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Comparison of Different Pre-Treatment Methods for Separating Hemicellulose from Straw during Lignocellulose Bioethanol Production

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Abstract

The interest in the development of methods to reduce greenhouse gases has increased enormously. Therefore, one focus is on the production of biofuels like advanced generation bioethanol from lignocellulosic agricultural residues such as wheat straw. The main focus of this work is the specific separation of hemicellulose into soluble xylose and its oligomers from pre-treated wheat straw in order to generate an additional carbon source for microorganisms. Wheat straw was pre-treated by either steam explosion, acid or alkaline pre-treatment and the amounts of xylose that were generated were compared. The best method for hemicellulose separation was the acid pre-treatment.

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Keywords: Wheat straw; Hemicellulose; Xylose; Steam Explosion pre-treatment; Acid pre-treatment; Alkaline pre-treatment

1. Introduction

The world's depleting energy resources are mainly based on non-renewable fossil fuels like oil, coal and natural gas. Because of the emitted non-biogenic CO_2 and the production of additional greenhouse gases, the interest in the development of methods to reduce or avoid those gases has increased enormously. One of the main research topics that aims to reduce greenhouse gases is the production of advanced bioethanol from lignocellulosic material like wheat straw [1, 2]. The three main components of lignocellulose are cellulose (a polysaccharide from glucose), hemicellulose (a heteropolysaccharid from xylose, mannose, glucose and galactose), and lignin (a phenolic macromolecule) [3]. These three main

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components are tightly associated with each other, so that the ligncellulosic material is less accessible for microorganisms or enzymes [3]. Consequently, the lignocellulosic structure has to be broken up by pre-treatment for better enzyme accessibility before the raw material can be used for bioethanol production. In further steps, the wheat straw is enzymatically hydrolysed for the conversion of cellulose to glucose. Finally, the acquired glucose can be metabolised by yeast, mainly *Saccharomyces cerevisiae*, to ethanol [1, 4].

Because of the preferential glucose utilisation of wild-type *Saccharomyces cerevisiae*, the components of hemicellulose, mainly xylose, are hardly utilisable by-products. A separation of the hemicelluloses prior to ethanol fermentation, offers the possibility to use this carbon source for xylose-fermenting microorganisms and, thereby increasing the overall yield [5, 6].

In general, the pre-treatment methods can be classified into four different categories: physical, physio-chemical, chemical and biological processes (Fig. 1.) [2, 7].

