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BIOREFINERY APPROACH FOR THE VALORIZATION OF VINE PRUNING RESIDUE

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Abstract – Lignocellulosic biomass (namely vine pruning residue, VPR) conversion into biofuels is considered a promising alternative to replace fossil fuels. In this work, VPR was submitted to hydrothermal treatment under isothermal conditions (180 and 200°C for 10-90min), in order to assess the effect of the treatment on the VPR fractionation. Liquid phase was analyzed for oligosaccharides composition and solid phase was submitted to enzymatic saccharification at liquid to solid ratio of 25 g/g and enzyme to substrate ratio of 25 FPU/g for glucose production. 62% of xylan was solubilized into xylose and xylooligosaccharides (2 and 11 g/L) and 70-98% of glucan remained in the pretreated VPR. Enzymatic saccharification of pretreated VPR was improved with the hardness of treatment. Overall, hydrothermal process (180°C, 60 min) was suitable for the production of xylose and xylooligosaccharides and to obtain a susceptible treated biomass for glucose production (13 g/L with 73% of conversion).

1. INTRODUCTION

In Portugal, agro-industrial wastes from wine sector represent an important part of lignocellulosic biomass generated specially residues derived of vine pruning (Brito et al., 2013). Approximately 1.2-3.5 t/ha of vine pruning residues (VPR) are estimated to be produced per year. Considering the harvested area of vine in Portugal (300,000 ha), more than 350,000 t of VPR are produced per year, which are usually burnt in the field (Rivas et al., 2007). The revalorization of this lignocellulosic residue into energy or products manufacturing by biorefinery development is considered of great importance to attain a sustainable growth (Lin et al., 2014).

Normally, the fractionation of lignocellulosic biomass needs a pretreatment to facility the access to lignocellulosic fractions (such as cellulose, hemicellulose and lignin). Currently, no single pretreatment exists that fulfils all the necessary requirements for an “ideal” lignocellulosic processing (Romaní et al., 2010). Nevertheless, treatment with water (without other reaction catalyst) at high temperatures (also known as hydrothermal treatment or autohydrolysis) is considered an eco-friendly process that allows the hemicellulose solubilization into hemicellulose-derived compounds (such as xylo-oligosaccharides and xylose) and the almost total recovery of cellulose and lignin in the solid



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fraction. Autohydrolysis treatment is considered as the first step for a biorefinery (Ruiz et al., 2013). In autohydrolysis treatments, a suspension of lignocellulosic biomass with water is heated to cause the controlled hydrolytic degradation of hemicelluloses by means of reactions catalyzed first by the hydronium ions from water autoionization and, in later reaction stages, by hydronium ions coming from organic acids generated *in situ* (Ruiz et al., 2013; Nitzsche et al., 2016).

In this work, VPR was submitted to hydrothermal treatment under isothermal conditions at 180 and 200 °C for 10-90 min, corresponding to severity factor (S_o) between 3.36-4.90, in order to assess the effect of the pretreatment on the fractionation of VPR. Liquid phase was analyzed for oligosaccharides composition and solid phase was submitted to enzymatic saccharification at liquid to solid ratio of 25 g/g and enzyme to substrate ratio of 25 FPU/g for glucose production.

2. MATERIAL AND METHODS

2.1. Raw Material

Vine pruning residue (VPR) was the raw material used in this study and provided from a local producer. These residues were air-dried, milled to pass an 8 mm screen, homogenized in a single lot to avoid differences in the composition among aliquots, and stored at room temperature in a dark and dry place until use.

2.2. Analysis of the raw material

The raw material was analyzed by TAPPI standards for extractives, moisture, ashes and quantitative acid hydrolysis with 72% w/w sulphuric acid (T-264-cm-07; T-211-cm-93; T-249-em-85). Liquors from quantitative acid hydrolysis were analyzed by HPLC for sugars (glucose, xylose, arabinose) and acetic acid, using a Refractive Index detector and a 87H (300x7.8 mm) Metacarb-Varian column eluted with 0.005 M H_2SO_4 at a flow rate of 0.7 mL/min. The content of polysaccharides (cellulose, xylan, arabinan) and acetyl groups were calculated from HPLC data. The Klason lignin content of VPR was gravimetrically measured from the insoluble solid residue obtained in the quantitative acid hydrolysis step.

