fermentation

MDPI

Article

Valorization of Byproducts from the Sugarcane Industry Through Production of 1,3-Propanediol by *Lentilactobacillus diolivorans*

Rayssa Karla Silva [1], Sophia Bezerra da Silva [1], Giselle Alves da Paixão [1], Fábio Gabriel Ferreira Júnior [1], Michelle Fernandes Araújo [2], Sarita Cândida Rabelo [3], Carlos Eduardo de Araújo Padilha [4,*], Emmanuel Damilano Dutra [4], Aline Carvalho da Costa [2], Marcos Antônio de Morais Júnior [5] and Rafael Barros de Souza [1,*]

[1] Laboratory of Microbial Metabolism, Institute of Biological Sciences, University of Pernambuco, Recife 50100-130, PE, Brazil; rayssa.karla@ufpe.br (R.K.S.); giselle.alvesp@ufpe.br (G.A.d.P.); fabiogabrielfarmaceutico@gmail.com (F.G.F.J.)
[2] School of Chemical Engineering, University of Campinas, Campinas 13083-852, SP, Brazil; al286899@unicamp.br (A.C.d.C.)
[3] School of Agricultural Sciences, São Paulo State University, Botucatu 18603-970, SP, Brazil; sarita.rabelo@unesp.br
[4] Laboratory of Bioenergy and Environment, Federal University of Pernambuco, Recife 50740-540, PE, Brazil
[5] Laboratory of Microbial Genetics, Department of Genetics, Federal University of Pernambuco, Recife 50670-420, PE, Brazil; marcos.moraisjr@ufpe.br
* Correspondence: carlospadilha.eq@gmail.com (C.E.d.A.P.); rafael.souza@upe.br (R.B.d.S.)

Abstract

This study aims to evaluate the use of sugarcane industry byproducts (bagasse and molasses), as well as glycerol from the liquor of the organosolv pretreatment of bagasse, to bioproduce 1,3-propanediol (1,3-PDO). For this purpose, *Lentilactobacillus diolivorans* was used, which can produce 1,3-PDO from a mixture of glycerol and sugars. First, experiments were conducted using the MRS medium to investigate the effect of various carbohydrate sources and their mixtures on the growth profile and 1,3-PDO production by *L. diolivorans*. Subsequently, the carbohydrates in the MRS medium were replaced with sugarcane byproducts. The results showed that the type of carbohydrate plays a crucial role in growth kinetics and 1,3-PDO production. Xylose and glucose showed the best results; however, sucrose was not enough to support biomass formation. The presence of xylose increased sucrose assimilation, resulting in a 4.5-fold increase in the concentration of 1,3-PDO from 1.3 to 6.18 g/L. An auspicious outcome was observed when the liquor of the acid pretreatment of sugarcane bagasse, molasses, and organosolv liquor were used as substrates, resulting in a production of 5.47 g/L 1,3-PDO with an efficiency of 82.77%. Therefore, producing a high-value chemical such as 1,3-PDO from the sugarcane byproducts seems to be a very promising strategy.

Keywords: biorefinery; sugarcane by-products; bioproduction; organosolv; glycerol

check for updates

Academic Editor: Konstantinos G. Kalogiannis

Received: 26 August 2025
Revised: 23 September 2025
Accepted: 25 September 2025
Published: 26 September 2025

Citation: Silva, R.K.; da Silva, S.B.; da Paixão, G.A.; Júnior, F.G.F.; Araújo, M.F.; Rabelo, S.C.; Padilha, C.E.d.A.; Dutra, E.D.; da Costa, A.C.; de Morais Júnior, M.A.; et al. Valorization of Byproducts from the Sugarcane Industry Through Production of 1,3-Propanediol by *Lentilactobacillus diolivorans*. *Fermentation* **2025**, *11*, 554. https://doi.org/10.3390/fermentation11100554

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The sugarcane-based industry settled in Brazil can be considered a self-sufficient industry and can be framed in the context of biorefineries in a circular economy [1]. Sugarcane juice can be boiled to produce crystal sugar in the sugar mills or fermented to produce fuel ethanol in the autonomous distilleries [2]. Moreover, ethanol can also be produced from crystal sugar byproduct, molasses, in the sugar mills-associated distilleries. Both processes produce the so-called first-generation (1G) ethanol. After ethanol distillation, the

byproduct vinasse is used for irrigation of the sugarcane fields or anaerobic digesters to produce biogas. Finally, the cellulose-rich bagasse accumulated from sugarcane crushing is generally burned to produce steam for the distillers and for electric generators to fulfill the energy requirement of the industry [2].

