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Integral valorization of vine shoots: Production of xylitol, butanol and 2,3-butanediol in a biorefinery context

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ABSTRACT

Vine shoots, a carbohydrate-rich lignocellulosic residue, represent a promising feedstock for producing xylitol, butanol, and 2,3-butanediol (2,3-BDO) in an integrated biorefinery. In this work, dilute sulfuric acid steam explosion (190 °C, 5 min, 1.6% w/v H_2SO_4) was used for the pretreatment of vine shoots. Xylitol was subsequently produced from the vine shoots hemicellulosic hydrolysate using *C. boidinii* and *C. guilliermondii*; while butanol and 2,3-BDO were generated from the remaining solid fraction of vine shoots using *C. beijerinckii* and *P. polymyxa*, respectively. Furthermore, two process configurations for butanol and 2,3-BDO generation were evaluated: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF), with comparisons conducted at high solids loadings (10% and 15% w/v). As a result, 8.4 g/L xylitol were achieved from the hemicellulosic fraction with *C. boidinii*. From the cellulosic fraction, the SHF configuration at the highest solids loading (15% w/v) yielded 8.8 g/L of butanol (corresponding to 12.4 g/L of total ABE) and 13.8 g/L of 2,3-BDO. Therefore, these results demonstrate the potential of vine shoots as a versatile feedstock for an integrated biorefinery.

Introduction

Wine is one of the most important beverages in the world, with a global consumption in 2024 of 214.2 million hectoliters. A great surface area of vineyard can be found throughout the world, standing in 2024 at around 7.1 million hectares. In this respect, Spain is considered to have one of the largest areas of vineyard in the world, with 930 kha in 2024. Regarding wine production, 225.8, 138.3 and 31.0 million of hectoliters were produced in 2024 throughout the world, the European Union and Spain, respectively, Spain being considered the third largest wine producer globally [1].

Different residues, such as vine shoots, grape pomace, grape stalks, and wine lees, are generated in viticulture practices and the winemaking industry [2], vine shoots being the most important. These are derived from pruning operations, with an estimated annual production of about 1–2 tonnes per hectare [3]. Although vine shoots can be used as organic fertilizer, their applications are quite limited and they are usually burned in the fields to prevent plagues [4]. However, due to their interesting composition (holocellulose, ≥60%; lignin, ≥20%; and extractives, ~3%), vine shoots could be considered a potential raw material in the production of several bioproducts [5] including xylitol, 2,3-butanediol (2,3-BDO) or biofuels (such as bioethanol and biobutanol), in an integrated biorefinery context, under the framework of the circular economy [2].

Xylitol, a sugar alcohol known for its great sweetening properties, but with fewer calories than sucrose and, at the same time, a lower risk of dental caries, has many interesting applications, such as in foods for diabetic patients, toothpaste, the pharmaceutical industry, the treatment of illnesses (anaemia, diabetes, muscle inflammation, and osteoporosis), and the cosmetics industry, among others [6,7]. Although

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xylitol has traditionally been obtained in chemical processes, biotechnological processes, where bacteria and/or yeast convert xylose into xylitol, can be highlighted as a fascinating method for the economically viable production of xylitol, which can be applied to hemicellulosic biomass [7–10] and, in particular, to vine shoots hydrolysate [11]. Among the xylitol-producing microorganisms, *Candida boidinii* and *Candida guilliermondii* are considered some of the best microorganism producers of xylitol due to their documented robustness and natural resilience to the inhibitory environment of lignocellulosic hydrolysates [12,13]. While genetically engineered strains often offer higher theoretical yields, they frequently exhibit sensitivity to the complex mixture of acetic acid, furfural, and phenolic compounds typical of steam-exploded viticultural residues. Selecting strains with high tolerance to inhibitory compounds is pivotal for developing economically robust biorefineries, as it enables the direct fermentation of crude hydrolysates while reducing process complexity [14]. Specifically, *C. boidinii* and *C. guilliermondii* were chosen for their superior ability to maintain xylose-to-xylitol conversion efficiency in the presence of high acetic acid concentrations (>5 g/L), which often inhibit other pentose-fermenting yeasts [15,16].

Regarding biofuels, biobutanol is of great interest, since it has a high energy density, a low vapor pressure, and it is compatible with gasoline engines [17]. In addition, compared to bioethanol, biobutanol's volatility, explosiveness, hygroscopicity and solubility in water are all lower [18]. Biobutanol can be obtained via fermentation through the ABE (Acetone-Butanol-Ethanol) process, by Clostridia strains. For instance, *Clostridium beijerinckii* is highlighted for their metabolic versatility and proven performance in non-detoxified or minimally processed media, making them ideal candidates for a cost-effective, integrated biorefinery framework where feedstock-specific inhibitor tolerance is a critical operational requirement [19,20].

2,3-BDO is an important industrial platform, bulk, and fine and commercial chemical, with diverse applications in the polymer, cosmetics, fuel, and painting industries. It can thus be employed as an antifreeze, liquid fuel, food additive, and fumigant [21]. 2,3-BDO can be produced biologically by different bacteria, such as *Klebisella*, *Bacilus*, *Enterobacter*, *Ralstonia*, *Paenibacillus*, and *Serratia marcescens*, or even *Saccharomyces cerevisiae* mutant yeasts, among others. Among them, *Paenibacillus polymyxa* is a prominent candidate for 2,3-BDO production, largely due to its classification as a non-pathogenic, class 1 strain. Its high fermentation yields and Biosafety Level 1 (BSL-1) status facilitate seamless industrial-scale implementation by minimizing the regulatory and infrastructure requirements associated with more restrictive pathogenic strains [22].

The objective of this study was the use of vine shoots as the raw material in an integrated biorefinery, for the production of different products, such as xylitol, biobutanol and 2,3-butanediol (among others), using both liquid and solid fractions from steam explosion pretreatment. Two different process strategies were compared: SHF and SSF. In addition, in order to attain high concentrations of the fermentation products (xylitol, biobutanol and 2,3-BDO), two different solid loadings (10 and 15%, w/v) were also investigated, which is very interesting for implementing in industrial scale processes. Moreover, in the case of biobutanol, in order to remove the fermentation product in situ and enhance production, the fermentation process was carried out in a bioreactor coupled to a gas stripping system in fed batch mode. To the best of our knowledge, this is the first work on the valorization of this lignocellulosic residue in the context of an integrated multi-product biorefinery for the simultaneous production of xylitol and butanol (or 2,3-BDO).

Materials and methods

Raw material and steam explosion pretreatment

Vine shoots from the pruning of vineyards were air dried (up to 10.3% moisture content), milled to a particle size of 1 cm using a laboratory hammer mill (Retsch, SM 100, Fisher Scientific S.L., Madrid, Spain), and stored until use. The composition was (% w/w dry matter): 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 [23].

The steam explosion pretreatment of the vine shoots was carried out in a 4 L custom-built pilot unit. 400 g of dried and milled vine shoots were impregnated in 1.6% (w/v) sulfuric acid (2 L, for 12 h), heated with saturated steam up to 190 °C, maintained for 5 min, and then quickly depressurized to atmospheric pressure. The pretreatment conditions were previously optimized by Castro et al. [24] to simultaneously maximize both hemicellulose sugar recovery in the prehydrolysate and enzymatic hydrolysis yield. Following the pretreatment, the resulting slurry was vacuum filtered to separate the liquid and solid phases. The liquid fraction (hemicellulosic hydrolysate) was analyzed for sugars and inhibitor compounds (acetic and formic acids, 5-hydroxymethylfurfural (HMF) and furfural), and then used as the medium in xylitol fermentations. The solid fraction was washed several times to eliminate the acidity until a neutral pH was reached, dried at 35 °C, analyzed for its content in cellulose, hemicellulose and lignin, and finally employed as substrate in different process configurations (separate hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF)) for butanol and 2,3-butanediol (2,3-BDO) production.

Microorganism and culture media

The yeasts *Candida boidinii* NCAIM Y.01308 and *Candida guilliermondii* NCAIM Y.01050 (both from the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary)), and the bacteria *Clostridium beijerinckii* DSM 6422 and *Paenibacillus polymyxa* DSM 365 (both from the German collection of microorganisms (DSMZ, Leibniz, Germany)), were the microorganisms employed in this work. Microbial cells were stored as glycerol stock (40% (v/v)) at −80 °C.

C. beijerinckii, the culture was grown in RCM (Reinforced Clostridial Medium), under anaerobic conditions (35 °C and 135 rpm for 24 h), as previously described [20]. *P. polymyxa* inoculum was grown in 250 mL Erlenmeyer flasks, with a working volume of 100 mL (medium Häßler), at 37 °C and 200 rpm for 24 h [25]. The xylitol producing yeast (*C. boidinii* and *C. guilliermondii*) were grown for 24 h at 30 °C and 220 rpm in 250 mL Erlenmeyer flasks, with a working volume of 50 mL [26].

Xylitol production from hemicellulosic hydrolysate

Raw hemicellulosic hydrolysate was detoxified by vacuum evaporation and activated carbon adsorption. Before detoxification, the hemicellulosic hydrolysate was adjusted to a pH of 3 with KOH (initial pH approximately 1.5).

Vacuum evaporation: The hydrolysate was evaporated for 2.5 h at 50 °C and 60–65 mbar until the volume was reduced to half (Rotavapor R-210, BÜCHI, Switzerland). Then, distilled water was added until the initial volume was restored.

