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Research article

Environmentally friendly technologies for obtaining high sugars concentrations from invasive woody species

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Abstract: The efficient utilization and conversion of inexpensive invasive raw materials into bioethanol following a biorefinery approach is a priority in the research field of renewable fuel. With this purpose, *Acacia dealbata* wood samples were pretreated with 1-ethyl-3-methylimidazolium acetate under optimized conditions, and the resulting solids were employed as a substrate for enzymatic hydrolysis. Enzymatic assays were performed according to a complete factorial experimental design, in which the effects of two independent variables (liquor to solid ratio and enzyme to substrate ratio) on the kinetics and yields of the xylan and cellulose saccharification were assessed. The Response Surface Methodology was employed for optimizing the experimental conditions. High sugar concentrations (around 80 g/L), and favorable polysaccharide conversions (CCG = 79.4% and XnCX = 77.9%). were predicted by the model under the selected operational conditions (6 g liquor/g substrate, 22 FPU/g). The results reported in this work compare well with other studies dealing with either other ionic liquids or classical pretreatments, using the same raw material or other woody substrates.

Keywords: *Acacia dealbata* wood; ionic liquid; pretreatment; enzymatic hydrolysis; biomass biorefinery

Abbreviations

LSR: liquor to solid ratio; ESR: enzyme to substrate ratio; EHS: enzymatic hydrolysis substrate; FPU: filter paper units; IU: international units; *t*: reaction time.

1. Introduction

Nowadays, there is an increased scientific and commercial interest in the development of viable biorefining strategies suitable for converting renewable raw materials into biofuels and platform chemicals, boosted by environmental concerns, depleting fossil resources and public awareness [1,2]. This goal can be achieved in biomass biorefineries, conceived as integrated chemical and biotechnological processes enabling the selective separation and the efficient utilization of the major feedstock components [3,4].

The feedstock cost is one of the economic bottlenecks in the biotechnological production of chemicals and fuels. *Acacia dealbata*, an invasive Australian woody legume, has become a serious environmental problem because it modifies the structure of different native ecosystems and threatens native aboveground [5]. *Acacia dealbata* is an attractive biorefinery raw material from environmental, economic and chemical points of view, because it is renewable, cheap, abundant, and rich in polysaccharides [6]. To our knowledge this promising raw material has not been yet extensively studied. Therefore further research is required to develop different green biorefinery schemes.

Owing to their complex chemical and structural nature, the woody materials are not directly suitable for bioconversion into fuels and chemicals [7]. The recalcitrance of lignocelullosic materials can be reduced by a suitable pretreatment [8], which is one of the key stages of biorefineries.

Desirably, pretreatments should meet a number of requirements, including [9–12]: (i) be simple and cost effective; (ii) allow high carbohydrates recovery; (iii) result in high digestibility of the polysaccharides in the subsequent enzymatic hydrolysis; (iv) avoid the formation of inhibitory byproducts hindering the subsequent hydrolysis and fermentation; (v) lead to high concentration of released sugars in the liquid fraction; (vi) be able to produce high quality lignin with small amounts of waste.

Several types of pretreatments have been explored in the last decades, and they have been extensively reviewed along with their key advantages and disadvantages [13,14]. In recent years, ionic liquids (ILs) have received attention as promising green solvents for the fractionation of lignocellulosic biomass [8,15–18]. In particular, halogen-free ILs of low viscosity can dissolve polysaccharides efficiently [19]. The ILs bearing an acetate group, like 1-ethyl-3-methylimidazolium acetate (EMIMAc), are promising solvents due to its favorable properties, including low melting temperature, low viscosity, non-toxicity and non-corrosive character [20,21]. Additionally, EMIMAc showed high capacity to reduce the crystallinity of cellulose and significantly improve the enzymatic saccharification of lignocelullosic materials [22].

Unlike chemical processes, the use of enzymes has the following advantages [23–25]: (1) operation can be performed under moderate pressure and temperature in non-corrosive media; (2) operation is “clean”, since no pollutants or substances hazardous to the environment or human health are used; (3) hydrolysis is specific, avoiding the generation of glucose degradation products; (4) high

hydrolysis yields can be achieved; and (5) no fermentation inhibitors are generated.

The objective of this work was to optimize the recovery fermentable sugars in the enzymatic hydrolysis of *Acacia dealbata* wood pretreated with EMIMAc. An experimental plan (in which the liquor to solid ratio (LSR) and the enzyme to substrate ratio (ESR) employed in enzymatic hydrolysis were employed as independent variables) was carried out to assess their effects on the kinetics and yields of enzymatic hydrolysis, using the Response Surface Methodology. The model predictions enabled the identification of the optimal operational conditions.