Alternatively, sugarcane bagasse can be pretreated and hydrolyzed to release sugars, especially glucose, which can be fermented to produce the second-generation (2G) ethanol. In this context, the pretreatment of the lignocellulosic biomass is an important step to solubilize lignin and hemicellulose in the liquid fraction, resulting in a solid phase with a high cellulose proportion [3]. Organosolv represents a modern technology of pretreatment that uses organic solvents for lignocellulosic biomass hydrolysis. One of the promising solvents that can be used is crude glycerol, the byproduct of biodiesel production [4]. After organosolv pretreatment, the liquid fraction (liquor) has high concentrations of glycerol, which has the potential to be reused as a substrate for other industrial processes, such as biogas production [4]. Moreover, sugarcane bagasse can also be pretreated with inorganic acid and provide a liquid fraction with xylose as the main sugar [5].

Despite this well-balanced process, there is still much untapped potential since bagasse and molasses can be better explored for high-added-value products that could increase the biorefinery profit margin [6]. To this end, 1,3-propanediol (1,3-PDO) is an interesting chemical compound that could expand the range of products in the sugarcane industry. 1,3-PDO is an organic compound ($C_3H_8O_2$) widely used in industrial applications, ranging from the textile industry to the materials industry [7]. Currently, it is synthesized through chemical synthesis with raw materials derived from petroleum.

Some species of bacteria were identified as having the ability to produce this compound from glycerol. Bacteria species such as *Klebsiella pneumoniae* and *Clostridium butyricum* can produce 1,3-PDO from glycerol; however, their industrial use can be onerous due to the pathogenic potential of the first and the difficulty in manipulating them since they are strictly anaerobic bacteria [8,9]. In this scenario, *Lentilactobacillus diolivorans* has stood out for its simple handling, being neither pathogenic nor strictly anaerobic. *L. diolivorans* has a higher capacity for 1,3-PDO production, reaching concentrations close to 80 g/L in fed-batch processes [10–12].

Particularly, *L. diolivorans* demand co-assimilation of sugars. In its metabolic pathway, the molecule of glycerol is dehydrated to 3-hydroxypropionaldehyde (3-HPA) by the enzyme glycerol dehydratase, which is further reduced to 1,3-PDO by the NADH-dependent 1,3-propanediol reductase. In this case, sugar metabolism provides the reducing equivalent for the second enzyme reaction as well as the carbon building block for cell synthesis. Previously, it was demonstrated that *L. diolivorans* can produce 1,3-PDO using industrial byproducts, such as lignocellulosic-rich biomass and crude glycerol [13].

Therefore, to evaluate the production of 1,3-PDO by *L. diolivorans* in the context of using the sugarcane industry co-products, it is necessary to establish a sugar source and a glycerol source. For that, the study was focused in of the liquid fraction of two well-defined pretreatment methodologies of the sugarcane bagasse, acid pretreatment and organosolv pretreatment, both of those pretreatments can be performed before enzymatic hydrolysis of the cellulosic fraction of the sugarcane bagasse, once they can be used to reduce the lignin and hemicellulose fraction and results in others byproducts that be directed to more valuable applications [4,5,14]. The acid pretreatment yields a liquid fraction with a high xylose content, whereas the organosolv pretreatment produces a liquid fraction with a high glycerol content [5]. Moreover, molasses is also a byproduct that can supply carbohydrates, such as sucrose, glucose, and fructose [15]. However, this byproduct can carry toxic compounds since the sugarcane juice is submitted to a high temperature that hydrolyzes the sucrose, releasing glucose and fructose, and produces toxic compounds [16].

Therefore, the present work analyzed the effect of different carbohydrate sources on the growth and fermentation metabolism, and the effect of the sugarcane industry byproduct on the performance of the *L. diolivorans* during the 1,3-PDO production. The results of this work can be used to evaluate the potential of 1,3-PDO production in a sugarcane biorefinery. It could burst the profit of the industries, contributing to the reduction in petroleum-derived intermediates for the synthesis of an industrially high-added-value product. While previous works have demonstrated the 1,3-PDO from glycerol, few have been performed in an integrated process into the sugarcane biorefinery context. This work provides a novel perspective for valorizing byproducts and probably wastes from different facilities in a single process.