Activated carbon treatment: The hydrolysate was mixed with activated carbon (Sigma-Aldrich, 100 mesh) at a solid-to-liquid ratio of 5% (w/v). Then the suspension was agitated in an orbital shaker at 200 rpm and 50 °C for 30 min, and it was finally filtered through 0.45 μm membranes for solid separation.

Hemicellulosic hydrolysate was used before and after detoxification as a source of xylose for xylitol production. Raw hydrolysate and detoxified hydrolysates were fermented using two different yeasts, *C. boidinii* and *C. guilliermondii* (Fig. 1). Before fermentation, the hydrolysates were adjusted to a pH of 6 with KOH and supplemented with nutrients from the culture medium. An initial cell concentration of 5 g/L (dry weight) was used, for which the inoculum was centrifuged at 3000

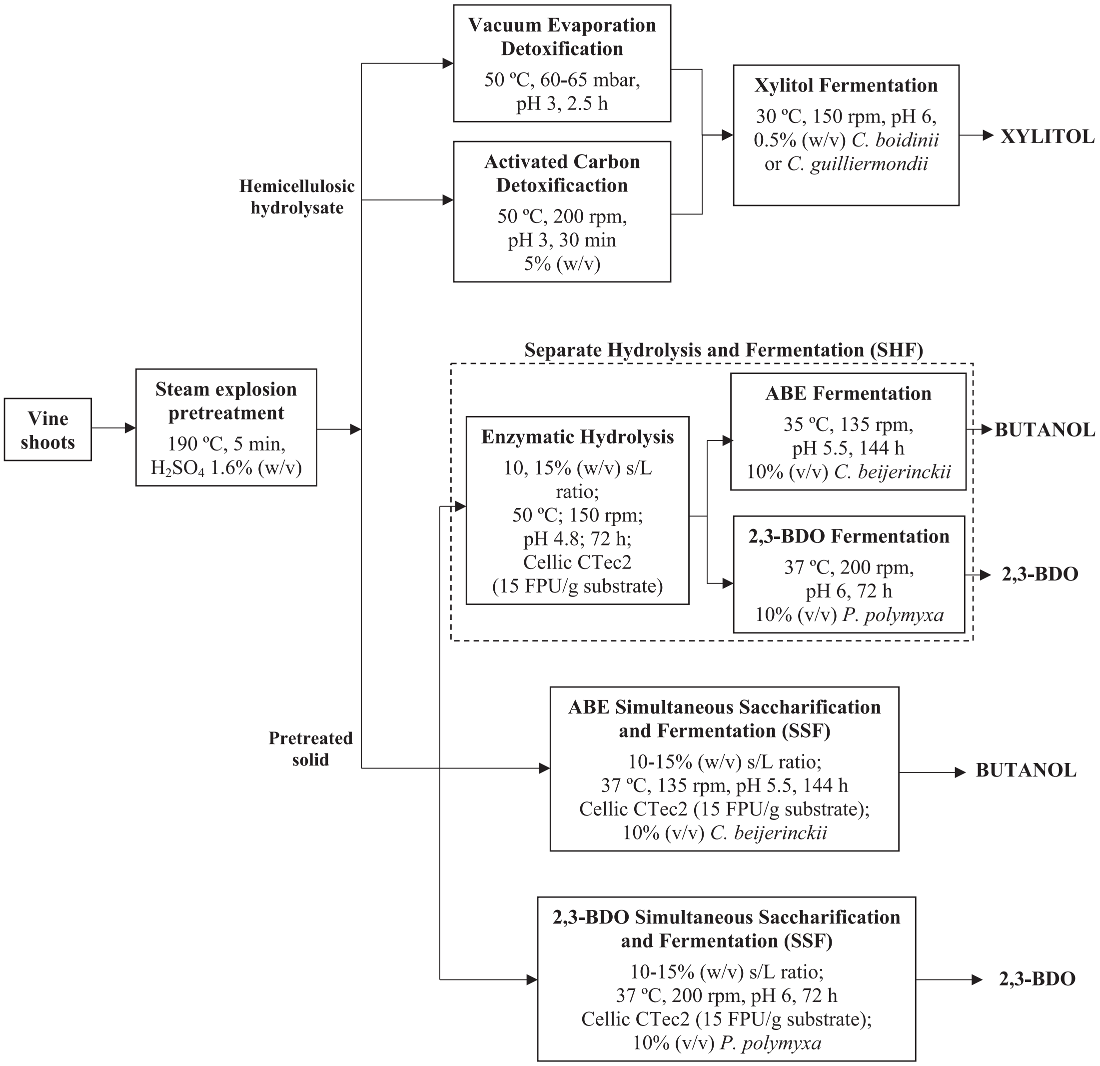

Fig. 1. Schematic process of vine shoots valorization for xylitol, butanol and 2,3-butanediol production.

rpm for 10 min, and the solid was resuspended in the hydrolysate. Fermentation was carried out in an orbital shaker at 30 °C and 150 rpm in 100 mL Erlenmeyer flasks with 40 mL of hydrolysate. Samples were taken daily for 4 days, analyzed by HPLC to determine the content of sugars, inhibitor compounds, ethanol, and xylitol. The xylitol yield was calculated as the ratio between the xylitol produced and the xylose consumed.

Enzymatic hydrolysis

To obtain an enzymatic hydrolysate from vine shoots which would be used later as fermentation medium, the enzymatic hydrolysis of steam explosion pretreated vine shoots was carried out at 10% w/v (30 g substrate and 300 mL enzymatic solution) and 15% w/v (45 g substrate and 300 mL enzymatic solution) substrate loading (Fig. 1), using 1000 mL Erlenmeyer flasks. Enzymatic hydrolysis was performed in an orbital shaker (Optic Ivymen Systems, Comecta, Barcelona, Spain), at 50 °C, 150 rpm, and pH 4.8 (adjusted with 10 M NaOH solution) for 72 h. Cellic CTec2 (15 FPU/g substrate), kindly donated by Novozymes A/S (Bagsvaerd, Denmark), was used. These conditions were selected based on previous results reported by Castro et al. [24]. The resulting enzymatic hydrolysates were separated from the residual solid by vacuum filtration, analyzed for their sugar content, pasteurized at 90 °C for 15 min, and used as fermentation media for ABE (acetone-butanol-ethanol) and 2,3-BDO production (Fig. 1).

ABE (acetone-butanol-ethanol) fermentation from steam explosion pretreated vine shoots

Separate hydrolysis and fermentation (SHF)

The enzymatic hydrolysates (obtained from enzymatic hydrolysis at 10 and 15% w/v substrate loading) of pretreated vine shoots were submitted to ABE fermentation. This was carried out at 35 °C, 135 rpm and pH 5.5 (without control during the fermentation), using 100 mL serum bottles (with rubber septum) with 50 mL of working volume, and under anaerobic conditions (flushing O_2 free nitrogen into the liquid). Vitamin, salt and acetate buffer solutions were also added, under the same conditions as those described by López-Linares et al. [27]. An inoculum loading of 10% (v/v) of *C. beijerinckii* was employed (Fig. 1).

Simultaneous saccharification and fermentation (SSF)

ABE SSF tests were carried out in 100 mL serum bottles (with rubber septum), using the pretreated vine shoots as substrate and a total volume of 50 mL distilled water (pH 5.5, adjusted with 10 M NaOH solution), containing vitamin, salt and acetate buffer, as described above for SHF. The substrate loadings used were 10 and 15% w/v (Fig. 1). Cellic CTec2 (15 FPU/g substrate) and *C. beijerinckii* inoculum (10% v/v) were simultaneously added at the beginning of SSF process (t = 0). ABE SSF essays were carried out under anaerobic conditions (flushing O_2 free nitrogen into the liquid). The ABE SSF experiments were performed at 37 °C and 135 rpm for 144 h.

In situ gas stripping fermentation

The enzymatic hydrolysate (at 15% w/v substrate loading) of the pretreated vine shoots was also subjected to ABE fermentation in a bioreactor coupled to a gas stripping system, in fed batch mode, following the protocol described by López-Linares et al. [20]. A Biostat B Plus reactor (Sartorius®) was used, at 50 rpm and using 600 mL of working volume. In order to alleviate the butanol inhibition, both the gas stripping technology and the fed-batch mode were begun at 48 h of fermentation, and finished at 165 h of process. The gas stripping mechanism was carried out using a flow rate of 1.0 vvm. The above mentioned enzymatic hydrolysate (at 15% w/v substrate loading) was also used as the feed hydrolysate in the fed-batch mode, with a feed flow of 6 mL/h.

ABE fermentation tests were carried out at least in duplicate. Samples were withdrawn during fermentation, centrifuged (at 13,500 rpm for 10 min) and analyzed to determine the sugar consumption, and acetone, butanol and ethanol production.

2,3-butanediol (2,3-BDO) fermentation from steam explosion pretreated vine shoots

Separate hydrolysis and fermentation (SHF)

Enzymatic hydrolysates (at 10 and 15% w/v substrate loading) of pretreated vine shoots were also subjected to 2,3-BDO fermentation. Fermentation tests were carried out at 37 °C, 200 rpm, 72 h, and pH 6 (Fig. 1), using a rotary shaker and 250 mL Erlenmeyer flasks (with 100 mL of working volume). The enzymatic hydrolysates were supplemented with the same nutrients, potassium phosphate buffers and trace elements employed in the pre-culture medium Häßler, except glucose. An inoculum loading of 10% (v/v) of *P. polymyxa* was used, and the pH was not controlled during the fermentation.