2. Materials and Method

2.1. Raw material

Acacia dealbata wood samples were collected locally, debarked and chipped. Air-dried *Acacia dealbata* wood samples were milled (Retsch SM1 74075 instrument, Germany) and sieved (Retsch AS200 Basic, Germany operating at an amplitude of 80 for 30 min) to select the fraction of particles with size in the range 0.25–1 mm. The resulting material was mixed in a single lot to avoid compositional differences, an assayed for composition using the methods listed below. Table 1 shows the chemical composition of the selected fraction of *Acacia dealbata* wood (expressed in g component/100 g oven-dry wood ± standard deviation).

Table 1. Chemical composition of *Acacia dealbata* wood and the substrate used in EH assays

	Content	
Component	*Acacia dealbata* wood (g /100 g dry wood)	EHS* (g/100 g dry EHS)
Cellulose	43.0 ± 1.31	46.2 ± 1.55
Xylan	15.6 ± 0.44	13.7 ± 1.04
Acetyl groups	3.26 ± 0.17	2.30 ± 0.39
Klason lignin	22.4 ± 0.83	21.9 ± 0.33
Extractives	5.10 ± 0.34	-
Ashes	0.63 ± 0.02	-
Others (by difference)	10.0	15.9

* Pretreated with EMIMAc at 150 ℃, 30 min and 20% of solid loading

2.2. Processing of Acacia dealbata

Wood samples and EMIMAc (from Sigma Aldrich) were dried overnight in an oven at 70 ℃ to remove moisture. Pretreatments were performed in stirred round-bottom flasks in absence of agitation, operating under the conditions (150 ℃ for 30 min at 20% solid loading) reported as optimal in an earlier study [6]. Temperature was controlled using a PID module. At the end of the treatments, cellulose was precipitated by adding water (as an anti-solvent) to the media under vigorous stirring. After 30 min, the suspension was filtered and the solid fraction was thoroughly washed with distilled water and dried overnight at 50 ℃, to yield the enzymatic hydrolysis substrate (EHS), which was assayed for composition and employed in saccharification experiments. The

composition of EHS (expressed in g component/100 g oven-dry EHS ± standard deviation) is also given in Table 1.

2.3. Enzymatic hydrolysis

Table 2 summarizes the experimental plan designed to assess the effects of the considered independent variables (LSR and ESR) on the enzymatic hydrolysis of EHS. Experiments were carried out in Erlenmeyer flasks with orbital agitation (150 rpm) using commercial enzymes (''Celluclast 1.5 L'' cellulases from *Trichodermareesei* and ''Novozym 188'' β-glucosidase from *Aspergillus niger*), kindly provided by Novozymes (Madrid, Spain). The cellulase activity of ''Celluclast 1.5 L'' was measured using the Filter Paper assay and expressed in terms of FPU/mL [26]. The β-glucosidase activity of ''Novozym 188'' was measured by the PNPG assay [27] and reported as IU/mL. Experiments were performed at 48.5 ℃ in media containing 0.05 N citric acid–citrate buffer (pH = 4.85), keeping a cellobiase to cellulase ratio of 5 IU/FPU. At given hydrolysis times, samples were withdrawn from the reaction media, centrifuged, filtered and analyzed by HPLC (see below).

Table 2. Experimental design employed to assess the enzymatic hydrolysis of pretreated *Acacia dealbata* wood, expressed in terms of the dimensional variables LSR and ESR and dimensionless variables x_1 and x_2.

	Dimensional independent variables		Dimesionless, normalized, independent variables	
Exp.	LSR (g/g)	ESR (FPU/g)	X_1	X_2
1	6	8	−1	−1
2	6	19	−1	0
3	6	30	−1	1
4	13	8	0	−1
5	13	19	0	0
6	13	19	0	0
7	13	19	0	0
8	13	30	0	1
9	20	8	1	−1
10	20	19	1	0
11	20	30	1	1

2.4. Analytical methods

2.4.1. Analysis of the raw material and pretreated solids

Wood and EHS samples were milled to particle sizes < 0.5 mm and subjected to the following analyses: extractives (TAPPI T-264-om-88m method); moisture (ISO 638:1978 method); ashes (T-244-om-93 method); cellulose, xylan, arabinan and acetyl groups (by HPLC determination of the glucose, xylose, arabinose and acetic acid contained in liquors from the TAPPI T13m assay); and

Klason lignin (by gravimetric determination of the solid from the TAPPI T13m assay). Determinations were carried out in triplicate.

2.4.2. High performance liquid chromatography analysis

Analyses were performed using an Agilent 1200 series chromatograph with a refractive index detector (temperature, 50 ℃). Other analysis conditions were: column, Aminex HPX-87H (BioRad, USA); mobile phase, 0.003 mol/L H_2SO_4 flow rate, 0.6 mL/min.