2. Materials and Methods

2.1. Microorganism and Culture Media

The bacterium *L. diolivorans* DSM14421 from the culture collection of the Leibniz Institute (DSMZ-GmBH) (Braunschweig, Germany) was used. The cells were preserved in Man, Rogosa and Sharpe (MRS) (Kasvi, Pinhais, Brazil) culture medium with 30% (v/v) glycerol at −80 °C. For the growth and fermentation tests, it was prepared in-house, following the concentration of components present in the commercial MRS (Kasvi), used as a reference condition, with a change in the carbohydrate source. The following carbohydrates were tested: glucose, sucrose, fructose, xylose, maltose, galactose, mannose, lactose, cellobiose, and trehalose. The concentration of all the carbohydrates was 20 g/L, and 10 g/L glycerol was added.

2.2. Acid and Organosolv Pretreatment of Sugarcane Bagasse

For the acid pretreatment, the process was carried out in an autoclave at 121 °C for 30 min, using sugarcane bagasse at 10% (w/v) solids in a 1.5% (v/v) sulfuric acid [5]. At the end of the reaction, the material was filtered using a qualitative filter to remove the solid fraction, and the hydrolysate was recovered and stored under refrigeration.

In turn, the organosolv pretreatment was conducted using crude glycerol, following the methodology described by [4]. The process was carried out in 500 mL stainless steel reactors at 210 °C for 30 min, with a solid loading of 30% (dry weight basis) and a crude glycerol-to-water ratio of 70:30. The reactors were immersed in a thermal fluid bath (Marconi, MA 159/BB, Contagem, Brazil), and upon completion of the reaction, they were rapidly cooled in an ice bath to terminate the process. Subsequently, the reactors were opened, and water was added at a ratio of 10 mL/g of raw sugarcane bagasse before centrifugation to recover the glycerol-rich liquor. The liquor was then stored under refrigeration.

The sugarcane molasses was obtained from the sugarcane industry Trapiche (Pernambuco, Brazil).

All the substrates were characterized for posterior use in the experiments. The carbohydrate, glycerol and acetic acid content were quantified using High-Performance Liquid Chromatography (HPLC). For molasses, the density and ash content were also determined. For the density, a 5 mL glass pycnometer, previously calibrated with distilled water, was used and after was determined by the following equation:

$$Density = \frac{(Wc - Wv)}{Vr} \quad (1)$$

where Wc = mass of the pycnometer with molasse; Wv = mass of the empty pycnometer and Vr = real volume of the pycnometer.

For the ash content, 5 g of the molasse were heated in a muffle furnace at 550 °C for 15 min, in a previously tared porcelain capsule, to remove all the moisture and other

compounds. After that, the capsule with the samples was placed in the desiccator before weighing. The result was determined by the following equation:

$$Ash\ (\%) = 100\ \times\ \frac{N}{P} \quad (2)$$

where N = weight (in grams) after drying and P = weight (in grams) before drying.

2.3. Growth and Fermentation

L. diolivorans cells were cultivated in MRS broth for 48 h at 37 °C without agitation to restart the cells. Then, the cells were recovered by centrifugation and washed with saline solution (0.85%). For the growth tests, the recovered cells were used to reinoculate the MRS medium with different carbohydrates. The testes were carried out under static conditions, at 37 °C, and with 20 g/L initial sugar concentration in a 96-well plate with 200 µL of working volume and performed using a microreader Synergy HT (Biotek, Basel, Switzerland) with an initial concentration of *L. diolivorans* of 0.1 (optical density at 600 nm), and with measurements every 2 h at 600 nm. The fermentation tests were performed as described previously [13]. Batch fermentations were carried out by mixing 243 mL of medium with 27 mL of bacterial seed culture (10% v/v). The flasks were incubated at 37 °C for 24 h without agitation. Samples were taken at the end to quantify the consumption of the sugars and the glycerol, and the metabolites produced, such as lactate, acetate, ethanol, and 1,3-PDO.

To evaluate the effect of each carbohydrate, fermentation tests were carried out with a single carbohydrate in the medium with an initial concentration of 20 g/L. Subsequently, the co-consumption of sucrose, glucose, and xylose was tested, according to the following combination: sucrose + glucose (SG mix); sucrose + xylose (SX mix); glucose + xylose (GX mix), and sucrose + glucose + xylose (SGX mix). In all the combinations, each sugar was used at 10 g/L, except for the SGX mix, which used 6.66 g/L for each sugar. Therefore, all the media contained an initial sugar concentration of 20 g/L with 10 g/L glycerol.