2.3. Autohydrolysis pretreatment of vine pruning residue

The VPR was submitted at autohydrolysis treatments under conditions listed in Table 1. For this, water was mixed with VPR at liquid to solid ration of 8 g/g, placed in stainless steel reactor and submersed in silicone oil bath at different temperatures (180-200°C) and different incubation time (10-90 min). The intensity of autohydrolysis treatments can be expressed in terms of “severity” (S_o), defined as the logarithm of the severity factor R_o (Lavoie et al., 2010), which was calculated using the expression:

$$S_o = \text{Log } R_o$$

$$S_0 = \log R_0 = \log(R_{0\text{HEATING}} + R_{0\text{COOLING}}) = \log \left[\int_0^{t_{\text{MAX}}} \exp \cdot \left(\frac{T(t) - T_{\text{REF}}}{\omega} \right) \cdot dt \right] + \left[\int_{t_{\text{MAX}}}^{t_{\text{F}}} \exp \cdot \left(\frac{T'(t) - T_{\text{REF}}}{\omega} \right) \cdot dt \right] \quad (1)$$

According to this expression, R_0 is the severity factor, t_{MAX} (min) is the time needed to achieve the target temperature T_{MAX} ($^{\circ}\text{C}$), t_{F} (min) is the time needed for the whole heating-cooling period, and $T(t)$ and $T'(t)$ represent the temperature profiles in the heating and cooling stages, respectively. Calculations were made using the values reported usually for ω and T_{REF} (14.75°C and 100°C , respectively).

Table 1. Autohydrolysis conditions for the fractionation of Vine Pruning Residue and Solid Phase Composition after treatment (g of component/100 g of autohydrolyzed VPR oven-dry basis)

Temperature ($^{\circ}\text{C}$)	time (min)	S_0 (-)	Solid Yield (g/100 g)	Glucan (g/100g)	Xylan (g/100g)	Klason lignin (g/100g)
180	10	3.36	82.3	36.3	14.1	30.6
180	20	3.66	80.3	37.3	13.2	31.4
180	30	3.83	71.8	33.3	11.1	35.8
180	40	3.96	67.9	38.3	9.1	36.6
180	50	4.05	65.5	40.3	7.9	40.6
180	60	4.13	62.1	42.6	5.4	42.5
180	70	4.20	63.7	45.6	6.1	43.8
180	80	4.26	73.7	44.5	5.3	42.9
200	30	4.42	69.4	43.5	5.9	48.1
200	40	4.55	66.8	43.6	5.5	45.7
200	60	4.72	65.3	43.0	5.3	53.2
200	90	4.90	71.8	40.7	5.1	51.0

After autohydrolysis treatment, solid and liquid phases were separated by filtration for determination of chemical composition. An aliquot of autohydrolysis liquors (liquid phase) was filtered through $0.45 \mu\text{m}$ membranes and used for direct HPLC determination of glucose, xylose, arabinose, acetic acid, hydroxymethylfurfural (HMF) and furfural (F), using the same method specified above. A second aliquot was subjected to quantitative posthydrolysis with 4% w/w

sulphuric acid at 121°C for 30 min, filtered through 0.45 µm membranes, and analyzed by HPLC for oligosaccharides concentration. Solid phase from autohydrolysis was washed with distilled water, air-dried and employed for solid yield determination (expressed as g autohydrolyzed VPR/100 g VPR, on dry basis). Pretreated VPR by autohydrolysis was analyzed for glucan, xylan and Klason lignin using the analytical procedure described in section 2.2.

