

## IMPROVED PRETREATMENT PROCESS OF WHEAT STRAW WITH DIRECT STEAM INJECTION

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**ABSTRACT:** The rising demands for biofuels and other bio-based chemical products enlarge the demand of raw material feedstock. Lignocelluloses, especially from residual biomass like straw, have great potential to fit that gap. The processing of lignocelluloses by an enzymatic saccharification requires a set of pretreatment steps. Different kinds of pretreatment techniques are described in the literature. One mostly used, the diluted acid pretreatment with mineral acids, is a state of the art technique and used in this study. The purpose of this work was an effective diluted acid pretreatment of whole bale wheat straw with direct steam injection in pilot scale. Additional mechanical pretreatment by using a hammer or cutting mill was renounced. Mechanical pretreatment increases the energy demand and makes the process uneconomical. The results of the study showed the high effectiveness of the direct steam injection. The up-scale process in the 425 L-Reactor was successful. High sugar yields were reached in the pretreatment process of a complete straw bale. Almost 68 % of the total available sugar was released after the subsequent enzymatic hydrolysis.

**Keywords:** agricultural residues, biomass, lignocelluloses, steam, wheat straw

### 1 INTRODUCTION

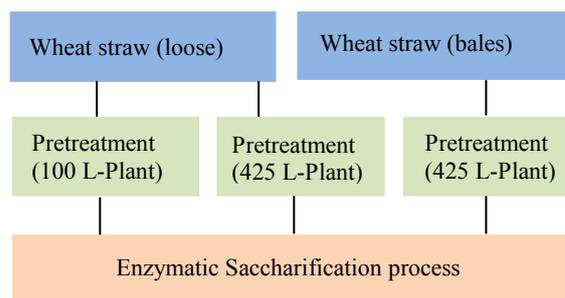
The demand for biobased products like biofuels or raw chemicals grows every year. Starch from corn or sugar cane are the dominant renewable feedstock today [1, 2]. The competition of corn or sugar cane as foodstuffs or renewable raw material in the non-food sector is very problematic aspect and center of current political or ethical controversies [3]. The rising demands of biobased products will intensify the problem and makes a reconsideration of alternatives mandatory for the future. Lignocelluloses may cover the future requirements of renewable feedstock. In Europe, wheat straw has the greatest potential of all agricultural residues because of its wide availability and low cost [4].

Lignocellulose is a complex composition of hemicelluloses, celluloses, lignin and inorganic components. Those structures protect the fiber structure of the lignocelluloses against microbial degradation [5]. Without pretreatment, the enzymatic saccharification works ineffective, because the accessibility for different kinds of cellulolytical activities is restricted. The accessibility depends on different factors, the crystalline structure of the celluloses and the lignin proportion of the lignocelluloses [6]. Crystalline structures restrict the enzymatic saccharification [7]. High sugar yields require an effective pretreatment of the lignocelluloses to decrease the crystallinity and remove the lignin.

Different pretreatment techniques are described in the literature [6]. One mostly used, the diluted acid pretreatment with mineral acids, is a state of the art technique for the pretreatment of biomass feedstock. The biomass is pretreated at moderate temperatures between 120 °C and 180 °C with acid concentrations between 0.25 % (v/v) and 1 % (v/v) [8, 9]. Previous studies showed promising results for the pretreatment of wheat straw in diluted acid. The disadvantages of many studies were the small laboratory scale and the mechanical pretreatment that was used for the pretreatment. Most of the studies used a hammer or cutting mill for granulation [10, 11]. Smaller particle size reduced the crystallinity and improved the enzymatic saccharification of the lignocelluloses [12]. The disadvantage of the mechanical pretreatment is the additional energy demand for the grinding process. The energy demand depends on the

chosen particle size of the wheat straw. The specific energy consumption for the milling process with hammer mill screen size of 0.8 and 3.2 mm were 51.6 and 11.4 kW h t<sup>-1</sup>[13]. In a study of the National Renewable Energy Laboratory one-third of total energy inputs was required for mechanical treatment of biomass in the bioethanol conversion [14]. Another critical point of wheat straw as feedstock in a biorefinery is the low bulk density. An economically efficient up-scaling process required great amounts of wheat straw. Complete wheat straw bales increased the loading capacity for pretreatment plants and reduced additionally the transportation cost for the biomass to the biorefinery [15].