In addition, to evaluate the media composed of the substrates coming from the sugarcane industry, the carbohydrate sources were replaced by the molasses and liquid fraction of the acid pretreatment of the sugarcane bagasse. Pure glycerol was replaced by the liquid fraction of the organosolv pretreatment of the sugarcane bagasse (PLO). Once again, all the industrial substrates were diluted to reach an initial sugar concentration of 20 g/L and a glycerol concentration of 10 g/L in the fermentation media. MRS in-house was used as a base to provide vitamins, amino acids, peptides, and minerals. Therefore, it was prepared three different media with pure carbohydrate and sugarcane byproducts: Formulation (1) is acid pretreatment of sugarcane bagasse, pure sucrose, and PLO; Formulation (2) is molasses, pure xyloses, and PLO; and Formulation (3) is acid pretreatment of sugarcane bagasse, molasses, and PLO. The content of the initial carbohydrate was adjusted to 20 g/L, and PLO was used for 10 g/L glycerol.

2.4. Analytical Methods and Statistical Analysis

All the chemical compounds (carbohydrates, organic acids, ethanol, glycerol, and 1,3-PDO) from the fermentation tests and the pretreatment of the bagasse were quantified by High-Performance Liquid Chromatography (HPLC—Agilent Technologies 1200 Series, Agilent Technologies, Santa Clara, CA, USA). An Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) was used and the mobile phase was a solution of sulfuric acid at 5 mM, using a flow rate of 0.6 mL/min, equipped with a Refractive Index Detector (RID) (Agilent, Santa Clara, CA, USA) to analyze carbohydrates, glycerol, acetic acid, 1,3-PDO and ethanol;

a Diode Array Detector (DAD) (Agilent, Santa Clara, CA, USA) using 210 nm wavelength was used for lactic acid quantification.

Yield calculation ($Y_{P/S}$) was carried out for all conditions. Glycerol and 1,3-PDO concentrations were used in the calculation, as can be seen in Equation (3). For efficiency (E), we used the theoretical conversion of glycerol into 1,3-propanediol (w/w), considering the total conversion of the substrate (Equation (4)).

$$Y_{\frac{P}{S}} = \frac{[1,3-PDO]}{[Glycerol_C]} \quad (3)$$

$$E(\%) = \frac{Y(g/g)}{0.82} \quad (4)$$

where [1,3-PDO] is the final 1,3-PDO concentration in g/L and [Glycerol$_C$] is the glycerol concentration consumed during the reaction. Both were evaluated at the end of the experiments. The value of 0.82 is the maximum theoretical yield.

All the experiments were performed in biological triplicate. The data were submitted to analysis of variance (ANOVA) and mean comparison test (TUKEY) using Minitab 17.1.0 with a 95% level of significance ($p < 0.05$).

3. Results and Discussion

3.1. Effects of Sugar Source on L. diolivorans Growth and Fermentation Performance

Among the monosaccharides, xylose and fructose promoted the fastest cell growth rates of 0.115 h^{-1} and 0.098 h^{-1}, respectively. They were followed by glucose with 0.060 $^{-1}$ and galactose with 0.048 h^{-1}. At the end of growth, all monosaccharides produced similar final biomass, except for mannose, which induced no growth (Figure 1A). The growth performance of *L. diolivorans* with xylose and glucose was also reported previously [11]. Therefore, *L. diolivorans* can assimilate the main sugars (glucose and xylose) derived from the processing of lignocellulosic biomass, such as sugarcane bagasse.

