factor

To compare the intensity of pretreatment, the severity factors of all pretreatment conditions were calculated. Table 2 shows the different factors relating to the pretreatment conditions. The factors are the same for steam explosion and steam pretreatment as there is no term for explosion in the basic equations.

**Table 2.** Severity factor calculated, based on the pretreatment condition

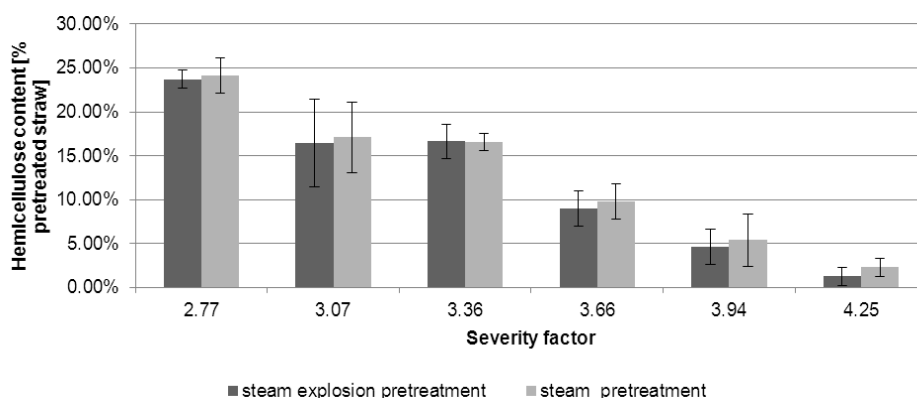
Pretreatment [°C]	Condition [min]	Severity factor
160	10	2.77
160	20	3.07
180	10	3.36
180	20	3.66
200	10	3.94
200	20	4.25

### Fibre content

During pretreatment, degradation of hemicellulose and lignin are possible. The mass loss of hemicellulose (containing xylose, arabinose and glucose) leads to a rising concentration of sugars in the liquid fraction. It should be noted that part of the hemicellulose is totally destroyed and cannot be detected as sugars.

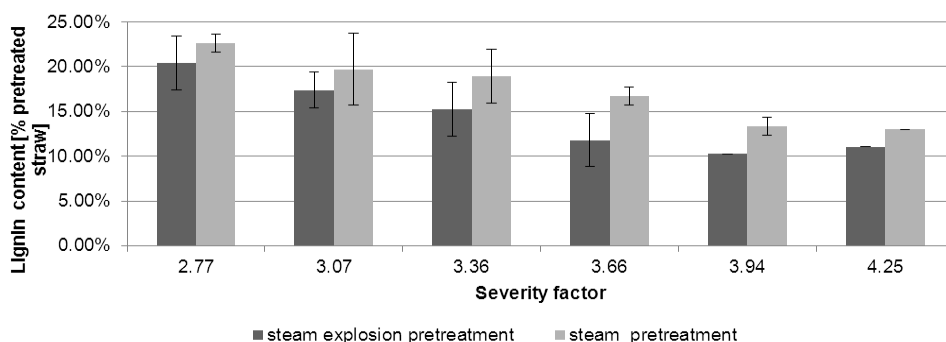
For comparison, the pretreatment effect on the fibre content based on pretreated straw was analyzed.

The influence of the degradation of hemicellulose based on the severity factor is shown in Fig. 2. All results are based on pretreated straw.



**Figure 2.** Degradation of hemicellulose based on the severity factor during pretreatment (steam and steam explosion).

Fig. 2 shows that the hemicellulose content decreases in direct relation to the severity factor, independent of pretreatment methods. Fig. 3 shows that by means of steam explosion more lignin is removed compared to treatment without the sudden pressure release via the steam explosion process.

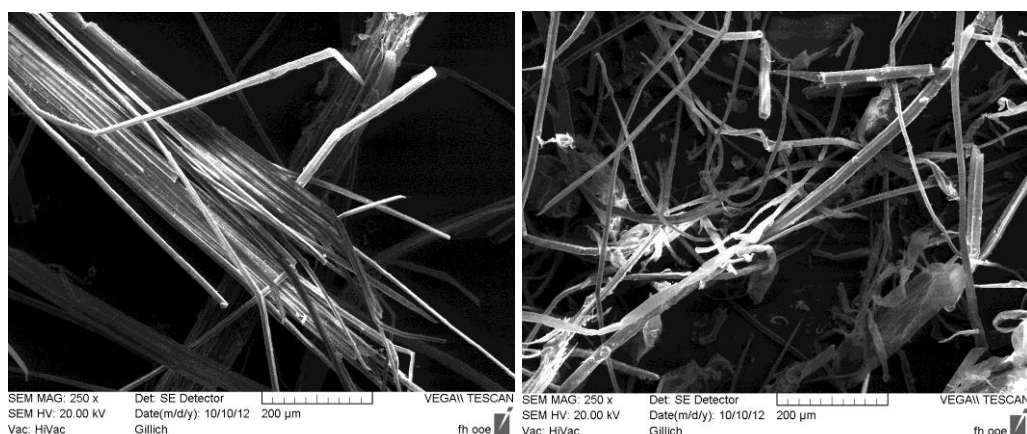


**Figure 3.** Degradation of lignin based on the severity factor during pretreatment.

The cellulose content in general remains stable at about 45% with a slight increase under more intensive pretreatment conditions. In general it can be said that the degradation of hemicellulose and lignin and also the stability of the cellulose content of steam explosion pretreated straw acted like steam pretreated straw.

