

Biological hydrogen and furfural production from steam-exploded vine shoots

Eulogio Castro^{a,b}, Camila A.B. Silva Rabelo^c, Carmen Padilla-Rascón^{a,b}, Alfonso M. Vidal^a, Juan C. López-Linares^a, Maria Bernadete A. Varesche^c, Inmaculada Romero^{a,b,*}

^a Dept. Chemical, Environmental and Materials Engineering, Universidad de Jaén, Paraje Las Lagunillas, 23071, Jaén, Spain

^b Center for Advanced Studies in Earth Sciences, Energy and Environment (CEACTEMA), Universidad de Jaén, 23071, Jaén, Spain

^c Laboratory of Biological Processes, Department of Hydraulics and Sanitation, Engineering School of São Carlos, University of São Paulo (EESC - USP) Campus II, São Carlos, SP, CEP 13563-120, Brazil

ARTICLE INFO

Keywords:

Vine shoots
Steam explosion
Microwave
Biohydrogen
Furfural

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 H₂. Vine shoots were subjected to steam explosion pretreatment, and its operational conditions were optimised as 190 °C and 1.6% H₂SO₄ 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 3550 mL per 100 g of raw vine shoots) and organic acids (1495.3 mg acetic acid/L and 1726.8 mg butyric acid/L). Based on furfural production, the chemical conversion of xylose in the prehydrolysate was optimised in a microwave reactor at 202 °C, using 0.195 M FeCl₃ as a catalyst, with a furfural production of 15 g/L and 73% yield.

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

Abbreviations: (GRL), Glucose recovery in liquid fractions; (HSRL), Hemicellulosic sugar recovery in liquid fractions; (EHY), Enzymatic hydrolysis yield.

* Corresponding author. Dept. Chemical, Environmental and Materials Engineering, Universidad de Jaén, Paraje Las Lagunillas, 23071, Jaén, Spain.

E-mail address: irromero@ujaen.es (I. Romero).

<https://doi.org/10.1016/j.renene.2023.119393>

Received 24 April 2023; Received in revised form 29 August 2023; Accepted 1 October 2023

Available online 3 October 2023

0960-1481/© 2023 Elsevier Ltd. All rights reserved.

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 valorisation, biorefinery development, and environmental conservation. Turning agricultural wastes into valuable resources can contribute to pave the way to circular bioeconomy, 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 (10.3% moisture content). Biomass was milled in a laboratory hammer mill (Retsch, SM 100, Fisher Scientific S. L., Madrid, Spain) and passed through a 1 cm screen. Biomass was homogenized, and stored at room temperature. On average, the raw material showed the following composition (% dry basis): cellulose, 33.9; hemicellulose, 18.5 (xylose, 18.0; arabinose, 0.8; galactose, 1.5; mannose, 0.5); acid-insoluble lignin, 22.1; acid-soluble lignin, 1.8; ash, 3.0; acetyl groups, 3.4; galacturonic acid, 1.1; extractives, 9.0; and glucose in extractives, 0.8 [12].

2.2. Steam explosion pretreatment

Vine shoots were pretreated by steam explosion in a custom-built

Table 1

Experimental conditions for steam explosion pretreatment of vine shoots.

Run	Temperature (°C)		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

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 sulfuric acid, and heated with saturated steam to reach the working temperature. The pretreatment time was fixed at 5 min, and once the time had elapsed, the reactor was rapidly depressurised to atmospheric pressure.

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

After pretreatment, the resulting slurry was vacuum filtered to separate the two phases. The pretreated solids were washed for acid removal until neutral pH, dried at 40 °C, and characterised as cellulose, hemicellulose, and lignin [13]. The resulting liquid fractions (prehydrolysates) were analysed for sugars and inhibitor compounds (Section 2.7.2). Recoveries of glucose and hemicellulosic sugars in the prehydrolysates were determined 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 N₂ atmosphere (100%) for 10 min for headspace gas exchange, closed with a silicone