The purpose of the work was the demonstration of an up-scaling process of the pretreatment with complete wheat straw bales. Figure 1 shows a graphical presentation of the wheat straw processing in this study. First, a pretreatment of loose wheat straw was performed in a 100 L-Reactor. Second, an up-scale process in a 425 L-Reactor was done. In a last step, a complete bale of wheat straw was pretreated.



**Figure 1:** Graphical presentation of the wheat straw processing scheme in this study.

### 2 MATERIALS AND METHODS

#### 2.1 Substrate Preparation and composition

Wheat straw was harvested in Bad Kreuznach (Southwest Germany) and provided by the farm Raeder. The straw was pressed in bales with pack density of 76.8 kg/ m<sup>3</sup> and stored protected against rain or humidity in a

storage container. No additional mechanical pretreatment was done. The determination of the dry matter content was performed at 105 °C for at least 12 h. The composition of the used wheat straw was analyzed by the procedure of the National Renewable Energy Laboratory (NREL) [16].

## 2.2 Enzymes

The enzymatic saccharification was performed with a commercial enzyme preparation of Novozymes (Cellic2®, Novozymes A/S, Denmark). The activity was determined in Filter Paper Units (FPU) right before usage. The determination of the FPU was done by a procedure of the NREL [17].

## 2.3 Diluted acid pretreatment of the wheat straw

The hydrothermal pretreatments were performed in the 100 L and 425 L stainless steel reactors from the Schmitt Company (Weselberg, Germany). Figure 2 shows both used pretreatment plants. The wheat straw was mixed with water and technical nitric acid (65 % (v/v)). The dry matter content (DM) was specified with 13.5 % (w/v). The final nitric acid concentration was adjusted to 0.32 % (v/v). The heating process was done with supercritical hot steam injected in the reactor floor. The pretreatment process was done for 30 min at 150 °C. Sudden expansion at 2 bar pressure separated the liquid and solid fraction. Samples of the liquid fraction were stored in a refrigerator for analysis. All tests were performed as duplicate.



**Figure 2:** Pretreatment plants with 100 L-Reactor (left) and 425 L-Reactor (right).

## 2.4 Enzymatic saccharification

The enzymatic saccharification was performed at 50 °C and a pH value of 5.0 in sodium-acetate buffer with 50 µg mL<sup>-1</sup> of the antibiotics gentamicin and chloramphenicol. The saccharification process was done with 15 g pretreated (see section 2.3) wheat straw in 150 mL buffer solution. The enzyme dosage depended on enzyme activity and dry matter content of the wheat straw. The determination of the dry matter content was done by 105 °C for at least 12 h. Saccharification experiments were performed at a dosage of 18.5 FPU/g dry matter content wheat straw for 24 h at 200 rpm in a shaking water bath. Samples were treated for 10 minutes in 100 °C to stop the enzymatic reaction. Samples of the liquid fraction were stored in a refrigerator for analysis. Sugar determination of the supernatant was performed by HPLC. All tests were performed as duplicate. In order to determine the proportion of celluloses, hemicelluloses and lignin after the enzymatic saccharification, the NREL compositional analysis was done again [16]. The pretreated wheat straw was washed with deionized water to remove the sugars from the surface.

## 2.5 Analysis of sugars and by-products

Samples from pretreatment and enzymatic saccharification were centrifuged at 13000 rpm for 10 min. The supernatant was analyzed for dissolved sugars (glucose and xylose) and common by products (Acetic acid, 5-HMF, and furfural) by HPLC, in a chromatograph equipped (Column Sepax HPLC, Carbomix H-Form, NP5, 5 µm, 7.8 x 300 mm) with a refractive index detector at 75 °C. 0.01 N H<sub>2</sub>SO<sub>4</sub> was used as eluent at a flow rate of 0.7 mL/min.

