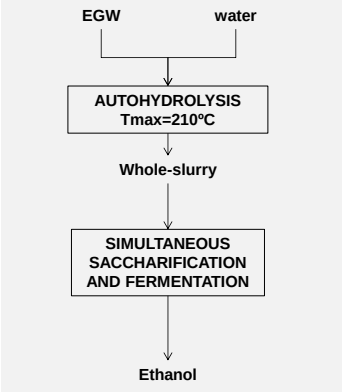
Use of whole-slurry from autohydrolyzed *Eucalyptus* wood for bioethanol production

P-EN2

A. Romaní[1,], H.A. Ruiz[2], F.B. Pereira[1], J.A. Teixeira[1], L. Domingues[1]. (1) CEB-Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal;, (2) School of Chemistry, Food Research Department, Autonomous University of Coahuila, Saltillo, Coahuila, México; [*]aloia@ceb.uminho.pt*



The development of a cost-effective process on large-scale is one of the most important targets in the second generation bioethanol. The use of pretreated whole-slurry allows savings in washing-steps and water consumption. In this work the whole-slurry from pretreated *Eucalyptus* wood (EW) was used for the bioethanol production by saccharification and fermentation process. Firstly, EW was submitted to autohydrolysis treatment and the slurry obtained was employed for the optimization of enzymatic saccharification using an experimental design. The optimized conditions were employed for bioethanol production using a robust industrial *Saccharomyces cerevisiae* strain. The highest ethanol concentration obtained was 50 g/L corresponding to an ethanol conversion of 95 %.

Introduction

The bioethanol is one of the most important liquid fuels obtained from processing of Lignocellulosic Materials (LCM) [1]. The processing or pretreatment of LCM is necessary to disrupt its structure and improve the accessibility of enzymes towards cellulose. Autohydrolysis is considered an environmentally friendly treatment that allows the solubilisation of hemicellulose fraction. Nevertheless, during the treatment some soluble derived compounds as furans, weak acids and phenolic compounds are generated and considered inhibitors of sequent saccharification and fermentation processes. One alternative to overcome this problem can be the use of enzymes and microorganisms that are able to act on the toxic compounds present in the hydrolysate [2]. The bioethanol production can be performed using simultaneous saccharification and fermentation (SSF) [3].

This work studies the use of whole-slurry from autohydrolyzed *Eucalyptus* wood (EW) in order to optimize the concentration of glucose. In the optimized conditions calculated, SSF assay was carried out to obtain high ethanol yields by a robust industrial strain of *Saccharomyces cerevisiae*.

Materials and Methods

The EW was mixed with water (1 kg of EW/8 kg of water) in a pressurized reactor at Tmax=210 ºC (non-isothermal regime). The solid fraction of pretreated EW was composed (g/100 g of raw material dry basis) by 43 of cellulose, 1.4 of xylan and 24 of Klason lignin. Moreover, the chemical composition of liquid phase (hydrolysate) was mainly xylose (7.3 %), xylooligosacharides (7.4 %), acetyl groups (2.1 %), acetic acid (2.6 %), hydroxymethylfurfural (0.3 %) and furfural (1.4 %), expressed as g of compound/100 of raw material dry basis. The solid fraction and hydrolysate (whole-slurry) were used as substrate in the optimization of enzymatic saccharification using an experimental Box-Behnken design. The studied variables were: Liquid to Solid Ratio (LSR, g/g), Cellulose to Substrate Ratio (CSR, FPU/g) and Hemicellulose to Substrate Ratio (HSR, UI/g). The operational conditions of experimental plan work are listed in Table 1.

S. cerevisiae PE-2 isolated from Brazilian bioethanol production plant was incubated at 30 ºC in an orbital shaker for 24 h. The biomass was recovered and added to SSF experiment. The SSF assay was carried out at 35ºC and 150 rpm. Samples from enzymatic saccharification and SSF were collected and analysed by HPLC.

