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Optimization of Operating Conditions in Enzymatic Hydrolysis of Pretreated Lignocellulosic Materials

María Teresa García-Cubero*, Mónica Marcos, Silvia Bolado, Mónica Coca
Gerardo González-Benito
Univ. Valladolid, Dep. Chemical Engineering and Environmental Technology
Dr. Mergelina s/n, 47011 Valladolid, Spain
maite@iq.uva.es

This work studies the influence of dry solids load, enzymes ratio and reaction time in sugars concentration and yield of enzymatic hydrolysis of different steam exploded grain straw. Enzymatic hydrolysis was performed using a mixture of cellulase, β-glucosidade and xylanase, enzymes kindly donated by Novozymes, in test flasks shaken in a rotary incubator at 300 rpm, 50 ºC and pH of 4.8. After 24 h of reaction time, the higher sugars concentration in the hydrolizates was obtained when a 10 % DS was tested (24 g/L for rye straw and 28.1 g/L for wheat straw). When a 10 % DS was tested, maximum glucose and xylose relase after 24 h (84.9 and 19.1 % respectively) were obtained for 1.5:0.5:1.5 enzyme ratio for rye straw, whereas for wheat straw, maximum yield for glucose and xylose (78 % and 29.5 % respectively) were obtained for 1.5:1:1.5, after 24 h of hydrolysis.

1. Introduction

Despite the efforts made to decrease the production cost of ethanol, this is still too high compared with petroleum derived fuels. A lot of researchers have focused on the exploitation of lignocellulosic materials, an abundant and renewable source of sugar substrate, which would guarantee process self-sufficiency. The use of lignocellulosic materials can significantly reduce the cost of raw materials for ethanol production The main obstacle for a better use of lignocellulosic materials to produce ethanol is their low digestibility because of the tight association between their components: cellulose, hemicellulose and lignin.

Therefore, a pre-treatment step has to be included because of the high crystallinity of the cellulose and the presence of lignin, which makes the cellulose recalcitrant to degradation. The pre-treatment step should improve the accessibility of the cellulose component to hydrolytic enzymes while avoiding degradation of solubilised hemicellulose and cellulose. Sugar degradation not only decreases the final ethanol yield but also produces degradation products that are inhibitory to the yeast used in the subsequent fermentation.

One of the pre-treatment methods most commonly used for lignocellulosic is steam explosion, in which raw material is exposed to pressurised steam, followed by rapid

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reduction in pressure. This results in a substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulose fraction, depolymerisation of the lignin components and defibration (Cara et al., 2006).
After pre-treatment, the material obtained is readily hydrolysed by hydrolytic enzymes, which aims to release the monomeric sugars contained in the cellulose and hemicellulose. Enzymatic hydrolysis releases the fermentable monosugars from the polymeric cellulose and hemicellulose. The combined use of enzymes, cellulases and ß-glucosidases, is necessary to obtain high cellulose conversion and prevent cellobiose accumulation. Cellulase breaks the branched cellulose chains while the cellobiose formed is broken down with the action of ß-glucosidases producing glucose. The combined action of both enzymes considerably reduces the time required for hydrolysis. The use of other auxiliary enzymes can improve the performance of the enzyme mixture. Xylanases and pectinases have led to enhanced cellulase performance, increasing cellulose conversion (Berlin et al., 2005, 2007) and xylosidase attack, xylooligosaccharides and release of xylose (Sorensen et al., 2007). Depending on the raw materials and the pretreatment technology, the enzyme mixture must be designed to the specific substrate, and the use of compounds to improve the enzyme-substrate interaction needs to be studied.
Enzymes currently entail a relevant process cost, a reduction in enzyme loading thus proving essential to improve biofuel profitability. Despite abundant and growing research, manufacturers report evidence only of substantial progress in the development of more efficient and cheaper enzymes only for cellulose hydrolysis. Reaction time, high substrate concentration and lignin content also impact hydrolysis yield.
High solid loads are required to achieve the necessary fermentable sugar concentration after the hydrolysis process. Nevertheless, using a high solid load causes some mixing and material transfer problems. It can generate an accumulation of cellobiose which is a strong inhibitor of ß-glucosidases. In addition, acetic acid, furfural, HMF, vanillin, etc…produced at the pretreatment stage can inhibit enzyme and microorganism action. In pilot scale plants, a maximum of 15-20 %DM has been reported (Talebnia et al., 2009), although initial substrate concentration higher than 10-15 % DM is not available at lab scale due to high viscosity and bad mixing in conventional equipment.
The aim of this work was to investigate the influence of the operating conditions such as solids load, enzyme ratio and hydrolysis time in order to increase the concentration of glucose and xylose in the fermentation medium.

2. Materials and Methods

2.1 Raw material

Wheat and rye straw were kindly donated by the Castilla y León Institute of Technological Agriculture. The straw was ground in a blender, sieved to obtain a particle size of 20 mm and kept in an oven at 45 °C. Characterization of both raw materials are summarised in Table 1.

