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A thermomechanical pretreatment to improve enzymatic hydrolysis of wheat straw

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Abstract
Wheat straw was pretreated with a thermomechanical process developed in our laboratory to increase the enzymatic hydrolysis extent of potentially fermentable sugars. This process involves subjecting the lignocellulosic biomass for a short time to saturated steam pressure, followed by an instantaneous decompression to vacuum at 50 mbar. Increasing of the heat induced by the saturated steam result in intensive vapour formation in the capillary porous structure of the plant material and the subsequent release of the pressure to vacuum allows fixing the structure. The process parameters tested in this study were the processing pressure between 3 and 7 bar and the particle size of crushed wheat straw. Enzymatic hydrolysis was performed on the pretreated solids by celluclast (1.5L). The results showed that the hydrolysis yield of wheat straw treated at pressure levels of 3, 5 and 7 bar for a processing time of 15 min was strongly improved compared to hydrolysis yield of un-pretreated wheat straw.

Keywords: pretreatment, wheat straw, enzymatic hydrolysis, glucose

1. Introduction

Currently, corn is the primary raw material for ethanol production in the United States (Silverstein et al., 2007) and sugarcane in Brazil (Solomon et al, 2007). However, the corn and sugarcane to ethanol industry draws its feedstock from food and is quite mature with little possibility of process improvement. Biofuels from food sources are known as first-generation biofuels. Lignocellulosic biomass is an interesting and necessary enlargement of the biomass used for the production of renewable biofuels. It can be used as a potential substrate to produce ethanol which is considered as one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilisation of fossil fuels (Cardona and Sanchez, 2007). These oils are known as second-generation biofuels. Lignocellulosic materials to be considered for ethanol production include wood, crops from annual plants, agricultural residue and waste paper (Tabka et al., 2006). However, there are a lot of challenges and obstacles such as cost, technology and environmental issues that need to be overcome. Considerable research efforts have been made to improve conversion yields of lignocellulosic materials by the insertion of pretreatment step before to enzymatic hydrolysis. The purposes of pretreatment is increasing of porosity as well as removing the lignin to make lignocellulosic materials more accessible to enzymes by destroying the cell structure and break down the various physical and chemical barriers (Cara et al, 2008; Mosier et al, 2005). These pretreatments can be physical, physico-chemical, chemical or biological (Hu and Al, 2008) and can be combined between them, with the objective to obtain a high yield from enzymatic hydrolysis, to generate small quantities of co-products and inhibitors of fermentation while reducing costs.