Simultaneous saccharification and fermentation (SSF)

2,3-BDO SSF tests were also performed at 10 and 15% w/v of substrate loadings (Fig. 1). In this case, 250 mL Erlenmeyer flasks were employed, using the pretreated vine shoots as substrate and a total volume of 100 mL distilled water (pH 6, adjusted with 10 M NaOH solution), containing the nutrients, potassium phosphate buffers and trace elements described above for SHF. Cellic CTec2 (15 FPU/g substrate) and *P. polymyxa* inoculum (10% v/v) were simultaneously added at the beginning of the SSF process ($t = 0$). 2,3-BDO SSF experiments were performed at 37 °C and 200 rpm for 72 h.

Samples from the SSF essays were taken each 24 h, centrifuged (at 13,500 rpm for 10 min), and analyzed for their contents in sugars, 2,3-BDO, ethanol, acetoin, and cells. Fermentation tests were performed at least in duplicate.

Analytical methods

National Renewable Energy Laboratory (NREL) methodology was used to determine the content of the structural carbohydrates, lignin, and ash in the steam explosion pretreated vine shoots [28,29]. Sugar content (glucose, xylose, galactose, arabinose and mannose) was analyzed by high-performance liquid chromatography (HPLC) using a Waters 2695 liquid chromatograph (Mildford, MA, USA) equipped with a refractive index detector (Waters 2414) and a Transgenomic CHO-782 carbohydrate analysis column at 70 °C, with ultrapure water as the mobile phase employed (0.6 mL/min).

Inhibitor compounds (formic and acetic acids, furfural and 5-hydroxymethylfurfural (HMF)) in hemicellulosic hydrolysate of vine shoots, as well as the content of sugars (glucose, xylose + galactose + mannose, and arabinose) and fermentation products (butanol, ethanol, acetone, 2,3-BDO, acetoin, acetic and butyric acids, among others) in the case of the SHF and SSF experiments, were also analyzed by HPLC with the equipment described above, but using in this case an Aminex HPX-87H column (at 30 °C) and 0.01 N H_2SO_4 (0.6 mL/min) as the mobile phase. Cell content was analyzed by the Bradford method [30], adding 1.5 mL Coomassie Plus Reagent (Bradford reagent) to the sample (0.05 mL), incubating samples for 10 min at room temperature, and measuring its absorbance at 595 nm. Albumin was used as a standard. Prior to the Bradford method, the SHF and SSF samples were centrifugated (at 13,500 rpm for 7 min), washed with 0.5 M NaOH solution (1 mL), resuspended in 1 M NaOH solution (1 mL), and subjected to ultrasound technology for 2 min (70% amplitude).

All analytical determinations were carried out in triplicate, and the average results are shown.

Data analysis

Variance analysis (ANOVA), at a confidence level of 95% ($p < 0.05$), was used to calculate statistical differences. The Tukey multiple range test was employed to find significantly different means.

Results and discussion

Vine shoots were pretreated by dilute sulfuric acid steam explosion, resulting in both hemicellulosic hydrolysate and pretreated solid. The hemicellulosic hydrolysate from the vine shoots was converted to xylitol, while the pretreated solid was converted to butanol and 2,3-butanediol employing two process configurations (SHF and SSF), studying the configuration influence on the process performance. In addition, different substrate loadings were investigated in both SHF and SSF configurations.

Composition of the hemicellulosic hydrolysate and pretreated solid of vine shoots

In this study, the composition of the vine shoots was significantly affected due to the dilute sulfuric acid steam explosion pretreatment. The composition of the pretreated vine shoots is shown in Table 1. The sulfuric acid steam explosion pretreatment resulted in a high solubilization of hemicellulose and extractive fractions from the vine shoots; while a pretreated solid enriched in glucan (41.9%) was obtained, which was employed as substrate for butanol and 2,3-butanediol production in the different process configurations (SHF and SSF). The sulfuric acid steam explosion pretreatment thus demonstrated its high effectiveness, as the hemicellulose solubilization and cellulose enrichment are some of the main aims of the pretreatment stage [31].

The composition of the hemicellulosic hydrolysate from the vine shoots is also displayed in Table 1. As can be seen, a hemicellulosic

Table 1
Composition of pretreated solid and hemicellulosic hydrolysate from vine shoots after dilute acid steam explosion pretreatment (190 °C, 5 min, 1.6% w/v H_2SO_4).

Component	Concentration
Pretreated solid (%)	
Cellulose	41.9 ± 0.5
Xylan	3.4 ± 0.5
Other sugars	n.d.
Lignin	38.9 ± 1.5
Hemicellulosic hydrolysate (g/L)	
Sugars	
Glucose	14.2 ± 0.5
Xylose	23.6 ± 0.8
Galactose	3.8 ± 0.2
Arabinose	3.6 ± 0.2
Mannose	0.9 ± 0.1
Inhibitors	
Furfural	1.4 ± 0.1
HMF	0.2 ± 0.0
Formic acid	0.6 ± 0.1
Acetic acid	9.5 ± 0.3

nd: Not detected; HMF: hydroxymethylfurfural.

hydrolysate with a total sugar content as high as 46.1 g/L was achieved from the sulfuric acid steam explosion pretreatment of the vine shoots, with xylose being the main sugar (23.6 g/L), corresponding to 51.2% of the total sugars contained in the hemicellulosic hydrolysate. It is worth noting that a considerable presence of oligomeric sugars (23.2%) was detected, representing a fraction of lost sugars as the yeasts used lack hydrolytic activity. On the other hand, as can be seen in Table 1, a considerable presence of inhibitor compounds (such as formic and acetic acids, furfural and HMF) was determined in the hemicellulosic hydrolysate, mainly acetic acid (9.5 g/L), which could be due to the high hemicellulose solubilization.

Xylitol production from hemicellulosic hydrolysate of vine shoots

Raw hemicellulosic hydrolysate was fermented before and after detoxification with *C. boidinii* and *C. guilliermondii*. Two detoxification methods were employed to remove toxic compounds, primarily acetic acid and furfural, from the hydrolysate and enhance its fermentability. Thus, the raw hydrolysate (RH) was subjected to vacuum evaporation (evaporated hydrolysate, EH) and treated with activated charcoal (activated charcoal hydrolysate, ACH) as described in Section 2.3.

Evaporation is considered an efficient method for removing organic acids, such as acetic acid. In this work, acetic acid was partially volatilized during the vacuum evaporation of the hydrolysate, resulting in a final concentration of 4.8 g/L, which means a 49% decrease in concentration (Table 1). Furfural, with a concentration of 1.4 g/L in the RH, was entirely removed by vacuum-evaporation. However, the evaporation process did not affect the concentrations of formic acid and HMF, although their initial concentrations were as low as 0.6 and 0.2 g/L, respectively (Table 1). Likewise, RH was detoxified with activated charcoal, which was similarly effective in completely removing furfural and also enabled the production of a hydrolysate without HMF. However, the concentration of acetic acid in the hydrolysate was reduced by only 19%, remaining at a final concentration of 7.6 g/L, compared to 4.8 g/L after vacuum evaporation. According to Felipe et al. [32], acetic acid can be potentially toxic for *C. guilliermondii* in concentrations above 3 g/L, while lower concentrations may have a positive effect on xylose uptake, xylitol yield, and cell growth. Acetic acid present in the medium becomes more toxic to the cell at lower pH values, whilst its inhibition can be alleviated at medium pH $\geq$ 4.5, as yeasts can adapt to weak acids under these conditions in a medium containing glucose [11,33]. In this regard, Lima et al. [34] studied the effect of acetic acid on the activities of xylose reductase and xylitol dehydrogenase in *C. guilliermondii* using bagasse hydrolysates and determined that acetic acid present in a medium with a pH of 5 did not affect sugar metabolism or xylitol formation by this yeast.