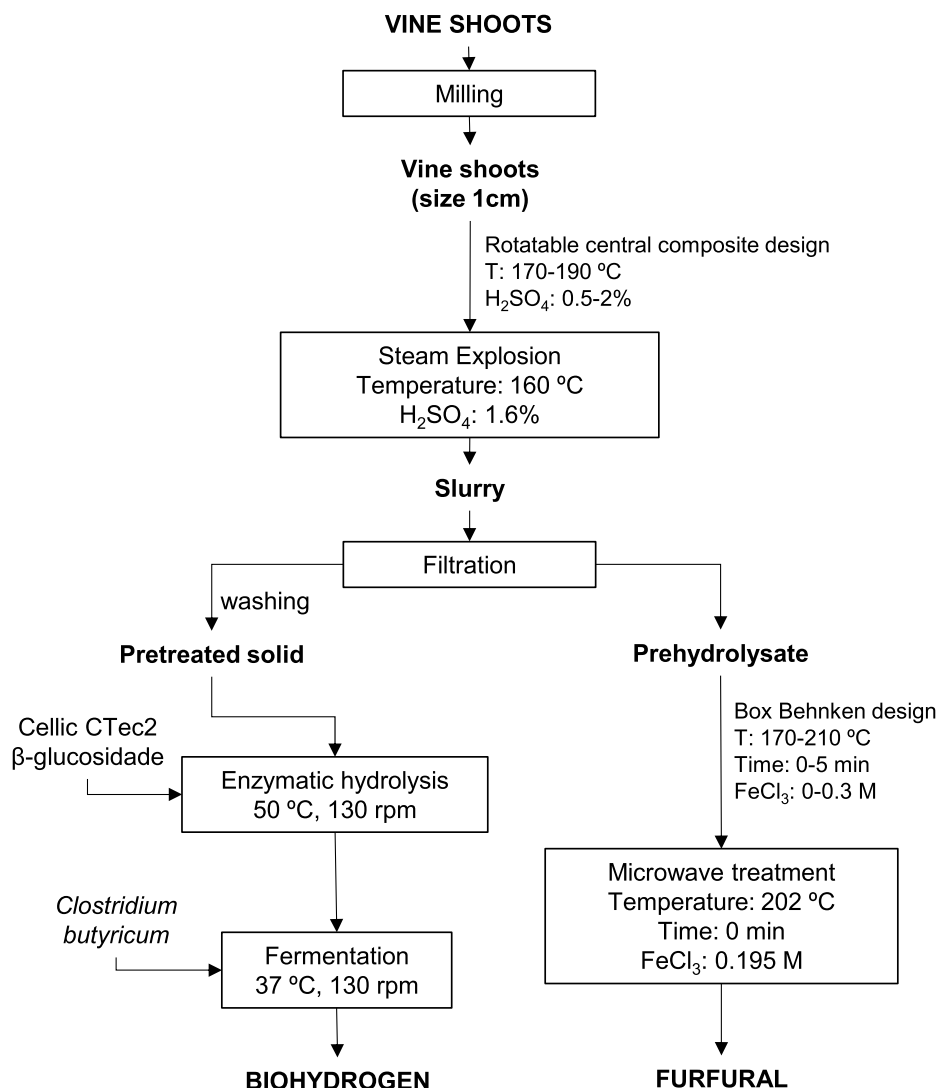


Fig. 1. Process scheme performed for the conversion of vine shoots into biohydrogen and furfural under optimised conditions for steam explosion pretreatment. (160 °C, 1.6% H₂SO₄).

cap and plastic screw, and incubated at 37 °C and 130 rpm for 48 h. After bacterial growth, liquid samples were centrifuged (4000 rpm, 6 min) and the supernatant was discarded. The pellet obtained was inoculated in the fermentative reactors.

2.5. Biohydrogen production from pretreated solid

The pretreated solid obtained at optimised conditions of steam explosion was saccharified with enzymes as described in Section 2.3 then converted into biohydrogen (H₂) with *Clostridium butyricum* (Fig. 1). The fermentative assays were conducted in the same flasks of the enzymatic hydrolysis using the final slurry as the substrate. Thereby, meat extract (5 g/L), yeast extract (1.5 g/L), and peptone (5 g/L) were added as macronutrients [14], and the pH was adjusted to 6.8 with NaOH. The inoculum (5.7 g total volatile solids/L) was added to the flasks, which were then subjected to N₂ atmosphere (100%) for 5 min, closed with a silicone cap and plastic screw, and incubated at 37 °C and 130 rpm. The control assay was performed under the same conditions mentioned above but without vine shoots.

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 H₂ 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 H₂ production data were adjusted to the modified Gompertz model [15] to estimate the maximum H₂ production potential (*P*), maximum H₂ production rate (*R_m*), and time of initial H₂ 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 H₂ 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 °C can be reached. The heating ramp in the microwave reached a temperature of 170 °C in 5 min then followed a ramp of 10 °C/30 s until reaching 210 °C. Capped

Table 2

Biomass recovery after steam explosion pretreatment of vine shoots and pretreated solids composition (%).

Run	Temp. (°C)	H ₂ SO 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

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

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. FeCl₃, acting as a Lewis acid, and H₂SO₄, acting as Brønsted acid, were used as catalysts. Nevertheless, only FeCl₃ was added to the liquor because the biomass had been impregnated with sulfuric acid before steam explosion. A pH of 1.4 was determined in the prehydrolysate.

A Box-Behnken experimental design was adopted for the microwave treatment, with a total of 17 experiments, including one point and four replicates at the center of the domain selected for each factor under study. Center values and intervals were chosen based on previous experience with another lignocellulosic residue [16]. The Box-Behnken design constitutes a methodology for determining the values of the variables (also called factors) at which a series of experiments should be performed to obtain, with a reduced or minimal experimental work load, the dependence of the response on the factors. The resulting data are then used to fit a second-order polynomial equation that models the relationship between the variables and the responses. This equation can be analysed and optimised to identify the optimal conditions for the desired outcome.

The factors selected were the temperature (170–210 °C), FeCl₃ concentration (0–0.3 M), and reaction time (0–5 min). Design-Expert 12.0.3.0 software was used to process the data. After microwave treatment, the final liquors were analysed by HPLC to determine their content in residual sugars and furfural (Section 2.7.2).

2.7. Analytical methods

2.7.1. Gas chromatography

The hydrogen measurement was conducted via gas chromatography.

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.

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

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

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).

Table 3 shows the composition of the prehydrolysates in sugars and inhibitors. In general, the main sugar in these liquids was xylose, although the presence of glucose was detected in all experiments. The solubilisation of glucose even at the mildest pretreatment conditions (9.5%; 170 °C, 0.5% H₂SO₄, run 1) can be due to the presence of glucose as part of the hemicelluloses [17] or the presence of amorphous cellulose in the composition 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 °C [8].

Maximum sugar concentration in the prehydrolysate (48.4 g/L) was determined from vine shoots impregnated with 2% H₂SO₄ before steam explosion at 190 °C (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 to the mild pretreatment conditions used in these experiments (Table 3). Senila et al. [9] determined a maximum value of hemicellulose recovery (33.5%) in the liquid fraction from vine shoots pretreated by autohydrolysis at 180 °C. In addition to sugars, sugar degradation compounds were detected in the prehydrolysates. Nevertheless, in general, their concentrations were not noticeable, being lower than 1 g/L for formic acid, 0.4 g/L for hydroxymethylfurfural, and 1.4 g/L for furfural. Only the presence of acetic acid, from the hydrolysis of hemicelluloses, stands out as an inhibitor in these liquids, reaching a maximum concentration of 7.7 g/L (run 11) (Table 3).

