Biological hydrogen and furfural production from steam-exploded vine shoots

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Abstract

Vine shoots are an agricultural waste rich in carbohydrates that can be considered as a promising energy source alternative. The objective of this work was to propose a process strategy for the valorisation of this residual biomass, including the chemical conversion of solubilised sugars into furfural and the biological conversion of cellulosic glucose into H_2. Vine shoots were subjected to steam explosion pretreatment, and its operational conditions were optimised as 190 ºC and 1.6% H_2SO_4 impregnated biomass. These pretreatment conditions allowed to recover 68.2% of the hemicellulose sugars and 18.2% of glucose in the prehydrolysate and 45.3% glucose by enzymatic hydrolysis. Thus, the pretreated solid obtained under optimised conditions was subjected to enzymatic

hydrolysis and the slurry generated was used as a substrate by *Clostridium butyricum* for fermentation into biohydrogen (830.7 mL/L and a yield of 3,550 mL per 100 g of raw vine shoots) and organic acids (1,495.3 mg acetic acid/L and 1,726.8 mg butyric acid/L). Based on furfural production, the chemical conversion of xylose in the pre-hydrolysate was optimised in a microwave reactor at 202 ºC, using 0.195 M $FeCl_3$ as a catalyst, with a furfural production of 15 g/L and 73% yield.

Keywords

Vine shoots, steam explosion, microwave, biohydrogen, furfural.

Abbreviations

Glucose recovery in liquid fractions (GRL)

Hemicellulosic sugar recovery in liquid fractions (HSRL)

Enzymatic hydrolysis yield (EHY)

1. Introduction

Residual biomass has received great interest as a low-cost feedstock for the production of a wide range of products, particularly including biofuels. In addition to the benefits of converting these lignocellulosic materials from an environmental point of view (compared to the usual practices of direct burning), a relevant feature is the ubiquitous availability in virtually any part of the world, making them an interesting option compared to the defined and limited locations of fossil fuels.

In the specific case of the viticulture practices and winemaking industry, several wastes are generated, such as vine shoots, grape pomace, grape stalks, and wine lees [1]. Vineyards are widespread throughout the world, with around 7.5 million ha being

distributed amongst different countries. In the European Union (EU), 2020 wine production was 166 million of hectoliters, and the estimate for 2021 was 145 million of hectoliters (excluding juices and musts), totalling almost half of the world's wine production [2]. This outstanding European production also brings large amounts of by-products with high environmental impacts, and vine shoots are among the main waste generated with the practice of viticulture [3]. Due to their lignocellulosic nature, any valorisation scheme of vine shoots must include the conversion of the sugar fraction, which requires a pretreatment step.

Pretreatment is required to fragment the complex structure in order to favour the release of sugars for a greater availability and prepare the biomass for the bioconversion to building-block chemicals or energy. An inadequate pretreatment can cause the formation of undesirable compounds, such as organic acids resulting from the degradation of sugars or phenols from lignin degradation [4]. Furthermore, the polymerisation of lignin and hemicellulose block the sugars' accessibility. Therefore, the elimination of lignin facilitates the bioconversion of lignocellulose materials. In addition, lignin can be used to produce bio-based products [5]. Steam explosion (SE) is a widely used physicochemical pretreatment method. SE produces the solubilisation of sugars' hemicellulose in the liquid and enlarges the surface for a better hydrolysis of solids [6]. The hydrolysate and the solid obtained in SE can be used as a subsequent stage flow.

A limited number of research papers dealing with the use of sugars that can be obtained from vine shoots are available in scientific literature. Dávila et al. [7] reported on the production of oligosaccharides as a result of hydrothermal pretreatment of vine shoots; depending on the pretreatment severity, a maximum concentration of 12 g

xylooligosaccharides was obtained. In another study from the same research team, a comparison between conventional and microwave-assisted hydrothermal pretreatment concluded that there were little differences on the production of oligosaccharides, but energy balance and time used were favourable to the second option. Rivas et al. [8] performed a 1-butanol-catalysed organosolv pretreatment to fractionate vine shoots into lignin, cellulose, and hemicellulose, from which furfural was obtained following a microwave-assisted treatment. Senila et al. [9] determined that 6 g ethanol per 100 g of vine shoots can be obtained following a process including autohydrolysis at 165 °C, chlorite delignification, and simultaneous saccharification and fermentation by *Saccharomyces cerevisiae* YSC2. However, reports on the production of energy carriers, such as a hydrogen, are missing and those addressing the integral conversion of sugars present in vine shoots are also scarce.

Furfural is a platform compound consisting of a five-carbon furan ring from which more than 80 different compounds can be obtained. The dehydration of xylose coming from the hemicellulose fraction of biomass is one of the main routes for production of furfural [10]. In turn, the glucose contained in the cellulose fraction of biomass can be directed to a wide range of products, including energy carriers such as hydrogen, following an anaerobic fermentation scheme [11].

Producing hydrogen and furfural from vine shoots holds significant relevance for sustainable energy production, waste valorization, biorefinery development, and environmental conservation. Turning agricultural wastes into valuable resources can contribute to pave the way to circular bioecomony, and aligns with global goals of reducing carbon emissions and advancing renewable energy technologies. In addition,

using this agricultural residue for producing hydrogen and furfural contribute clearly to technology innovation and can exert a beneficial change of the usual disposal method (direct burning, with the associated economic and environmental costs). For the first time, dilute sulfuric acid steam explosion is optimised for vine shoots as a fractionation strategy that improved their enzymatic digestibility, while furfural production from hemicelluloses was also optimised through microwave-assisted technology. As another novelty, the simultaneous production of hydrogen and furfural, using respectively the cellulosic and the hemicellulosic fractions of pretreated vine shoots was carried out in this work. The objective of this work was to propose a process scheme for the valorisation of this residual biomass, including the chemical conversion of xylose into furfural and the biological conversion of cellulosic glucose into hydrogen.

2. Materials and methods

2.1 Raw material

Vine shoots were collected after pruning of the vineyards. Biomass was milled in a laboratory hammer mill (Retsch, SM 100, Fisher Scientific S. L., Madrid, Spain) and passed through a 1 cm screen. Their moisture content was determined (10.3%), then they were characterised according to the National Renewable Energy Laboratory (NREL, Golden, CO, USA) methodology [12]. The milled biomass was stored at room temperature.