Additionally, when a detoxification method is used to remove toxic compounds from the hydrolysate, it is crucial to consider the potential loss of sugars. In this work, no sugar losses were detected with any of the detoxification methods used, which results in very advantageous

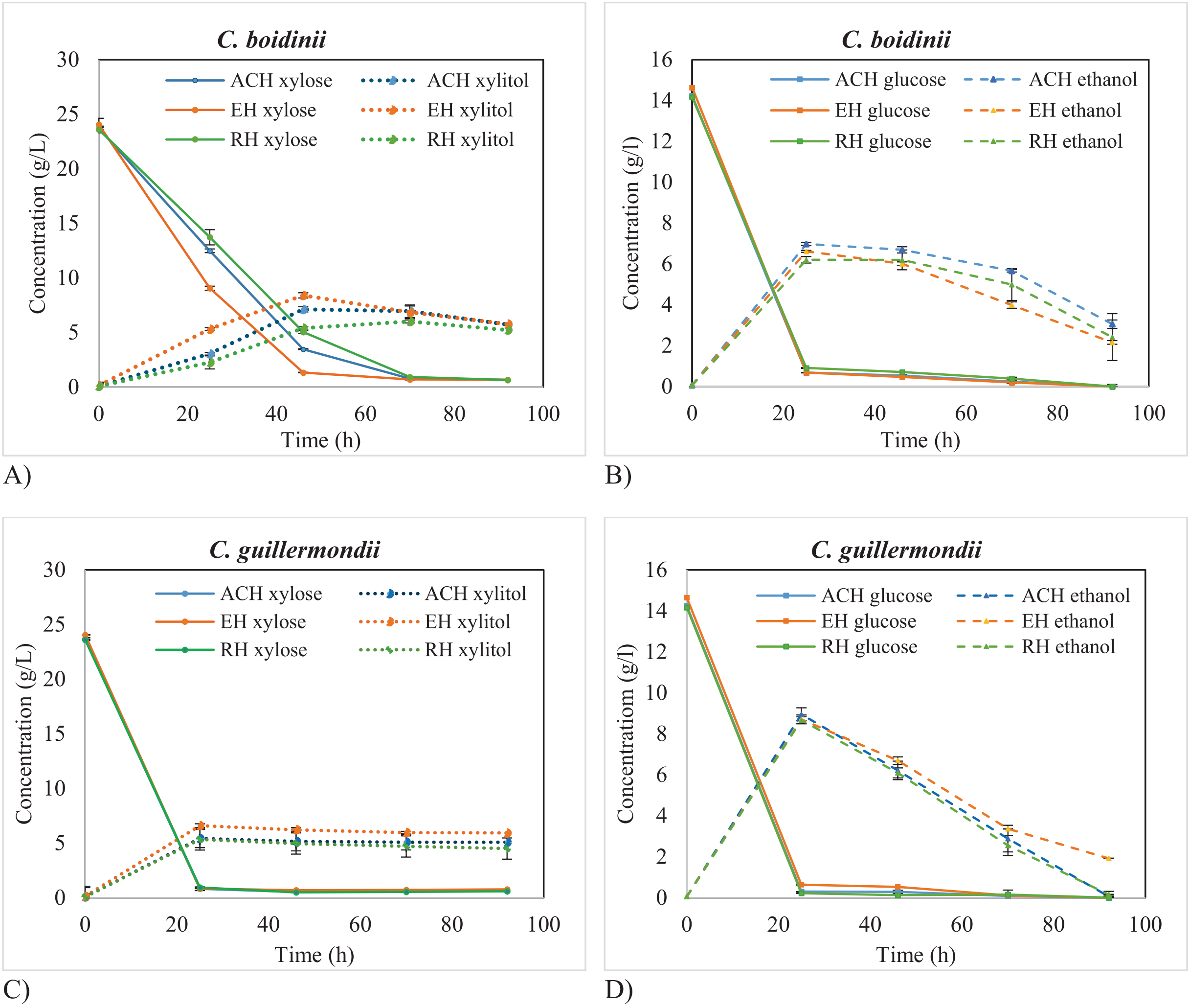

Fig. 2. Time profile for the fermentation of raw hydrolysate (RH), evaporated hydrolysate (EH), and activated charcoal-treated hydrolysate (ACH) with *C. boidinii* (A) (B) and *C. guilliermondii* (C) (D). Xylose and glucose consumption (continuous lines), and xylitol and ethanol production (dashed lines).

conditions.

Fig. 2A and 2B compare the fermentation of the raw hydrolysate (RH) and the hydrolysates detoxified by vacuum evaporation (EH) and activated charcoal treatment (ACH) with *C. boidinii*. As can be observed, *C. boidinii* began to consume xylose from the start and was able to assimilate all the xylose in the fermentation of the three hydrolysates (Fig. 2A). Nevertheless, the fermentation of detoxified hydrolysates was more efficient, especially after vacuum evaporation. Thus, the maximum xylitol production, 8.4 g/L, was achieved in the fermentation of EH after 46 h. In this case, xylose consumption was faster, and only 1.3 g/L remained unconsumed in the medium at that time. *C. boidinii* continued to consume xylose, and a residual xylose concentration of less than 1 g/L remained at 70 h of fermentation in all three cases. However, this did not result in a higher xylitol concentration in the medium. On the contrary, a slight decrease in xylitol concentration was observed, which decreased to 6 g/L in the fermentation of EH, which means that xylitol was consumed by the yeast itself (Fig. 2A). This behavior of the yeast has previously been observed in other xylitol-producing strains. Zhang et al. [35] studied the metabolic engineering of *C. tropicalis* for xylitol production from commercial xylose and determined the oxidation of xylitol to xylulose by the xylitol dehydrogenase gene, which prevents the accumulation of xylitol in the medium. In this work, since after 46 h practically all the sugars had been consumed by the yeast, the fermentation time could be shortened to that time.

In addition to xylose, the presence of glucose in the vine shoot hydrolysates is noteworthy, 14.2 g/L *versus* 23.6 g/L of xylose (Table 1), which means a glucose/xylose ratio of 0.6. According to Kumari et al. [7], the presence of glucose in the fermentation medium can be beneficial, since it can be utilized by the microorganism as an energy source, allowing xylose consumption to be directed exclusively to the production of xylitol as the final product. However, when the glucose/xylose ratio exceeds 10%, glucose inhibits xylose transport into the cell [33] and represses the induction of relevant enzymes [36]. Queiroz et al. [33] studied the effects of glucose/xylose ratio on xylitol bioproduction by *Candida tropicalis* using semi-defined medium containing these sugars. These authors varied the glucose/xylose ratio and observed a 70% drop in xylitol yield after 48 h when the ratio was increased from 0.2 to 5. In this work, given that vine shoot hydrolysates have a glucose/xylose ratio of 0.6, the fermentation performance of the yeasts could be negatively affected by the high glucose concentration in the medium.

As can be observed in Fig. 2A and 2B, the uptake of glucose and xylose by *C. boidinii* occurred simultaneously from the beginning of the fermentation. However, glucose uptake by *C. boidinii* was faster, and only 0.9 g/L remained in the medium after 25 h of fermentation in the three experiments. At that same time, a maximum ethanol of around 6.5 g/L was also produced as a by-product in the three fermentations, due to glucose in the hydrolysate medium, which was later observed to be partly consumed by the microorganism itself. López-Linares et al. [15] reported ethanol production and consumption during the fermentation of exhausted olive pomace hydrolysates with *C. boidinii*; although their glucose/xylose ratio was 0.21 and the ethanol production was lower than that determined in this work. This behavior has been observed with *C. tropicalis* in the fermentation of wheat straw hydrolysates to xylitol [37].

When the vine shoot hydrolysates were fermented with *C. guilliermondii*, the effect of detoxification was less pronounced. As can be observed in Fig. 2C, the performance of the fermentation of the undetoxified hydrolysates and the detoxified hydrolysates was very similar. Compared to *C. boidinii*, xylose was assimilated in a shorter time by *C. guilliermondii*, and only 0.9 g/L remained unconsumed in the medium at 25 h, in all three cases. As occurred with *C. boidinii*, the maximum xylitol production was determined in the fermentation of EH, 6.6 g/L *versus* 5.3 g/L obtained from RH. Likewise, simultaneous consumption of glucose and xylose occurred from the beginning, and approximately 8.5 g/L ethanol was determined at 25 h (Fig. 2D). Garai et al. [38], using an acid hydrolysate of corncobs, also obtained a higher concentration of ethanol than xylitol with *C. tropicalis*. This can be attributed to the composition of the vine shoot biomass, which contains a part of glucose easily hydrolysable that is incorporated into the hydrolysate during the pretreatment, resulting in hydrolysates with high glucose/xylose ratios [24,39–41]. In this work, the sugar composition of the vine shoot hydrolysates was determined to be a key factor in the performance of the fermentation. The metabolism of the yeasts could be negatively affected by the high glucose/xylose ratio in the hydrolysate.

When comparing the performance of *C. boidinii* and *C. guilliermondii* in the fermentation of the undetoxified hydrolysate and detoxified hydrolysates, both yeasts achieved their best results in terms of xylose consumption and xylitol production in the fermentation of EH. The highest xylitol production corresponded to *C. boidinii* with a yield of 0.55 g/g and a productivity of 0.18 g/L•h at 46 h, while *C. guilliermondii* achieved a xylitol yield of 0.43 g/g and a productivity of 0.26 g/L•h at 25 h. The former achieved a higher xylitol production, although it required a longer time, and this resulted in a higher productivity for *C. guilliermondii*. These results are comparable with those reported on the production of xylitol from synthetic xylose with *C. boidinii* (0.45 g/g and 0.26 g/L•h) [42] or *Arundo donax* hydrolysates with *C. tropicalis* after 96 h (0.54 g/g and 0.27 g/L•h) [43]. Other authors reported higher xylitol yields and productivities using hydrolysates with a glucose/xylose ratio lower than 0.1 from olive stones with *C. boidinii* after 46 h (0.63 g/g and 0.42 g/L•h) [26] and 0.3 from wheat straw with *C. guilliermondii* after 54 h (0.88 g/g and 0.57 g/L•h) [44]. Furthermore, when comparing these findings with other studies specifically utilizing vine shoots as the feedstock, the performance of *C. boidinii* and *C. guilliermondii* remains competitive. For instance, Rivas et al. [45] reported a xylitol yield of 0.53 g/g (0.46 g/L•h) using *Debaryomyces hansenii* from vine shoot hemicellulosic hydrolysates, which is slightly lower than the 0.55 g/g achieved by *C. boidinii* in this work. However, higher xylitol yields and productivities (0.66 g/g and 0.71 g/L•h) were achieved by Portilla et al. [46] from vine shoot hemicellulosic hydrolysates, using also *D. hansenii*. It is worth mentioning that in both studies with *D. hansenii*, prior to xylitol fermentation of hydrolysate, a first step of fermentation by *Lactobacillus acidophilus* for lactic acid production was carried out to the selective consumption of glucose. More recently, Baptista et al. [11] using a genetically modified *Saccharomyce cerevisiae* strain on vine shoots hemicellulosic hydrolysates attained xylitol yields ranging from 0.26 to 0.40 g/g (0.11 to 0.17 g/L•h), which are lower than those obtained in this study. The variations in yield across these studies can be attributed to differences in the detoxification efficiency, the type of strain used and the glucose/xylose ratios of the resulting hydrolysates. Our results demonstrate that *C. boidinii* is particularly robust, achieving high xylose-to-xylitol conversion despite the significant glucose concentration present in our steam-exploded vine shoot hydrolysate.