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

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 (%) = $+54.08 + 8.8 A$ $+17.36 B - 6.38$ $A \cdot B - 3.19 A^2 -$ $6.66 B^2$	9.46	0.9608	0.9328	<0.0001	34.32	0.36
EHY (%) = $+34.33$ $+ 10.00 A + 4.36$ $B - 2.77 A \cdot B -$ $4.14 B^2$	5.59	0.9777	0.9665	<0.0001	87.52	0.34

HSRL: hemicellulosic sugars recovery in liquids; EHY: enzymatic hydrolysis yield; A: temperature (°C); B: H₂SO₄ concentration (% w/v).

3.2. Optimisation of the steam explosion pretreatment of vine shoots

The results obtained in the 13 pretreatment experiments of the central composite design were statistically processed and analysed. The responses HSRL and EHY were processed according to ANOVA for a quadratic model. The equations obtained, in terms of coded factors, for both responses are included in Table 5. The values of R² and adjusted R² in 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 HSRL 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).

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% H₂SO₄, 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, considering total sugars measured in the liquor and glucose recovered by enzymatic hydrolysis, 65% of sugars in raw vine shoots were recovered by steam explosion and hydrolysis. Semwal et al. [21] achieved a glucan conversion of 88% from acid impregnated rice straw pretreated by steam explosion and enzymatically hydrolysed at 15% solids.

3.3. Bioconversion of pretreated vine shoots into biohydrogen

The optimised conditions for steam explosion pretreatment of vine shoots yielded a solid fraction with 43.43 ± 0.55% cellulose, 2.58 ± 0.10% hemicellulose, and 41.62 ± 0.89% lignin. This pretreated solid showed great potential to be used as a substrate in biological processes, due to (1) a large part of fermentable sugars in the form of cellulose and (2) absence of inhibitors of the fermentation process. Thus, this biomass was used as a source of sugars to produce biohydrogen. For this purpose,

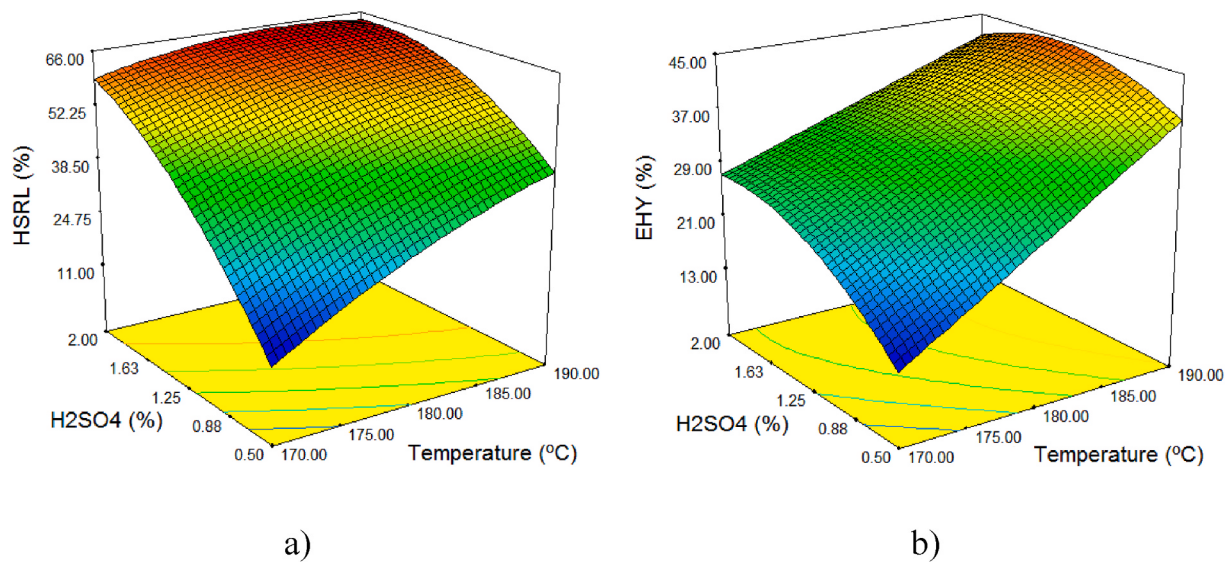


Fig. 2. Response surfaces and contour plots for (a) hemicellulose sugars recovery and (b) enzymatic hydrolysis yield as a function of pretreatment temperature and H_2SO_4 concentration.

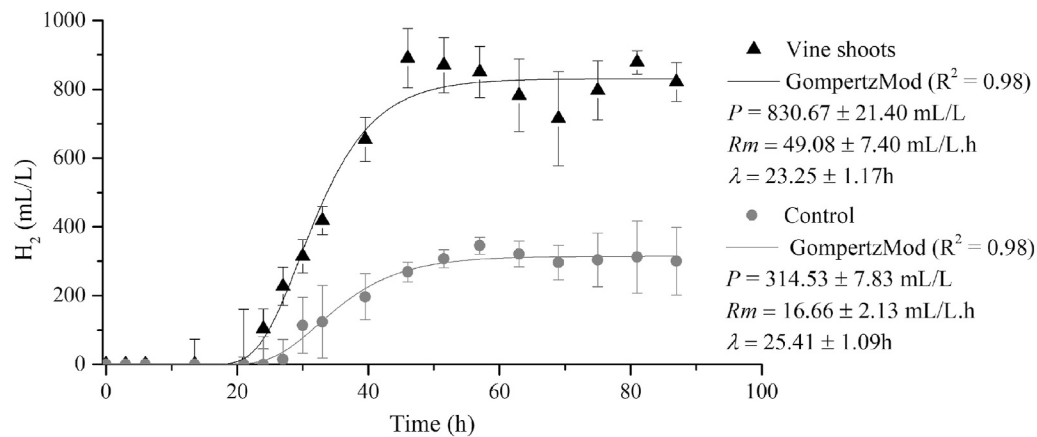


Fig. 3. Cumulative H_2 production from vine shoots assay and control assay. P : maximum H_2 production potential, R_m : maximum H_2 production rate, λ : time of initial H_2 production.