2.2. Steam explosion pretreatment

Vine shoots were pretreated by steam explosion in a custom-built pilot unit equipped with a 4 L capacity vessel. The reactor was filled with 400 g of dry biomass, soaked for 12 h in 2 L of diluted sulphuric acid, and heated with saturated steam to reach the working

117 temperature. The pretreatment time was fixed at 5 min, and once the time had elapsed,
118 the reactor was rapidly depressurised to atmospheric pressure.

119 Steam explosion pretreatment of raw material was performed according to a rotatable
120 central composite experimental design (α = 1.414) with a total of 13 experiments,
121 including one point and 4 replicates at the central point as shown in Table 1. Center values
122 and intervals for both pretreatment temperature and acid concentration were chosen based
123 on previous experience. Commercial software (Design-Expert 12.0.3.0, Stat-Ease Inc.,
124 Minneapolis, USA) was used to analyse the experimental results.

125

126 **Table 1**. Experimental conditions for steam explosion pretreatment of
127 vine shoots.

Run	Temperature (ºC)		Acid concentration (%w/v)	
	Coded value	Real value	Coded value	Real value
1	-1	170	-1	0.50
2	+1	190	-1	0.50
3	0	180	0	1.25
4	0	180	+1.414	2.30
5	0	180	0	1.25
6	0	180	0	1.25
7	0	180	-1.414	0.20
8	-1	170	+1	2.00
9	0	180	0	1.25
10	-1.414	166	0	1.25
11	+1	190	+1	2.00
12	0	180	0	1.25
13	+1.414	194	0	1.25

128

129 After pretreatment, the resulting slurry was vacuum filtered to separate the two phases.
130 The pretreated solids were washed for acid removal until neutral pH, dried at 40 ºC, and
131 characterised as cellulose, hemicellulose, and lignin [12]. The resulting liquid fractions
132 (pre-hydrolysates) were analysed for sugars and inhibitor compounds (Section 2.7.2).
133 Recoveries of glucose and hemicellulosic sugars in the pre-hydrolysates were determined
134 as a percentage of the sugar content in the raw material.

2.3. Enzymatic saccharification tests

After SE pretreatment, pretreated solids were washed and enzymatically hydrolysed at 5% (w/v dry basis) solid loading. Enzymatic hydrolysis was carried out with Cellic CTec2 complex, supplied by Novozymes (Bagsværd, Denmark), with an enzyme load of 15 filter paper units/g substrate. β-glucosidase (Novozymes A/S), at 15 international units/g substrate, was added to supplement β-glucosidase activity of the enzymatic complex. 25 mL of solution 0.05 M sodium citrate that acts as a buffer with a pH of 4.8, and the enzymes were added to 100 mL Erlenmeyer flasks. Saccharification tests were performed in an orbital shaker at 150 rpm for 72 h with an incubation temperature of 50 °C. Glucose concentration in the hydrolysates was determined by HPLC (Section 2.7.2).

2.4. Microorganism and inoculum

A pure culture of *Clostridium butyricum* (CCT7470) obtained from the Tropical Culture Collection of the Andre Tosello Foundation (Campinas, SP, Brazil) was used as the fermentative inoculum. Cells were reactivated in autoclaved modified medium (sodium acetate 3 g/L, agar 0.5 g/L, starch 1 g/L, sodium chloride 5 g/L, meat extract 10 g/L, yeast extract 3 g/L, peptone 10 g/L, glucose 2.5 g/L, and xylose 2.5 g/L) with a pH of 6.8. The culture medium (250 mL), together with the inoculum, were added to 500 mL Erlenmeyer flasks, subjected to N_2 atmosphere (100%) for 10 min for headspace gas exchange, closed with a silicone cap and plastic screw, and incubated at 37 °C and 130 rpm for 48 h. After bacterial growth, liquid samples were centrifuged (4,000 rpm, 6 min) and the supernatant was discarded. The pellet obtained was inoculated in the fermentative reactors.

2.5. Biohydrogen production from pretreated solid

157 The pretreated solid obtained at optimised conditions of steam explosion was saccharified
158 with enzymes as described in Section 2.3 then converted into biohydrogen (H_2) with
159 *Clostridium butyricum* (Fig. 1). The fermentative assays were conducted in the same
160 flasks of the enzymatic hydrolysis using the final slurry as the substrate. Thereby, meat
161 extract (5 g/L), yeast extract (1.5 g/L), and peptone (5 g/L) were added as macronutrients
162 [13], and the pH was adjusted to 6.8 with NaOH. The inoculum (5.7 g total volatile
163 solids/L) was added to the flasks, which were then subjected to N_2 atmosphere (100%)
164 for 5 min, closed with a silicone cap and plastic screw, and incubated at 37 °C and 130
165 rpm. The control assay was performed under the same conditions mentioned above but
166 without vine shoots.