Butanol production from steam explosion pretreated vine shoots

Comparison of SHF and SSF processes

Steam explosion pretreated vine shoots were used as a substrate for butanol production by ABE fermentation; these being studied with two different process configurations (SHF and SSF) and substrate loadings (10 and 15% w/v) (Fig. 1). The results obtained are shown in Table 2 and Fig. 3.

Regarding the SHF configuration, two stages were carried out: a first stage of enzymatic hydrolysis, followed by a second stage of ABE fermentation of enzymatic hydrolysates. After the enzymatic hydrolysis, enzymatic hydrolysates with a total sugars content of 25.9 g/L (glucose, 24.7 g/L; xylose, 1.2 g/L) and 40.3 g/L (glucose, 38.5 g/L; xylose, 1.8 g/L) were achieved for 10 and 15% (w/v) substrate loadings, respectively, after the enzymatic hydrolysis of steam explosion pretreated vine shoots. Glucose recovery (g glucose by enzymatic hydrolysis/100 g glucose (contained as cellulose) in pretreated vine shoots) of about 60% was attained. Both 10 and 15% (w/v) enzymatic hydrolysates obtained

Table 2
ABE fermentation of vine shoots in SHF and SSF configurations (at 10 and 15% (w/v) of substrate loading). Sugar uptake; butanol, ABE, acetic acid and butyric acid concentrations (g/L); butanol and ABE yields ($Y_{BUT/sugars}$, $Y_{ABE/sugars}$ expressed as g/g sugars consumed); and butanol and ABE productivities (Q_{BUT}, Q_{ABE} expressed as g/L·h) at the time of maximum production of butanol and ABE.

Process conf.	Solids loading (%, w/v)	Time (h)	Sugar uptake(%)	Butanol (g/L)	ABE (g/L)	Acetic acid (g/L)	Butyric acid (g/L)	$Y_{BUT/sugars}$(g/g)	$Y_{ABE/sugars}$(g/g)	Q_{BUT}(g/L·h)	Q_{ABE}(g/L·h)
SHF	10	144	100	5.9 ± 0.0	8.1 ± 0.1	1.3 ± 0.0	1.5 ± 0.1	0.24	0.34	0.041	0.056
	15	144	98.4	8.8 ± 0.0	12.4 ± 0.0	1.3 ± 0.0	0.7 ± 0.0	0.23	0.33	0.061	0.086
SSF	10	144	100	4.7 ± 0.2	8.0 ± 0.2	1.2 ± 0.1	0.9 ± 0.4	0.20	0.33	0.033	0.055
	15	144	84.5	5.7 ± 0.0	9.6 ± 0.0	0.8 ± 0.0	0.3 ± 0.0	0.17	0.30	0.039	0.067

were then subjected to the ABE fermentation process. As shown in Fig. 3A, sugars were completely consumed for both 10 and 15% substrate loadings. Considering butanol production, 5.9 and 8.8 g/L were obtained for SHF 10 and 15%, respectively, at the end of fermentation (t = 144 h) (Fig. 3B and Table 2). Then, the use of a higher substrate loading (15 vs 10% w/v) allowed a 49.2% increase in butanol production from the vine shoots, with a high butanol yield (0.23 vs 0.24 g/g sugars consumed). This is very interesting for implementation in the industrial scale process, as well as considering the profitability of the process. ABE concentrations attained were also significantly ($p < 0.05$) higher when a solid loading of 15% was used (12.4 vs 8.1 g/L) (Fig. 3C and Table 2), which corresponds to an ABE yield of 0.33 g/g sugars consumed (vs 0.34 g/g) and ABE productivity of 0.061 g/L·h (vs and 0.041 g/L·h) (Table 2). Then, significant differences ($p < 0.05$) were observed in butanol and ABE production for both SHF 10 and 15%.

Concerning the SSF configuration, in this case, both enzymatic hydrolysis and ABE fermentation stages were carried out simultaneously. As can be appreciated in Fig. 3A, although *C. beijerinckii* was not able to metabolize the totality of sugars generated in the enzymatic hydrolysis during the first 24 h of SSF; in general, a very low sugars concentration (< 2.5 g/L) remaining in the fermentation broth was observed after 48 h for both SSF 10 and 15%. However, it is worth noting that, in SSF 15%, sugars started to be accumulated in the fermentation broth after 72 h of the process, probably due to the partial cell death of *C. beijerinckii* after this process time (Fig. 3A). On the other hand, not much difference ($p > 0.05$) was observed in butanol (4.7–5.7 g/L) and ABE (8.0–9.6 g/L) production in SSF tests for both 10 and 15% substrate loadings (Fig. 3B and 3C, and Table 2). Similar butanol and ABE yields (0.17–0.20 g/g and 0.30–0.33 g/g, respectively), and butanol and ABE productivities (0.033–0.039 and 0.055–0.067 g/L·h, respectively) were also achieved for both substrate loadings (Table 2).

On the other hand, as can be seen in Table 2, not very high butyric acid concentrations (< 1.5 g/L) were detected at the end of fermentation (t = 144 h) in both SHF and SSF processes. This fact shows the effectivity of *C. beijerinckii*, as in a first stage (acidogenic phase) butyric acid is produced, later becoming butanol in a second stage (solventogenic phase) [47].

Comparing both SHF and SSF configurations, as Table 2 and Fig. 3B and 3C show, only when a substrate loading of 15% (w/v) was used, were significant differences ($p < 0.05$) found in butanol and ABE production, achieving the best results for SHF configuration (8.8 g/L butanol (vs 5.7 g/L-SSF 15%) and 12.4 g/L ABE (vs 9.6 g/L-SSF 15%), respectively). Higher yields of butanol (0.23 vs 0.17 g/g) and ABE (0.33 vs 0.30 g/g), as well as higher productivities of butanol (0.061 vs 0.039 g/L·h) and ABE (0.086 vs 0.067 g/L·h), were also attained in the SHF configuration (at 15% w/v solid loading) (Table 2). Nevertheless, when a substrate loading of 10% (w/v) was employed, although higher butanol concentrations were yielded in the SHF configuration (5.9 g/L-SHF vs 4.7 g/L-SSF), no significant difference ($p > 0.05$) was detected in this case. In addition, similar ABE productions (8.0–8.1 g/L) were observed in this case (Fig. 3B and Table 2). Therefore, taking into account these results and the advantages of the SSF technology, such as the use of a unique reactor vessel, higher energy efficiency and cost-effectiveness, easy scaling up, and lower capital cost [48], the use of SSF configuration could be beneficial for low substrate loadings of vine shoots (≤10% w/v).

In short, the best results obtained in this work from vine shoots (8.8 g/L butanol and 12.4 g/L ABE; 3.5 g butanol/100 g vine shoots and 4.9 g ABE/100 g vine shoots), with a butanol yield and productivity of 0.23 g/g and 0.061 g/L•h, respectively (ABE yield and productivity of 0.33 g/g and 0.086 g/L•h, respectively), were achieved using the SHF configuration at 15% (w/v) of substrate loading. Comparing the best results obtained in this work with those reported in the literature, a lower ABE production (8.5 g/L) was achieved from a slurry of green macroalgae *Enteromorpha intestinalis* pretreated with sulfuric acid (121 °C, 60 min, 270 mM H_2SO_4) by fermentation with *C. acetobutylicum* [49]. Lower solvents concentrations (6.7 g/L butanol and 10.4 g/L ABE) to those obtained in this work were also yielded in the fermentation by *C. beijerinckii* of spent coffee grounds enzymatic hydrolysate (after dilute acid pretreatment: 160.47 °C, 5 min and 1.5% H_2SO_4) [20]. Nimbalkar et al. [50] also reported much lower butanol and ABE levels (4.4 and 6.7 g/L, respectively) from press mud pretreated slurry (121 °C, 15 min and 1.5% H_2SO_4) by C. *acetobutylicum*. López-Linares et al. [51] also attained lower butanol and ABE concentrations (7.4 and 11.5 g/L, respectively) from carrot discard enzymatic hydrolysate (without using pretreatment), using the same microorganism employed in this work (*C. beijerinckii*).