The determination of the phenolic components concentration was done with a spectrometric method. 1 mL of the supernatant was mixed with 0.5 mL Folin-Ciocalteu reagents (1:2 diluted with deionized water) and incubated for 15 min at 25 °C. Next, 5 mL of a 2 % (v/v) sodium carbonate solution (dissolved in 0.1 M sodium hydroxide) was added and mixed again. After 30 min the adsorption at 730 nm wavelength was measured. A calibration curve was created with vanillin. The total concentration of all phenolic components was determinate.

## 3 RESULTS

### 3.1 Substrate compositional analysis

The used wheat straw had a dry matter content of 96 % (w/w). Table I showed the celluloses, hemicelluloses and lignin proportion of the wheat straw based on the compositional analysis of the NREL [16].

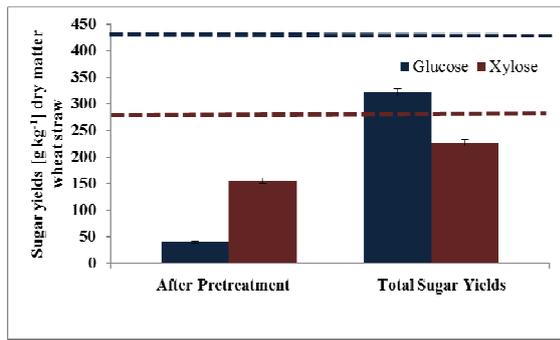
**Table I:** Wheat straw Composition based on the compositional analyses of the NREL.

Component	Dry solids (% w/w)
Celluloses	39.6 %
Hemicelluloses	28.9 %
Lignin	17.2 %

The theoretical yields for glucose and xylose were calculated based on the celluloses and hemicelluloses proportion in table I. The calculated yield for the glucose was 429 g kg<sup>-1</sup> DM and 280 g kg<sup>-1</sup> DM wheat straw (WS) for the xylose. The total sugar yield was 709 g kg<sup>-1</sup> DM wheat straw.

### 3.2 Diluted acid pretreatment of loose wheat straw in a 100 L-Reactor

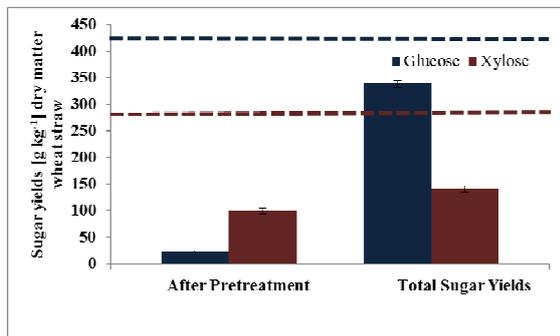
Figure 3 shows the glucose and xylose yields after the pretreatment process of loose straw in the 100 L-Reactor and after enzymatic saccharification based on untreated wheat straw. The dotted lines mark the theoretical yield for the glucose (blue) and xylose (red). After the pretreatment process, almost 50 % of the theoretical contained xylose was released in the liquid fraction. In the enzymatic saccharification, most of the celluloses were degraded into glucose. Based on 1 kg DM WS, 321 g was released, that corresponds with 74 % of the theoretical glucose yield. The total xylose yield after the enzymatic saccharification was 74 % of the theoretical yield. The total sugar yield after the pretreatment and enzymatic saccharification was 574 g carbohydrates kg<sup>-1</sup> DM WS, what corresponds with 81 % of the theoretical total sugar yield.



**Figure 3:** Glucose and xylose yields after the pretreatment of loose wheat straw in 100 L-reactor and subsequently enzymatic saccharification. The dotted lines mark the theoretical glucose and xylose yields. The error bars show the standard deviation. All experiments were done as duplicate.