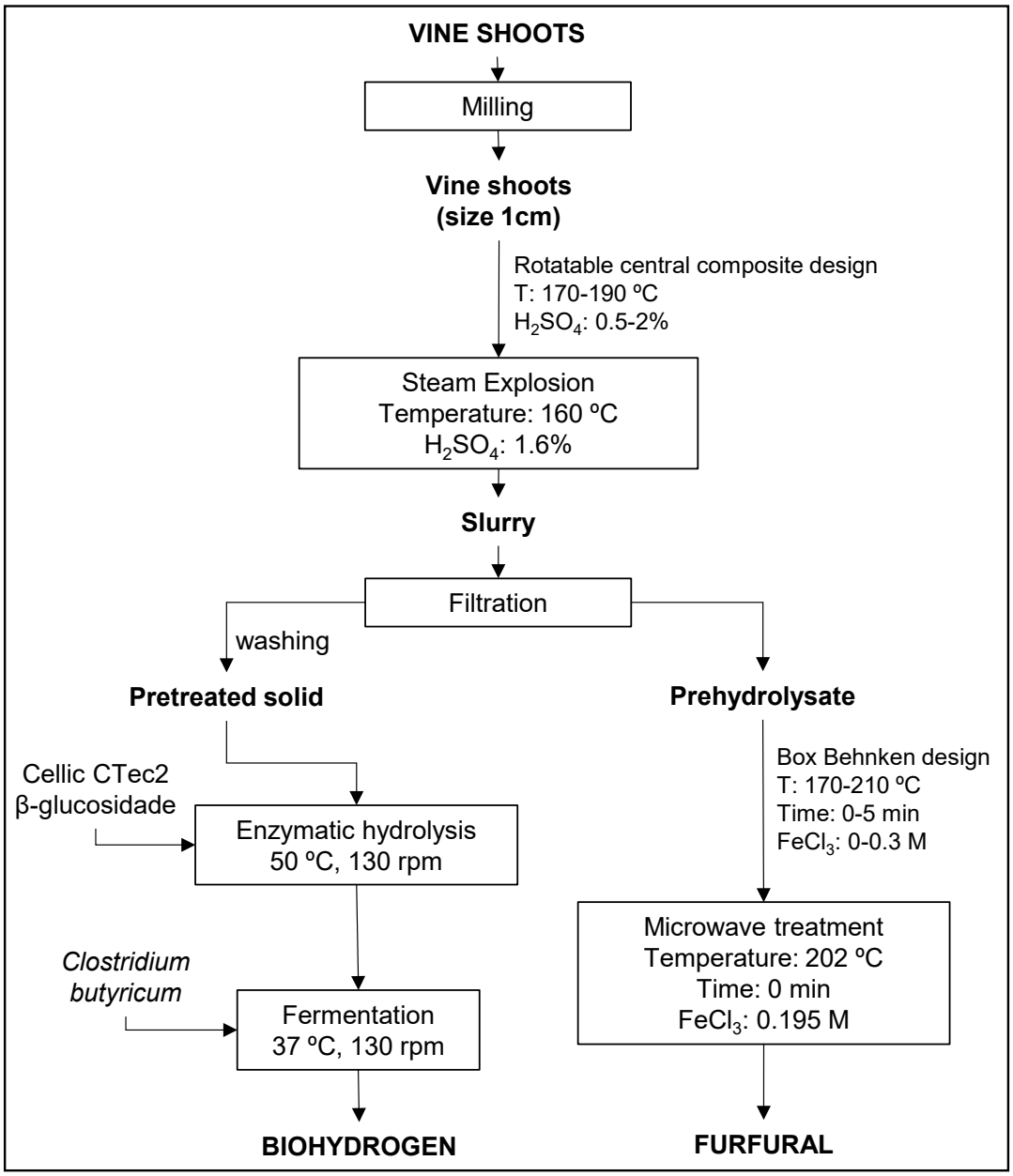


Figure 1. Process scheme performed for the conversion of vine shoots into biohydrogen and furfural under optimized conditions for steam explosion pretreatment. (160ºC, 1.6% H_2SO_4).

Gaseous samples (1.0 mL) were collected directly from the headspace of the flasks with a syringe with a push button valve and transferred to 2 mL vials with vacuum. The H_2

content of the biogas was determined using a gas chromatograph (GC), and the final production of soluble metabolites was analysed by HPLC as described in Section 2.7.1.

The H_2 production data were adjusted to the modified Gompertz model [14] to estimate the maximum H_2 production potential (*P*), maximum H_2 production rate (*Rm*), and time of initial H_2 production (λ) (Eq. 1):

$$H = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where *H* is the cumulative H_2 production (mL/L), *t* is the operation time (h), and *e* is the Euler number (2.71828). The parameters were calculated using OriginPro 9.0 software (OriginLab Corp., Northampton, MA, USA).

2.6. Microwave dehydration of the prehydrolysate

The liquid fraction obtained under optimal conditions of steam explosion pretreatment was used as the substrate for furfural production (Fig. 1). Microwave treatment was carried out in an Anton Paar microwave reactor (Monowave 400, Graz, Austria), equipped with an infrared sensor for temperature control. The reactor has a maximum power of 850 W, and a maximum temperature of 300 ºC can be reached. The heating ramp in the microwave reached a temperature of 170 ºC in 5 min then followed a ramp of 10 °C/30 s until reaching 210 ºC. Capped 10 mL glass tubes filled with 4 mL of prehydrolysate were used and heated to the desired temperature and time, then cooled with compressed air. The liquor used for the reaction was previously filtered through 0.45 µm filters. $FeCl_3$, acting as a Lewis acid, and H_2SO_4, acting as Brønsted acid, were used as catalysts. Nevertheless, only $FeCl_3$ was added to the liquor because the biomass had

194 been impregnated with sulfuric acid before steam explosion. A pH of 1.4 was determined
195 in the prehydrolysate.

196 A Box-Behnken experimental design was adopted for the microwave treatment, with a
197 total of 17 experiments, including one point and four replicates at the center of the domain
198 selected for each factor under study. Center values and intervals were chosen based on
199 previous experience with another lignocellulosic residue [15]. The Box-Behnken design
200 constitutes a methodology for determining the values of the variables (also called factors)
201 at which a series of experiments should be performed to obtain, with a reduced or minimal
202 experimental work load, the dependence of the response on the factors. The resulting data
203 are then used to fit a second-order polynomial equation that models the relationship
204 between the variables and the responses. This equation can be analyzed and optimized to
205 identify the optimal conditions for the desired outcome.

206 The factors selected were the temperature (170–210 ºC), $FeCl_3$ concentration (0–0.3 M),
207 and reaction time (0–5 min~~)~~ Design-Expert 12.0.3.0 software was used to process the
208 data. After microwave treatment, the final liquors were analysed by HPLC to determine
209 their content in residual sugars and furfural (Section 2.7.2).

210 *2.7 Analytical methods*

211 2.7.1 Gas chromatography

212 The hydrogen measurement was conducted via gas chromatography. The equipment used
213 was a Shimadzu (Shimadzu Corp., Kyoto, Japan) model GC-2010, equipped with a
214 thermal conductivity detector. The carrier gas was argon, and the injector temperature
215 was 90 °C in split mode (1:20) for 2 min. The injection volume was 40 µL. The oven

temperature was 130 °C for 4.2 min then increased 30 °C/min until reaching 135 °C, which was maintained for 2 min. The column flow was 1.2 mL/min. The capillary column was a Carboxen 1010 PLOT (30 m length, 0.32 mm diameter, 0.3 μm film thickness), supplied by Supelco (Bellefonte, PA, USA). The detector was maintained at 180 °C, employing a current of 20 mA and a negative polarity. The makeup gas of the detector was argon. The GC was equipped with a PAL autosampler (Zwingen, Switzerland).

2.7.2 Liquid chromatography

The sugar and inhibitor concentrations were analysed by HPLC (model Agilent 1260 Infinity; Agilent Technologies, Santa Clara, CA, USA), using the ICSep ICE-COREGEL 87H3 (Transgenomic, Inc., Santa Clara, CA, USA) column. The oven temperature was 65 °C. The mobile phase was a solution of 5 mM sulphuric acid with a flow of 0.6 mL/min. The samples were filtered through 0.45 μm nylon membranes.