In situ gas stripping fermentation. In order to enhance the butanol generation and simultaneously mitigate the inhibition of this fermentation product, the configuration where the best results were achieved (SHF-15% w/v) was subjected to a fed-batch fermentation system with in situ gas stripping. In this way, the enzymatic hydrolysate (at a 15% w/v substrate loading) was used as both initial fermentation and fed-batch broths. Profiles of sugars (glucose and xylose) (A) and products (butanol, ethanol and acetone) (B) during fermentation are displayed in Fig. 4. As can be appreciated, both glucose and xylose were completely metabolized by *C. beijerinckii* at 48 h of the process. Once the fed-batch mode was initiated (at 48 h fermentation), even though glucose partially continued to be metabolized, a progressive accumulation was detected throughout the fermentation, stabilizing between 72 and 144 h of the process, and increasing at the end of fermentation (t > 144 h) (Fig. 4A). This could be due to the high concentration of sugars (about 40 g/L) in the enzymatic hydrolysate (at 15% w/v substrate loading). Thus, the feeding flow of the enzymatic hydrolysate should be investigated in greater depth. As can be observed in Fig. 4B, solvents (considering both solvents accumulated in the gas stripping condensate and solvents remaining in the fermentation broth) produced during the fermentation increased significantly ($p < 0.05$) after 72 h of process, reaching levels of 8.0 g/L butanol and 10.7 g/L ABE. Nevertheless, in order to try to

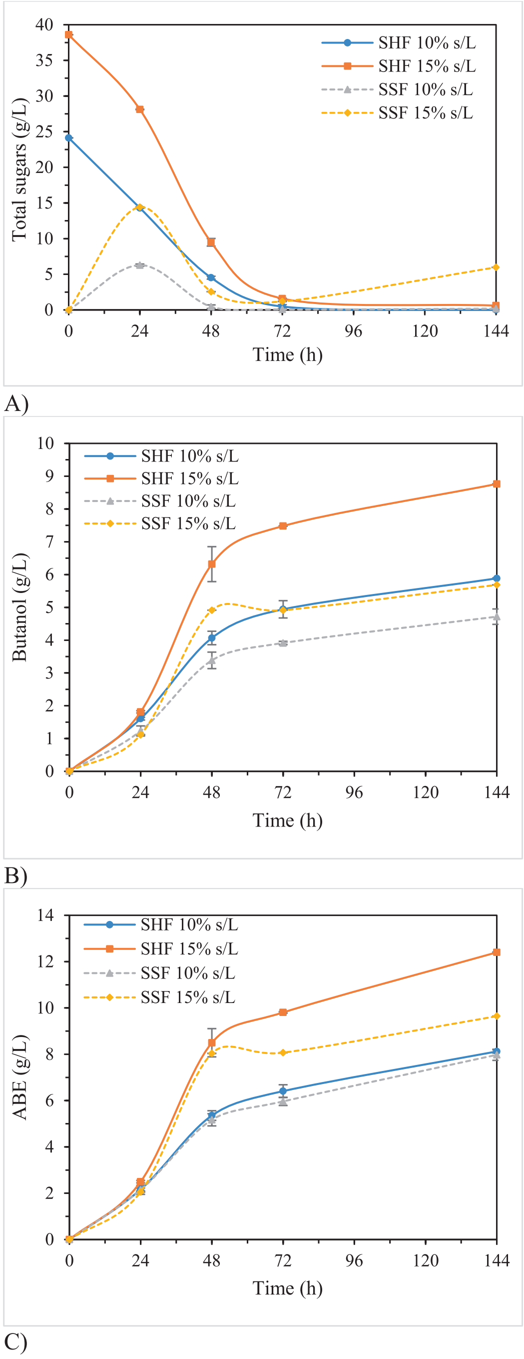

Fig. 3. ABE fermentation kinetics of vine shoots in SHF (continuous lines) and SSF (dashed lines) configurations (at 10 and 15% (w/v) of substrate loading). Total sugars consumption (A), and butanol (B) and ABE (acetone-butanol-ethanol) (C) production.

enhance these results, further research into operating strategies with stripping (such as flow rate used, pulse feeding, or intermittent stripping, among others) should be carried out.

2,3-BDO production from steam explosion pretreated vine shoots

Another alternative for the valorization of vine shoots was 2,3-butanediol production by *P. polymyxa* from dilute acid pretreated vine shoots. In this case, similarly to those previously described in butanol production from pretreated vine shoots (Section 3.3.), two different process configurations (SHF and SSF) and substrate loadings (10 and 15% w/v) were studied (Fig. 1). Fig. 5 and Table 3 display the results obtained for sugar consumption and product formation.

Concerning the SHF configuration, as described in Section 3.3., firstly, two enzymatic hydrolysates were obtained from vine shoots for both 10 and 15% (w/v) solid loadings (composed by 25.9 and 40.3 g/L total sugars), which were later fermented by *P. polymyxa* in a second stage. As can be observed in Fig. 5A, sugars were quickly consumed by *P. polymyxa* in both cases, with sugar uptakes higher than 87% (Table 3) after 24 h of fermentation, metabolizing completely at 48 h of process. In addition, high maximum 2,3-BDO production was achieved for both 10 and 15% (w/v) substrate loadings (8.1 and 13.8 g/L, respectively) (Fig. 5B and Table 3); the 2,3-BDO formation being significantly increased ($p < 0.05$) up to 70.4% when the substrate loading was increased from 10 to 15%. This is very interesting for implementation in the industrial scale process. It is worth highlighting that the maximum 2,3-BDO production was reached in both cases at times of fermentation of only 24 h (Fig. 5B), which could be very interesting for the economic profitability of the process. As can be seen in Table 3, high yields (0.42–0.45 g/g sugars consumed) and productivities (0.338–0.574 g/L·h) of butanol were obtained for both solid loadings, without these parameters being negatively affected by an increase in the solid loading.

With regards to the SSF configuration, all sugars hydrolyzed by enzymes were completely consumed by *P. polymyxa* for both 10 and 15% solid loadings, not being accumulated at any time during the fermentation (Fig. 5A). In this configuration, as can be appreciated in Fig. 5B and Table 3, the maximum 2,3-BDO concentration reached increased by 45.9% (reaching a maximum value of 10.8 g/L) when substrate loading increased from 10 to 15%, this increase being statistically significant ($p < 0.05$). In this case, as for the SHF configuration, butanol yields and productivities were not negatively affected by the increase in solid loading, with values of 0.25–0.28 g/g and 0.310–0.448 g/L·h, respectively (Table 3).

Comparing both SHF and SSF configurations, not much difference ($p > 0.05$) in the maximum 2,3-BDO production (7.4–8.1 g/L) was observed for the lowest substrate loading (10%) (Fig. 5B and Table 3). Therefore, in this case, the SSF strategy could be a good option, since a unique reactor vessel is used; while energy efficiency and cost-effectiveness are higher, scaling up is easier and capital cost is lower [48]. Nevertheless, when a higher substrate loading was used (15%), significant differences ($p < 0.05$) were found in 2,3-BDO production, reaching the highest 2,3-BDO concentration (13.8 vs 10.8 g/L), yield (0.45 vs 0.25 g/g) and productivity (0.574 vs 0.448 g/L·h) for the SHF configuration (Fig. 5B and Table 3).

In brief, in this case, the best results attained in this study from vine shoots (13.8 g/L 2,3-BDO and 5.5 g 2,3-BDO/100 g vine shoots (Fig. 6)), with a 2,3-BDO yield and productivity of 0.45 g/g (90% of its theoretical value, considering 0.5 g/g the theoretical 2,3-BDO yield [52]) and 0.574 g/L•h, respectively, were obtained using the SHF configuration at 15% (w/v) of substrate loading. Comparing the best results obtained in this study with those reported in the literature, a similar 2,3-BDO yield (0.43 g/g) but much lower 2,3-BDO productivity (0.26 vs 0.574 g/L·h) was achieved in the fermentation of carrot discard enzymatic hydrolysate, also using the SHF configuration and *P. polymyxa* DSM 365 [25]. A lower 2,3-BDO yield (0.36 vs. 0.45 g/g) was also attained from fruit waste (plums, apples, and pears) extract by *Enterobacter ludwigii* FMCC 204 [53]. Białkowska et al. [54] reported a slightly lower concentration (12.8 vs 13.8 g/L) and much lower yield (0.17 vs 0.45 g/g) of 2,3-BDO in the fermentation from apple pomace enzymatic hydrolysate by *Bacillus subtilis* LOCK 1086. López-Linares et al. [55] achieved a much lower 2,3-BDO concentration (3.7 g/L), yield (0.14 g/g) and productivity (0.10 g/L·h) than those achieved in this work in the co-culture fermentation by *P. polymyxa* DSM 365 and *Rhodococcus* sp. of brewer's spent grain hemicellulosic hydrolysate. Much lower 2,3-BDO levels (5.2–5.9 vs 13.8 g/L) and slightly lower 2,3-BDO yields (0.38–0.41 vs 0.45 g/g) were obtained from aqueous solutions of pepper, pineapple, and cabbage waste by *Bacillus licheniformis* YNP5-TSU [56]. Lower or similar 2,3-BDO