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	<i>E. coli</i> mutant strains	240 mL/g sugar	324.0	[27]
Sugarcane bagasse	Enzymatic hydrolysis	<i>C. butyricum</i>	148.8 mL/L	635.9	[23]
Sugarcane bagasse	Enzymatic hydrolysis	<i>Paraclostridium</i> sp.	166.8 mL/L	695.0	[24]
De-oiled jatropa waste	No	Sewage sludge	10.6 mL/g VS	1543	[28]
<i>Opuntia</i> spp.	Organic extraction	Mixed anaerobic sludge	41.0 mL H_2 /g VS	2990	[29]
Vine shoots	Steam explosion + enzymatic hydrolysis	<i>C. butyricum</i>	830.7 mL/L	3550.0	This work
<i>Cladophora</i> sp. biomass	No	Anaerobic sludge	54.7 mL H_2 /g VS	3560	[30]
Wheat stalk	No	Digested dairy manure	37.0 mL/g VS	3700 (/g VS)	[31]
<i>Chlorella</i> sp. biomass	Enzymatic hydrolysis	Wastewater sludge	43.16 mL H_2 /g VS	4003	[32]
Sweet sorghum stover	Enzymatic hydrolysis	Sewage sludge	402.01 mL	4021	[25]
Algal biomass	No	Granular digester sludge	45 mL/g biomass	4500	[33]
Corn stover	Steam explosion	<i>C. cellulolyticum</i> + <i>Citrobacter amalonaticus</i>	51.9 L/kg TS	5190	[34]
Citrus peel waste	Alkaline delignification	Autochthonous consortium + sludge	7.27 mmol/L	5426.7	[35]
Citrus peel waste	Hydrothermolysis	Autochthonous consortium + sludge	8.19 mmol/L	6116.7	[35]

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

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.

it was enzymatically hydrolysed and the resulting slurry was fermented by *C. butyricum* (Fig. 1).

Islam et al. [22] also pretreated and enzymatically hydrolysed the biomass, followed by fermentation to obtain better conditions for biohydrogen production. These authors used an alkaline treatment of sweet sorghum stems, hydrolysis with the addition of cellulase, and fermentation by *Clostridium thermosaccharolyticum* and reported a production of 839.3 mL H₂/L (6.37 mmol/g-substrate), 95% more than the fermentation of biomass without any treatment.

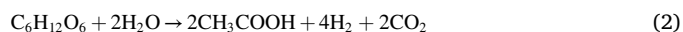
The biohydrogen production from vine shoots after steam explosion pretreatment and enzymatic hydrolysis is shown in Fig. 3. The time of initial H₂ production (λ) was 23.25 h, probably due to bacterial adaptation to the new growth conditions. But after this adaptation period, a production rate of 49.08 mL H₂/L·h was observed. The fermentation of vine shoot slurry with *C. butyricum* resulted in H₂ production of 830.67 mL/L, which corresponds to a yield of 3550 mL/100 g biomass.

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. [23] and Rabelo et al. [24] used a slurry of enzymatically pretreated sugarcane bagasse as a substrate for H₂ production. Both authors used pure cultures as the inoculum (*Clostridium* and *Paraclostridium*, respectively) and obtained 635.9 and 695.0 mL H₂/100 g biomass, respectively. On the other hand, when using mixed culture as the inoculum, Shanmugam et al. [25] obtained 4021 mL H₂/100 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 sugarcane bagasse (cellulose 31.4%, hemicellulose 36.6%, and lignin 24.8% [26]) and sweet sorghum stover (cellulose 37.4%, hemicellulose 20.4%, and lignin 16.3% [25]) and, therefore, the results of metabolite production will also be different in quantity and quality. Nonetheless, the H₂ production yield (3550 mL H₂/100 g biomass) from steam-exploded vine shoots is comparable with other lignocellulosic substrates (Table 6).

In addition to the microbial composition, other factors affect the biological processes, such as the fermentative production of H₂ from lignocellulosic biomass, namely temperature, pH, substrate concentration [36,37], nutrient concentration [38–40], concentration of inhibitors [41,42], and substrate pretreatment conditions [43,44]. For vine shoot fermentation, factors recognised as suitable for the H₂ production were used, such as pH close to neutral [11], for substrate pretreatment [45] combined with enzymatic hydrolysis [23,24]. Therefore, from the results obtained (3550.0 mL H₂/100 g-biomass), it is possible to use vine shoots in fermentative H₂ production and open the range for the

optimisation of the process in future works.

The biological H₂ production from vine shoots was characterized as a fermentation of the acetic-butyric-type, since acetic acid (1495.3 mg/L) and butyric acid (1726.8 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 H₂ and CO₂ (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 [46].



These organic acids are bioactive secondary metabolites that have applications in the pharmaceutical, food, and chemical industries as a precursor to biofuels [47]. Furthermore, supernatants rich in organic acids together with the previously digested biomass can serve as a substrate for sequential methane (CH₄) production during the last step of anaerobic digestion. Braga et al. [48] used a two-stage process in batch reactors to produce H₂ and CH₄ from pretreated sugarcane bagasse. These authors obtained 123.2 mL H₂/L and the acidified fermentation supernatant was used to produce 170.2 mL CH₄/L. Rabelo et al. [49] also used acidified supernatant for sequential CH₄ production and reported that the metabolites accumulated during fermentation (9140.5 mg HAc/L) were converted into 870 mL/L of CH₄ 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 H₂ 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.