2.4.3. Fitting of data

The experimental data were fitted to the proposed models using commercial software (Solver, Microsoft Excel, Microsoft, USA).

3. Results and Discussion

3.1. Chemical composition and pretreatment of Acacia dealbata wood

The data in Table 1 confirm the high polysaccharide content of *Acacia dealbata* wood (close to 60%), as well as its limited lignin content (22.4%). Other components of minor importance for the purposes of this study include extractives and acetyl groups, which accounted for 5.10 and 3.26 wt% of the dry wood, respectively. These results are in the range reported in literature [6,28–30].

Table 1 also lists compositional data of EHS. As indicated in an earlier work [6], the EMIMAc pretreatment led to favorable polysaccharide preservation, resulting in solid fractions (EHS) with increased contents of cellulose and xylan (46.2 and 13.7%, respectively).

3.2. Enzymatic hydrolysis

3.2.1 Experimental plan

The enzymatic hydrolysis is strongly affected by LSR and ESR. To assess their effects, a complete, factorial experimental design (in which both variables, whose variation ranges were 6–20 g/g and 8–30 FPU/g, respectively, were evaluated at three levels) was performed. The same general philosophy has been successfully employed in literature to assess the enzymatic hydrolysis of a variety of substrates [31–33].

Table 2 shows the set of experiments, whose structure corresponds to a complete, factorial, centered, experimental design (2^3 plus two additional replications at the central point of the experimental domain). The dimensionless, normalized, independent variables (x_1 and x_2), with variation ranges (−1, 1), are linearly related to the dimensional independent ones (liquor to solid ratio (LSR) and enzyme to substrate ratio (ESR)), respectively. The dimensionless ones of independent variables were calculated following the equation:

$$x_1 = 2\ \frac{LSR_i - LSR_{mean}}{LSR_{max} - LSR_{min}} \qquad (1)$$

$$x_2 = 2\ \frac{ESR_i - ESR_{mean}}{ESR_{max} - ESR_{min}} \qquad (2)$$

where the subscripts *i* is the considered experiment, mean is the average value of the variable min and max and min and max mean minimum and maximum values of the respective variation ranges, respectively.

In order to allow a generalized interpretation of the effects caused by the reaction time, concentration profiles were determined for enzymatic assays performed under the conditions of experiments 1–11. As representative examples, Figure 1 shows the concentration profiles determined for glucose and xylose in assays 1, 2, 3, 6, 9 and 11, as well as the conversions calculated from them (the cellulose conversion into glucose, denoted CCG, and the xylan conversion into xylose, denoted XnCX). The concentration profiles of both components follow similar patterns, beyond the obvious differences resulting from the relative cellulose and xylan contents of EHS. The highest concentrations of glucose and xylose reached after 48h of hydrolysis, varied in the ranges of 18.5–71.1 g/L and 6.59–18.4 g/L, respectively.

The highest glucose concentration (71.1 g/L) was obtained in experiment 3, operating at LSR = 6 g/g and ESR = 30 FPU/g after 48h of hydrolysis, whereas high xylose concentrations were reached at prolonged reaction times in experiments 1–3 (Figure 1d). In comparison, limited sugar concentrations were noticed in experiment 9 (18.5 g glucose/L and 6.59 g xylose/L at the end of the assay).

