Optimization of glycerol-organosolv pretreatment for improving enzymatic saccharification of Eucalyptus wood
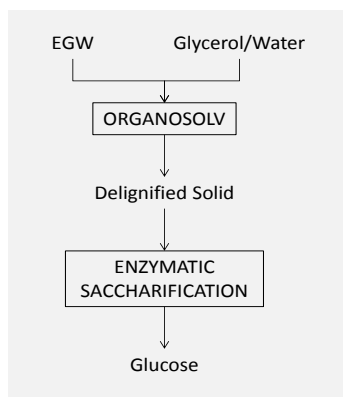
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This work contributes to the improvement of biomass pretreatment technologies and shows an efficient pretreatment by glycerol -water with a good lignocellulose biomass fractionation and an enhanced enzymatic susceptibility of pretreated solid. The results show that solubilized wood fraction after glycerol treatment is composed by 15.9 and 13.2 g of lignin and xylose / 100 g of raw material in the liquid phase, respectively, whereas the solid phase was hydrolyzed by enzymes (achieving up to 90 % conversion of cellulose to glucose). So, the use of glycerol in organosolv treatment is a suitable alternative for the use of an industrial by-product and the structural modification of biomass for second generation bioethanol production.

Introduction

The 2nd generation (2G) bioethanol or ethanol from Lignocellulosic Materials (LCM) is an interesting and promising alternative to fossil fuels. Nevertheless, significant changes in the structure of LCM are required in order to enable a techno-economically viable process. A typical 2G process includes at least 3 steps: i) pretreatment; ii) saccharification and iii) fermentation. The efficient pretreatment demands a high fractionation for the economic use of this renewable resource [1] and should satisfy several requirements: i) avoide size reduction, ii) preserve hemicellulose fraction, iiii) limit formation of inhibitors, iv) minimize energy input, and v) be cost-effective [2].

Organolsov pulping has been employed in pulp and paper production as alternative to kraft or sulphite processes. In this context, organosolv treatment can be considered as a good strategy to improve enzymatic saccharification of LCM. Moreover, the crude glycerol is a by-product that is generated in the biodiesel industry and represents a 10 % of total biodiesel output. Consequently, the use of glycerol in organosolv treatment is a suitable alternative for the use of an industrial by-product and the structural modification of biomass for 2G bioethanol production [3].

The aim of this work is to study the influence of operational conditions (temperature, time and percentage of glycerol-water) on saccharification of pretreated *Eucalyptus globulus* wood (EGW) by glycerol-organosolv process.

Materials and Methods

EGW was composed by 45 % of cellulose, 16 % of xylan, 25 % of klason lignin and 3 % of acetic acid. Organosolv treatment of EGW was carried out in a 160 mL total volume batch cylinder stainless reactor that was submerged in an oil bath with temperature control under conditions listed in Table 1.

Table 1. Operational conditions (expressed in terms of dimensionless and dimension independent variables) and dependent variable concerning enzymatic hydrolysis [4]

Run	T_D (ºC)	GW (%)	t_D (min)	T_D (ºC)	GW (%)	t_D (min)	G_{72h} [a] (g/L)
				x_1	x_2	x_3	y_1
1	180	40	65	-1	-1	0	32.8
2	200	40	65	1	-1	0	43.2
3	180	80	65	-1	1	0	17.5
4	200	80	65	1	1	0	38.3
5	180	60	40	-1	0	-1	9.4
6	200	60	40	1	0	-1	38.0
7	180	60	90	-1	0	1	36.7
8	200	60	90	1	0	1	44.4
9	190	40	40	0	-1	-1	32.5
10	190	80	40	0	1	-1	22.6
11	190	40	90	0	-1	1	38.6
12	190	80	90	0	1	1	36.5
13	190	60	65	0	0	0	39.2
14	190	60	65	0	0	0	39.5
15	190	60	65	0	0	0	39.4

The evaluation of operational conditions of process-Temperature of delignification (T_D=180-200 ºC), time of delignification (t_D=40-90 min) and glycerol-water solutions (GW=40-80 %) - was carried out using Box-Behnken design (three replicates in the central point). The delignified solids from organosolv-pretreatment were used as substrate on enzymatic saccharification at liquid solid ratio (LSR) of 20 g/g and enzyme to substrate ratio (ESR) of 20 FPU/g (carried out in an orbital shaker at 48.5ºC, 150 rpm and pH 4.5). Samples were withdrawn and analysed by HPLC for glucose concentration.