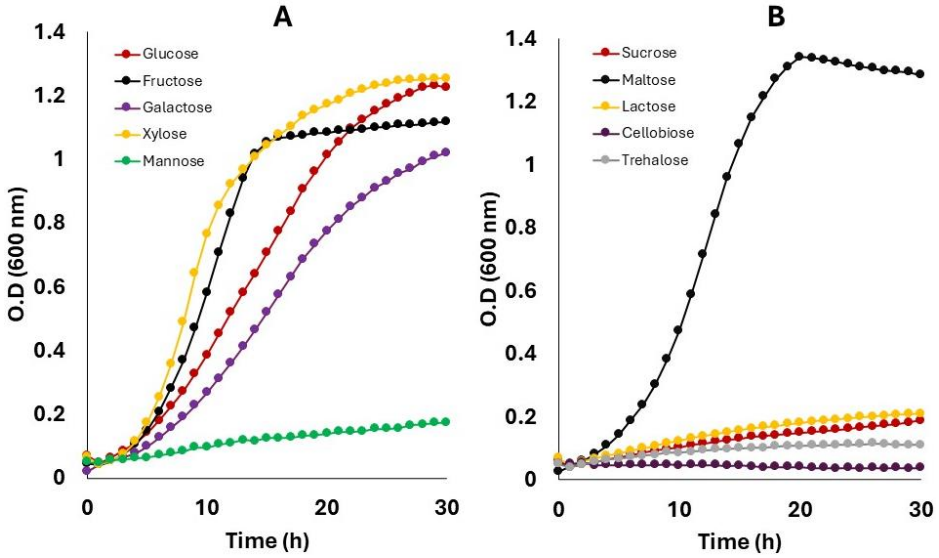

Figure 1. Growth profile of the bacteria *Lentilactobacillus diolivorans* in different carbon sources: (**A**) growth in MRS in-house medium with glucose (red circle, fructose (black circle), galactose (purple circle), xylose (yellow circle) and mannose (green circle); (**B**) growth in medium with sucrose (red circle), maltose (black circle), lactose (yellow circle), cellobiose (purple circle) e trehalose (gray circle).

On the other hand, the bacteria did not utilize sucrose, a disaccharide extensively employed in 1G ethanol production and derived from sugarcane juice and molasses (Figure 1B).

In addition, cellobiose, lactose, and trehalose were not used by the bacterial cells, while maltose promoted cell growth at a rate similar to fructose (Figure 1B). It is important to highlight that cellobiose, maltose, lactose, and trehalose were used in this work to compare the sugar metabolism of *L. diolivorans* with the main sugars present in the sugarcane industry. These results indicated that *L. diolivorans* produces a glycosidase capable of hydrolyzing the α-1,4 glucosyl bond present in maltose but not the α-1,1 and β-1,4 glucosyl bonds in trehalose and cellobiose, respectively. Surely, this bacterium cannot take advantage of the incomplete hydrolysis of cellulose that produces cellobiose, but it could be very useful in the fermentation of by-products of the cereal-based substrates and food waste that contains maltose-enriched partially hydrolyzed starch.

After having determined the effect of the carbon source on aerobic bacterial growth, it was evaluated that their influence on the conversion of glycerol to 1,3-PDO in oxygen-limited fermentation tests (Table 1). It is important to mention that *L. diolivorans* depend on sugar catabolism for the generation of reduction equivalent NADH to produce 1,3-PDO [17,18]. All monosaccharides were practically consumed (>85% consumption) after 24 h. The difference relied on the 1,3-PDO production that exceeded 8.0 g/L hexoses and remained below 2.0 g/L xylose. It resulted in conversion efficiencies greater than 90% for hexoses and below 50% for xylose. Interestingly, xylose induced the highest growth rate, indicating that most of its carbon was assimilated into bacterial cells. This low production of 1,3-PDO from xylose was also reported previously [11].

Table 1. Final parameters of the 24 h batch fermentations of *Lentilactobacillus diolivorans* in MRS in-house media with different carbohydrates supplemented with pure glycerol. The initial concentration of all sugars was 20 g/L.

Parameter	Glucose	Fructose	Galactose	Xylose	Maltose	Sucrose
Carbohydrate consumed (g/L)	17.99 ± 0.23 ^{b}	18.32 ± 0.93 ^{b}	18.34 ± 0.25 ^{b}	17.30 ± 0.82 ^{b}	21.29 ± 0.10 ^{a}	3.97 ± 0.11 ^{c}
Acetic acid (g/L)	3.21 ± 0.51 ^{bc}	4.28 ± 0.51 ^{ab}	3.66 ± 0.60 ^{ab}	4.50 ± 0.21 ^{a}	3.34 ± 0.4 ^{abc}	2.30 ± 0.30 ^{c}
Lactic acid (g/L)	11.68 ± 0.43 ^{b}	8.60 ± 0.23 ^{c}	11.53 ± 0.15 ^{b}	13.06 ± 0.20 ^{a}	11.12 ± 0.55 ^{b}	0.00 ± 0.00 ^{d}
Ethanol (g/L)	1.26 ± 0.19 ^{a}	0.00 ± 0.00 ^{b}	1.15 ± 0.09 ^{a}	0.00 ± 0.00 ^{b}	1.01 ± 0.12 ^{a}	0.00 ± 0.00 ^{b}
Mannitol (g/L)	0.00 ^{b}	2.86 ± 0.25 ^{a}	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}
Glycerol consumed (g/L)	8.36 ± 0.28 ^{a}	9.01 ± 0.45 ^{a}	8.64 ± 0.13 ^{a}	3.58 ± 0.31 ^{b}	8.95 ± 0.54 ^{a}	2.40 ± 0.24 ^{c}
1,3-Propanediol (g/L)	6.22 ± 0.18 ^{b}	7.05 ± 0.17 ^{a}	7.07 ± 0.18 ^{a}	1.51 ± 0.10 ^{c}	7.15 ± 0.14 ^{a}	1.30 ± 0.14 ^{c}
Y, 1,3-PDO	0.75 ± 0.03 ^{a}	0.78 ± 0.06 ^{a}	0.82 ± 0.02 ^{a}	0.42 ± 0.05 ^{b}	0.80 ± 0.05 ^{a}	0.54 ± 0.05 ^{b}
E (%)	90.20 ± 3.9 ^{a}	95.00 ± 6.85 ^{a}	99.01 ± 2.69 ^{a}	51.24 ± 5.85 ^{b}	96.96 ± 5.90 ^{a}	65.73 ± 6.39 ^{b}