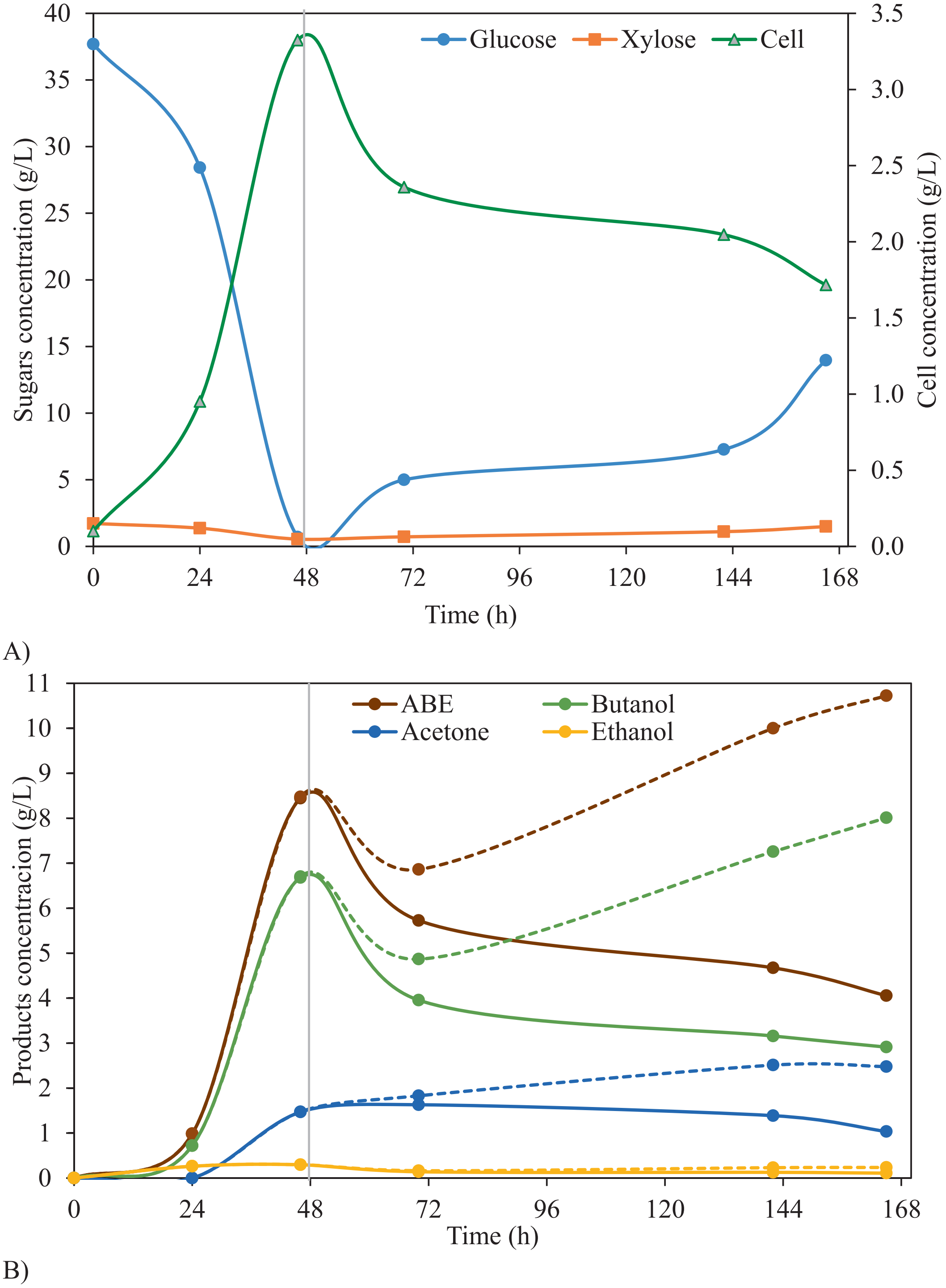

Fig. 4. ABE fermentation fed-batch profiles with in situ gas stripping of vine shoots in SHF configuration (at 15% (w/v) of substrate loading). The fed-batch mode was performed using the enzymatic hydrolysate (obtained from enzymatic hydrolysis at 15% w/v substrate loading) of pretreated vine shoots. Continuous lines represent the production of solvents in the fermentation broth, while dashed lines represent the solvent total production (solvents from the gas stripping condensate plus solvents in the fermentation broth). The continuous vertical line (in gray colour) represents the beginning of gas stripping and feed-batch processes (at 48 h of fermentation).

yields (0.40–0.47 g/g), but much lower 2,3-BDO productivity (0.36–0.42 vs 0.574 g/L·h) were reported by Fernández-Delgado et al. [57] and Barrios et al. [58] from enzymatic hydrolysates of banana peel and discarded red beetroot, respectively, by *P. polymyxa* DSM 365, also using the SHF strategy.

Besides 2,3-BDO, other by-products are generated in the fermentation by *P.polymyxa*, such as acetoin (Fig. 5 C) and ethanol (Fig. 5D). Ethanol and acetoin are originated from pyruvate (an intermediate in 2,3-BDO fermentation), ethanol being formed through successive pyruvate–formate lyase, acetaldehyde dehydrogenase, and ethanol dehydrogenase pathways, and acetoin by successive α-acetolactate synthase and 4,α-acetolactate decarboxylase pathways [59]. In this way, as can be seen in Table 3, besides 2,3-BDO, 1.2–2.8 g/L ethanol and 0.7–2.7 g/L acetoin were produced at the time of maximum 2,3-BDO production (24

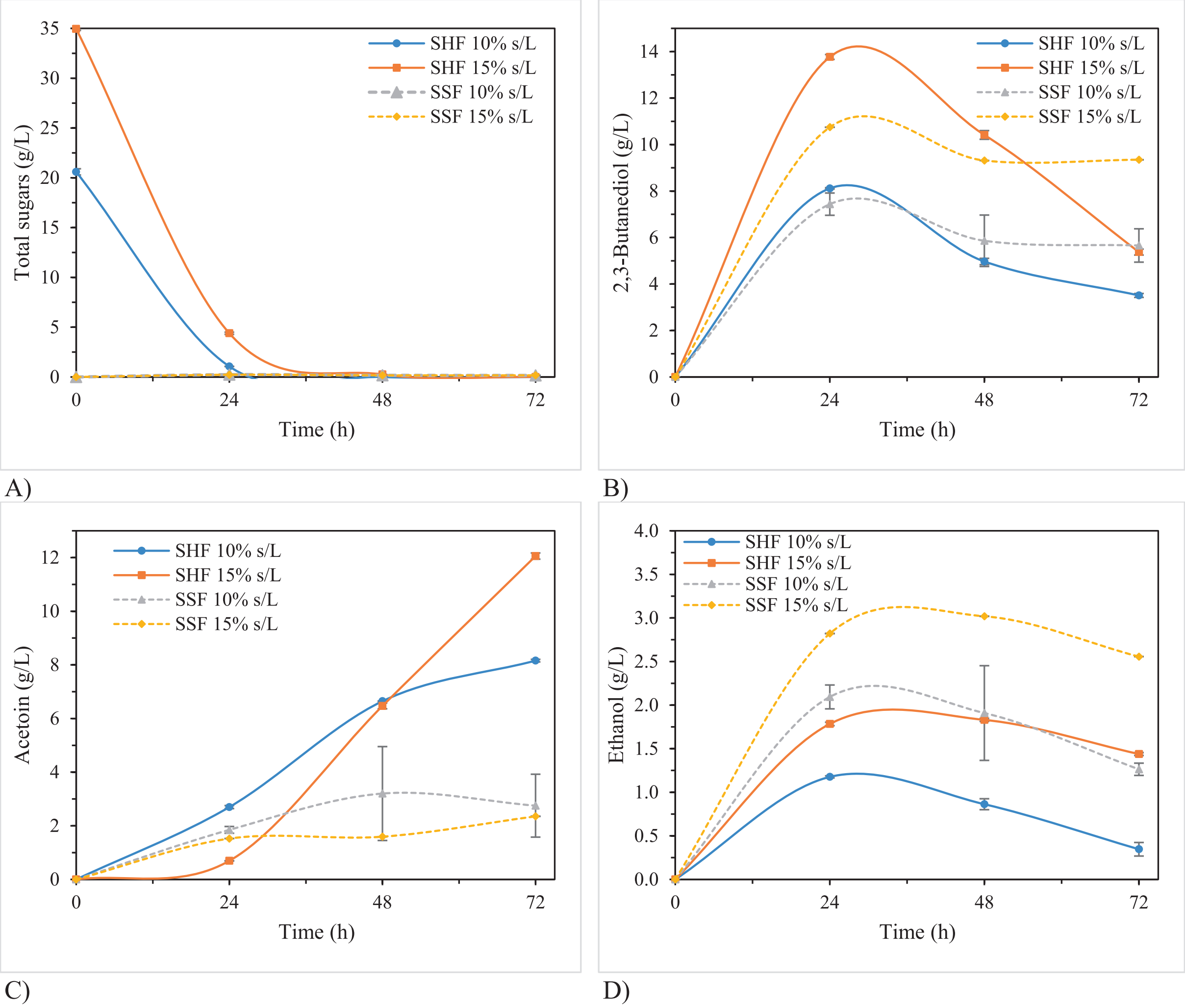

Fig. 5. 2,3-butanediol fermentation kinetics of vine shoots in SHF (continuous lines) and SSF (dashed lines) configurations (at 10 and 15% (w/v) of substrate loading). Total sugars consumption (A), and 2,3-butanediol (B), acetoin (C) and ethanol (D) production.