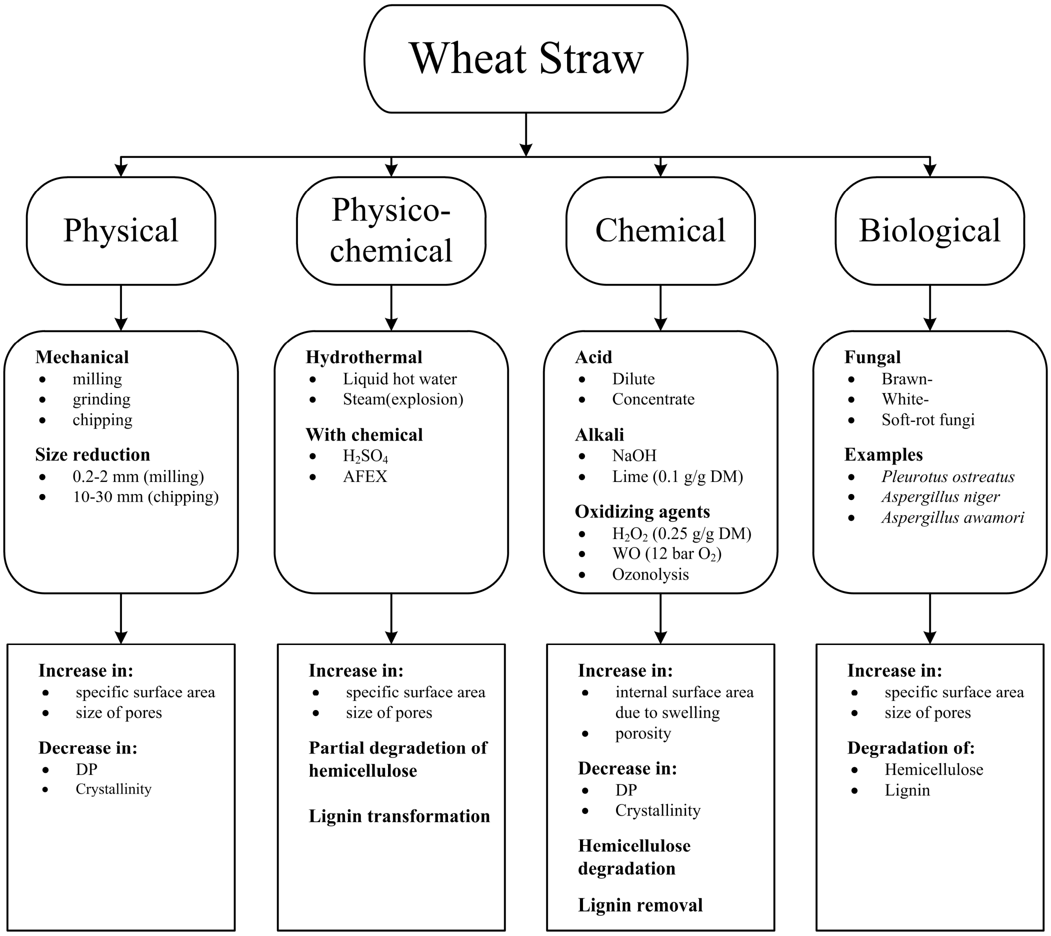

Fig. 1: Different pre-treatment methods and their possible effects on wheat straw [2]

Physical pre-treatments (like milling, chipping and grinding) provoke an increase of the specific surface area. When performing physio-chemical processes like steam explosion or liquid hot water pre-treatment, hemicellulose partially degrades and lignin can be depolymerised. The addition of acidic or alkaline solutions causes lignin to dissolve and hemicellulose to degrade. Biological processes in fungi are also able to degrade hemicellulose and lignin. All pre-treatment processes result in a better accessibility of the substrates for enzymes during bioethanol production [2, 7]. The separation of the hemicellulose from the lignocellulose complex can be affected during pre-treatment with chemicals (water, acid or alkaline solution) or/and by high temperature [7, 8]. These conditions lead to splitting up the hemicellulose into oligomers and xylose by auto hydrolysis. The oligomers and the xylose are present in solution in the liquid fraction [2, 8, 9].

In this study, three different pre-treatment methods were analysed for their ability to solubilise hemicellulose for xylose separation. The methods were steam explosion, acidic and alkaline pre-treatment.

Steam explosion is a very common method during which lignocelluloses are treated with superheated steam for a specific period of time. A sudden pressure release induces an explosive decompression of the steam, thereby breaking up the wheat straw [2]. The combination of temperature and incubation time leads to partial auto hydrolysis of hemicellulose to soluble sugars (oligomer and xylose) and the production of potential inhibitors like acetic acid [7, 10].

During acid pre-treatment, sulphuric acid is added to wheat straw and the mixture is heated. The addition of the acid has a catalytic effect, allowing lower pre-treatment temperatures [2, 5]. The treatment leads to hemicelluloses that are cracked into fermentable sugars by enzymatic hydrolysis [5, 11]. Inhibitors can also be produced by this pre-treatment method [11].

Pre-treatment with alkaline solutions (like sodium hydroxide) just allows a less effective solubilisation of hemicellulose. A better accessibility for enzymes can be achieved [2, 12]

In this study, the sugar content of the pre-treated material was determined in order to draw conclusions about the effectiveness of the pre-treatment methods.