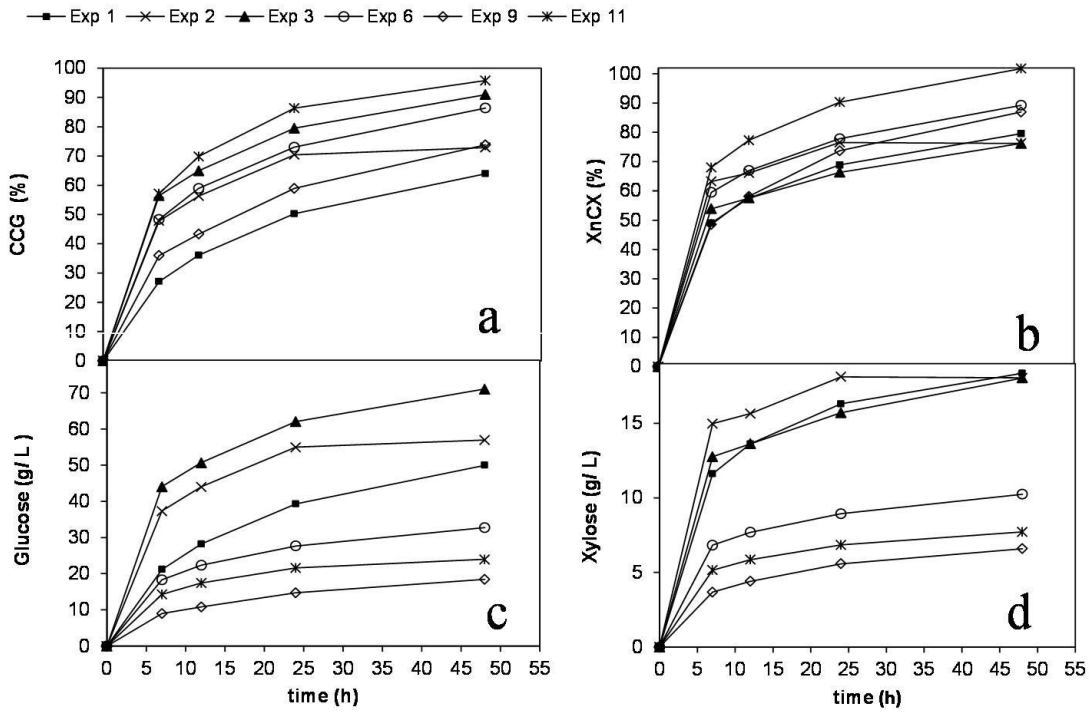

Figure 1. **Time courses of cellulose and xylan conversions (CCG and XnCX) and glucose and xylose concentrations obtained in selected enzymatic hydrolysis assays (experiments 1, 2, 3, 6, 9 and 11)**

The susceptibility of EHS to enzymatic saccharification was confirmed by the high conversions (above 70%) reached after 24 h in almost all the experiments (see Figures 1a and b). High xylan conversions were reached in the zone of the experimental domain defined by LSR in the range 13–20 g/g and ESR ≥ 19 FPU/g. Complete xylan conversion was achieved in experiment 11 (performed at LSR = 20 g/g and ESR = 30 FPU/g) after 48 h of hydrolysis. In comparison 24.0 g glucose/L (corresponding to 95.8% cellulose conversion into glucose) was achieved at the end of experiment 11.

In comparative terms, experiment 3 (performed at LSR = 6 g/g with ESR = 30 FPU/g) provided high sugar concentrations at all hydrolysis times (for instance, 50.7 g glucose/L after 12 h, corresponding to 64.9% cellulose hydrolysis; or 89.1 g total sugars/L after 48 h, corresponding 91.0 and 76.2% hydrolysis of cellulose and xylan, respectively (see Figure 1). On the other hand, operating at the intermediate LSR with the highest enzyme charges assayed (experiment 8) led to lower sugar concentrations (46 g/L at 48 h) but to saccharification conversions, in the range 92.6–98.6 %, which are quite close to the highest values obtained.

3.2.2. Generalized interpretation of data

Different equations have been proposed in the literature to describe the effects of the reaction time on enzymatic hydrolysis of cellulosic substrates. Among them, the model suggested by Holtzapple, et al. (1984) [34] stands out for its simplicity and ability to provide a close interpretation of experimental data [12, 35]. The form of the equation is:

$$G = G_{max}\,\frac{t}{t + t_{1/2G}} \qquad (3)$$

where G is the glucose concentration achieved at time t, G_{max} is the maximum glucose concentration that would be reached at an infinite reaction time, and $t_{1/2G}$ is the time needed to achieve $G = G_{max}/2$. Based on the similar shape of the concentration profiles, the same equation has been employed in the mathematical modeling of xylose generation along the experiments:

$$X = X_{max}\,\frac{t}{t + t_{1/2X}} \qquad (4)$$

where X is the xylose concentration achieved at time t, X_{max} is the maximum xylose concentration that would be reached at an infinite reaction time, and $t_{1/2x}$ is the time needed to achieve $X = X_{max}/2$.

In this work, the concentration profiles determined for glucose and xylose in the various experiments were fitted to the Holtzapple's model, with the restrictions $G_{max} \leq G_{POT}$ and $X_{max} \leq X_{POT.}$ Table 3 shows the results determined for the parameters G_{max}, $t_{1/2G}$, X_{max} and $t_{1/2X.}$ The high values reached by the parameter R^2 when fitting the data from the various experiments (average value > 0.995) confirmed the suitability of the proposed model for data interpretation.

According to the data in Table 3, the $t_{1/2G}$ varied in the range 4.45–16.05 h, reaching its highest values in experiments performed at the lowest enzyme charge considered (8 FPU/g). However, as can also be seen in the cited table, lower values were found for the $t_{1/2X}$, within the range 2.04–11.51 h.