2.2 Steam explosion pre-treatment

The steam explosion was carried out in a 5L stainless steel batch reactor in which the straw was loaded at the top and heated to the desired temperature (210 °C) with

saturated steam. When the pre-set residence time concluded (10 min), the steam-treated biomass was released from the reactor by rapid depressurisation of the vessel. After the pre-treatment, the product was washed with warm water and the residual solid was separated by filtration. The solid portion was dried in an oven at 45 °C, stored in a freezer and used for enzymatic hydrolysis.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis was performed using a mixture of cellulase complex (NS50013) and β-glucosidase (NS50010) and xilanase (NS50030), enzymes kindly donated by Novozymes (Denmark). The hydrolysis step operated at 50 °C for a period of 24 and/or 48 h. Test flasks were shaken in a rotary incubator at 300 rpm. After hydrolysis, 600 μL samples were withdrawn, passed through a 0.22 μm filter and stored for sugar analysis. The influence of solid load was tested modifying dry matter content between 10 and 15 % with different enzymes ratio of cellulase:β-glucosidase:xilanase. Every test was conducted in triplicate. The mean value and standard deviation were calculated.

Table 1: Characterization of raw material (in %;w/w)

	Wheat straw	Rye straw
Cellulose (as glucose, %)	32.4	25.9
Hemicellulose (as xylose, %)	19.1	21.5
Acid Lignin (%)	21.3	27.1
Ash (%)	6.4	3.1
Moisture (%)	6.9	5.9

2.4 Analytical methods.

Acid insoluble lignin, acid soluble lignin, cellulose and hemicellulose in the raw material were estimated following NREL laboratory analytical procedures Lap 003, 004 and 002 respectively. A Bio-Rad HPX-87C ion-exclusion column was used to measure sugar concentrations. The mobile phase was water at a flow rate of 0.6 mL min^{-1} and 60 °C. The detector was based on the refraction index measurement. Sugars from enzymatic hydrolysis were also analysed by HPLC using the Aminex HPX-87C column (Bio-Rad, Hercules, CA) under the operating conditions previously indicated.

3. Results and Discussion

3.1 Influence of dry solids content

Table 2 shows the main results obtained in the different trials. The highest glucose concentration in rye hydrolysate (15 g/L after 24 h and 18,3 g/L after 48h) was obtained when a 15 % DS was tested. However, hydrolysis yield is higher when a 10 % DS was tested (43.9 % and 58.0 % after 24 and 48 h respectively). The same effect was found for wheat straw hydrolysis, a maximum glucose concentration of 15.0 and 25.7 g/L was obtained when a 15% DS was tested, whereas higher hydrolysis yield was attained when a 10%DS was fixed: 40.5 and 69.7% after 24 and 48 h respectively.

However, the effect with xylose release was the opposite: the highest xylose release (5.0 g/L for rye straw and 7.5 g/L for wheat straw) was found for the higher solid load tested (15 % DS).

Table 2: Enzymatic hydrolysis of rye and wheat straw with a mixture of cellulase and β-glucosidase for different dry solid content

		10% DS		15% DS	
		t = 24h	t = 48 h	t = 24h	t = 48 h
Wheat straw	Glucose (g/L)	14.6	25.0	15.0	25.7
	Xylose (g/L)	4.5	5.7	3.2	7.5
Rye straw	Glucose (g/L)	12.6	16.,7	15.0	18.3
	Xylose (g/L)	2.9	4.0	4.1	5.0

Enzymatic hydrolysis was carried out during 24 and 48 h in order to determine the optimal reaction time. Results showed that an increase in the reaction time led to a scarce increase in sugar concentrations for both dry solid contents tested. For rye straw and 10% DS, glucose and xylose concentrations increased from 12.6 g/L and 2.9 g/L respectively after 24 h to 16.7 g/L and 4.0 g/L after 48 h, whereas when a 15% DS was tested, glucose and xylose concentrations increased from 15 g/L and 4.1 g/L respectively after 24 h to 18.3 g/L and 5.0 g/L after 48 h. The same effect was found when wheat straw was tested: glucose increase from 14.6 g/L to 25 g/L when reaction time increase from 24h to 48 h and xylose released increased from 4.5 g/L at 24 h to 5.5 g/L after 48 h of hydrolysis.

3.2 Influence of xylanase addition

Figure 1 shows the results derived from experiments with rye straw (10%DS) and different cellulose-β-glucosidase-xylanase ratios. Glucose and xylose concentration in the hydrolyzed liquid was higher when xylanase was employed in the enzyme mixture. After 24 h of enzymatic hydrolysis, hydrolysis yield increased from 43.9 % when a mixture of cellulose:β-glucosidase was used, to 59.5 % when xylanase was included into the enzyme mixture. The same effect was obtained after 48h of reaction time: enzymatic hydrolysis yield increased from 58 % to 77 % when xylanase was introduced in the enzyme mixture.