3. Results and discussion

3.1 Vine shoots composition

The composition of raw vine shoots used in this work was determined as follows (% dry basis): cellulose, 33.9 ± 0.2; hemicellulose, 18.5 ± 0.4 (xylan, 15.9 ± 0.3; arabinan, 0.7 ± 0.1; galactan, 1.3 ± 0.1; mannan, 0.5 ± 0.0); acid-insoluble lignin, 22.1 ± 0.5; acid-soluble lignin, 1.8 ± 0.0; ash, 3.0 ± 0.0; acetyl groups, 3.4 ± 0.0; galacturonic acid, 1.1 ± 0.1; extractives, 9.0 ± 0.4; and glucose in extractives, 0.8 ± 0.0. These results are mean values of three replicates. The high content of structural carbohydrates (58%), mainly glucose, stands out. The hemicellulose consisted of 86.5% xylose, which was the main sugar in this fraction. The composition of vine shoots determined in this work is in the range reported by other authors [9, 16].

3.2 Effect of steam explosion pretreatment on vine shoots

Steam explosion pretreatment meant a partial solubilisation of the biomass and its recovery ranged between 85% (runs 1 and 7) and 58% (runs 11 and 13), depending on the harshness of the pretreatment. These latter experiments resulted in solids with the lowest hemicellulose content, since the solubilisation corresponded mainly to extractives and the hemicellulose fraction. This biomass solubilisation yielded cellulose-enriched solids, with cellulose contents between 36% and 43.5%. Likewise, the percentage of lignin in the pretreated solids was also increased from 23.9% (in raw vine shoots) to 42.5% (runs 11 and 13) (Table 2).

248 **Table 2**. Biomass recovery after steam explosion pretreatment of vine shoots and
249 pretreated solids composition (%).

Run	Temp. (ºC)	H_2SO_4 conc. (%)	Biomass recovery	Cellulose	Hemicellulose	AIL	ASL
1	170	0.50	85.77	35.96	18.40	26.20	1.77
2	190	0.50	72.35	39.85	12.61	31.50	1.56
3	180	1.25	63.46	43.00	7.10	37.81	1.41
4	180	2.30	60.89	43.48	5.45	37.92	1.38
5	180	1.25	67.27	40.35	8.93	36.41	1.47
6	180	1.25	68.52	40.85	10.40	34.23	1.52
7	180	0.20	85.17	36.13	17.60	27.87	1.60
8	170	2.00	67.80	36.63	7.17	35.34	1.33
9	180	1.25	68.23	39.71	8.89	36.11	1.25
10	166	1.25	75.63	35.68	13.62	32.69	1.51
11	190	2.00	58.34	43.87	3.68	41.22	1.27
12	180	1.25	68.24	38.95	10.03	35.42	1.49
13	194	1.25	58.59	41.47	3.45	41.33	1.32

250 Mean values of three replicates, standard deviations <0.05; AIL: Acid insoluble lignin; ASL: Acid
251 soluble lignin.
252

253 Table 3 shows the composition of the prehydrolysates in sugars and inhibitors. In general,
254 the main sugar in these liquids was xylose, although the presence of glucose was detected
255 in all experiments. The solubilisation of glucose even at the mildest pretreatment
256 conditions (9.5%; 170 ºC, 0.5% H_2SO_4, run 1) can be due to the presence of glucose as
257 part of the hemicelluloses [17] or the presence of amorphous cellulose in the composition
258 of vine shoots, which is easily hydrolysed. With the same feedstock, other authors have

obtained a sugar solution containing 24% glucose and 63% xylose after organosolv treatment in a microwave-heated reactor at 190 ºC [8].

Table 3. Composition of prehydrolysates in sugars and inhibitors (g/L) and sugars recovery (%).

Run	Glucose	Xylose	Gal	Arab	Man	Formic acid	Acetic acid	Furfural	HMF	GRL	HSRL
1	0.54	0.00	0.27	0.86	0.00	0.32	0.29	0.00	0.00	9.48	14.20
2	1.66	1.37	1.00	1.64	0.06	0.65	1.03	0.22	0.11	12.92	43.51
3	12.19	12.88	3.48	3.39	0.74	0.78	3.04	0.44	0.18	15.99	62.17
4	14.63	22.04	4.21	3.22	1.30	0.92	6.75	0.95	0.26	15.90	63.56
5	11.27	12.64	3.08	3.02	0.69	0.67	3.14	0.47	0.17	14.72	54.68
6	6.59	6.86	1.99	2.38	0.33	0.52	1.79	0.30	0.11	14.67	53.41
7	0.64	0.19	0.27	0.49	0.01	0.61	0.61	0.03	0.03	6.50	14.17
8	13.57	19.82	3.93	3.24	1.07	0.60	5.51	0.41	0.14	16.04	61.48
9	10.14	11.28	2.62	2.58	0.50	0.66	2.89	0.44	0.16	13.86	52.83
10	5.33	4.02	1.69	2.50	0.18	0.28	1.30	0.14	0.06	12.38	32.62
11	15.53	23.61	4.42	3.33	1.46	1.09	7.66	1.41	0.39	17.36	65.26
12	5.83	6.32	2.04	2.34	0.37	0.53	1.61	0.32	0.11	12.79	47.32
13	13.14	16.66	3.56	3.19	0.96	0.99	4.89	1.17	0.40	15.07	59.00

Mean values of three replicates, standard deviations <0.05. Gal: galactose; Arab: arabinose; Man: mannose; HMF: hydroxymethylfurfural; GRL: glucose recovery in liquid fractions HSRL: hemicellulosic sugar recovery in liquid fractions.

Maximum sugar concentration in the prehydrolysate (48.4 g/L) was determined from vine shoots impregnated with 2% H_2SO_4 before steam explosion at 190 ºC (run 11), where glucose and xylose accounted for 32% and almost 50% of the total sugar content, respectively. This experiment reached the highest hemicellulose sugar recovery (65.3%), while runs 1 and 7 only recovered 14% of hemicellulose sugars in the prehydrolysate due

271 to the mild pretreatment conditions used in these experiments (Table 3). Senila et al. [9]
272 determined a maximum value of hemicellulose recovery (33.5%) in the liquid fraction
273 from vine shoots pretreated by autohydrolysis at 180 ºC. In addition to sugars, sugar
274 degradation compounds were detected in the prehydrolysates. Nevertheless, in general,
275 their concentrations were not noticeable, being lower than 1 g/L for formic acid, 0.4 g/L
276 for hydroxymethylfurfural, and 1.4 g/L for furfural. Only the presence of acetic acid, from
277 the hydrolysis of hemicelluloses, stands out as an inhibitor in these liquids, reaching a
278 maximum concentration of 7.7 g/L (run 11) (Table 3).