2. Material und Methods

2.1. Raw Material

Wheat straw (*Triticum aestivum*) was obtained from local producers in Austria. The air-dried straw was chopped using 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 %.

2.2. Steam Explosion Pre-treatment

Straw was pre-soaked with distilled water for 15 minutes with a mass ratio of one part distilled water to one part wheat straw. The steam explosion pre-treatment was carried out at the following pre-treatment conditions: incubation time 20 minutes and pre-treatment temperatures 180 °C, 190 °C and 200 °C.

The steam explosion pre-treatment 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 pre-soaked straw. Saturated steam from a high-voltage steam generator was injected within 3 minutes when wheat straw was heated to the pre-treatment temperature under pressure. Then, the temperature was held for 20 minutes. The pressure was released suddenly by opening a ball valve causing the wheat straw to be transferred to the collecting and steam expansion vessel. From there, the pre-treated straw was withdrawn (see Fig.2).

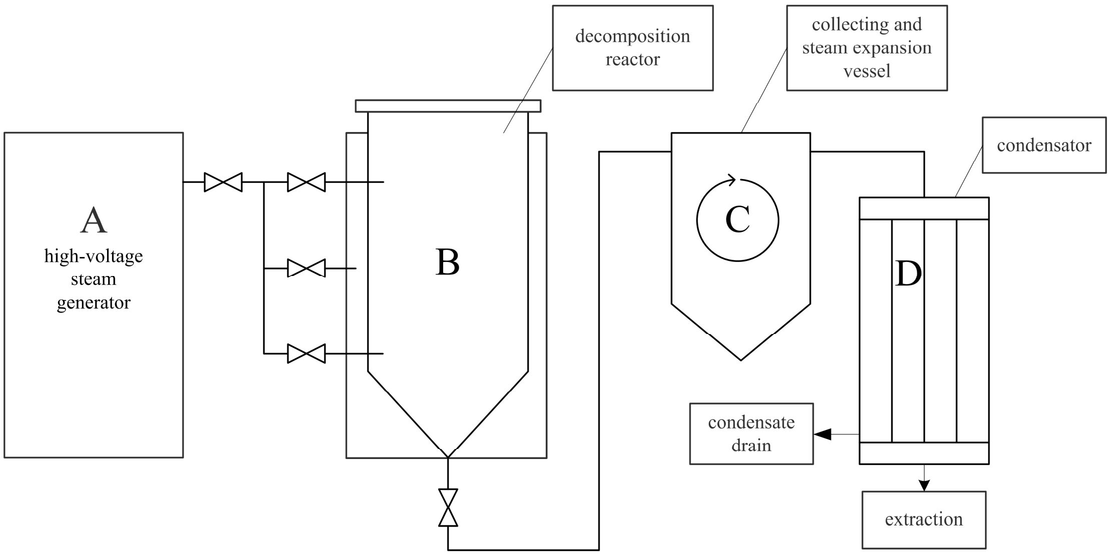

Fig. 2: Laboratory scale steam explosion unit. Main equipment: (A) high-voltage steam generator, (B) decomposition reactor, (C) collecting and steam expansion vessel and (D) condensator.

2.3. Acid Pre-treatment

10 g wheat straw were weighed into a 250 mL flask and pre-soaked for 15 minutes with 90 g sulphuric acid (H_2SO_4) solution in different concentrations (0 %, 0.5 % 1 %, 5 % and 10 %). The pre-treatment was carried out in an 18 L table-top autoclave (CertoClav, Traun, Austria). The following pre-treatment condition was applied: incubation time of 30 minutes, pre-treatment temperature of 100 °C.

2.4. Alkaline Pre-treatment

10 g wheat straw were pre-soaked with 90 g sodium hydroxide (NaOH) solution in different concentrations in a 250 mL flask. The pre-treatment was carried out in an 18 L table-top autoclave (CertoClav, Traun, Austria). The following pre-treatment condition was applied: incubation time of 30 minutes, pre-treatment temperature of 100 °C.