As a general trend, for a given LSR, increased ESR led to decreased $t_{1/2}$, whereas the lowest values of $t_{1/2G}$ and $t_{1/2X}$ (4.45 and 2.04 h, respectively) corresponded to experiments 11 and 2. On the

other hand, average values of 4.90 h and 3.43 h were obtained for $t_{1/2G}$ and $t_{1/2X}$, respectively, in assays carried out at ESR = 30 FPU/g (experiments 3, 8 and 11).

Concerning variables G_{max} and X_{max} (see Table 3), the highest results (76.5 and 20.2 g/L, respectively) were obtained in experiments 3 and 1, confirming the high susceptibility of both substrates towards enzymatic saccharification (also reflected in the fast hydrolysis kinetics observed in the initial reaction stages).

Table 3. Kinetics parameters deduced from experiments 1–11.

Experiment	y_1 or $t_{½G}$* (h)	y_2 or G_{max}** (g/L)	y_3 or $t_{½X}$* (h)	y_4 or X_{max}** (g/L)
1	15.5	65.60	5.49	20.20
2	5.07	64.20	2.04	19.00
3	5.43	76.50	3.86	18.80
4	16.05	36.70	11.51	11.50
5	7.91	35.90	4.89	11.40
6	7.56	37.10	4.66	11.00
7	7.95	36.70	4.48	10.80
8	4.92	37.10	3.34	11.50
9	12.1	22.70	8.03	7.58
10	8.07	24.50	3.79	7.58
11	4.45	24.50	3.10	7.58

* time needed to achieve 50% G_{max} or X_{max}

** maximum glucose or xylose concentration predicted for time = ∞

3.2.3. Dependence of the kinetic parameters on LSR and ESR

The set of variables G_{max}, $t_{1/2G}$, X_{max} and $t_{1/2X}$ allows a generalized interpretation of the enzymatic hydrolysis, and have been included in this study as dependent variables (denoted y_1, y_2, y_3, and y_4, respectively), which were correlated with the independent variables (LSR and ESR) by empirical modelling.

A great amount of experimental work would be needed to assess the effects of each independent variable on each dependent variable in a systematic way. Alternatively, the Response Surface Methodology can serve for optimization based on relatively small experimental designs. This approach was employed in the present study.

The interrelationships between dependent and independent variables were established by the following empirical equation:

$$y_j = b_{0j} + \sum_{i=1}^{2} b_{ij} x_i + \sum_{i=1}^{2} \sum_{k=1}^{2} b_{ikj} x_i x_k \quad (5)$$

where y_j (j = 1, 2, 3 and 4) are the dependent variables (G_{max}, $t_{1/2G}$, X_{max} and $t_{1/2X}$, respectively); x_i or x_k (i or k: 1–2, $k \geq i$) are the dimensionless normalized, independent variables measuring the values of LSR and ESR; and b_{0j}...b_{ikj} are regression coefficients calculated from experimental data by multiple regression using the least-squares method. Table 4 lists the values determined for the

regression coefficients, as well as, for the statistical parameters measuring the correlation (R^2) and significance of models (based on Fisher's F-test).

Table 4. Regression coefficients and significance (based on t-test) and statistical parameters measuring the correlation and significance of models obtained for variables y_1 to y_4 in set of experiments 1–11.

Regression Coefficients				
Coefficient	y_1 or $t_{½G}$ (h)	y_2 or G_{max} (g/L)	y_3 or $t_{½X}$ (h)	y_4 or X_{max} (g/L)
b_{0j}	7.78[a]	36.01[a]	4.77[a]	11.09[a]
b_{1j}	−0.23	−22.42[a]	0.51	−5.89
b_{2j}	−4.81[a]	2.16	−2.53[a]	−0.23
b_{12j}	0.61	−2.27	0.71	0.34
b_{11j}	−1.17	9.15[a]	−1.98[b]	2.16[a]
b_{22j}	2.74[b]	1.75	2.52[b]	0.34
Statistical Parameters Measuring the Correlation and Significance of the Models				
R^2	0.937	0.989	0.889	0.997
F_{exp}	14.80	89.43	8.01	471.16
Significance level (%)	> 99	> 99	> 98	> 99

[a] *Coefficients significant at the 99% confidence level*

[b] *Coefficients significant at the 95% confidence level*

The values of coefficients and the model predictions obtained for the various dependent variables are discussed in the following paragraphs.

A) $\boldsymbol{t_{1/2G}}$—The values of the regression coefficients listed in Table 4 describing the behavior of variable $t_{1/2G}$ (denoted y_1), show that ESR and its quadratic term were the most influential terms affecting the model response. To facilitate the interpretation of results, Figure 2 shows the calculated dependence of $t_{1/2G}$ on LSR and ESR. The variation pattern was defined by the lowest values (4.2–4.8 h) of the dependent variable at the higher enzyme loadings assayed, in both extremes of the LSR considered, and by a marked drop with ESR. On the other hand, the highest values of $t_{1/2G}$ (> 15 h), were predicted for operation at the lowest ESR at intermediate or low LSR. The latter variable caused just limited effects on $t_{1/2G}$.