279 After pretreatment, bioconversion processes require a saccharification step in which
280 enzymes break down the polysaccharides into monomeric sugars [18]. Enzymatic
281 hydrolysis tests using pretreated solids as substrate were carried out to determine their
282 enzymatic digestibility. The highest glucose concentration in the hydrolysate (15 g/L)
283 was determined when the pretreatment was carried out at 194 ºC using 1.25% acid-
284 impregnated raw material (run 13). This glucose concentration corresponds to 66%
285 saccharification efficiency and an enzymatic yield of 47% (Table 4). These results
286 compare favourably with those reported from 2% acid-impregnated olive tree pruning
287 biomass pretreated by steam explosion at 190 ºC [19]; however, Dávila et al. [20]
288 achieved complete cellulose conversion from vine shoots pretreated with 12% NaOH at
289 124 °C for 105 min, although hemicellulose recovery was not evaluated in this work.

290 **Table 4**. Results of enzymatic saccharification of pretreated solids.

Run	Glucose concentration (g/L)	Saccharification efficiency (%)	EH yield (%)
1	2.99	15.13	13.77
2	10.12	46.18	39.30
3	10.38	43.87	35.33
4	9.67	40.42	31.58
5	9.08	40.90	32.76
6	8.99	40.03	33.07
7	4.20	21.12	19.18
8	7.69	38.15	27.97
9	10.38	47.54	38.02
10	4.65	23.72	18.89
11	13.55	56.15	42.42
12	9.20	42.93	33.67
13	15.00	65.77	47.17

291 Mean values of three replicates, standard deviations <0.05. Saccharification
292 efficiency: g glucose by enzymatic hydrolysis/100 g glucose in substrate; EH
293 yield: g glucose by enzymatic hydrolysis/100 g glucose in raw material.
294

295 *3.3 Optimisation of the steam explosion pretreatment of vine shoots*

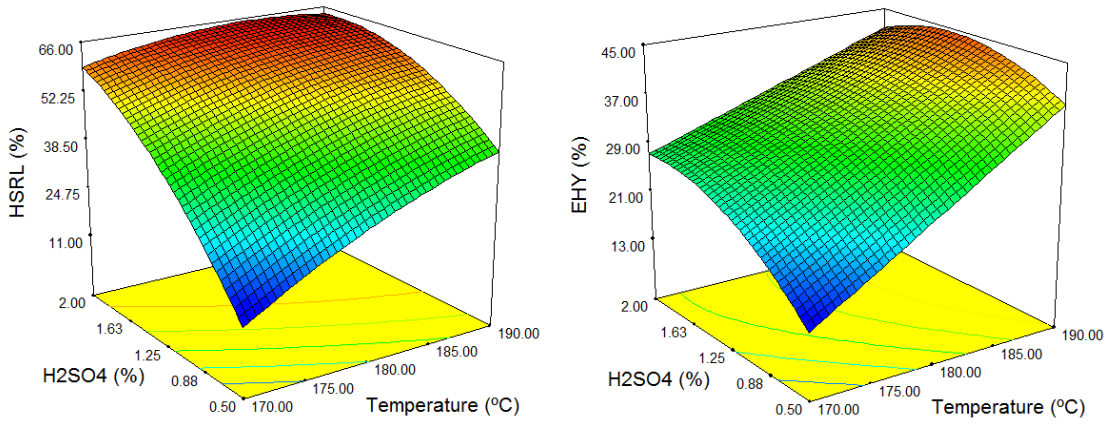
296 The results obtained in the 13 pretreatment experiments of the central composite design
297 were statistically processed and analysed. The responses HSRL and EHY were processed
298 according to ANOVA for a quadratic model. The equations obtained, in terms of coded
299 factors, for both responses are included in Table 5. The values of R^2 and adjusted R^2 in
300 both equations show a good agreement between the experimental and predicted data.

From the coefficients of these equations, it can be determined that the acid concentration used for biomass impregnation was the most significant factor on HSRL. In contrast, the temperature was the most influential parameter on EHY, although both responses were affected by the interaction between factors. Fig. 2a illustrates the response surface and contour plots obtained from the model applied to HSRL, and the positive influence of both factors in this response is clear, although at the simultaneous maximum level of both factors a slight decrease on HSR can be observed. In the case of EHY, it can be observed that this response achieved its maximum level at intermediate levels of acid concentration then EHY did not increase (Fig. 2b).

Table 5. Model equations and statistical parameters for steam explosion pretreatment of vine shoots.

Equation	CV (%)	R^2	R^2 adjust	p-value	F-value	Lack of fit
HSRL (%) = +54.08 + 8.8·A +17.36·B - 6.38·A·B -3.19·A^2 - 6.66·B^2	9.46	0.9608	0.9328	<0.0001	34.32	0.36
EHY (%) = +34.33 +10.00·A + 4.36·B -2.77·A·B - 4.14·B^2	5.59	0.9777	0.9665	<0.0001	87.52	0.34

HSRL: hemicellulosic sugars recovery in liquids; EHY: enzymatic hydrolysis yield; A: temperature (ºC); B: H_2SO_4 concentration (% w/v)



a) b)

Figure 2. Response surfaces and contour plots for (a) hemicellulose sugars recovery and (b) enzymatic hydrolysis yield as a function of pretreatment temperature and H_2SO_4 concentration.

The mathematical model that was developed from the experimental results is able to predict the operational conditions that should be used in the steam explosion pretreatment of vine shoots to optimise model responses. In this study, the optimisation focused on the simultaneous maximisation of both responses for HSRL and EHY.

The model predicted the following as the optimal conditions for steam explosion pretreatment of vine shoots: 190 °C and 1.63% H_2SO_4, with a desirability of 0.936. The values predicted by the model for the responses were 63.5% (HSRL) and 44.1% (EHY). To validate the model, a new experiment was carried out (in triplicate) at the optimised conditions. The experimental values obtained were 68.2 ± 1.0% (HSRL) and 45.3 ± 0.8% (EHY). The experimental results are very close to the values predicted by the statistical model and within the limits of variability in a 95% confidence level. In addition to hemicellulose sugars, 18.2% of glucose was recovered in the prehydrolysate. Therefore,

332 considering total sugars measured in the liquor and glucose recovered by enzymatic
333 hydrolysis, 65% of sugars in raw vine shoots were recovered by steam explosion and
334 hydrolysis. Semwal et al. (2019) achieved a glucan conversion of 88% from acid
335 impregnated rice straw pretreated by steam explosion and enzymatically hydrolysed at
336 15% solids.