2.5. Separation

The pre-treated wheat straw was separated into solid and liquid fractions by vacuum filtration (Filter paper MN 640 w, Düren, Germany). The solid fractions were washed with distilled water until the wash water was neutral.

2.6. Enzymatic Hydrolysis

Enzymatic hydrolysis is the main step for conversion of oligosaccharides into C5-sugars (Xylose) and C6-sugars (Glucose). For the enzymatic hydrolysis of the liquid fraction after pre-treatment, 5 mL of the liquid fraction was mixed with 4.7 mL citrate buffer, c = 50 mmol L^{-1} (pH 5.0: adjusted with NaOH). The conversion of oligosaccharides into C5-sugars was carried out using the enzyme mixture Accellerase 1500 from Genencor®. The amount of enzyme used was 0.06 mL $mL^{-1}_{liquid\ fraction}$. The activity of the enzymes according to the manufacturer was 47 FPU mL^{-1}. The incubation was carried out for 72 h at 50 °C in a shaking incubator at rotational speed of 2.5 s^{-1}.

The liquid fraction after acid pre-treatment was not enzymatically hydrolysed as the pre-treatment with sulphuric acid directly achieved the quantitative conversion of oligosaccharides into xylose.

The conversion of the remaining oligosaccharides in the solid fraction was also carried out by enzymatic hydrolysis. 10 g pre-treated and washed wheat straw was mixed with 87 g citrate buffer, c = 50 mmol L^{-1} (pH 5.0: adjusted with NaOH). 30 FPU $g^{-1}_{cellulose}$ of the enzyme mixture Acellerase 1500 from Genencor® were added and the reaction was incubated for 72 h at 50 °C in a shaking incubator at rotational speed of 2.5 s^{-1}.

2.7. Chemical Analysis

The dry substance content was analysed with an IR-moisture analyser (Ohaus MB 45, Parsippany, USA). The quantitative determination of the total sugar content (glucose, xylose) from the solid fraction of pre-treated wheat straw and untreated wheat straw was analysed according to the method for determination of carbohydrates in biomass by high performance liquid chromatography (HPLC) by ASTM E1758-95.

Saccharides, organic acids, ethanol and furans from liquids were quantified by HPLC, using an 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 data acquisition was performed per refractive index detection and UV–detection at 210 nm.

3. Results

The main focus of this study is the specific degradation of hemicellulose in utilisable and soluble sugars (oligomers and xylose). In order to achieve this wheat straw was pre-treated by different methods (steam explosion, acid and alkaline pre-treatment) and separated into a liquid and a solid fraction. In the liquid fraction mainly hemicellulosic components like oligomers or xylose should be contained. Hence, the acquired sugar concentrations of the hydrolysed, separated liquid fractions were measured. Additionally, the remaining hemicellulose in the solid fractions also was quantified.

The sum of the sugar concentrations in the solid and in the liquid fractions of each pre-treatment condition shows the hemicellulose/xylose loss during pre-treatment. In addition to the detected degradation and xylose loss during pre-treatment, the bioethanol potential of the different pre-treated wheat straw solids was determined by enzymatic hydrolysis of the solid fraction.

3.1. Steam Explosion Pre-treatment

Different Steam Explosion pre-treated wheat straws were separated into their liquid and their solid fraction. The sugar contents of the fractions were determined. The sum of the determined xylose concentration in the liquid and in the solid fraction is in direct correlation to the mass loss of xylose during pre-treatment. As point of reference, the total xylose concentration of untreated wheat straw was defined. These results are shown in Fig.3.