Results

The glucose concentration obtained at 96 h of saccharification was used to calculate the cellulose to glucose conversion (CGC_{96h}) of enzymatic hydrolysis assays by the following equation:

$$GGC_{96h}=100\ G_{96h}/Gp \quad \textbf{Equation (1)}$$

where G_{96h} is the glucose at 96h of saccharification and Gp is the potential glucose calculated from maximal glucose obtained taking in account the glucan of substrate.

The CGC_{96h} was listed in Table 1 and correlated with the independent variables by second order polynomial equation (as follows):

$$y_1=96.2 + 17.0x_1 + 14.8x_2 + 6.1x_3 - 17.4x_1^2 - 13.3x_2^2 - 0.7\,x_3^2 + 2.7x_1x_2 - 2.6x_1x3 - 7.8\,x_2x_3 \quad \textbf{Equation (2)}$$

Figure 1 shows the graphical representation of equation 2 in which can be observed the influence of LSR and CSR on CGC_{96h}. Conversions above 90 % were obtained for CSR in the range 19.23 FPU/g and LSR>9 g/g.

Table 1. Operational conditions (expressed in terms of dimensionless and dimension independent variables) and dependent variable concerning enzymatic hydrolysis

Run	LSR (g/g)	CSR (FPU/g)	HSR (UI/g)				CGC_{96h} [a] (%)
				x_1	x_2	x_3	y_1
1	4	8	300	-1	-1	0	37.8
2	25	8	300	1	-1	0	64.8
3	4	30	300	-1	1	0	60.9
4	25	30	300	1	1	0	98.8
5	4	19	100	-1	0	-1	56.0
6	25	19	100	1	0	-1	97.0
7	4	19	500	-1	0	1	64.7
8	25	19	500	1	0	1	95.1
9	14.5	8	100	0	-1	-1	48.8
10	14.5	30	100	0	1	-1	94.8
11	14.5	8	500	0	-1	1	85.3
12	14.5	30	500	0	1	1	100
13	14.5	19	300	0	0	0	96.2
14	14.5	19	300	0	0	0	96.2
15	14.5	19	300	0	0	0	96.2

[a](CGC_{96h}): cellulose to glucose conversion at 96 h

The optimized conditions were chosen considering the maximal concentration and conversion of glucose (93 g/L and 86%, respectively). The predicted conditions were CSR=22 FPU/g, HSR=500 UI/g and LSR=6.4 g/g. Under these conditions, the SSF process was carried out. The results obtained can be seen in Figure 2.

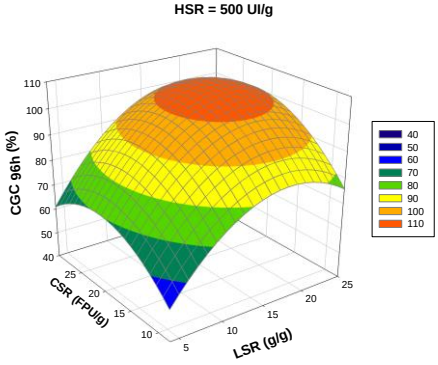


Figure 1. Response surface of CGC_{96h} (%) on CSR (FPU/g) and LSR (g/g). Results calculated from HSR fixed to 500 UI/g.

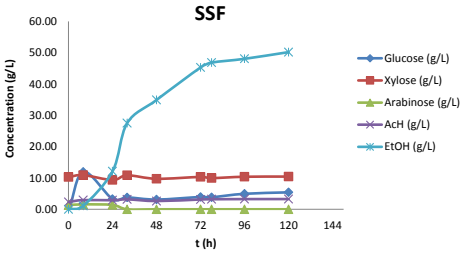


Figure 2. Time-course of SSF process.

After fermentation, 50 g/L of ethanol were achieved corresponding to 94 % of conversion and productivity of 0.6 g/Lh at 72 h.

Conclusions

The results obtained in this work confirmed the use of whole-slurry from autohydrolyzed *Eucalyptus* wood for bioethanol production. The good performance of fermentation with high productivity and elevated concentration of ethanol was demonstrated.

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