Results

The glucan present in the delignified solids from different conditions of pretreatment varied in the range of 54 to 83 g glucan/100 g. The maximal value of glucan in the solid implied a recovery of glucan of 98 % in respect to raw material and a delignification percentage of 72 %. These results show the selectivity of pretreatment to preserve the cellulose in the solid and solubilisation of lignin. The susceptibility of pretreated solids to enzymatic saccharification was evaluated. Figure 1 shows the glucose-profile of selected experiments and the difference of saccharification among substrates can be also seen. An 80 % of cellulose to glucose conversion was obtained in assays carried out at T_D=200ºC and t_D>65 min at 21 h of saccharification process.
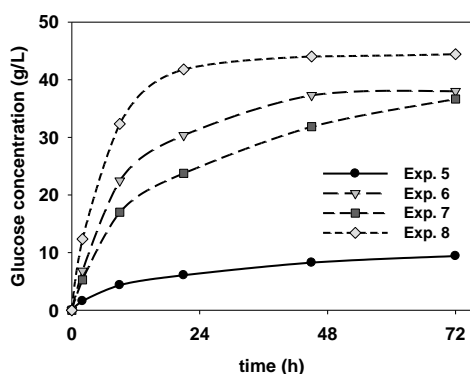

Figure 1. Time-course of enzymatic saccharification of selected experiments.

The glucose concentration at 72 h (G_{72h}) of all experiments was listed in Table 1. These results

can be correlated with the independent variables by the second order polynomial equation and the regression coefficients can be calculated, as it is shown in the following equation:

$$y_1 = 39.4 + 8.5x_1 - 4.0x_2 + 6.7x_3 - 3.4x_1^2 - 3.0x_2^2 - 3.8x_3^2 + 2.6x_1x_2 - 5.2x_1x_3 + 2.0x_2x_3 \quad \textbf{Equation (1)}$$

Figure 2 displays the response surface of predicted values of G_{72h} for t_D (min) and GW (%) with the most influential variable in the glucose concentration being t_D.
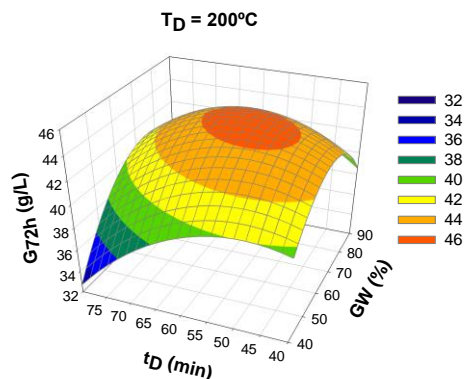

Figure 2. Response surface of glucose after 72 h of enzymatic saccharification (G_{72h}, g/L) on time delignification (t_D, min) and glycerol-water (GW, %). Results calculated for temperature of delignification (T_D) fixed to 200 ºC.

Glucose concentration higher than 44 g/L was achieved working at T_D=200 ºC, GW between 48-64 % and t_D between 63-70 min. The model was used for calculation of the maximum concentration of glucose employing the following operational conditions: T_D=200 ºC, GW=56% and t_D=68.8 min.

Conclusions

The results of this work revealed that the organosolv-pretreatment using a mixture of glycerol-water is an efficient process for improving the enzymatic saccharification of EGW. The empirical model allowed the optimization of variables for a maximum concentration of glucose (42 g/L) corresponding to a conversion yield of cellulose to glucose higher than 98 %.

References

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