2.4. Enzymatic hydrolysis of VPR from autohydrolysis

In order to evaluate the enzymatic susceptibility of cellulose remained in pretreated vine pruning residues from autohydrolysis treatment, enzymatic hydrolysis assays were carried out using commercial enzymes Cellic Ctec2 (cellulase) and Htec2 (hemicellulase), kindly provided by Novozymes (Denmark). Enzymatic activities for celluloses and hemicellulases were determined (Ghose, 1986; Bailey et al., 1992) corresponding to 120 FPU/ml and 1690 IU/mL, respectively. The enzymatic hydrolysis was performed at liquid solid ratio (LSR) of 25 g/g, enzyme loading of 25 FPU/g of substrate, in a 0.05 N sodium citrate buffer (pH 4.85), at 50°C, and 150 rpm. Samples were withdrawn between 0-72h and analyzed by HPLC for glucose concentration. All determinations were performed in duplicate.

3. RESULTS

3.1. Vine pruning residue composition

Residue from vine pruning was analyzed for structural and no structural components, expressed in g/100 g wood in oven-dry basis with three replicate determinations. The composition was displayed in Figure 1.

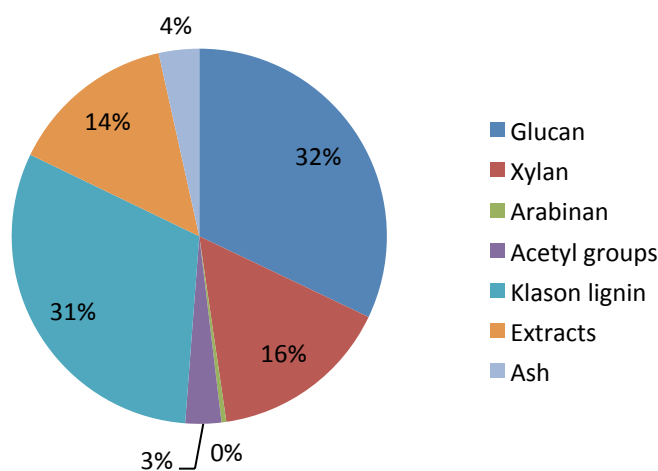


Figure 1 – Chemical composition of vine pruning residue

3.2. Autohydrolysis treatment of vine pruning residue

Residue from vine pruning was treated under conditions listed in Table 1 and analyzed for its chemical composition (see Table 1). As can be observed from results of Table 1, pretreated VPR was composed by 33.3-45.6 g of glucan/100 g, corresponding to glucan recovery between 78-99 % for all of conditions studied in this work. On the other hand, 27.6-79.1 % of xylan was solubilized (calculated as difference of xylan in raw material and recovered xylan in solid phase of pretreated VPR).

The hemicellulose solubilization was represented in Figure 2. At 180 °C and 60 min ($S_0=4.13$), the maximum concentration of xylose and xylooligosaccharides (2 and 11 g/L) was achieved which corresponded to a solubilization of xylan of 62 %.

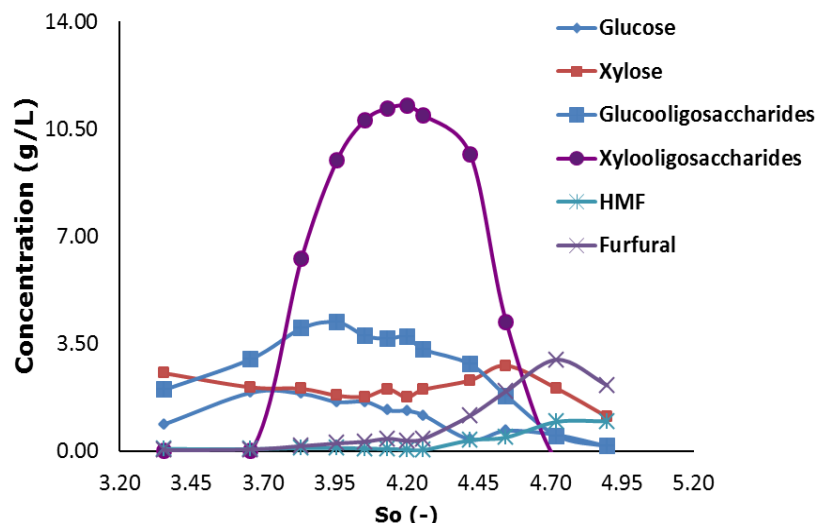


Figure 2 – Liquid phase composition shown in concentration from pretreated VPR by autohydrolysis (S_0 : 3.36-4.90).