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The purely physical processes of pretreatment can be summarized by an intense mechanical grinding in order to increase the accessible surfaces to enzymes, thus supporting the further hydrolysis and reducing the polymerization degree of cellulose. This kind of pretreatment can be interesting if the granulometry is very low, which limits its interest and its use due to the energy cost of grinding (< 2 mm), (Ogier et al., 1999). Steam explosion pretreatment consists to expose the biomass up to high pressure (15 to 50 bar) and temperature (180 to 250 °C) in presence of steam during a determined time, up to 90 minutes (Sassner et al., 2008), followed by a rapid reduction in pressure, in order to breakdown the lignocellulosic structure. This technology was implemented at industrial scale with batch processes (Stake and Iotech) (Ballerini and Alalzadrd-Toux, 2006). The treatment leads to a partial self-hydrolysis of hemicelluloses, depolymerisation of lignin and a destructuration of cellulose, largely dependent on treatment temperature. However, the yields of enzymatic hydrolysis are about 50 % and some inhibiting compounds of the fermentation appeared following the pyrolysis of cellulose. Steam explosion is often combined with acidification with H_2SO_4 or SO_2 (Silverstein et al., 2007; Rodriguez et al., 2007), to improve the yield of hydrolysis (90% of potential glucose and nearly 100% of sugars resulting from hemicelluloses) at moderate temperatures (150 to 200 °C against 250 °C in absence of catalyst) during 15 to 20 minutes, thus minimizing the formation of sugar degradation products. Steam explosion with acid catalysis (H_2SO_4) exhibited better results than other techniques such as the Afex process (Ammonia Fiber EXplosion) developed in USA (Balan et al, 2008). Among the advantages of steam explosion, it was noted (Ballerini and Alalzadrd-Toux, 2006) a low energy consumption, small quantities of generated undesirable products, a simplicity of implementation in batch, low quantities of chemical reagents and a good adaptation to the big particles size such as shavings. The steam explosion is thus considered to be one of the most promising methods to make biomass more accessible to enzymes. In order to obtain a higher yields of enzymatic hydrolysis than those described in the literature and limiting the production of inhibitors of alcoholic fermentation, a thermomechanical pretreatment termed "D.I.C." process (in french: Détente Instantanée Contrôlée) was performed on wheat straw. This physical process is close to steam explosion technology (Zhang et al., 2008; Viola et al., 2008). The difference is that the D.I.C treatment comprises two additional steps: instauration of initial vacuum before injection of the steam. This step, allows to reduce the resistance of air and thus to facilitate the diffusion of steam into the product. Consequently, the time necessary to reach the steam equilibrium temperature is reduced. The second step consists to an abrupt decompression which carries out towards the vacuum (50 mbar) instead of atmospheric pressure like it is the case with the steam explosion process (Maache-Rezzoug et al., 2008). Due to the instantaneous character of this transformation as well as the adiabatic nature of the transition of steam inside the product, the water vaporisation induces a fast cooling. The temperature is quickly stabilized at a balance temperature of the considered final pressure, limiting the reactions of degradations. This study aims to assess the effect of processing steam pressure and processing time of the D.I.C pretreatment. The results indicate that the two parameters improve significantly the yield of fermentable sugar, with a strongest effect for the processing pressure. The yield increased from 10g glucose/100g d.m to 32 g glucose/100g d.m for non-pretreated wheat straw and wheat straw treated at 7 bar, respectively

2. Experimental procedures

2.1 Plant material

Wheat straw used in this study grown in Charente-Maritime region in France. It was ground in Gindomix (GM 200) Retsch crusher at 7500 RPM during 50 seconds and sieved to obtain two particle sizes: 600 to 1000 µm and 50 to 600 µm. The third size was obtained by cutting and calibrated in sieve to obtain a fraction ranging between 1000 and 8000 µm. Due to the low mechanical effect this fraction was qualified as “non crushed straw”. The raw moisture content, measured at 105 °C, with infrared balance (Sartorius MA 30) was about 10.6%. The non crushed straw (1000-8000 µm), the crushed straw of intermediate granulometry (600-1000 µm) and the finely crushed straw (50 - 600 µm), will be defined as NCS, ICS and FCS, respectively.

2.2 Experimental set-up for D.I.C. processing

The experimental set-up (fig. 1) was largely described in different previous publications (Rezzoug et al, 2000, Nouviaire et al., 2008, Rezzoug et al., 2008), it is composed of three main elements

- The processing vessel (2) where the samples were placed and treated.
- The vacuum system which consists mainly from a vacuum tank (4) with a volume (1600 l) 130 fold greater than the processing vessel (12 l, and a vacuum pump (5). The initial vacuum pressure of the vacuum container was maintained at 5 kPa in all experiments.
- A pneumatic valve (3) that separate the processing vessel from the vacuum tank. It can be opened in less than 0.2 seconds; this ensures a rapid decompression within the reactor.