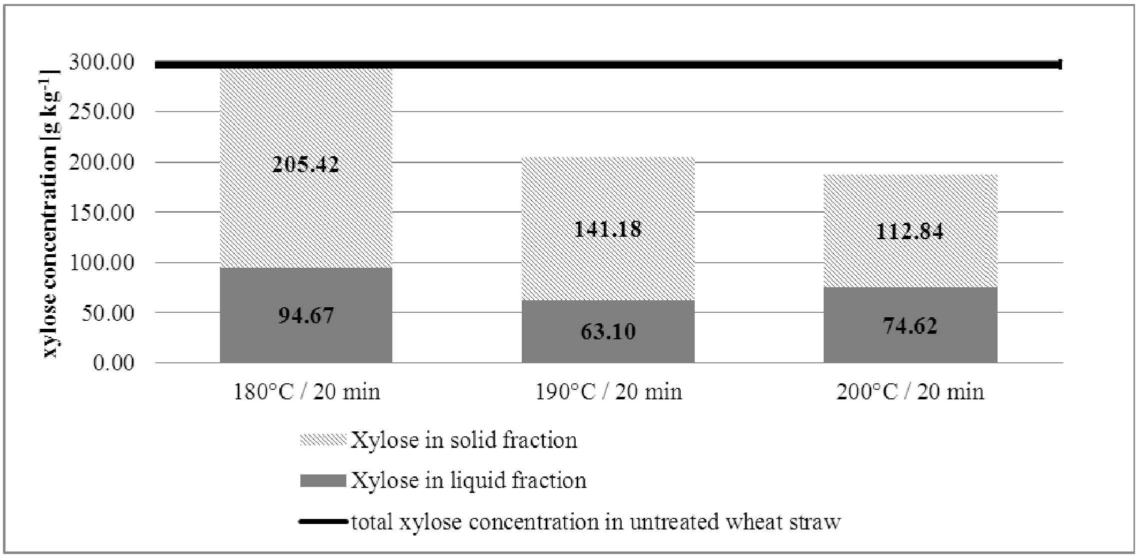

Fig. 3: Xylose concentration [g kg^{-1} straw] of the enzymatically hydrolysed liquid fraction of steam explosion pre-treated wheat straw. The following assay conditions were examined: 180 °C, 190 °C and 200 °C at 20 minutes

The highest amount of xylose in the liquid fraction was detected by a steam explosion pre-treatment at a temperature of 180 °C and a retention time of 20 minutes. This condition resulted in 94.67 g kg^{-1} xylose in the liquid fraction and 205.42 g kg^{-1} xylose in the solid fraction. The sum of these concentrations shows that at this pre-treatment condition no loss of xylose could be detected.

Depending on the severity of the steam explosion pre-treatment, the xylose concentrations in the solid and in the liquid fraction decrease significantly. So, the increasing severity of the steam explosion pre-treatment resulted in an increase xylose loss and a significantly decreased extraction of xylose in the liquid fraction.

A similar effect has been described by Liu et al [13] using chemical incubation with $FeCl_3$. In this study, the authors observed that the increasing severity also causes an increased xylose loss.

3.2. Acid Pre-treatment

During the acid pre-treatment, wheat straw was soaked with different concentrations of sulphuric acid for 30 minutes at 100 °C. The totalled xylose content of the solid and the liquid fraction was compared with the total xylose content of untreated wheat straw. Fig. 4 shows that the resulting xylose concentrations in the liquid and solid fractions are depending on the sulphuric acid concentrations.

The increasing addition of sulphuric acid causes an increasing dissolution of xylose in the liquid fraction, while the xylose concentrations in the solid fractions decrease. A maximum xylose concentration of 120.9 g kg^{-1} in the liquid fraction can be detected after an incubation in 10 % sulphuric acid. In general, it can be said that acid pre-treatment in general has the effect that part of the xylose is lost during pre-treatment.