### 3.3 Up scale of the pretreatment process in the 425 L-Reactor

After the successful establishment in the 100 L-Reactor, the pretreatment process was scaled up in the 425 L Reactor. Figure 4 shows the calculated glucose and xylose yields after the pretreatment process and the enzymatic saccharification based on untreated wheat straw. The dotted lines mark again the theoretical yields of glucose and xylose. Similar to the pretreatments in the 100 L scale, most of the contained glucose was released after the enzymatic saccharification. 339 g glucose kg<sup>-1</sup> DM WS was recovered after the enzymatic saccharification. That corresponded with 79 % of the theoretical yield. With 480 g total sugars kg<sup>-1</sup> dry mass wheat straw, 67 % of the theoretical sugar yield was reached. Compared with the pretreatment in the 100 L-Reactor, the glucose yield was higher, but the total xylose yield in the 425 L-Reactor was significantly lower. Only 50 % of the theoretical xylose yield was reached after the enzymatic saccharification. That explains the lower total sugar yield compared with the 100 L-Reactor pretreatment.

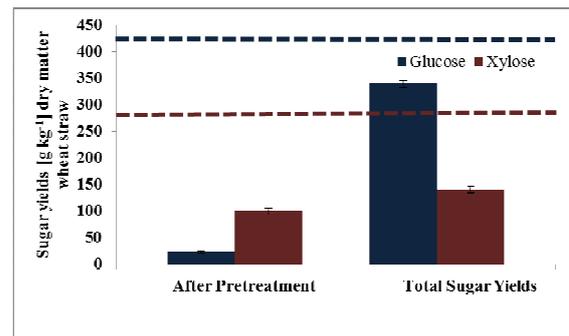


**Figure 4:** Glucose and xylose yields after the pretreatment of loose wheat straw in the 425 L-reactor and a subsequently enzymatic saccharification. The dotted lines mark the theoretical glucose and xylose yields. The error bars show the standard deviation. All experiments were done as duplicate.

### 3.4 Pretreatment of complete bales of wheat straw in the 425 L-reactor

Figure 5 shows the calculated glucose and xylose yields after the pretreatment process of a complete straw

bale in the 425 L-Reactor and the follow enzymatic saccharification based on untreated wheat straw. The released sugar yields were lower than in the previous experiments (see Figures 2 and 3). The total glucose yield after the enzymatic saccharification was 323 g glucose kg<sup>-1</sup> DM WS. That corresponds with 74 % of the theoretical calculated glucose yield. Similar to the previously experiments (see Figures 2 and 3), the reached xylose yield was significantly lower. Only 58 % of the theoretical xylose was released after the enzymatic saccharification. The total sugar yield present after the pretreatment and enzymatic saccharification was 487 g carbohydrates kg<sup>-1</sup> DM WS, what corresponds with 68 % of the theoretical yield.



**Figure 5:** Glucose and xylose yields after the pretreatment of a bale wheat straw in 425 L-reactor and a subsequently enzymatic saccharification. The dotted lines mark the theoretical glucose and xylose yields. The error bars show the standard deviation. All experiments were done as duplicate.

Table II summarizes the xylose and glucose of all three experiments. Comparable with the theoretical glucose yield, most of the contained glucose was released from the used wheat straw. There were no negative effects in the up-scaling or the pretreatment process of complete straw bales. In comparison to the 100 L-Reactor pretreatment, the xylose yields in the 425 L-Reactor pretreatments were significantly lower. Only two-third of the theoretical xylose was released after the enzymatic saccharification.

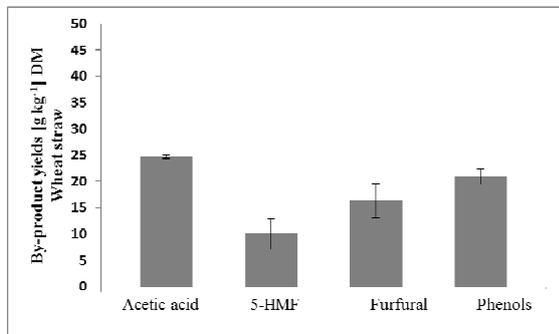
**Table II:** Summary of the released glucose, xylose and total sugar yields corresponds with the theoretical yields.