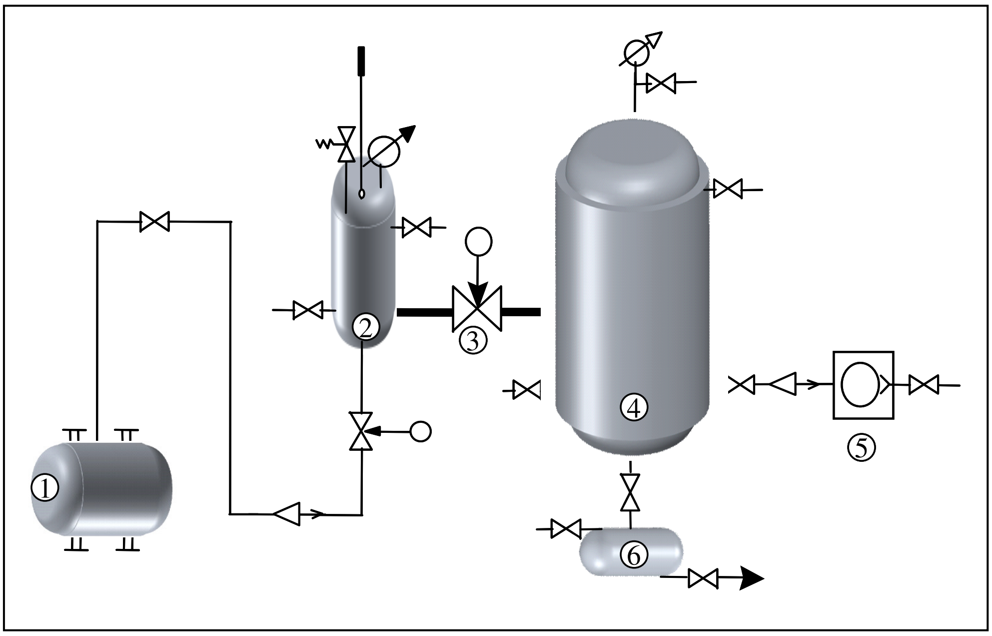

Figure 1. *Schematic of apparatus for extraction of essential oil from orange peel by Instantaneous Controlled Pressure Drop. 1. Boiler, 2. Pressure vessel, 3.Valve, 4. Vacuum tank, 5. Vacuum pump, 6. Extract container. All parts and valves of the apparatus are made in stainless steel.*

2.3 Protocol of wheat straw thermomechanical pretreatment

Wheat straw is firstly placed in the D.I.C. vessel (1) which is maintained under a vacuum (~ 50 mbar) through its connection to a vacuum container (fig. 2b). The vacuum allows a better diffusion of the heating fluid through the plant and consequently heat transfer between the steam and peels is improved and the time to reach the desired processing pressure (or processing temperature) is shortened. After closing the electropneumatic valve (3) which connects the reactor (2) to the vacuum tank (4), an atmosphere of saturated steam pressure (between 3 and 7 bar in this study) is created within the D.I.C. reactor (fig. 2c). After a processing time at fixed processing pressure (fig. 2d), the thermal treatment is followed by a rapid decompression resulting in a rapid drop in pressure (fig. 2e). The equilibrium pressure after decompression depends on the operating pressure: the higher the processing pressure, the higher the equilibrium pressure. The created steam wheat straw by autovaporization induces mechanical strength capable of causing deformations and micro cavities whose amplitude depends on rheological properties of the product at initial moisture content and temperature. The evaporation, which is effected in adiabatic conditions, induces a rapid cooling.