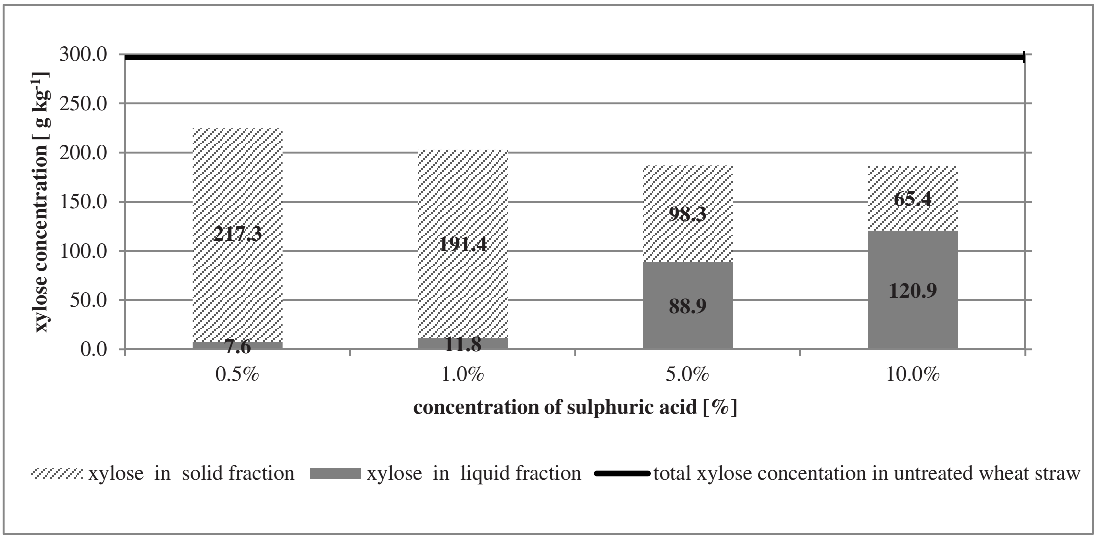

Fig. 4: Xylose concentration [g kg^{-1} straw] of the enzymatically hydrolysed liquid fraction [grey] and solid fraction [black-and-white dashed] of acid pre-treated wheat straw. Following concentrations of sulphuric acid solutions were tested: 0.5 %, 1 %, 5 % and 10 % at 100 °C for 30 minutes

3.3. Alkaline Pre-treatment

Wheat straw was pre-treated with different concentrations of sodium hydroxide at 100 °C. The xylose concentration of the solid and the liquid fraction were determined.

Fig.5 shows that a pre-treatment temperature of 100 °C and a sodium hydroxide concentration up to 5 % results in a xylose concentration increase up to 59.2 g kg^{-1} xylose in the liquid fraction. Beyond these conditions, the xylose concentration is decreasing. In comparison to the total xylose concentration of untreated wheat straw, it can be shown that increasing concentrations of sodium hydroxide lead to a xylose loss.