	Glucose yield [dry solid w/w]	Xylose yield [dry solid w/w]	Total sugar yields
Lose straw (100 L-reactor)	74 %	72 %	81 %
Lose straw (425 L-reactor)	78 %	50 %	64 %
Straw bale (425 L-reactor)	74 %	58 %	68 %

### 3.5 By-product formation during the pretreatment process

Figure 6 shows the calculated yields of the main by-products, formed in the pretreatment process of loose wheat straw in the 100 L-Reactor. The diluted acid

pretreatment supports the releasing and forming of different by-products. In this context the furans, furfural and 5-HMF, plays an important role. High temperatures in combination with acid benefits the formation of both furans [18]. In a chemical conversion, the xylose or glucose reacts to furfural and 5-HMF. High furan concentrations also inhibit the growth of microorganism [19]. Other important by-products are the acetic acid and a set of different phenolic compounds. Acetic acid was released in the degradation of the hemicelluloses. A couple of microorganisms, especially yeasts, are sensitive for higher acetic concentrations [20]. Lignin consists of different phenolic compounds. In the pretreatment process, parts of the lignin were removed. High concentrations of phenolic compounds also inhibit the growth of microorganism [21]. In order to avoid the sugar yield losses and the forming of growth inhabiting substances, the process parameters for the pretreatment must be adapted.



**Figure 6:** Yields of most common by-products after the pretreatment of wheat straw. The error bars show the standard deviation. All experiments were done as duplicate.

### 3.6 Composition of the wheat straw after the pretreatment and the enzymatic saccharification

Table III shows the results of the compositional analyses after the pretreatment process and the enzymatic saccharification. A sample of the pretreated wheat straw from the 100 L-Reactor was taken. The pretreatment process degraded more than 50 % of the hemicelluloses. As a consequence, the hemicelluloses proportion decreased and the celluloses in solid fraction rose up. After the subsequently enzymatic saccharification, the celluloses percentage was at almost 30 %. This suggests that the enzymatic saccharification was not complete. The hemicelluloses proportion was almost completely removed after the pretreatment process and the enzymatic saccharification. That means, parts of the released xylose could not be recovered during the pretreatment process.

**Table III:** Results for the compositional analyses of the wheat straw after the pretreatment and the enzymatic saccharification (ES) process. The analyses were done as triplicate.

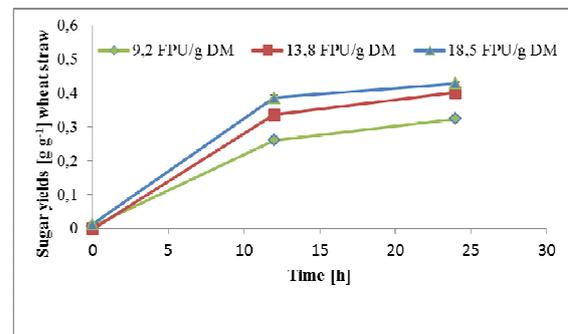
Component	Dry solids (% w/w)	
	After TDH	After ES
Celluloses	51,2 %	29,8 %
Hemicelluloses	13,8 %	4,5 %
Lignin	27,6 %	47,2 %

### 3.7 Balancing of the enzymatic saccharification as an example of the 100 L-Reactor

The compositional analyses in figure 3 show that the celluloses was not complete degraded after the enzymatic saccharification. The celluloses proportion after the pretreatment was 51.2 % (dry solid w/w). Based on that the theoretical glucose yield was 563 g kg<sup>-1</sup> DM WS. The Glucose yield after the enzymatic saccharification was 399 g kg<sup>-1</sup> DM pretreated WS, what corresponds with 70 % of the theoretical glucose yield. That confirmed the results of the compositional analyses in table 3 and showed the challenges in the enzymatic saccharification.

### 3.8 Enzymatic saccharification of the pretreated straw with different enzyme dosage

The results of the balancing after the enzymatic saccharification, indicates that the enzymatic degradation was incomplete. Couple parameters have influence on the enzymatic saccharification. An important one was the enzyme concentration used in the process. For the enzyme saccharification an enzyme preparation of Novozymes (Novozymes A/S, Denmark) was used. The enzyme activity was specified with 231,1 FPU mL<sup>-1</sup> [17]. With 9,2 FPU g<sup>-1</sup> DM WS, 13,8 FPU g<sup>-1</sup> DM WS and 18,5 FPU g<sup>-1</sup> DM WS three different enzyme dosage were tested. The enzymatic saccharification was done for 24 h at 50 °C and pH 5.5. Figure 7 shows the calculated glucose yields during 24 h incubation. The glucose yields increased with higher enzyme dosage. After 24 h 0.42 g glucose g<sup>-1</sup> DM WS was released by an enzyme dosage of 18.5 FPU g<sup>-1</sup> DM. Enzyme dosage of 9.2 FPU g<sup>-1</sup> DM decreased the glucose by almost 30 % to 0.31 g g<sup>-1</sup> DM WS. That implies an activity loss during the enzymatic saccharification, which was responsible for the incomplete degradation of the celluloses.



