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Influence of process parameters on the enzymatic hydrolysis of steam-exploded wheat straw

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The work studies the influence of different operating parameters, e.g. % dry solids loading (%ST), enzymes ratio and reaction time in the yield of enzymatic hydrolysis of wheat straw steam exploded (210ºC, 20 bar, 20 mm particle size and 10 min of reaction time). Enzymatic hydrolysis was performed using a mixture of cellulase complex (NS50013) and β-glucosidase (NS50010), both enzymes kindly donated by Novozymes (Denmark) in test flasks shaken in a rotary incubator at 150 rpm and 50 ºC. The maximum glucose concentration (25 g/L) in the hydrolysed has been obtained when a 15%dry solid content, an enzyme ratio of 5:1 and a reaction time of 24h.

1. Introduction

Lignocellulosic materials are an abundant and renewable source of sugar substrate that could be fermented to ethanol. Using lignocellulosic materials such as agricultural residues, forestry and municipal wastes and other low-cost biomasses, can significantly reduce the cost of raw materials for ethanol production and it has been estimated that this accounts for about 50% of the biomass in the world (Lin and Tanaka, 2006).

Wheat straw is one of the most abundant crop residues in European countries with a production of 170 million tonnes, and seems to be the cheapest and the most useful raw material for ethanol production (Tabka, 2006). Wheat straw is composed of a mixture of cellulose and hemicellulose (45% and 30% respectively) that are bound to lignin (approx. 25% DS) by hydrogen and covalent bonds. Cellulose, is a crystalline linear polymer composed of thousands of D-glucose linked by β-(1,4)-glycosidic bonds. Hemicellulose is an amorphous and partly crystalline polymer, formed mostly of β-(1,4)-xylose. Lignin is an aromatic polymer containing phenolic residues that binds the fibres together. (Zaldivar et al., 2001; Lin and Tanaka, 2006). Cellulose and hemicellulose can be hydrolysed to sugars, which can be fermented to ethanol. Lignin doesn't contribute fermentable carbon sources because there is no known microorganism able to transform it for ethanol production (Galbe and Zacchi, 2002).

In the conversion of lignocelulosics into ethanol, a pre-treatment step is therefore included because of the high crystallinity of the cellulose and the presence of lignin, which makes the cellulose recalcitrant to degradation (Wingren et al., 2005).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 results in degradation

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products that are inhibitory to the yeast used in the subsequent fermentation (Öhgren et al., 2007).

The steam explosion is one of the pre-treatment methods more commonly used with lignocellulosic materials. The raw material is exposed to pressurised steam, followed by rapid reduction in pressure resulting in substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulose fraction, depolymerisation of the lignin components and defibration (Cara et al., 2006). After the pre-treatment, the material obtained is readily hydrolysed by hydrolytic enzymes; the aim of this process is to release the monomeric sugars contained in the cellulose and hemicellulose.

At least three categories of enzymes are necessary to convert cellulose into soluble sugars. These include endoglucanase (EG) (EC 3.2.1.4), which hydrolyses internal β-1,4-glucosidic bonds randomly in the cellulose chain; cellobiohydrolase (CBH) (EC 3.2.1.91) which moves processively along the cellulose chain and cleaves off cellobiose units from the ends of the chain; and β-glucosidase (BG) (EC 3.2.1.21) which converts cellobiose and soluble cellodextrins into glucose. All these enzymes work synergistically to hydrolyse cellulose (Gusakov et al., 2007). The hydrolytic efficiency of a multi-enzyme mixture in the process of lignicellulose saccharification depends both on properties of individual enzymes and their ratio in the multi-enzyme cocktail (Berlin et al., 2007; Zhou et al., 2009)

It is important to get a glucose solution as concentrated as possible from the enzymatic hydrolysis step, from which a concentrated ethanol solution may be obtained as a result of fermentation. One way for obtaining concentrated glucose solutions is to use as much substrate for the enzymatic hydrolysis as possible, although problems related to material agitation and inhibition by glucose arise (Cara et al., 2007).

Determining the minimum reaction time is important, too. It is related with to energy consumption necessary for the shaking and the maintaining of a constant temperature (enzymatic activity depends on the temperature and pH, amongst other factors). Reaction time is also related with the process scale-up.

The aim of this study is to obtain the optimum cellulase:β-glucosidase enzymes ratio for a more efficient enzymatic hydrolysis of lignocellulose and a more rational utilization of enzymes (the high cost of enzymes is one reason of the low profitability of the process, Zhou et al., 2009). The influence of other operating parameters was also tested: % dry solids loading (%ST) and reaction time in the yield of enzymatic hydrolysis of steam-exploded wheat straw, in order to obtain the optimal conditions for the most profitable process.

2. Materials and Methods

2.1 Raw Material

Wheat straw was 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 45ºC. The composition (% w/w) of straw is shown in Table 1.

Table 1. Wheat straw composition

Component		% (w/w) dry matter
Cellulose (as glucosa)		29.6
Hemicellulose		20.9
	Xylose	17.5
	Galactose	1.0
	Arabinose	2.1
	Manose	0.3
Acid Insoluble Lignin (AIL)		23.8
Acid Soluble Lignin (ASL)		3.8
Extractives		19.1
Ash		9

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 (210ºC) 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 45ºC, stored in a freezer and used for enzymatic hydrolysis.

2.3 Enzymatic hydrolsysis

Hydrolysis was performed on each sample to determine the improvement in enzymatic saccharification under the different conditions applied. Enzymatic hydrolysis was performed using a mixture of cellulase complex (NS50013) and β-glucosidase (NS50010), both enzymes kindly donated by Novozymes (Denmark). The hydrolysis operated at 50ºC for a period of 24 and/or 48 h. Test flasks were shaken in a rotary incubator at 300 rpm. The different experiments carried out are summarised in Table 2.