3.4. Optimisation of furfural production from the hemicellulose prehydrolysate

The prehydrolysate obtained under optimal steam explosion conditions (190 °C and 1.63% H₂SO₄) 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

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: FeCl₃ concentration (M); C: time (min).

solution with a maximum pentose concentration of 17.5 g/L from the same feedstock pretreated with 52% n-butanol at 190 °C using 2% H₂SO₄ as the catalyst.

Xylose in vine shoot prehydrolysate was chemically converted into furfural by dehydration using FeCl₃ as a catalyst. The experiments were performed in a microwave reactor and the influence of the temperature, FeCl₃ 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 FeCl₃ concentration corresponded to the highest yield (72.9%), which was reached at 210 °C using 0.15 M FeCl₃ (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 °C, FeCl₃ 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.

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, FeCl₃ 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 R² values exceeding 0.99 and adjusted R² values of 0.99.

Fig. 4a shows the influence of FeCl₃ concentration and reaction time on furfural production. The reaction time only had a positive influence on furfural concentration at low levels of FeCl₃. However, the effect of FeCl₃ 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).

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 °C, and 0.195 M FeCl₃. 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. [16] obtained a solution with 18 g/L furfural and

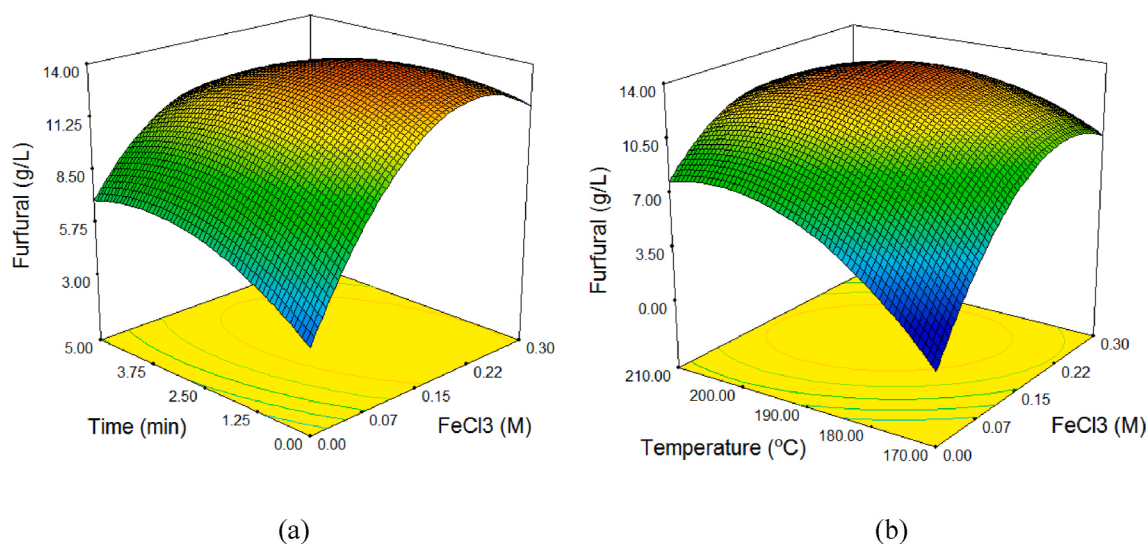


Fig. 4. Response surfaces and contour plots for furfural concentration as a function of (a) FeCl₃ concentration and reaction time and (b) temperature and FeCl₃ concentration.

63.3% yield from an hemicellulosic liquor of olive stones treated in a microwave reactor at 200 °C using 0.1 M FeCl₃ 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) [50]. 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 [16]. Brazdauskas et al. [51] reported a maximum furfural yield of 72% from deciduous wood pentosans after hydrolysis at 175 °C for 90 min using a mixture of H₃PO₄/NaH₂PO₄ as a catalyst.

4. Conclusions

Steam explosion pretreatment on vine shoots has been revealed as an efficient fractionation strategy. Operational optimised conditions (190 °C and 1.6% H₂SO₄) allowed to recover 37.8 g of sugars per 100 g of vine shoots, yielding a good substrate for biohydrogen production with *C. butyricum* (3550 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 will focus on the valorisation of the lignin-rich solid remaining after the conversion of cellulose into hydrogen and the techno-economic and environmental analysis of the global process.

Funding

This work was supported by the Ministry of Science and Innovation (Spain)/State Investigation Agency [Grant PID2020-112594RB-C31] and by “ERDF A way of making Europe”; São Paulo Research Foundation (FAPESP) [2018/26470-7] and [BEPE 2020/04000-9].

CRediT authorship contribution statement

Eulogio Castro: Conceptualization. **Camila Abreu B. Silva Rabelo:** Writing – original draft. **Carmen Padilla-Rascón:** Investigation. **Alfonso M. Vidal:** Investigation. **Juan C. López-Linares:** Investigation. **Maria Bernadete A. Varesche:** Conceptualization. **Inmaculada Romero:** Project administration, Supervision.

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.

Acknowledgements

Technical and human support provided by Centro de Instrumentación Científico-Técnica (CICT)- Servicios Centrales de Apoyo a la Investigación (SCAI)- Universidad de Jaén is gratefully acknowledged.