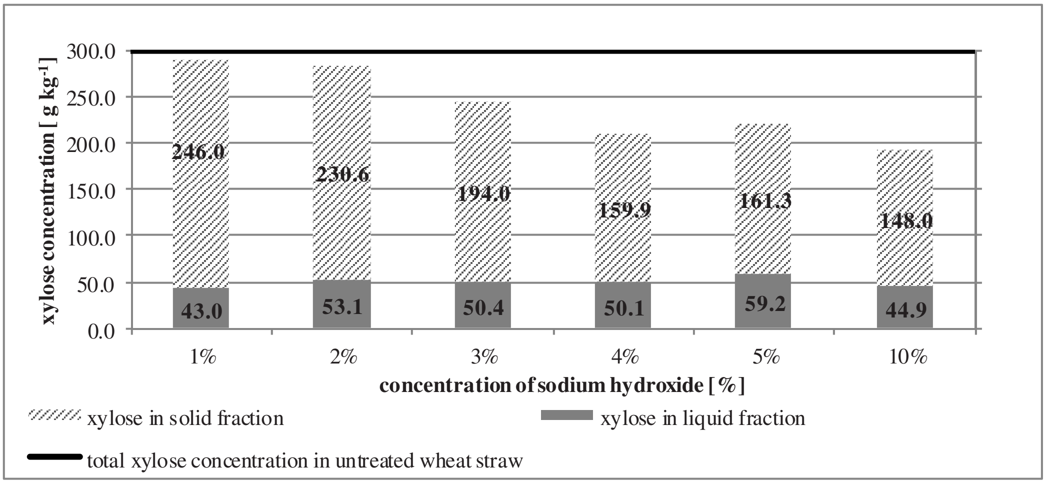

Fig. 5: Xylose concentration [g kg^{-1} straw] of the enzymatically hydrolysed liquid fraction [grey] and solid fraction [black-and-white dashed] of alkaline pre-treated wheat straw. Following concentrations of sodium hydroxide solutions were tested: 1 %, 2 %, 3%, 4%, 5 % and 10 % at 100 °C for 30 minutes

3.4. Comparison of Applied Pre-Treatment Methods

The best results from all applied pre-treatment methods (sections 3.1, 3.2 and 3.3) were compared to determine the most effective hemicellulose to xylose conversion process.

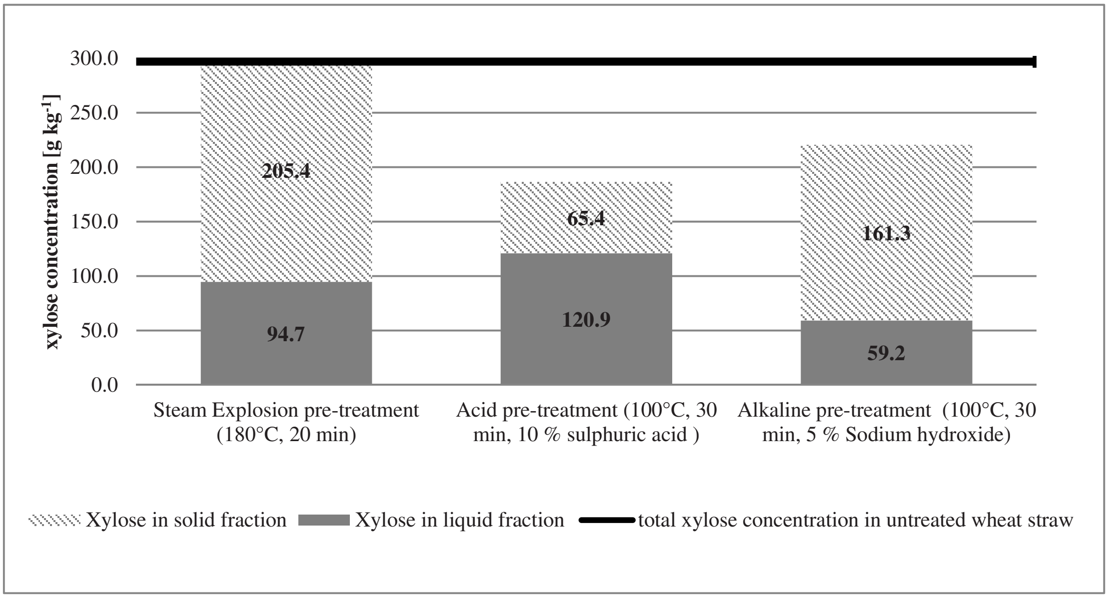

Fig. 6: Xylose concentration [g kg^{-1} straw] of the liquid fraction and solid fraction for evaluating the separation of hemicellulose: xylose concentration in g kg^{-1} straw of liquid fraction [grey], xylose concentration in g kg^{-1} straw of solid fraction [black-and-white dashed]

Fig.6 shows that acid pre-treatment leads to the highest xylose concentration of 120.9 g kg^{-1} (conditions: 100 °C, 30 min, 10 % sulphuric acid), followed by a xylose concentration of 94.7 g kg^{-1} using steam exploded wheat straw (conditions: 180 °C, 20 min). The worst yield is achieved by alkaline pre-treatment (conditions: 100 °C, 30 min, 5 % sodium hydroxide). In addition, the Steam Explosion pre-treatment causes no xylose loss. The addition of chemicals leads to a xylose loss of 100 g kg^{-1} xylose using acid pre-treatment and 80 g kg^{-1} xylose when using alkaline pre-treatment.