Table 3
2,3-butanediol fermentation of vine shoots in SHF and SSF configurations (at 10 and 15% (w/v) of substrate loading). Sugar uptake (%); 2,3-butanediol (2,3-BDO), ethanol, acetoin and cell concentrations (g/L); and butanediol yield ($Y_{BDO/sugars}$, expressed as g/g sugars consumed) and productivity (Q_{BDO}, expressed as g/L·h) at the time of maximum butanediol production. Data in parentheses refer to sugar uptake at the end of the fermentation process (72 h).

Process conf.	Solids loading (%, w/v)	Time (h)	Sugar uptake (%)	2,3-BDO (g/L)	Ethanol (g/L)	Acetoin (g/L)	Cell (g/L)	$Y_{BDO/sugars}$ (g/g)	Q_{BDO} (g/L·h)
SHF	10	24	94.9 (100)	8.1 ± 0.0	1.2 ± 0.0	2.7 ± 0.1	0.9 ± 0.0	0.42	0.338
	15	24	87.4 (100)	13.8 ± 0.1	1.8 ± 0.0	0.7 ± 0.0	1.6 ± 0.1	0.45	0.574
SSF	10	24	98.9 (100)	7.4 ± 0.5	2.1 ± 0.1	1.9 ± 0.1	4.1 ± 0.3	0.28	0.310
	15	24	99.3 (100)	10.8 ± 0.0	2.8 ± 0.0	1.5 ± 0.0	2.7 ± 0.2	0.25	0.448

h). It is worth noting that, as can be observed in Fig. 5C, considerable acetoin generation was initiated when sugar levels in the fermentation broth were low, the 2,3-BDO produced also being consumed after this time of fermentation (after t = 24 h) (Fig. 5B). This same behavior was also reported by other authors from the literature (such as Okonkwo et al., [60]; López-Linares et al. [25]; Fernández-Delgado et al., [57]) in the 2,3-BDO fermentation by *P. polymyxa* DSM 365 of non-detoxified wheat straw, carrot discard and banana waste hydrolysates, respectively. According to Maina et al. [61], 2,3-BDO can reversibly become acetoin, regenerating NADH, with a continuous oxidation–reduction state.

Comprehensive biorefinery assessment for vine shoots residue valorization

The integrated valorization strategy proposed in this study – targeting xylitol, butanol, and 2,3-BDO – aligns with the growing body of literature seeking to maximize the economic viability of viticultural by-products. Furthermore, multi-product biorefineries are being promoted rather than traditional biorefineries that focus on the production of a single product [62]. Previous studies on vine shoots have predominantly focused on the production of bioethanol [39,63–68] and the extraction of phenolic compounds [67,69–73]. For instance, Dávila et al. [64] proposed a biorefinery scheme comprising sequential processes of hydrothermal pretreatment (with a severity of 4.47) followed by a second stage of alkali delignification pretreatment, resulting in an ethanol

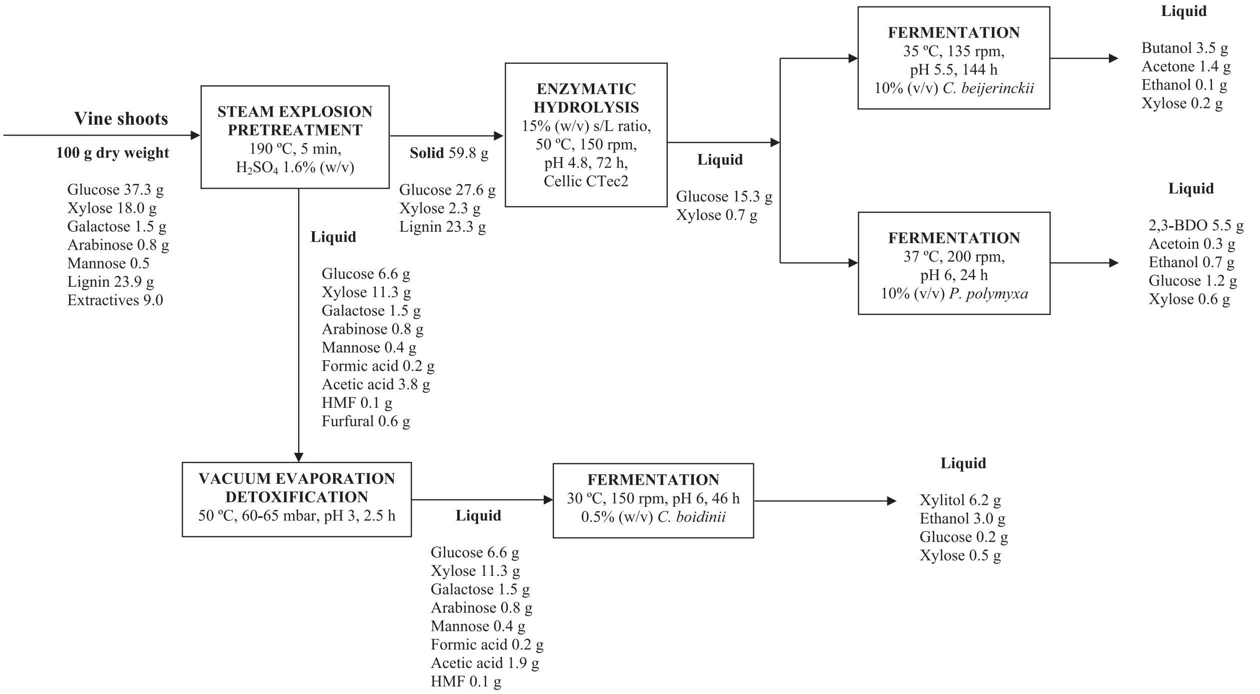

Fig. 6. Mass balance flow diagram of the overall process for xylitol production from steam explosion hemicellulosic hydrolysate of vine shoots (after vacuum evaporation detoxification) by *C. boidinii*, and butanol or 2,3-butanediol production from steam explosion pretreated vine shoots by *C. beijerinckii* and *P. polymyxa*, respectively, using SHF configuration (at 15% w/v).

generation of 13.3 g/L by a SHF process and in the extraction of high-quality lignin. Jesus et al. [63] also evaluated sequential autohydrolysis pretreatment (first stage at 180 °C for 60 min followed by a second stage at 200 °C for 30 min; total severity = 4.60) for the recovery of oligosaccharides in the liquid phase and the improvement of the enzymatic saccharification of the pretreated biomass, achieving 13.1 g of ethanol (19.1 g/L ethanol) by SHF configuration, 13.7 g of xylooligosaccharides, 3.1 g of phenolic compounds, and 27 g of lignin per 100 g of vine pruning residue. More recently, Cardoza et al. [67] also reported an innovative biorefinery approach, sequentially combining ohmic heating extraction (OHE) (80 °C for 60 min) followed by autohydrolysis (200 °C for 30 min), for the extraction of phenolic compounds (31.3 mg GAE/g vine shoots) from liquid fraction and ethanol production (20.57 g/L) from pretreated solid by SSF configuration. Maximal ethanol concentration of 10 g/L (0.16 g/g) and 50.5 g/L (0.32 g/g) were also obtained when pretreated vine shoots (autohydrolysis at 215 °C for 0 min; severity = 3.89) were supplemented with wine lees or grape must, respectively, in a SSF process [11].

While these established approaches prioritize high-volume commodities, our findings suggest that diversifying the product portfolio to include higher-value platform chemicals may offer a more robust economic framework. As quantified in the overall mass balance (Fig. 6), the proposed process yields 6.2 g of xylitol from the hemicellulosic fraction, while the cellulosic fraction can be diverted to produce either 3.5 g of butanol or 5.5 g of 2,3-BDO per 100 g of raw biomass (under SHF conditions at 15% w/v). Such a detailed mass balance underscores the high sugar recovery achieved during the steam explosion pretreatment and the robust performance of the subsequent detoxification and fermentation stages. Furthermore, compared to work by Buratti et al. [65], who utilized the same type of pretreatment (steam explosion) used in this study, but for bioethanol production (yielding 8.9 g/100 g vineyard pruning; pretreatment severity = 4.24; 210 °C and 20 bar of pressure for 10 min), our integrated configuration maintains comparable sugar utilization efficiency while synthesizing a more diverse array of specialized metabolites. By leveraging the distinct metabolic capabilities of *C. beijerinckii* and *P. polymyxa*, this multifaceted valorization not only addresses the carbohydrate fractions but also expands the potential revenue streams, enhancing the industrial feasibility of vine-shoot-based biorefineries.

Conclusions

This study demonstrated the comprehensive valorization of both cellulosic and hemicellulosic sugars derived from vine shoots. The simultaneous production of xylitol, butanol, and 2,3-BDO, among other fermentation by-products, was successfully implemented within a multi-product biorefinery framework. With this objective, different process configurations (separate hydrolysis and fermentation, SHF; and simultaneous saccharification and fermentation, SSF) at different solids loadings (10% and 15% w/v) were studied. Yields of 6.2 g xylitol, 4.9 g ABE and/or 5.5 g 2,3-BDO, among other fermentation by-products, per 100 g of vine shoots were achieved, displaying the potential of this lignocellulosic residue for use in an integrated biorefinery approach.

CRediT authorship contribution statement

Juan Carlos López-Linares: Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Juan Miguel Romero-García:** Methodology, Investigation. **Alfonso M. Vidal:** Methodology, Investigation. **Susana Lucas:** Supervision, Formal analysis, Conceptualization. **Mónica Coca:** Supervision, Formal analysis, Conceptualization. **M.Teresa García-Cubero:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Inmaculada Romero:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Eulogio Castro:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

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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