Average with different letters in the same column indicate a statistical difference ($p < 0.05$).

Acetic acid and lactic acid were produced at the same level from all monosaccharides, except for xylose and fructose, while ethanol was only slightly produced in all conditions (Table 1). As expected, mannitol was produced from the direct reduction in fructose, leaving less fructose to convert into the other metabolites. Maltose, the only disaccharide that promoted bacterial growth, was also consumed during fermentation, and the results were similar to glucose. Mannitol, lactate, and ethanol represent redox sinks from which the cells get rid of the excess NADH generated by glycolysis [10]. Mannitol represents the direct reduction in fructose by mannitol dehydrogenase in the Lactic Acid Bacteria (LAB) when fructose is the only sugar source or when it is in excess [19]. Lactate is the reduced form of pyruvate by lactate dehydrogenase and represents the most efficient way of NADH reoxidation among the LAB. Ethanol is only produced by heterofermentative bacteria such as *L. diolivorans* by decarboxylating the pyruvate to acetaldehyde and then reducing it by alcohol dehydrogenase.

Interestingly, in this work, ethanol represented less than 10% of the fermentation products in all conditions tested (Table 1). Therefore, the NADH to produce 1,3-PDO has

been diverted from ethanol. It can be concluded that in the presence of glycerol, *L. diolivorans* is forced to reduce the synthesis of ethanol and divert the carbon to the other products. In fructose, less ethanol was produced, and the explanation lies in the distribution of available reducing power to mannitol. Due to the higher cell growth observed, it is possible to conclude that maltose consumption benefits biomass production more. However, it did not impair 1,3-PDO production from glycerol (Table 1). This showed that the little sucrose that was consumed was directed to anabolic metabolism.

Sucrose is a relevant industrial carbon source due to its presence in molasses from sugarcane and sweet sorghum. Despite the low level of assimilation, ORF annotation in the automatic RAST system (https://www.theseed.org/, accessed on 20 May 2025) with the genome available indicated that *L. diolivorans* has a sucrose-specific PTS (EC 2.7.1.211) likely to be used to import sucrose [20]. PTS (Phosphotransferase System) is one of the three sugar transport systems of LAB, together with the binding protein-dependent ATP-Binding Cassette (ABC) and electrochemical cation-gradient-driven system [21]. In addition, *L. diolivorans* also has a sucrose phosphorylase (EC 2.4.1.7) that catalyzes the phosphorolysis of sucrose to yield α-glucose 1-phosphate and fructose. Therefore, it has the complete mechanism to assimilate this sugar. However, it is not clear why the consumption is much slower when compared to other sugars. This phenomenon in *L. diolivorans* can be a bottleneck for the use of several matrices that have sucrose as the main carbohydrate or at least have high concentrations of this sugar.

Being heterofermentative, *L. diolivorans* produces lactic acid, acetic acid, and ethanol from carbohydrates to maintain the redox balance by consuming NADH from the glycolytic pathway [10]. When glycerol is added to the medium, the first enzyme reaction of this assimilatory route produces 3-HPA that acts as an electron acceptor in the second reaction. Hence, it diverts part of the glycolytic NADH for 1,3-PDO synthesis [10,22–24]. Therefore, there is direct competition for the glycolytic NADH in the production of other metabolites of the glycolytic pathway. In general, hexoses can provide more NADH, allowing the excess of this cofactor to be diverted to the synthesis of 1,3-PDO without compromising the metabolism of the bacteria. Therefore, pentoses such as xylose provide less NADH, which is quickly reassimilated in the production of lactic acid, the main competitor with 1,3-PDO for glycolytic NADH [11].