Considering hemicellulose separation as a preliminary step to a bioethanol process based on cellulose to glucose conversion and subsequent fermentation, it is necessary to determine the glucose potential of the pre-treated solid fraction. In order to address this question, the solid residues from the different pre-treatment methods were enzymatically hydrolysed.

The results (Fig 7) show that the steam explosion pre-treated wheat achieves a glucose concentration of 224.5 g kg^{-1}, followed by 94.7 g kg^{-1} of alkaline pre-treated and 89.1 g kg^{-1} of acid pre-treated wheat straw. Just small amount of glucose were extracted from the solid fraction.

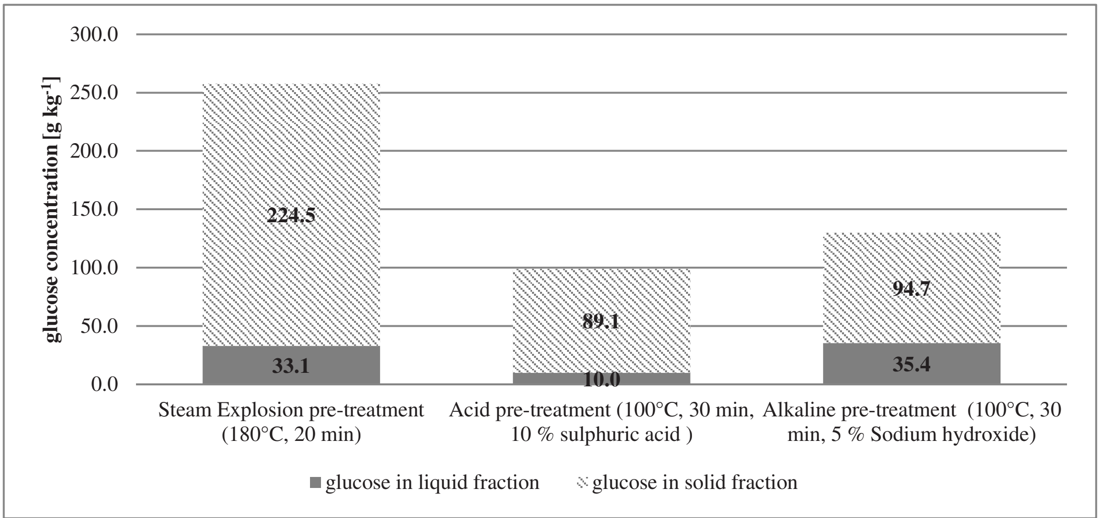

Fig. 7: Glucose concentration [g kg^{-1} straw] of the liquid fraction and solid fraction for evaluating the bioethanol potential: glucose concentration in g kg^{-1} straw of liquid fraction [grey], glucose concentration in g kg^{-1} straw of solid fraction [black-and-white dashed]

4. Conclusion

Concerning the highest yield of sugars, the steam explosion pre-treatment is the best method for obtaining glucose (also the summarized sugar yield) and the acid pre-treatment for obtaining xylose.

Based on our results, the acid-pre-treatment with sulphuric acid seems to be the best method for separation of hemicellulose in the liquid fraction, directly followed by the steam explosion pre-treatment.

If a combination of xylose separation and cellulose conversion to glucose should be pursued steam explosion pre-treatment achieves the highest concentration of sugars (sum of xylose and glucose). Generally, it can be said that the desired end product determines which pre-treatment method should be used in the process.

The combination of acid pre-treatment and steam explosion pre-treatment might allow a better xylose separation and a better conversion of cellulose to glucose. This hypothesis will be tested in further experiments.

Acknowledgements

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