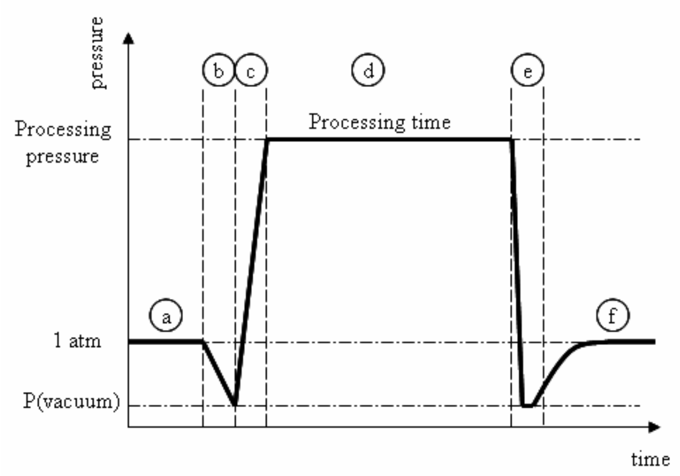

Figure 2. *Typical pressure-time profile for D.I.C processing cycle.*

2.4- Enzymes

Celluclast 1.5L, the enzyme concentrate used for cellulose hydrolysis, was a commercial *Trichoderma reesei* cellulase preparation contains *endo*-glucanases, *exo*-glucanases, cellobiohydrolases and *β*-glucosidases. This preparation was a brownish liquid with a density of approx. 1.20 g/ml and contained 191 mg protein/ml. The cellulasic activity of concentrate was 96 FPU/ml. One unit of FPU is defined as the enzyme amount which releases 1 μmol of glucose equivalents from Whatman n°1 filter paper in 1 min. Optimum conditions of activity were between 4.5 and 6 for pH and 50 to 60 °C for temperature.

2.5- Cellulose hydrolysis

Celluclast-1.5 L (96 FPU/ml) was added to 50 mM citrate-phosphate buffer (pH=4.6) and then mixed to the substrate (10g/l). The experiments were carried out in 100 ml Erlenmeyer flasks containing 10 ml total reaction volume (buffer enzyme mixture). The flasks were sealed and incubated in a rotary shaker at 600 rpm at 50 °C during 20 h. To follow the hydrolysis, a flask was withdrawn at different times and the liquid phase (hydrolysate) was immediately heated for 5 min on a boiling water bath to precipitate the proteins and prevent further hydrolysis. The mixture was then centrifuged at 14000 rpm for 2 min to remove solids. The cellulose hydrolysis yield of samples was determined by 3.5 dinitrosalicyclic acid method (DNS method). All reactions were carried out in triplicate.

3. Results and discussion

3.2. chemical composition of wheat straw

The chemical composition of the raw material is given in table 1.

Table 1. Chemical composition of wheat straw

Components	Composition of wheat straw (g/100 g d.m)
Cellulose[a]	31±1
Hemicellulose[b]	43±3
Lignin[b]	22±5
Ashes[c]	4±1

[a] determined by acid and enzymatic hydrolysis; [b] determined by acid hydrolysis; [c] ashes may include components as proteins and lipids

3.2. Effect of particle size on glucose production

As indicated by Ballesteros et al, (2000) for steam explosion pretreatment, the most important variables are processing time, temperature and chip size. The authors argued that, when larger chips are used, heat transfer problem overcook the exterior of the lignocellulosic structure and incompletely autohydrolyse the interior. Consequently, prior to steam explosion, particle size must be reduced, which requires a significant amount of energy. In this study, the raw material was milled to three different particle size (1000-8000 µm; 600-1000 µm; 50-600 µm) and the glucose production was evaluated. As expected from figure 3, it is clear that the particle size have a strong effect. For the largest chips, the maximum glucose production was about 10g/100 g d.m while for the smallest ones the maximum glucose production was about 15g/100 g d.m. according to Hendriks and Zeeman (2009), the reduction in particle size leads to an increase in available specific surface and a reduction of the degree of polymerisation (DP). These factors increase the total hydrolysis yield of the lignocellulose.