References

- [1] M.M. Contreras, J.M. Romero-García, J.C. López-Linares, I. Romero, E. Castro, Residues from grapevine and wine production as feedstock for a biorefinery, *Food Bioprod. Process.* 134 (2022) 56–79, <https://doi.org/10.1016/j.fbp.2022.05.005>.
- [2] IOV. International Organization of Vine and Wine, State of the World Vitivinicultural Sector in 2020, 2020. <https://www.oiv.int/public/medias/7909/oiv-state-of-the-world-vitivinicultural-sector-in-2020.pdf>. (Accessed 7 November 2022).
- [3] M. Troilo, G. Difonzo, V.M. Paradiso, C. Summo, F. Caponio, Bioactive compounds from vine shoots, grape stalks, and wine lees: their potential use in agro-food chains, *Food* 10 (2021) 342, <https://doi.org/10.3390/foods10020342>.
- [4] J.M. Romero-García, A. Susmozas, C. Padilla-Rascón, P. Manzanares, E. Castro, J. M. Oliva, I. Romero, Ethanol production from olive stones using different process strategies, *Renew. Energy* 194 (2022) 1174–1183, <https://doi.org/10.1016/j.renene.2022.06.022>.
- [5] S. Banu Jamaldeen, M.B. Kurade, B. Basak, C.G. Yoo, K.K. Oh, B. Jeon, T.H. Kim, A review on physico-chemical delignification as a pretreatment of lignocellulosic biomass for enhanced bioconversion, *Bioresour. Technol.* 346 (2022), 126591, <https://doi.org/10.1016/j.biortech.2021.126591>.
- [6] Y. Yu, J. Wu, X. Ren, A. Lau, H. Rezaei, M. Takada, X. Bi, S. Sokhansanj, Steam explosion of lignocellulosic biomass for multiple advanced bioenergy processes: a review, *Renew. Sustain. Energy Rev.* 154 (2022), 111871, <https://doi.org/10.1016/j.rser.2021.111871>.
- [7] I. Dávila, O. Gordobil, J. Labidi, P. Gullón, Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing, *Bioresour. Technol.* 211 (2016) 636–644, <https://doi.org/10.1016/j.biortech.2016.03.153>.
- [8] S. Rivas, L. López, C. Vila, J.C. Parajó, Organosolv processing of vine shoots: fractionation and conversion of hemicellulosic sugars into platform chemicals by microwave irradiation, *Bioresour. Technol.* 342 (2021), 125967, <https://doi.org/10.1016/j.biortech.2021.125967>.
- [9] L. Senila, E. Kovacs, D.A. Scurtu, O. Cadar, A. Becze, M. Senila, E.A. Levei, D. E. Dumitras, I. Tenu, C. Roman, Bioethanol production from vineyard waste by autohydrolysis pretreatment and chlorite delignification via simultaneous saccharification and fermentation, *Molecules* 25 (2020) 2606, <https://doi.org/10.3390/molecules25112606>.
- [10] Y. Zhao, K. Lu, H. Xu, L. Zhu, S. Wang, A critical review of recent advances in the production of furfural and 5-hydroxymethylfurfural from lignocellulosic biomass through homogeneous catalytic hydrothermal conversion, *Renew. Sustain. Energy Rev.* 139 (2021), 110706, <https://doi.org/10.1016/j.rser.2021.110706>.
- [11] C.A.B.S. Rabelo, L.A. Soares, I. Kimiko Sakamoto, M.B.A. Varesche, Bioconversion of cellulose into hydrogen, biogas and organic acids using microbial consortium from a pulp and paper mill wastewater treatment plant, *Quim. Nova* 41 (2018) 169–175, <https://doi.org/10.21577/0100-4042.20170157>.
- [12] C.A.B. Silva Rabelo, A.M. Vidal, R. Casanova-González, J.M. Romero-García, M.B. A. Varesche, I. Romero, E. Castro, Vine shoots pre-treatment strategies for improved hydrogen production and metabolites redistribution in *Clostridium butyricum*, *Ind. Crops Prod.* 204 (2023), 117317, <https://doi.org/10.1016/j.indcrop.2023.117317>.
- [13] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Structural Carbohydrates and Lignin in Biomass, NREL, 2011 (Revised in 2022); NREL/TP-510-42618, <https://www.nrel.gov/docs/gen/fy13/42618.pdf>.
- [14] A.G.L. Moura, C.A.B.S. Rabelo, C.H. Okino, S.I. Maintinguer, E.L. Silva, M.B. A. Varesche, Enhancement of *Clostridium butyricum* hydrogen production by iron and nickel nanoparticles: effects on hydA expression, *Int. J. Hydrogen Energy* 45 (2020) 28447–28461, <https://doi.org/10.1016/j.ijhydene.2020.07.161>.
- [15] M.H. Zwietering, I. Jongenburger, F.M. Rombouts, K. van 't Riet, Modeling of the bacterial growth curve, *Appl. Environ. Microbiol.* 56 (1990) 1875–1881, <https://doi.org/10.1128/aem.56.6.1875-1881.1990>.
- [16] C. Padilla-Rascón, J.M. Romero-García, E. Ruiz, I. Romero, E. Castro, Microwave-assisted production of furfural from the hemicellulosic fraction of olive stones, *Process Saf. Environ. Protect.* 152 (2021) 630–640, <https://doi.org/10.1016/j.psep.2021.06.035>.
- [17] I. Dávila, P. Gullón, J. Labidi, Influence of the heating mechanism during the aqueous processing of vine shoots for the obtaining of hemicellulosic oligosaccharides, *Waste Manage. (Tucson, Ariz.)* 120 (2021) 146–155, <https://doi.org/10.1016/j.wasman.2020.11.014>.
- [18] O. Rosales-Calderon, V. Arantes, A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol, *Biotechnol. Biofuels* 12 (2019) 240, <https://doi.org/10.1186/s13068-019-1529-1>.
- [19] C. Cara, E. Ruiz, M. Ballesteros, P. Manzanares, M.J. Negro, E. Castro, Production of fuel ethanol from steam-explosion pretreated olive tree pruning, *Fuel* 87 (2008) 692–700, <https://doi.org/10.1016/j.fuel.2007.05.008>.
- [20] I. Dávila, P. Gullón, M.A. Andrés, J. Labidi, Coproduction of lignin and glucose from vine shoots by eco-friendly strategies: toward the development of an integrated biorefinery, *Bioresour. Technol.* 244 (2017) 328–337, <https://doi.org/10.1016/j.biortech.2017.07.104>.
- [21] S. Semwal, T. Raj, R. Kumar, J. Christopher, R.P. Gupta, S.K. Puri, R. Kumar, S.S. V. Ramakumar, Process optimization and mass balance studies of pilot scale steam explosion pretreatment of rice straw for higher sugar release, *Biomass Bioenergy* 130 (2019), 105390, <https://doi.org/10.1016/j.biombioe.2019.105390>.
- [22] M.S. Islam, Z. Zhang, S. Qu, C. Liu, C. Guo, C. Liu, Coproduction of hydrogen and volatile fatty acids via integrated two-step fermentation of sweet sorghum stalks by alkaline and enzymatic treatment, *Biomass Bioenergy* 145 (2021), 105923, <https://doi.org/10.1016/j.biombioe.2020.105923>.
- [23] B.S. Dionizio, C.A.B.S. Rabelo, H.C. Ramos de Jesus, M.B.A. Varesche, D.H. Ferreira de Souza, The deconstruction of the lignocellulosic structure of sugarcane bagasse by laccases improves the production of H₂ and organic acids, *Appl. Biochem. Biotechnol.* 194 (2022) 3145–3166, <https://doi.org/10.1007/s12010-022-03905-9>.
- [24] C.A.B. Silva Rabelo, B.S. Dionizio, A.G.L. Moura, W. Malagó Jr., M.B.A. Varesche, Dilution strategy to obtain H₂ in the 2nd acidogenic phase from enzymatically pretreated sugarcane bagasse, *Int. J. Hydrogen Energy* 47 (2022) 32455–32472, <https://doi.org/10.1016/j.ijhydene.2022.07.131>.