### SEM-images

As mentioned before the different pretreatment methods had no influence on the fibre content. For further comparison the morphological structure of the steam explosion pretreated straw and steam pretreated straw was examined. In Fig. 4. SEM-images show that the steam pretreated wheat has more compact fibres which are less fractured, while the steam explosion pretreated straw has very fractured fibres.



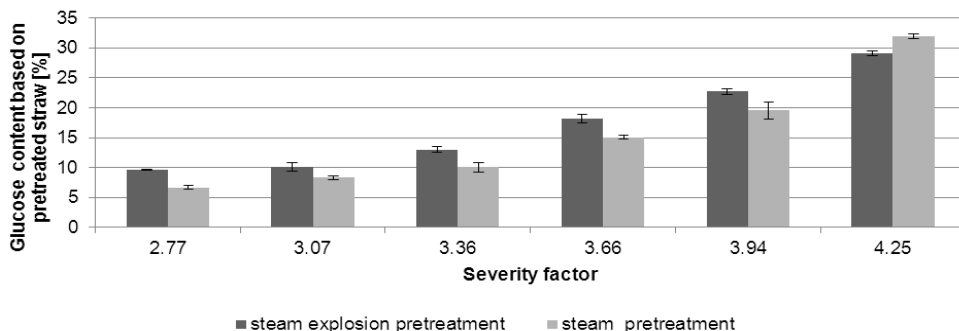
**Figure 4.** SEM-image of pretreated straw (pretreatment conditions: 200 °C and 20 min): left – steam pretreated wheat straw; right – steam explosion pretreated wheat straw.

### Enzymatic hydrolysis and fermentation of the pretreated wheat straws

For conversion of the compact cellulose into monosaccharids (mainly glucose) enzymatic hydrolysis was applied. As shown in Fig. 5 the conversion of cellulose into glucose rises in line with the severity factor. Generally it can be said that the conversion of steam pretreated up to a severity factor of 4.00 is less efficient than

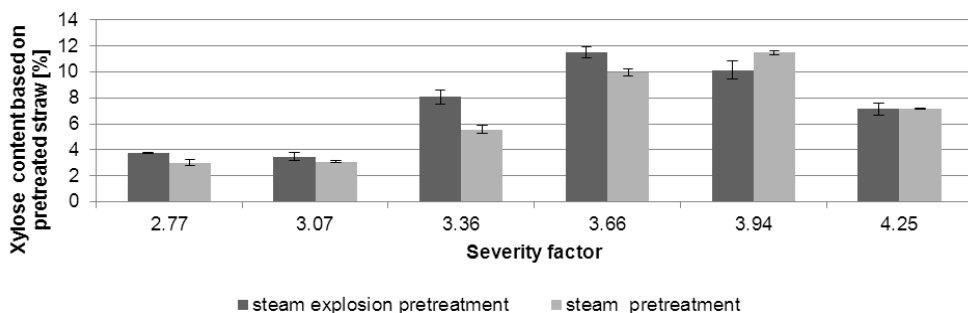
steam explosion pretreatment. Only at severity factors higher than 4.00 is the saccharification of steam pretreated wheat slightly higher compared to the steam explosion pretreatment.

After hydrolysis, glucose concentrations up to 300 g kg<sup>-1</sup> were reached both for the steam pretreated straw and steam exploded straw.



**Figure 5.** Glucose content after enzymatic hydrolysis based on pretreated wheat straw.

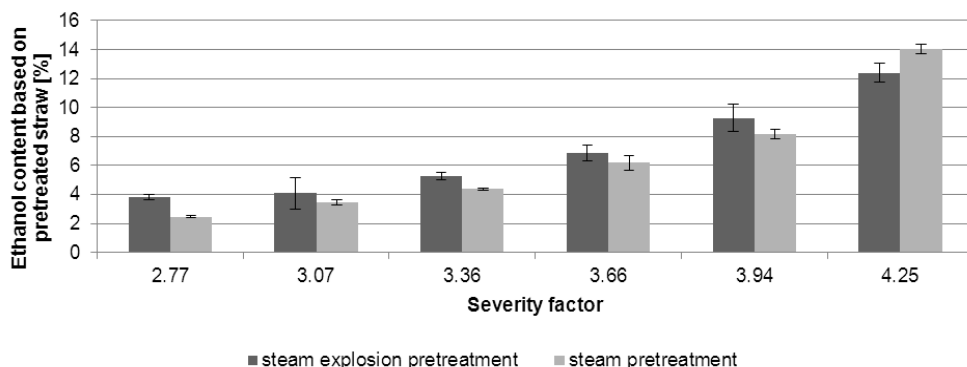
In addition to the glucose concentration after enzymatic hydrolysis, the xylose concentration was determined. Fig. 6 shows that both pretreatment methods had their maximum at xylose concentration 11.5% based on pretreated wheat straw. Steam pretreatment needs a higher severity factor (temperature and/or time) to reach an equal xylose concentration than the explosion pretreatment does. After the maximum is reached the xylose is reduced to small fragments which are not detected.