**Figure 7:** The glucose yields after an 24 hour enzymatic saccharification at different enzyme concentrations. The error bars show the standard deviation. All experiments were done as duplicate.

## 4 DISCUSSION

Wheat straw represents a well useable low cost feedstock for the rising demand of renewable raw materials. The highly complex structure of the lignocelluloses complicated an enzymatic saccharification. Especially the crystalline structures and the lignin proportion reduce the accessibility for the cellulolytic enzymes. As a consequence, a pretreatment process for the wheat straw was necessary. Different pretreatment techniques are described in the literature [8]. The hydrothermal diluted pretreatment, used in this

study, is a state of the art technique for the pretreatment of lignocelluloses. The purpose of the work was the demonstration of an effectiveness pretreatment process with direct steam injection to reached high sugar yields. The results show high sugars yields after the pretreatment and the enzymatic saccharification without additional mechanical pretreatment and in a pilot scale.

In the first step, the pretreatment process of loose wheat straw was performed in the 100 L-Reactor. Upper sugar yields after the enzymatic saccharification demonstrated the success of the direct steam injection pretreatment. Almost 81 % of the total carbohydrates were released after the enzymatic saccharification of the pretreated wheat straw. Primarily most of the celluloses were degraded into glucose. 72 % of the theoretical glucose yield was reached after the enzymatic saccharification. Next, the direct steam injection procedure was up-scaled from the 100 L-Reactor in the 425 L-Reactor. The results in Figure 4 show the general possibility of an up-scaling process. The released glucose yield reached 78 % of the theoretical glucose yield. 67 % of the theoretical sugar yield was released after the enzymatic saccharification. The last step described the pretreatment of complete wheat straw bale. The first two experiments were performed with loose straw. Wheat straw has a low density, an economically efficient procedure implies the use of complete bales [15]. The pretreatment process of complete bales was performed in the 425 L-Reactor. The results demonstrated the success of the direct steam injection pretreatment. 74 % of the contained glucose was released from the celluloses. Similar to experiment with loose straw in the 425 L-Reactor, the total xylose yield after the enzymatic saccharification was significantly lower and reached only 58 % of the theoretical yield.

The results showed the effectiveness of the direct steam injection. Previous studies [11, 22] showed in general the positive effect of the hydrothermal diluted pretreatment in the laboratory scale. Compared to other studies [11, 22, 23] in the here presented study no granulation process in a hammer or cutting mill was done. Saha et al. [11] released 58 % of the glucose after the enzymatic saccharification. In Pedersen et al. [10] only 25 % of the glucose was released. This comparison shows the high effectiveness of the steam pretreatment in this study. Two factors influenced the success of the steam pretreatment. The high temperatures in combination with the additional diluted acid reduced the crystalline structure and improved the enzymatic saccharification. The second factor implies the use of supercritical hot steam injected into the reactor for the heating process. The steam not only heated up the water-straw mix. It also disintegrated the structure of the lignocelluloses and improved the accessibility for the cellulolytical enzymes. Other studies confirmed that and showed the positive effect of steam pretreatment in the laboratory scale with previously mechanical pretreatment. Erdei et al. released almost 95 % of the contained glucose after the pretreatment process and enzymatic saccharification [23]. However, they used with 190 °C a much higher pretreatment temperature. High temperatures are critical, because a lot of energy is necessary, what has a negative economic efficiency effect on the pretreatment process. In the study of Erdei et al. the wheat straw was also mechanical pretreated, what also increased the energy demand. To explain how the steam pretreatment in the presented study work, another

variation of the steam pretreatment, the steam explosion (e.g. Ammonium fiber steam explosion (AFEX)) shows the mode of effect. High temperatures between 190 °C and 270 °C and high pressure were used to disintegrate the fiber structure in a couple minutes. The sudden change in pressure destroy the fiber and decreased the crystallinity structure of the celluloses [24]. In the diluted acid pretreatment, used in this study, the temperatures and pressure were lower; therefore the needed pretreatment times were longer.