337 *3.4 Bioconversion of pretreated vine shoots into biohydrogen*

338 The optimised conditions for steam explosion pretreatment of vine shoots yielded a solid
339 fraction with 43.43 ± 0.55% cellulose, 2.58 ± 0.10% hemicellulose, and 41.62 ± 0.89%
340 lignin. This pretreated solid showed great potential to be used as a substrate in biological
341 processes, due to (1) a large part of fermentable sugars in the form of cellulose and (2)
342 absence of inhibitors of the fermentation process. Thus, this biomass was used as a source
343 of sugars to produce biohydrogen. For this purpose, it was enzymatically hydrolysed and
344 the resulting slurry was fermented by *C. butyricum* (Fig. 1).

345 Islam et al. [21] also pretreated and enzymatically hydrolysed the biomass, followed by
346 fermentation to obtain better conditions for biohydrogen production. These authors used
347 an alkaline treatment of sweet sorghum stems, hydrolysis with the addition of cellulase,
348 and fermentation by *Clostridium thermosaccharolyticum* and reported a production of
349 839.3 mL H_2/L (6.37 mmol/g-substrate), 95% more than the fermentation of biomass
350 without any treatment.

351 The biohydrogen production from vine shoots after steam explosion pretreatment and
352 enzymatic hydrolysis is shown in Fig. 3. The time of initial H_2 production (λ) was 23.25
353 h, probably due to bacterial adaptation to the new growth conditions. But after this

adaptation period, a production rate of 49.08 mL H_2/L·h was observed. The fermentation of vine shoot slurry with *C. butyricum* resulted in H_2 production of 830.67 mL/L, which corresponds to a yield of 3,550 mL/100 g biomass.

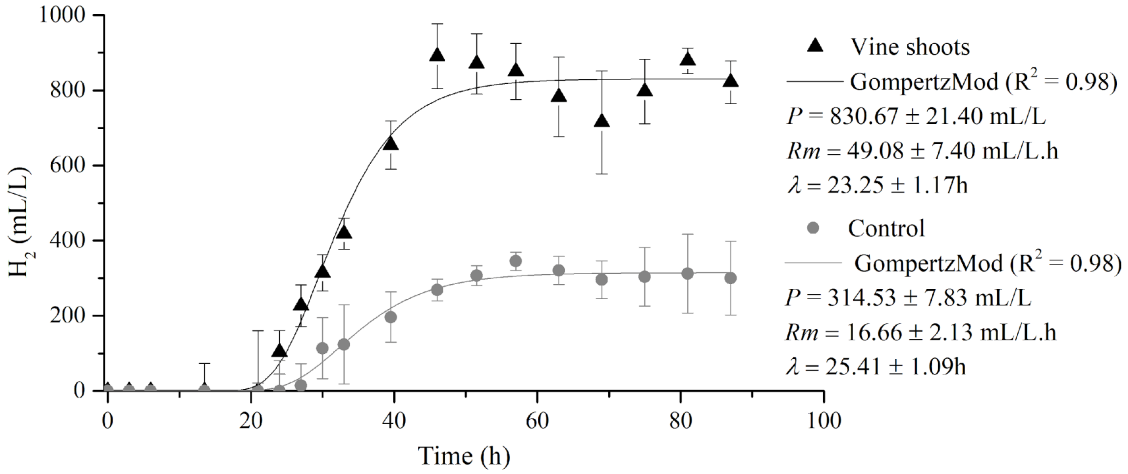


Figure 3. Cumulative H_2 production from vine shoots assay and control assay. *P*: maximum H_2 production potential, *Rm*: maximum H_2 production rate, λ: time of initial H_2 production.

The results obtained from vine shoots could not be compared with those reported by other researchers, because the use of this raw material in fermentation processes for biohydrogen production has not been reported; instead, comparisons with results obtained from other lignocellulosic biomasses are briefly described next. For instance, Dionizio et al. [22] and Rabelo et al. [23] used a slurry of enzymatically pretreated sugarcane bagasse as a substrate for H_2 production. Both authors used pure cultures as the inoculum (*Clostriduim* and *Paraclostridium*, respectively) and obtained 635.9 and 695.0 mL H_2/100 g biomass, respectively. On the other hand, when using mixed culture as the inoculum, Shanmugam et al. [24] obtained 4,021 mL H_2/100 g biomass with enzymatically pretreated sweet sorghum stover. Vine shoots (cellulose 33.9 ± 1.0%, hemicellulose 18.5 ± 0.4%, and lignin 23.9 ± 0.5%) are effectively different from

373 sugarcane bagasse (cellulose 31.4%, hemicellulose 36.6%, and lignin 24.8% [25]) and
374 sweet sorghum stover (cellulose 37.4%, hemicellulose 20.4%, and lignin 16.3% [24])
375 and, therefore, the results of metabolite production will also be different in quantity and
376 quality. Nonetheless, the H_2 production yield (3,550 mL H_2/100 g biomass) from steam-
377 exploded vine shoots is comparable with other lignocellulosic substrates (Table 6).

Table 6. Different types of biomass used as substrate for H_2 production.

Biomass	Pretreatment	Inoculum	P (unit reported by author)	mL H_2/100g biomass	Reference
Office waste paper	Acid hydrolysis	*E. coli* mutant strains	240 mL/g sugar	324.0	[18]
Sugarcane bagasse	Enzymatic hydrolysis	*C. butyricum*	148.8 mL/L	635.9	[14]
Sugarcane bagasse	Enzymatic hydrolysis	*Paraclostridium* sp.	166.8 mL/L	695.0	[15]
De-oiled jatropha waste	No	Sewage sludge	10.6 mL/g VS	1543	[19]
Opuntia spp.	Organic extraction	Mixed anaerobic sludge	41.0 mL H_2/g VS	2990	[20]
Vine shoots	Steam explosion + enzymatic hydrolysis	*C. butyricum*	830.7 mL/L	3550.0	**This work**
Cladophora sp. biomass	No	Anaerobic sludge	54.7 mL H_2/g VS	3560	[21]
Wheat stalk	No	Digested dairy manure	37.0 mL/g VS	3700 (/g VS)	[22]
Chlorella sp. biomass	Enzymatic hydrolysis	Wastewater sludge	43.16 mL H_2/g VS	4003	[23]
Sweet sorghum stover	Enzymatic hydrolysis	Sewage sludge	402.01 mL	4021	[16]
Algal biomass	No	Granular digester sludge	45 mL/g biomass	4500	[24]
Corn stover	Steam explosion	*C. cellulolyticum* + *Citrobacter amalonaticus*	51.9 L/kg TS	5190	[5]
Citrus peel waste	Alkaline delignification	Autochthonous consortium + sludge	7.27 mmol/L	5426.7	[25]
Citrus peel waste	Hydrothermolysis	Autochthonous consortium + sludge	8.19 mmol/L	6116.7	[25]