- [25] S. Shanmugam, A. Hari, P. Ulaganathan, F. Yang, S. Krishnaswamy, Y. Wu, Potential of biohydrogen generation using the delignified lignocellulosic biomass by a newly identified thermostable laccase from *Trichoderma asperellum* strain BPLMBT1, *Int. J. Hydrogen Energy* 43 (2018) 3618–3628, <https://doi.org/10.1016/j.ijhydene.2018.01.016>.
- [26] F. Ahmad, I.K. Sakamoto, M.A.T. Adorno, F. Motteran, E.L. Silva, M.B.A. Varesche, Methane production from hydrogen peroxide assisted hydrothermal pretreatment of solid fraction sugarcane bagasse, *Waste Biomass Valor* 11 (2020) 31–50, <https://doi.org/10.1007/s12649-018-0452-1>.
- [27] A. Poladyan, L. Margaryan, K. Trchounian, A. Trchounian, Biomass and biohydrogen production during dark fermentation of *Escherichia coli* using office paper waste and cardboard, *Int. J. Hydrogen Energy* 45 (2020) 286–293, <https://doi.org/10.1016/j.ijhydene.2019.10.246>.
- [28] G. Kumar, C. Lin, Bioconversion of de-oiled Jatropha Waste (DJW) to hydrogen and methane gas by anaerobic fermentation: influence of substrate concentration, temperature and pH, *Int. J. Hydrogen Energy* 38 (2013) 63–72, <https://doi.org/10.1016/j.ijhydene.2012.10.053>.
- [29] K.A. Carrillo-Verástegui, C. Escamilla-Alvarado, C.E. Escárcega-González, J. J. Cano-Gómez, D. Paniagua-Vega, I. Nava-Bravo, E. Ríos-Leal, Biohydrogen potential assessment of *Opuntia* spp.: effect of inoculum-to-substrate ratio and residual biomass evaluation, *Int. J. Hydrogen Energy* 47 (2022) 30085–30096, <https://doi.org/10.1016/j.ijhydene.2022.06.138>.
- [30] B. Taşkan, E.O. Köroğlu, E. Taşkan, *Cladophora* sp. as a sustainable feedstock for dark fermentative biohydrogen production, *Int. J. Hydrogen Energy* 47 (2022) 15410–15418, <https://doi.org/10.1016/j.ijhydene.2022.03.024>.
- [31] Y. Chu, Y. Wei, X. Yuan, X. Shi, Y. Chu, R. Guo, Bioconversion of wheat stalk to hydrogen by dark fermentation: effect of different mixed microflora on hydrogen yield and cellulose solubilisation, *Bioresour. Technol.* 102 (2011) 3805–3809, <https://doi.org/10.1016/j.biortech.2010.11.092>.
- [32] K. Sriyod, A. Reungsang, P. Plangklang, One-step multi enzyme pretreatment and biohydrogen production from *Chlorella* sp. biomass, *Int. J. Hydrogen Energy* 46 (2021) 39675–39687, <https://doi.org/10.1016/j.ijhydene.2021.09.232>.
- [33] P. Sivagurunathan, G. Kumar, T. Kobayashi, K. Xu, S. Kim, D.D. Nguyen, S. W. Chang, Co-digestion of untreated macro and microalgal biomass for biohydrogen production: impact of inoculum augmentation and microbial insights, *Int. J. Hydrogen Energy* 43 (2018) 11484–11492, <https://doi.org/10.1016/j.ijhydene.2018.02.193>.
- [34] S. Zhang, Q. Lai, Y. Lu, Z. Liu, T. Wang, C. Zhang, X.H. Xing, Enhanced biohydrogen production from corn stover by the combination of *Clostridium cellulolyticum* and hydrogen fermentation bacteria, *J. Biosci. Bioeng.* 122 (2016) 482–487, <https://doi.org/10.1016/j.jbiosc.2016.03.014>.
- [35] F.P. Camargo, I.K. Sakamoto, I.C.S. Duarte, M.B.A. Varesche, Influence of alkaline peroxide assisted and hydrothermal pretreatment on biodegradability and biohydrogen formation from citrus peel waste, *Int. J. Hydrogen Energy* 44 (2019) 22888–22903, <https://doi.org/10.1016/j.ijhydene.2019.07.011>.
- [36] D. Infantes, A. González del Campo, J. Villaseñor, F.J. Fernández, Influence of pH, temperature and volatile fatty acids on hydrogen production by acidogenic fermentation, *Int. J. Hydrogen Energy* 36 (2011) 15595–15601, <https://doi.org/10.1016/j.ijhydene.2011.09.061>.
- [37] C.A.B.S. Rabelo, L.A. Soares, I.K. Sakamoto, E.L. Silva, M.B.A. Varesche, Optimization of hydrogen and organic acids productions with autochthonous and allochthonous bacteria from sugarcane bagasse in batch reactors, *J. Environ. Manag.* 223 (2018) 952–963, <https://doi.org/10.1016/j.jenvman.2018.07.015>.