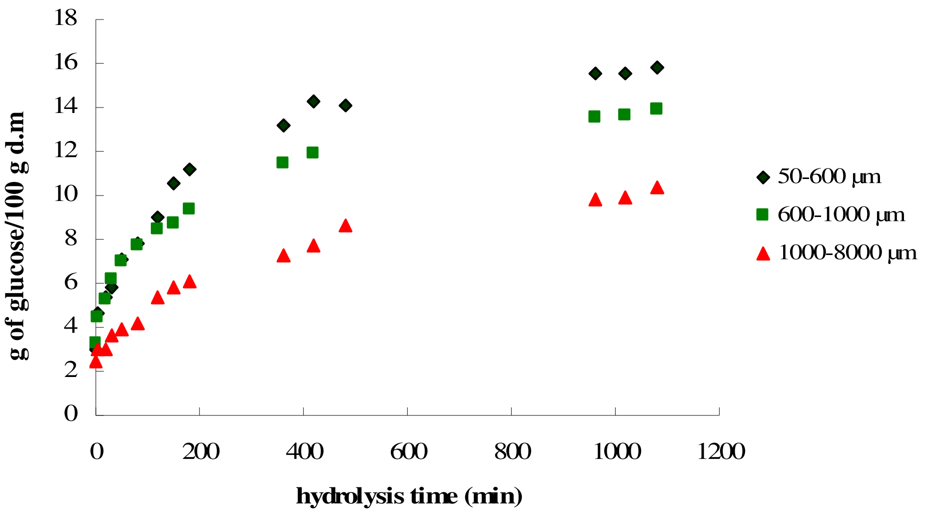

Figure 3. *Kinetics of glucose production versus wheat straw particle size for unpretreated samples*

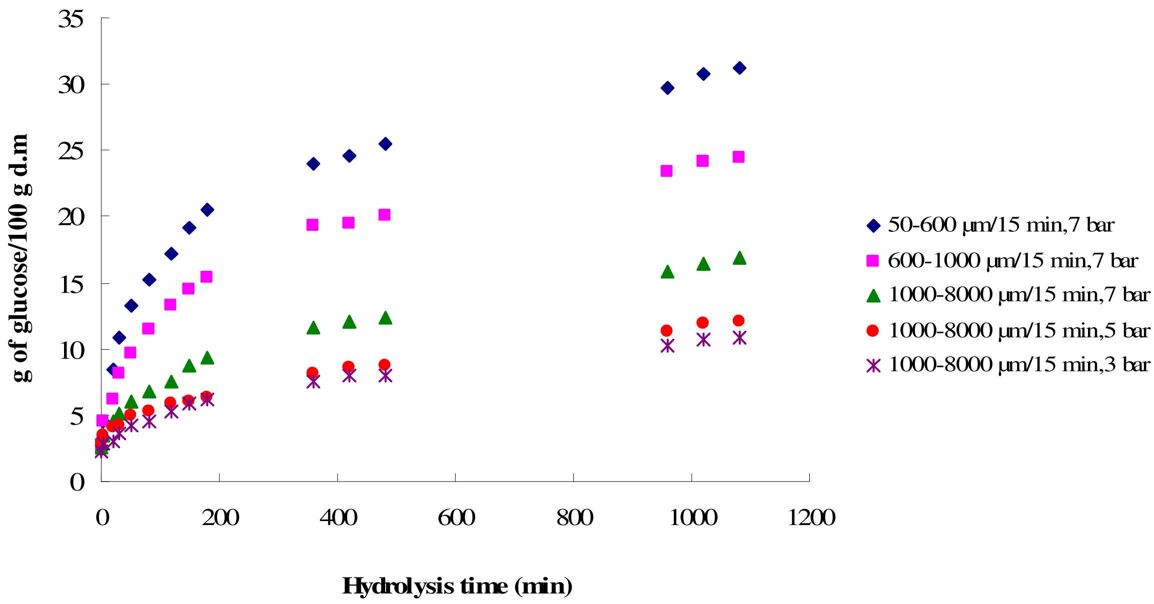

Figure 4. *Kinetics of glucose for samples pretreated by D.I.C thermomechanical process at different processing pressures*