The evaluation of these physiological parameters serves as a basis for predicting 1,3-PDO production yields from different industrial substrates and serves to identify bottlenecks in these yields. The biofactory concept conceived in the strategy of this work considers the possibility of using products from the sugar and alcohol industry, such as molasses (rich in sucrose) and/or sugarcane bagasse hydrolysate (rich in glucose and xylose), together with the glycerol residue from liquor organosolv, to produce 1,3-PDO. Therefore, the limitation of 1,3-PDO production from xylose and sucrose could make the application of these proposed substrates unfeasible. However, the strategy of co-consumption of sugars mixed in the substrate has been shown for pentose and hexose in the simultaneous fermentation process [25]. Therefore, effect of co-assimilation of these sugars by *L. diolivorans* in the 1,3-PDO production was evaluated.

3.2. Evaluation of Fermentation with Co-Consumption of Carbohydrates

A set of fermentation tests was carried out in MRS medium containing a mixture of the most common carbohydrates found in sugarcane bagasse and molasses: sucrose + glucose (SG mix), sucrose + xylose (SX mix), glucose + xylose (GX mix), and sucrose + glucose + xylose (SGX mix) (Table 2). The final concentration of total carbohydrates was 20 g/L, with the same proportion for all sugars present in the medium. All the fermentation was supplemented with 10 g/L pure glycerol. The results showed that all carbohydrates in the

mixtures were consumed, except for sucrose in the SG mix, which had only partial uptake (50%). In the SX mix, all the sucrose present was consumed, almost double that of the condition when sucrose and glucose are present together in the medium.

Table 2. Final parameters of batch fermentation (24 h) of *L. diolivorans* in MRS media composed of a mix of carbohydrates, in a concentration of around 20.0 g/L and pure glycerol at 10.0 g/L.

Parameter	SG Mix	SX Mix	GX Mix	SGX Mix
Sucrose consumed (g/L)	4.58 ± 0.50 ^{c}	9.32 ± 0.21 ^{a}	-	5.87 ± 0.25 ^{b}
Glucose consumed (g/L)	10.90 ± 0.04 ^{a}	-	10.89 ± 0.08 ^{a}	7.62 ± 0.05 ^{b}
Xylose consumed (g/L)	-	11.27 ± 0.08 ^{a}	10.27 ± 0.83 ^{b}	7.32 ± 0.06 ^{c}
Carbohydrates consumed (g/L)	15.48 ± 0.54 ^{c}	20.58 ± 0.16 ^{b}	21.16 ± 0.76 ^{a}	20.82 ± 0.29 ^{ab}
Acetic acid (g/L)	3.78 ± 0.28 ^{c}	6.27 ± 0.03 ^{a}	5.55 ± 0.06 ^{b}	5.27 ± 0.04 ^{b}
Lactic acid (g/L)	9.96 ± 0.15 ^{d}	14.70 ± 0.60 ^{b}	16.28 ± 0.47 ^{a}	13.19 ± 0.31 ^{c}
$Y_{p/s}$ Lactic acid	0.95 ± 0.06 ^{a}	0.71 ± 0.02 ^{bc}	0.77 ± 0.03 ^{b}	0.63 ± 0.02 ^{c}
Ethanol (g/L)	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{c}	1.82 ± 0.13 ^{a}	1.34 ± 0.09 ^{b}
Glycerol consumed (g/L)	10.52 ± 0.58 ^{a}	7.99 ± 0.51 ^{b}	5.27 ± 0.22 ^{c}	8.20 ± 0.32 ^{b}
1.3-Propanediol (g/L)	7.63 ± 0.61 ^{a}	6.18 ± 0.17 ^{b}	4.31 ± 0.06 ^{c}	6.59 ± 0.04 ^{ab}
$Y_{p/s}$ 1.3-PDO	0.73 ± 0.02 ^{b}	0.77 ± 0.04 ^{ab}	0.82 ± 0.03 ^{a}	0.80 ± 0.04 ^{a}

Average with different letters in the same column indicate a statistical difference ($p < 0.05$). SG mix (sucrose + glucose), SX mix (sucrose + xylose), GX mix (glucose + xylose), SGC mix (sucrose + glucose + xylose).