3.3. Enzymatic saccharification of autohydrolyzed vine pruning residue

Enzymatic saccharification of pretreated VPR was shown in Figure 3 in which the effect of autohydrolysis pretreatment can be seen. The glucose concentration varied in the range of 3-18 g/L corresponding to 18-91% of glucan to glucose conversion for $S_0 = 3.36$ and 4.90, respectively.

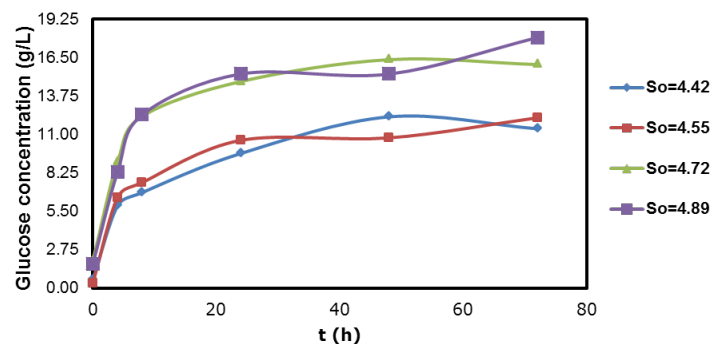
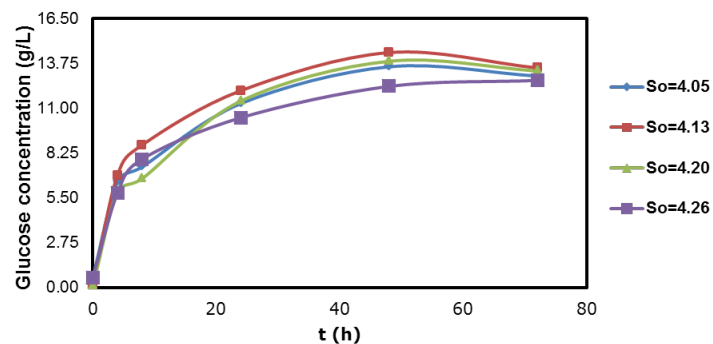
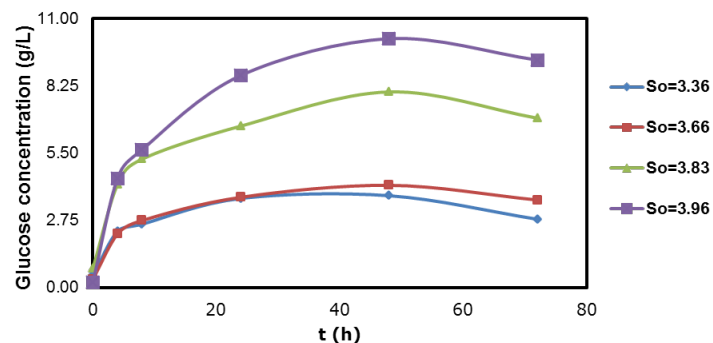


Figure 3 – Enzymatic saccharification of pretreated VPR by autohydrolysis. Glucose concentration release through time is shown for the different severity factors (S_0).

4. CONCLUSIONS

Overall, the autohydrolysis process at 180°C and 60 min ($S_0=4.13$) was suitable for the recovery of hemicellulosic fraction in liquid phase as xylose and xylooligosaccharides and to obtain a susceptible pretreated lignocellulosic biomass for glucose production (13 g/L with 73 % of glucan to glucose conversion).



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