3.2. Influence of processing pressure on glucose production

By comparing figure 3 and 4, it can be seen that the proposed pretreatment enhance the yield of produced glucose, probably by increasing the contact stubstrate-enzymes. For the higher particle size, which constitute for us the "uncrushed" samples, the maximum of glucose production was about 10 g/100g d.m (33 % of the theoretical cellulose value) while it was about 16g/100 g d.m (53 % of the theoretical cellulose value) for the same samples pretreated during 15 min under 7 bar as processing pressure. This effect can be clearly confirmed for the other sizes used. It can be also seen that the processing pressure have a strong effect on the yield of fermentable sugar. At the same conditions of processing time, the higher the processing pressure the higher the evaporation which leads to a better accessibility during the hydrolysis. It should be noted that processing pressures of 3, 5 and 7 bar correspond to a temperatures of 135, 152 and 165 °C, respectively. The totality of the theoretical cellulose was hydrolysed for the lower size sample (50-600 μm) pretreated at 7 bar during 15 minutes since the maximum glucose produced was about 30 g/100 g d.m. From figure 4, it can be also observed that the initial rates of cellulose hydrolysis increased with the particle size and the processing pressure during pretreatment. For the three samples pretreated at 3, 5 and 7 bar , it was about 0.18 g/l, 0.22 g/l and 0.57 g/l of reducing sugars released in initial step of hydrolysis, respectively. The higher initial rate was obtained for the lower particle size sample pretreated at 7 bar during 15 minutes.

3.3. Influence of processing time on glucose production

Three experiments were performed to evaluate the effect of processing time. From table 4 it can be seen that the processing time have also a strong affect on glucose production since at moderate processing pressure (4.5 bar) 70 % of theoretical cellulose was hydrolysed for 62 minutes processing time during the pretreatment.

Table 2. Effect of processing time during pretreatment on glucose production

Processing time (min)*	Maximum glucose produced (g/100 g d.m)
3	10.37
32	16.29
62	21.70

*the pretreatements were performed at fixed processing pressure (4.5 bar) and fixed moisture content (25 %) and for particle size of 1000-8000 μm

3.4. Conclusion

From this first work on a new pretreatment to produce fermentable sugars from lignocellulosic biomass, it can be concluded that both processing time and processing pressure have a strong effect on the yield. As showed by figure 5, the proposed pre-treatment enhance the accessibility of enzymes in the substrate due to the flash expansion provoked by the sudden release of steam pressure. However, to evaluate the effect of the different parameters of this pretreatment simultaneously a complete experimental design must be conducted

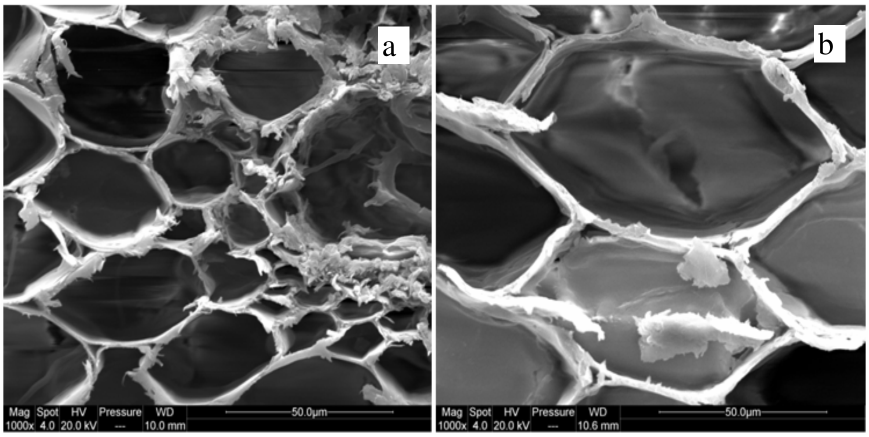

Figure 5. *Microstructure of wheat straw (1000-8000 μm) non pretreated (a) and pretreated (b) during 15 min at 7 bar. (magnification X 1000)*

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