- [38] C.M. Pan, Y.T. Fan, Y. Xing, H.W. Hou, M.L. Zhang, Statistical optimization of process parameters on biohydrogen production from glucose by *Clostridium* sp. Fanp2, *Bioresour. Technol.* 99 (2008) 3146–3154, <https://doi.org/10.1016/j.biortech.2007.05.055>.
- [39] L.A. Soares, J.K. Braga, F. Motteran, I.K. Sakamoto, E.L. Silva, M.B.A. Varesche, Design and optimization of hydrogen production from hydrothermally pretreated sugarcane bagasse using response surface methodology, *Water Sci. Technol.* 76 (2017) 95–105, <https://doi.org/10.2166/wst.2017.183>.
- [40] L.A. Soares, C.A. Borges Silva Rabelo, I.K. Sakamoto, T.P. Delforno, E.L. Silva, M.B. A. Varesche, Metagenomic analysis and optimization of hydrogen production from sugarcane bagasse, *Biomass Bioenergy* 117 (2018) 78–85, <https://doi.org/10.1016/j.biombioe.2018.07.018>.
- [41] S.R. Chaganti, D. Kim, J.A. Lalman, W.A. Shewa, Statistical optimization of factors affecting biohydrogen production from xylose fermentation using inhibited mixed anaerobic cultures, *Int. J. Hydrogen Energy* 37 (2012) 11710–11718, <https://doi.org/10.1016/j.ijhydene.2012.05.036>.
- [42] S.R. Shanmugam, S.R. Chaganti, J.A. Lalman, D.D. Heath, Statistical optimization of conditions for minimum H₂ consumption in mixed anaerobic cultures: effect on homoacetogenesis and methanogenesis, *Int. J. Hydrogen Energy* 39 (2014) 15433–15445, <https://doi.org/10.1016/j.ijhydene.2014.07.143>.
- [43] Z. Lai, M. Zhu, X. Yang, J. Wang, S. Li, Optimization of key factors affecting hydrogen production from sugarcane bagasse by a thermophilic anaerobic pure culture, *Biotechnol. Biofuels* 7 (2014) 119, <https://doi.org/10.1186/s13068-014-0119-5>.
- [44] A.C. Villa Montoya, R.C.S. Mazareli, E.L. Silva, M.B.A. Varesche, Improving the hydrogen production from coffee waste through hydrothermal pretreatment, co-digestion and microbial consortium bioaugmentation, *Biomass Bioenergy* 137 (2020), 105551, <https://doi.org/10.1016/j.biombioe.2020.105551>.
- [45] L.A. Soares, C.A.B.S. Rabelo, P. Seleguim Jr., M.B.A. Varesche, Effect of hydrothermal pretreatment on sugarcane bagasse to enhance the production of hydrogen and organic acid, *Int. J. Environ. Waste Manag.* 29 (2022) 162–178, <https://doi.org/10.1504/ijewm.2022.10045331>.
- [46] F.A. Rainey, Class II. "Clostridia", in: Vos Paul, George M. Garrity, Dorothy Jones, R. Noel, Krieg Wolfgang Ludwig, Fred A. Rainey, Schleifer Karl-Heinz, William B. Whitman (Eds.), *Bergey's Paul Vos, George M. Garrity, Dorothy Jones, Noel R. Krieg, Manual of Systematic Bacteriology*, 3, The Firmicutes, New York: Springer-Verlag, 2009, p. 736, <https://doi.org/10.1007/978-0-387-68489-5>.
- [47] M. Dwidar, J. Park, R.J. Mitchell, B. Sang, The future of butyric acid in industry, *Sci. World J.* 2012 (2012), 471417, <https://doi.org/10.1100/2012/471417>.
- [48] J.K. Braga, F. Motteran, I.K. Sakamoto, M.B.A. Varesche, Bacterial and archaeal community structure involved in biofuels production using hydrothermal and enzymatic-pretreated sugarcane bagasse for an improvement in hydrogen and methane production, *Sustain. Energy Fuels* 2 (2018) 2644–2660, <https://doi.org/10.1039/C8SE00312B>.
- [49] C.A.B.S. Rabelo, F.P. Camargo, I.K. Sakamoto, M.B.A. Varesche, Metataxonomic characterization of an autochthonous and allochthonous microbial consortium involved in a two-stage anaerobic batch reactor applied to hydrogen and methane production from sugarcane bagasse, *Enzym. Microb. Technol.* 162 (2023), 110119, <https://doi.org/10.1016/j.enzmictec.2022.110119>.
- [50] C. Sánchez, L. Serrano, M.A. Andres, J. Labidi, Furfural production from corn cobs autohydrolysis liquors by microwave technology, *Ind. Crop. Prod.* 42 (2013) 513–519, <https://doi.org/10.1016/j.indcrop.2012.06.042>.
- [51] P. Brazdauskas, D. Godina, M. Puke, Direct furfural production from deciduous wood pentosans using different phosphorus-containing catalysts in the context of biorefining, *Molecules* 27 (2022) 7353, <https://doi.org/10.3390/molecules27217353>.