The results also showed the significant lower xylose yields after the enzymatic saccharification, especially after the scale up into the 425 L-Reactor. Only two-third of the theoretical xylose yield was reached. Different declarations are possible. The results of the compositional analysis in table 3 showed that the proportion of hemicelluloses decreased fewer than 5 % after the enzymatic saccharification. That shows, almost the complete hemicelluloses was released during the pretreatment process and the following enzymatic saccharification. The hydrothermal diluted acid pretreatment of biomass like wheat straw forms a couple of different by-products. Figure 6 showed the yields for the common by-products. Particularly the both furans furfural and 5-HMF, which formed of glucose or xylose, decreased the released sugar yields. High furan concentration not only reduced the xylose yields. Also the growths of microorganisms were inhibiting. Different factors effects the effectiveness of the pretreatment and the forming of by-products. One of them, the pretreatment time has a great influence of the xylose yields. Results from other experiments performed in our institute (Data not shown) showed a correlation between the pretreatment times with the reached xylose yields and the forming of furans, which was also demonstrated Pedersen et al. [22]. They pretreated the wheat straw for only 10 min and released almost 90 % of the contained xylose. Lower pretreatment durations increased the xylose yield and reduced the concentration of furans like 5-HMF and furfural.

Significant proportion of celluloses was not degraded after the enzymatic saccharification. One third of the contained glucose was not recovered. The experiments with different enzyme dosages give a hint for the reasons. Low enzyme concentrations decreased the reached glucose yields. The results imply a significant activity loss during the enzymatic saccharification. Ximenes et al. [25] and Berlin et al. [26] described similar effects. Two factors play an important role for the stability of enzymes activity. First, the heat instability of enzymes and second the interaction between the cellulolytical enzymes and the lignin proportion lignocelluloses. Couple of studies described the negative effect of the lignin to the activity of the enzymes preparations [25, 26]. The enzymes adsorb on the lignin and irreversible lost their activity. Own experiments (data not shown), showed the high activity losses in the first hours of the enzymatic saccharification because of the lignin interaction. The results of the saccharification show the high enzyme dosage are necessary for a complete saccharification process. High enzyme concentrations increased the cost for products based on lignocelluloses. Some studies showed the positive effect of some reagents like Tween 80 or BSA [27, 28] on the enzymatic activity. But the application of additional reagents induces more cost.

## 5 CONCLUSION

Many previous studies for the diluted acid pretreatment of wheat straw were performed only in the laboratory scale [22, 23, 29]. They also done mechanical pretreatment with hammer or cutting mill for reduced the particle size to improve the enzymatic saccharification. That increases the energy consumption and makes the process uneconomical. The here presented results show the effectiveness of the diluted acid pretreatment with steam injection and the possibility by using whole bales of straw in a pilot scale. No mechanical pretreatment was necessary. Almost 68 % of the contained sugars were released after the pretreatment of a whole straw bale and the following enzymatic saccharification. Upper sugar recovery yields were prevented by highly enzyme activity losses in the enzymatic saccharification. The heat instability and the activity loss by interaction with the lignin proportion of the lignocelluloses were the reasons. For complete glucose recovery after the enzymatic saccharification greater amounts of enzyme preparations are necessary. In this case, the cost for enzyme preparations had to come down. Improve processes in the enzyme production would be necessary. Another possibility for lower pretreatment cost could be an enzyme engineering to reduce the heat instability or sensitivity against lignin components.

The enzymatic saccharification in this work was performed in a small scale. Further works are necessary to upscale this process and reach high sugar yields. The energy demand should be as low as possible.

## 6 ACKNOWLEDGEMENTS

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## 8 LOGO