B) $\mathbf{G_{max}}$—According to the values of the regression coefficients (see Table 4), all of the independent variables affected significantly G_{max} (denoted y_2), the major effects being associated to linear and quadratic terms of LSR. Figure 3 shows the calculated dependence of G_{max} on LSR and ESR. Decreased LSR resulted in steadily increased of G_{max}, which reached its maximum value (73.8 g/L) operating at the highest ESR with the lowest LSR. On the other hand, the minimum G_{max} value (22.7 g/L) was found operating at ESR = 19 FPU/g with LSR = 20 g/g. Favorable values of G_{max} (in the range of 60–73.8 g/L) were predicted for LSR around 6 g/g, no matter the ESR considered.

C) $\boldsymbol{t_{1/2X}}$—Similarly to the variation pattern observed for $t_{1/2G}$, the most influential terms affecting $t_{1/2X}$ (or y_3) were ESR and its quadratic term. In this variable, LSR (through the quadratic term of the equation) was more influential than in the case of $t_{1/2G}$. This fact is reflected in Figure 4, which shows the calculated dependence of $t_{1/2X}$ on LSR and ESR. Increased ESR led to decreased

$t_{1/2X}$, up to reach minimum values close to 2 h in the range 22–26 FPU/g. Operation at higher ESR resulted in a slight increase of the dependent variable. Regarding the effects caused by LSR, the maximum values of $t_{1/2X}$ were achieved in both extremes of the response surface operating at medium LSR. Similarly to the behavior observed for $t_{1/2G}$, short $t_{1/2X}$ were predicted for operation at LSR around 6 or 20 g/g and high enzyme loadings (22–26 FPU/g).

D) **X_{max}**—All the independent variables caused significant effects on X_{max} (denoted y_4). Among them (similarity to the case of G_{max}) the most influential one was LSR (through its linear and quadratic terms). Figure 5 shows the calculated dependence of X_{max} on LSR and ESR, which presented general variation patterns related to the ones described above for G_{max}. However, operating at LSR about 6 g/g, increased enzyme loadings resulted in slightly decreased X_{max}, (but in increased G_{max}). The maximum X_{max} value (20.1 g/L) was predicted for the lowest LSR (6 g/g) and enzyme loading (8 FPU/g), whereas the maximum G_{max} was achieved at higher ESR. Interestingly, $X_{max} > 16$ g/L were predicted for operation at low LSR (6–8 g/g) in the whole ESR range considered.

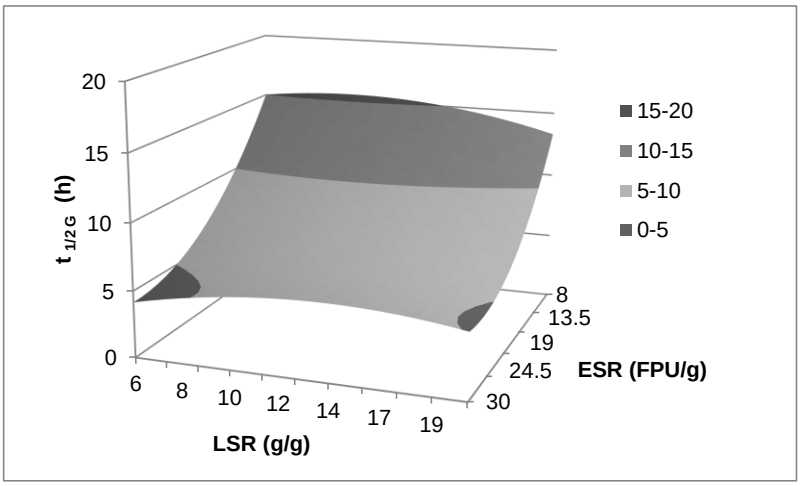

Figure 2. Dependence of the kinetic parameter $t_{1/2G}$ on both the liquid to solid ratio (LSR) and the enzyme to substrate ratio (ESR).