After hydrolysis, 600 μL samples were withdrawn, passed through a 0.22 μm filter and stored for sugar analysis. Every test was conducted in triplicate and the mean value and standard deviation was calculated.

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. However, a Bio-Rad HPX-87C ion-exclusion column was used to measure sugar concentration. The mobile phase was water at a flow rate of 0.6 mL min-1 and 60 ºC. 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.

Table 2. Experimental design

Run test	% DS	t (h)	Cellulase:β-glucosidase) ratio
E.1	10	24/48	1:1
E.2	15	24/48	1:1
E.3	20	24	1:1
E.4	25	24	1:1
E.5	30	24	1:1
E.6	10	24/48	1.5:0.5
E.7	10	24/48	2:1
E.8	10	24/48	2:2
E.9	10	24/48	5:1
E.10	10	24/48	10:2
E.11	15	24/48	1.5:0.5
E.12	15	24/48	2:1
E.13	15	24/48	2:2
E.14	15	24/48	5:1
E.15	15	24/48	10:2

*(1:1) = (0.11 g cellulase:0.05 g β-glucosidase)/gcellulose

3. Results and discussion

3.1 Influence of dry solids content

Enzymatic hydrolysis was carried out working with a dry solid content between 10 and 25% (w/w). Results obtained for glucose and xylose in the hydrolysed liquid are summarised in Figure 1. As it is shown, the concentration of glucose in the liquid was slightly higher at 15%DS (15,0 g/L) than at 10%DS (14,6 g/L), but hardly diminished when higher dry solid content were studied.

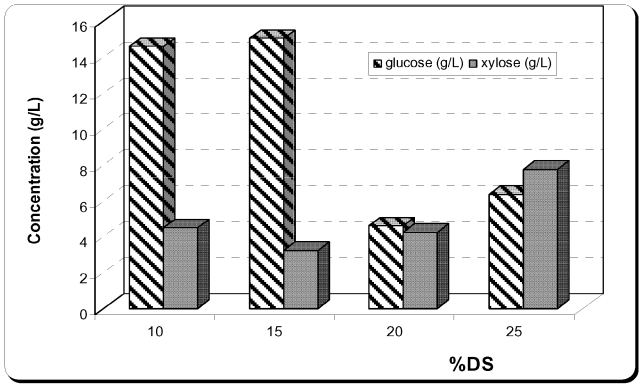

Figure 1. Influence of dry solids content on hydrolysed sugar concentration

This fact can be explained because reaction rate generally increases whith substrate concentration, but when higher concentrations are tested, a possible substrate inhibition can occur, diminishing the reaction rate and, therefore, the hydrolysis yield. Cara et al. (2007), pointed out that mixing problems occur for some materials, when the

consistence is high, explaining the low yield obtained at high load of solids. However, the effect observed for the xylose is opposite: the higher the dry solids load, the higher the xylose concentration after 24 h of hydrolysis.

3.2 Influence of cellulase:β-glucosidase ratio

Figure 2 summarises results obtained when different enzyme ratios were tested at 10 and 15%DS respectively. Both graphss show a maximum glucose concentration when a ratio of 5:1 was employed, because of the higher amount of cellulase available to the enzymatic hydrolysis. On the other hand, the cocktail of enzymes used doesn't significantly affect the xylose concentration after hydrolysis. The use of enzymes other than cellulases and β-glucosidases should be tested in order to increase the total sugar concentration in the hydrolysed stream. (Tabka et al., 2006).

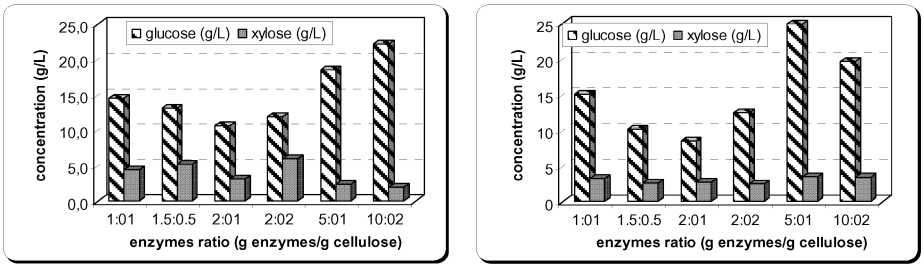

Figure 2. Influence of enzyme ratios on sugar concentration at 10% and 15% DS

3.3 Influence of reaction time

As it is shown in Figure 3, an increase in the reaction time slightly increased sugar concentration when a 15% dry solid content was tested. The same effect was observed when 10%DS was tried, but the differences obtained are not statistically significant.

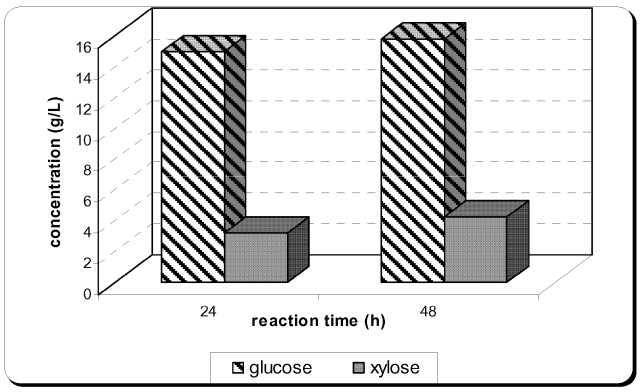

Figure 3. Influence of reaction time on sugar concentration for 15%DS

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 lowest time reaction and the maximum load of solid material must be prioritised because they have a direct influence on the production of fermentable sugars as well as on the economics of the development. Moreover, a optimal knowledge of kinetics and enzyme synergy is the key to achieving this aim.

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