**Figure 6.** Xylose content after enzymatic hydrolysis based on pretreated wheat straw.

After fermentation, ethanol concentrations ranging from 120–140 g kg<sup>-1</sup> were achieved. Results suggest that the explosion process slightly favors the solubilisation of sugars and, therefore, enhances ethanol production. Only at higher temperature and longer incubation time does the explosion process not seem to be necessary.

The fermentative conversion of glucose to ethanol is shown in Fig. 7. This graph shows that conversion of glucose is similar to the conversion of cellulose to glucose.



**Figure 7.** Ethanol content after fermentation based on pretreated wheat straw.

### Bioethanol production capacity in Austria

To be able to evaluate the bioethanol production capacity in Austria we determined the optimal pretreatment conditions for the major energy crops cultivated in Austria. Table 3 shows the cellulose content of the respective crops, the theoretical and the practical bioethanol yield. For these experiments the steam explosion method was applied. Longer pretreatment times resulted in higher ethanol yields. Surprisingly, the highest ethanol yields were achieved using rye and not wheat straw. The fact that wheat straw is suggested for use in industrial plants (Pschorn, 2012) may be due to the fact that wheat is the major crop cultivated in Europe.

**Table 3.** Ethanol yield of different crops at different pretreatment conditions

Substrate	Pretreatment Condition	Cellulose [kg t <sup>-1</sup> ]	Ethanol [kg] theor.	Ethanol [kg] pract.	Yield [%]
Oat	200 °C 10 min	417	208.5	108	52
Oat	200 °C 20 min	457	228.5	141	62
Corn	190 °C 10 min	433	216	95	44
Rye	200 °C 10 min	493	246	108	44
Rye	200 °C 20 min	487	243	169	70
Wheat	200 °C 10 min	441	221	93	42
Wheat	200 °C 20 min	479	240	124	52

On the basis of the annual crop yield in Austria and the amount of straw (see Table 1), and on the ethanol yield from the respective crops (Table 4) we calculated Austria's bioethanol production potential from straw. About 319,000 tons of bioethanol could be produced from these lignocellulosic materials. In Austria 1,800,000 t of gasoline was consumed in 2011. Therefore 25% of this could be replaced by bioethanol from straw. As a result, we can show that even in a country not focused on agricultural production, all the bioethanol needed for E 10 can easily be provided using less than half of the available straw. Only about half of the straw would have to be used.



**Table 4.** Ethanol yield of different crops

Substrate	Straw p.a. [t]	Ethanol [kg t <sup>-1</sup> ]	Ethanol [t a <sup>-1</sup> ]
Oat	60,394	208.5	17,031
Corn	1,226,566	216	233,048
Rye	90,901	243	30,725
Wheat	1,425,470	240	176,473
Total			457,276

These calculations could be taken to easily determine the lignocellulosic bioethanol production capacity of different countries worldwide.

## CONCLUSIONS

Straw from the major crops in Austria was successfully pretreated, enzymatically hydrolyzed and fermented to ethanol. The two methods of pretreatment, steam treatment and pretreatment by steam explosion treatment, were compared. As a result it can be stated that the sudden pressure release is not absolutely necessary for optimized bioethanol production. Scanning electron microscopy showed that the steam pretreated wheat has more compact fibres which are less fractured, while the steam explosion pretreated straw has very fractured fibres.

When different crops were compared it was shown that, surprisingly, rye was the best substrate for bioenergy production, giving a yield of up to 70% of the theoretically possible value. We presented a calculation tool which can be used to determine the bioethanol potential from lignocellulosic feedstocks for each specific country, simply knowing the crop yield of the respective country. For all substrates there remains huge optimization potential. It has to be taken into account that only ‘standard enzymes’ were applied for the saccharification and simple baker's yeast was used for fermentation.

Nevertheless it still turned out that the gasoline requirement for the E 10 (replacement of 10% of gasoline by bioethanol) could easily be provided by lignocellulosic bioethanol. For straw from wheat a market already exists and there could be a shortage in the market. Straw from corn is more or less completely ploughed into the ground immediately after harvest.

A proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL amending Directive 98/70/EC relating to the quality of petrol and diesel fuels seems to support the production of lignocellulosic bioethanol. This indicates that research and development on lignocellulosic bioethanol is still very important.

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