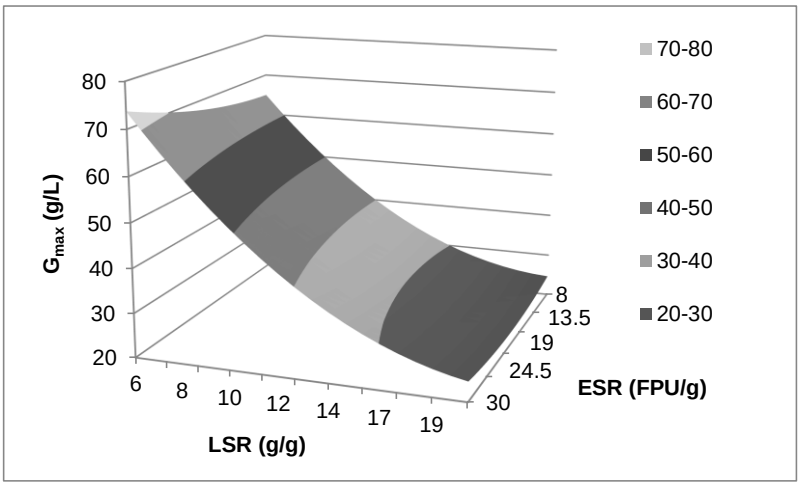

Figure 3. Dependence of the kinetic parameter G_{max} on both the liquid to solid ratio (LSR) and the enzyme to substrate ratio (ESR).

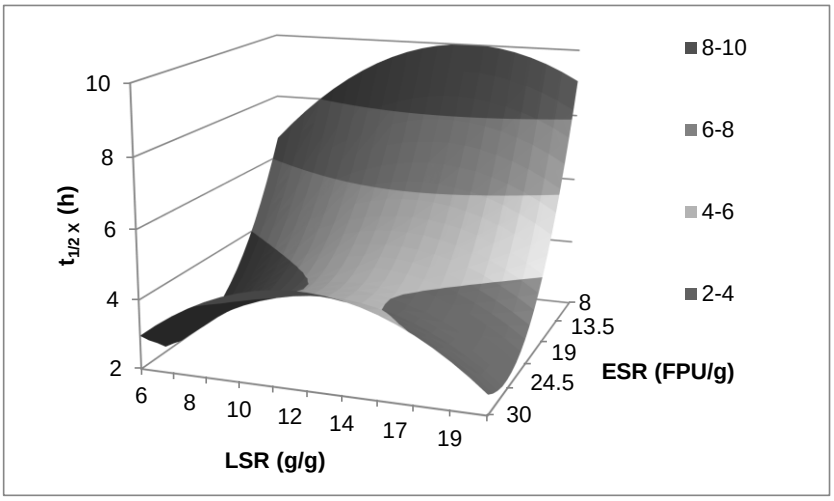

Figure 4. Dependence the kinetic parameter $t_{1/2X}$ on liquid to solid ratio (LSR) and enzyme to substrate ratio (ESR).

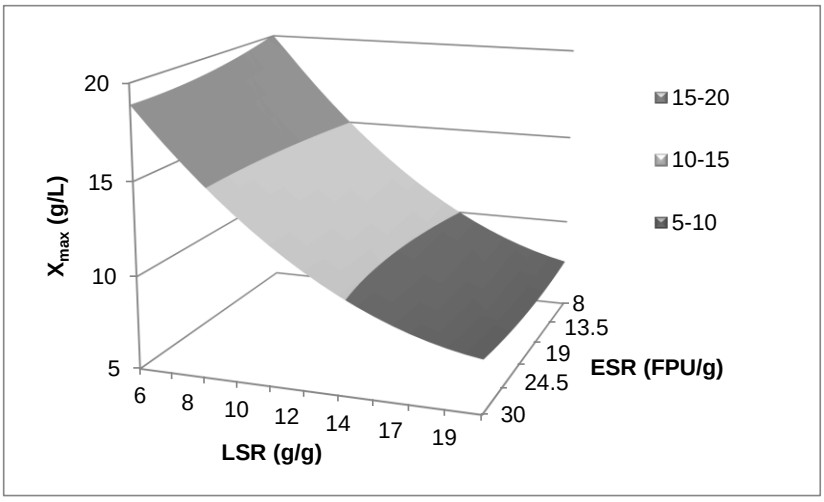

Figure 5. Dependence of the kinetic parameter X_{max} on both the liquid to solid ratio (LSR) and the enzyme to substrate ratio (ESR).

From the calculated response surfaces shown in Figures 2–5, it can be observed that the maximum G_{max} and the minimum $t_{1/2G}$ (73.8 g/L and 4.17 h, respectively) were predicted for conditions defined by LSR = 6 g/g and ESR= 30 FPU/g; which also led to X_{max} and $t_{1/2X}$ of 18.9 g/L and 2.97 h, respectively (both of them close to the maximum ones). Under these conditions, the model also predicted the highest sugar concentration (87.3 g/L) at favorable polysaccharide conversions (CCG = 88.6% and XnCX = 76.9%).