Moreover, best results for glucose were obtained when a higher amount of cellulase and xylanase was tested, with a maximum glucose concentration of 24 g/L (hydrolysis yield: 84%) for both enzymes ratios (1.5:0.5:1.5 and 1.5:1:1.5 for cellulose:β–glucosidase:xylanase respectively) after 24 h. After 48h, results showed a higher glucose released (29 g/L) when a mixture 1.5:1:1.5 for cellulose:β–glucosidase:xylanase was employed, with yields higher than the theoretical 100 %. This fact is probably due to the presence of cellulose in the xylanase supplied and also due to the break down of glucose linkages in the hemicellulosic structure.

Related with xylose, the use of xylanase slightly increased xylose release and no significantly differences were observed with the different enzymes ratio tested. Maximum xylose concentration was around 5 g/L after 48h of reaction time.

Results for wheat straw (Figure 2) were similar than that obtained for rye straw. Glucose and xylose released increased when xylanase was added to the enzyme mixture. After 24 h of enzymatic hydrolysis, hydrolysis yield increased from 40.5% when a mixture of cellulose:β-glucosidase was used, to 54.8% when xylanase was included into the enzyme mixture. The same effect was obtained after 48h of reaction

time: enzymatic hydrolysis yield increased from 69.7% to 88.4% when xylanase was introduced in the enzyme mixture.

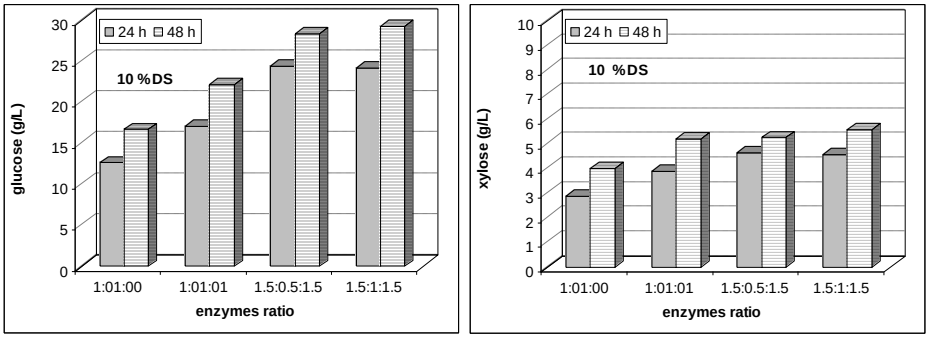

Figure 1: Glucose and xylose released from rye straw after 24 and 48 h of enzymatic hydrolysis(10% DS) with different cellulose:β-glucosidase:xylanase ratios.

However, the use of xylanase didn't increase significantly glucose concentration. Afer 24 h, a maximum glucose concentration of 20 g/L and 28.1 g/L (hydrolysis yield 55.6 and 78%) was obtained for both enzymes ratios (1.5:0.5:1.5 and 1.5:1:1.5 for cellulose:β–glucosidase:xylanase respectively). After 48h, results showed a higher glucose released (32.9 g/L) when a mixture 1.5:1:1.5 for cellulose: β–glucosidase:xylanase was employed, with lower yields (90% aprox.) than the obtained with rye straw, probably due to the higher cellulose content of raw material..
The use of xylanase slightly increased xylose release and no significantly differences were observed with the different enzymes ratio tested. Maximum xylose concentration was around 6 g/L after 24h of reaction time.

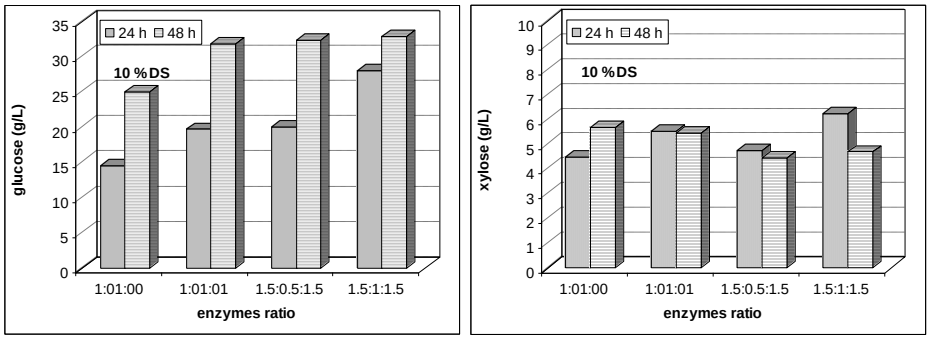

Figure 2: Glucose and xylose released from wheat straw after 24 and 48 h of enzymatic hydrolysis(10% DS) with different cellulose:β-glucosidase:xylanase ratios.

4. Conclusions

Optimization of process parameters in enzymatic hydrolysis must be considered in detail in order to diminish the high cost and increase the yield of the process. To find the optimal enzyme ratio, the best enzyme mixture and the lowest time reaction must be

prioritised in order to maximize the production of fermentable sugars (glucose and xylose). The maximum load of solid material is another parameter to take into account in the process economy. Moreover, the use of enzymes able to break down efficiently hemicellulose structure must be studied.

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