P: maximum H_2 production potential; VS: volatile solids; TS: total solids

In addition to the microbial composition, other factors affect the biological processes, such as the fermentative production of H_2 from lignocellulosic biomass, namely temperature, pH, substrate concentration [26, 27], nutrient concentration [28-30], concentration of inhibitors [31, 32], and substrate pretreatment conditions [33, 34]. For vine shoot fermentation, factors recognised as suitable for the H_2 production were used, such as pH close to neutral [11], for substrate pretreatment [35] combined with enzymatic hydrolysis [22, 23]. Therefore, from the results obtained (3,550.0 mL H_2/100 g-biomass), it is possible to use vine shoots in fermentative H_2 production and open the range for the optimisation of the process in future works.

The biological H_2 production from vine shoots was characterized as a fermentation of the acetic-butyric-type, since acetic acid (1,495.3 mg/L) and butyric acid (1,726.8 mg/L) were produced as the main by-products. Soluble sugars from enzymatic hydrolysis enter the glycolytic pathway, forming acetic and butyric acids along with H_2 and CO_2 (Equations 2 and 3, respectively). The production of both acids was consistent with the fermentation of bacteria of the Clostridia class used as the inoculum [36].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \quad (3)$$

These organic acids are bioactive secondary metabolites that have applications in the pharmaceutical, food, and chemical industries as a precursor to biofuels [37]. Furthermore, supernatants rich in organic acids together with the previously digested biomass can serve as a substrate for sequential methane (CH_4) production during the last step of anaerobic digestion. Braga et al. [38] used a two-stage process in batch reactors

to produce H_2 and CH_4 from pretreated sugarcane bagasse. These authors obtained 123.2 mL H_2/L and the acidified fermentation supernatant was used to produce 170.2 mL CH_4/L. Rabelo et al. [39] also used acidified supernatant for sequential CH_4 production and reported that the metabolites accumulated during fermentation (9140.5 mg HAc/L) were converted into 870 mL/L of CH_4 with the addition of a methanogenic sludge rich in hydrogenotrophic and acetoclastic archaea. The use of acidified liquid waste for methane production can also reduce the organic load, being an alternative for the treatment and reuse of fermentation wastes.

The biological H_2 production is an alternative for the increasing energy demand, and the feasibility of the process is at the centre of the most current discussions on the subject. In the present study, vine shoots, a largely available agricultural waste in the EU, were used as a substrate for the production of biohydrogen, presenting a sustainable alternative for recycling this waste together with the production of renewable energy.

3.5 Optimisation of furfural production from the hemicellulose prehydrolysate

The prehydrolysate obtained under optimal steam explosion conditions (190 °C and 1.63% H_2SO_4) was used as a substrate for furfural production. The chemical composition of this liquor was determined as follows: xylose, 26.6 g/L; glucose, 17.6 g/L; galactose, 4.0 g/L; arabinose, 5.2 g/L; mannose 0.4 g/L; formic acid, 1.1 g/L; acetic acid, 7.8 g/L; HMF, 0.4 g/L; and furfural, 1.2 g/L. Rivas et al. [8] obtained a sugar solution with a maximum pentose concentration of 17.5 g/L from the same feedstock pretreated with 52% n-butanol at 190 ºC using 2% H_2SO_4 as the catalyst.

Xylose in vine shoot prehydrolysate was chemically converted into furfural by dehydration using $FeCl_3$ as a catalyst. The experiments were performed in a microwave reactor and the influence of the temperature, $FeCl_3$ concentration, and reaction time was evaluated. Table 7 shows a summary of the results obtained, detailing the residual sugar concentration, furfural concentration and yield, xylose conversion, and selectivity obtained at different conditions of the Box-Behnken experimental design. It can be noted that the furfural concentration in the raw liquor (1.2 g/L) was subtracted from the furfural concentration determined at the end of each experiment, and the resulting concentrations are shown in Table 7. Furfural concentrations ranged from 2 (run 6) to 14.9 g/L (run 12). The latter $FeCl_3$ concentration corresponded to the highest yield (72.9%), which was reached at 210 ºC using 0.15 M $FeCl_3$ (run 12). Under these conditions, a xylose conversion of 94.9% was determined, close to the maximum conversion reached in this work (95.6%: 2.5 min, 210 ºC, $FeCl_3$ 0.3 M, run 17). Thus, the maximum values of furfural concentration and yield and conversion were determined under conditions of high harshness, which was necessary to dehydrate the xylose contained in the liquor. Nevertheless, these conditions yielded the lowest values of selectivity due to the loss of furfural by degradation.

Table 7. Box Behnken experimental design: factors and responses for furfural production from vine shoots.

Run	Temp. (°C)	$FeCl_3$ (M)	Time (min)	XGM (g/L)	Furfural (g/L)	Yield (%)	Conversion (%)	Selectivity (%)
1	170	0.30	2.50	15.18	9.27	42.97	59.66	72.02
2	170	0.15	0.00	30.18	3.55	12.61	11.99	100
3	190	0.30	0.00	7.39	12.70	61.20	83.29	73.47
4	190	0.00	5.00	25.61	6.18	26.57	25.76	100
5	190	0.15	2.50	7.46	13.23	64.04	82.96	77.19
6	170	0.00	2.50	33.08	2.00	4.37	2.36	100
7	190	0.30	5.00	1.75	8.69	39.88	95.79	41.63
8	210	0.00	2.50	16.52	10.42	49.09	54.52	90.05
9	190	0.15	2.50	5.85	13.01	62.83	87.53	71.78
10	170	0.15	5.00	18.44	9.12	42.20	49.27	85.65
11	190	0.15	2.50	5.31	14.15	68.92	88.98	77.45
12	210	0.15	0.00	2.83	14.91	72.93	94.85	76.89
13	190	0.15	2.50	6.24	13.34	64.58	86.51	74.66
14	190	0.00	0.00	32.19	2.62	7.65	5.36	100
15	190	0.15	2.50	6.14	13.95	67.83	86.74	78.20
16	210	0.15	5.00	1.39	7.51	33.65	95.62	35.19
17	210	0.30	2.50	1.63	5.51	23.00	94.84	24.25

Mean values of three replicates, standard deviations <0.05. XGM: Sum of xylose, galactose and mannose.