However, the enzyme cost still represents one of the primary expenses in the processes for biomass into chemicals and fuels [36]. The development of viable and efficient biorefinery processes requires operation with reduced enzyme dosages and high possible solid loadings, in order to optimize the utilization of substrates and to reduce the operational costs by increasing the sugar concentrations. According to the model predictions, hydrolysates containing about 80 g sugars/L can be obtained operating at LSR near 6 g/g and at an enzyme loading of 22 FPU/g. Under these

conditions, the values predicted for the dependent variables (G_{max} = 68.9 g/L, $t_{1/2G}$ = 5.56 h, X_{max} = 19.0 g/L and $t_{1/2X}$ = 1.93 h) are close to the optimal ones found in this work.

3.2.4. Generalized interpretation of kinetic data and evaluation of the predictive ability of the models

The empirical modeling performed in this study allowed the prediction of the kinetics of glucose and xylose generation by enzymatic hydrolysis along the whole experimental domain, through the following calculation scheme: (i) for given values of LSR and ESR, the dimensionless independent variables (x_1, x_2) can be calculated using equations 1 and 2; (ii) the dependent variables (G_{max}, $t_{1/2G}$, X_{max} and $t_{1/2X}$) can be then calculated using equation 5 and the set of regression coefficients (listed in Table 4); and (iii) with this information, the concentrations of glucose and xylose achievable at the desired hydrolysis time can be calculated using equations 3 and 4.

Figure 6 allows an evaluation of the correspondence between the predicted and experimental values of the concentrations of glucose and xylose concentrations after 7, 12, 24 and 48 h of enzymatic saccharification. The close agreement between calculated and experimental data confirms the validity of the models for quantitative calculations and modelling.

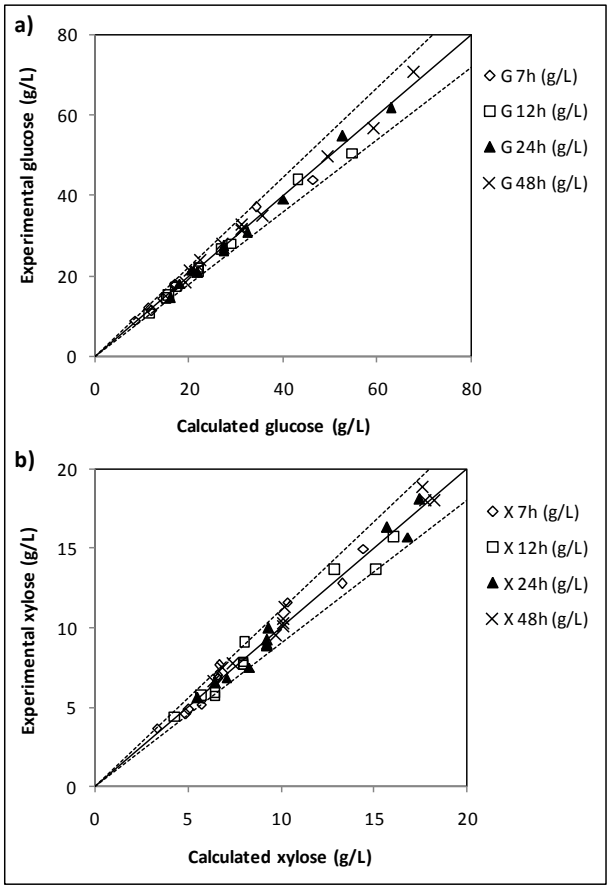

Figure 6. Correspondence between predicted and experimental values of glucose (a) and xylose (b) concentrations achieved in hydrolysates after 7, 12, 24 and 48 h of enzymatic saccharification

4. Conclusion

Samples of *A.dealbata* wood were pretreated with EMIMAc under operational conditions reported as optimal (150 ℃, 30 min and 20% of solid loading), and the pretreated solids (EHS) were employed as substrates for enzymatic hydrolysis. The effects of the reaction time were assessed for a set of experiments, from which kinetic parameters measuring the kinetics of cellulose and xylan hydrolysis were calculated. These parameters were employed in a Surface Response Methodology assessment to describe their dependence on two selected operational variables (LSR and ESR). High sugar concentrations and polysaccharide conversions were obtained operating at low LSR and medium to high ESR.

Unlike other classical pretreatments, such as acid pretreatment, alkaline pretreatment or steam explosion, the pretreatment with EMIMAc showed high capacity to disrupt the structure of crystalline cellulose allowing the preservation of high contents of cellulose and hemicelluloses in the raw material and the coproduction of high glucose and xylose concentrations by enzymatic hydrolysis under the optimized conditions cited above.

The best EHS behaved as susceptible hydrolysis substrates are being successfully employed for further optimization of fermentation process producing ethanol by simultaneous saccharification and fermentation (SSF) strategies. On the other hand, alternative recycling strategies for EMIMAc are also under study.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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