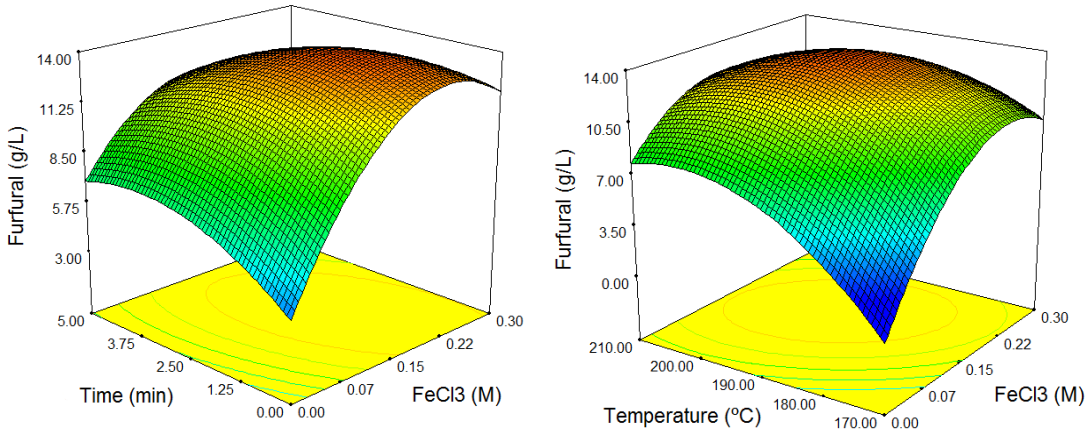
The results obtained were statistically analysed by Design-Expert software to determine the influence of the study factors on the responses: furfural concentration, furfural yield, xylose conversion, and selectivity. Table 8 includes the equations in terms of coded values for the four responses and their statistical parameters. As can be observed, $FeCl_3$ concentration was the most significant factor for all responses, followed by temperature, and the least influential by far was the reaction time. The value of CV was lower than 5% in all cases, indicating that the responses are influenced by the study factors. The fit of the models is good, with R^2 values exceeding 0.99 and adjusted R^2 values of 0.99.

Table 8. Model equations and statistical parameters for furfural production from vine shoots.

Equation	CV (%)	R^2	R^2 adjust	p-value	F-value	Lack of fit
Furfural (g/L) = 13.54 +2.44·A +3.15·B -0.28·C -4.32·A·B -3.24·A·C -1.89·B·C -4.03·A^2 -5.26·B^2 -0.73·C^2	4.97	0.9949	0.9857	<0.0001	108.28	0.98
Yield (%) = 4.18 +0.46·A +0.39·B +0.0036·C -0.76·A·B -0.40·A·C -0.63·B·C -0.38·A^2 -0.74·B^2 -0.38·C^2	1.28	0.9991	0.9974	<0.0001	589.85	2.59
Conversion (%) = 4.46 +0.89·A +0.93·B +0.53·C -0.67·A·B -0.54·A·C -0.45·B·C-0.49·A^2 -0.60·B^2 -0.29·C^2	0.92	0.9996	0.9990	<0.0001	1487.28	5.83
Selectivity (%) = 76 -14.70·A -23.64·B -10.58·C -9.45·A·B -10.26·A·C -10.56·B·C -4.70·A^2	2.71	0.9954	0.9908	<0.0001	215.82	0.08

A: temperature (°C); B: $FeCl_3$ concentration (M); C: time (min).

Fig. 4a shows the influence of $FeCl_3$ concentration and reaction time on furfural production. The reaction time only had a positive influence on furfural concentration at low levels of $FeCl_3$. However, the effect of $FeCl_3$ concentration on furfural production was positive until an intermediate level of about 0.2 M, probably due to furfural degradation. For the same reason, the highest furfural concentration was achieved at intermediate values of both temperature and salt concentration (Fig. 4b).



(a) (b)

Figure 4. Response surfaces and contour plots for furfural concentration as a function of (a) $FeCl_3$ concentration and reaction time and (b) temperature and $FeCl_3$ concentration.

After studying the effect of the main variables on furfural production, the optimal conditions at which the furfural concentration is maximised were determined. According to the model obtained from the experimental design, the conditions that maximised the furfural concentration were 0 min, 202 ºC, and 0.195 M $FeCl_3$. Under these conditions, the model predicted a furfural production of 15.3 g/L, and a yield of 72.9%. The optimised conditions were experimentally replicated and resulted in a liquor with a concentration of 14.9 ± 0.4 g/L furfural, corresponding to a yield of 73%, a xylose conversion of 89%, and a selectivity of 82%. Padilla-Rascón et al. [15] obtained a solution with 18 g/L furfural and 63.3 % yield from an hemicellulosic liquor of olive stones treated in a microwave reactor at 200ºC using 0.1 M $FeCl_3$ as a catalyst.

The furfural production obtained in this work compares favourably with those reported previously, using a microwave reactor, from organosolv pretreated vine shoots with a maximum yield of 65% [8] or corn cobs pretreated by autohydrolysis (13.2 g/L and 37% yield) [40]. In a previous work carried out with acid hydrolysate of olive stones, a higher

furfural concentration was obtained (18 g/L), but its yield was lower (63.3%), which indicates that the reaction was less efficient [15]. Brazdausks et al. [41] reported a maximum furfural yield of 72% from deciduous wood pentosans after hydrolysis at 175 ºC for 90 min using a mixture of H_3PO_4/NaH_2PO_4 as a catalyst.

4. Conclusions

Steam explosion pretreatment on vine shoots has been revealed as an efficient fractionation strategy. Operational optimised conditions (190 ºC and 1.6% H_2SO_4) allowed to recover 37.8 g of sugars per 100 g of vine shoots, yielding a good substrate for biohydrogen production with *C. butyricum* (3,550 mL/100 g raw material). The pretreatment liquid stream can be used as a source of xylose to obtain furfural by chemical conversion in a microwave reactor with a yield of 73%. The process scheme proposed in this work involved the integral valorisation of sugars in vine shoots to obtain products highly demanded in the bioenergy sector. This work contributes to a circular economy model through the production of green energy based on residual biomass. Future research would focus on the valorisation of the lignin-rich solid remaining after the conversion of cellulose into hydrogen and a techno-economic and environmental analysis of the global process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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