It is noteworthy that sucrose consumption was stimulated by the presence of xylose in both SX mix and SGX mix. It was shown that xylose uptake in *L. diolivorans* occurs through a H+ proton-symporter (XylE) mechanism, which tends to acidify the bacterial cytoplasm [26]. In consequence, there might be an increase in the maintenance energy with the cells expending more ATP to activate the H+-ATPase to pump out these protons. Then, the increased flow of carbon through the glycolytic pathway may supply the necessary ATP [27]. Therefore, this increased flow may be correlated with faster assimilation of sucrose in the media with co-assimilation with xylose. Lactate and acetate production and yield were comparable to those of single monosaccharide-based substrates (Table 1). Ethanol was not produced in the presence of sucrose unless glucose and xylose were concomitantly present in the substrate. Once again, the demand for NADH for reducing glycerol seems to divert the reducing cofactor from ethanol production.

Opposite to ethanol production, glycerol consumption and 1,3-PDO production were stimulated by sucrose. Moreover, the negative effect of xylose on glycerol consumption and 1,3-PDO production was partially surpassed by the presence of sucrose and/or glucose in the substrate. However, the yield values were in the same range for all four sugar mixtures, as was seen for single hexoses and maltose (Table 1). It suggests that the low production of 1,3-PDO in xylose, and probably in sucrose, might be related to some sort of impairment in the glycerol assimilation mechanism. The catabolism of hexoses and pentoses uses different metabolic pathways, with the hexose being metabolized through the EMP (Embden–Meyerhof–Parnas pathway or glycolysis) and the pentoses being metabolized through the PPP (Pentose Phosphate Pathway). These metabolic pathways supply the cells with levels of NADH [28]. It seems that pentoses do not favor the production of 1,3-PDO due to the low availability of glycolytic NADH [11]. In this context, the use of residues that provide hexoses appears promising to complement the supply of glycolytic cofactors. That would be an adequate scenario for the use of hydrolysates of complete bagasse biomass, which might contain a mixture of glucose and xylose coming from the hydrolysis of cellulose and hemicellulose, respectively. However, one may take into consideration the results with the GX mix that did not result in high 1,3-PDO, despite the high yield. Alternatively, it seems that the use of molasses should fulfill all needs in energetic terms, coming from sucrose, glucose, and fructose in its composition [15]. The blending of molasses with a

xylose-rich hemicellulose hydrolysate can be an excellent strategy to produce 1,3-PDO, as was observed for the SGX mix substrate tested herein.

3.3. Characterization of the Sugarcane Industry Byproducts

All the substrates used for the medium formulation were characterized to see the presence or not of compounds that can be toxic for the bacterial metabolism and to quantify the sugar and glycerol present to be diluted when necessary. As expected, in the sugarcane bagasse acid hydrolyzed (SBH), xylose is the most abundant carbohydrate, followed by glucose, which is present at less than 4 g/L in the medium (Table 3). After the production of the SBH, the pH was adjusted to 4.8, and it was observed that acetic acid was present in the SBH with a concentration of 2.60 g/L. Molasses was used as another carbon source, and showed high concentration of sucrose, followed by fructose and glucose.

Table 3. Characterization of the sugarcane molasses and sugarcane bagasse acid hydrolyzed (SBH) used in the fermentations to produce 1,3-propanediol.

Parameter	Molasses	SBH
Sucrose (g/L)	255.48 ± 0.11	0.0
Glucose (g/L)	23.48 ± 1.36	3.60 ± 0.38
Fructose (g/L)	31.55 ± 1.21	0.0
Xylose (g/L)	0.00	14.77 ± 1.42
Acetic acid (g/L)	0.00	2.60 ± 0.09
pH	6.0	4.8
Density	1.35	N.D.
Ashes (%)	6.03	N.D.

N.D. (not determined).

The sugarcane industry has different byproducts that microorganisms can use. The sugarcane bagasse is formed from cellulose, hemicellulose, and lignin. Naturally, the substrates cannot be used by the cells, and they need to undergo several treatments to make the sugar available for cell assimilation. After the acid pretreatment, normally the solid phase goes to enzymatic hydrolysis, and then for the fermentation for 2G ethanol production, but the liquid part is rich in hemicellulose, and part of this polymer is degraded by the acid and generates xylose. In the SBH, it is possible to observe that xylose is the most abundant carbohydrate (Table 3). Pentoses are not used for ethanol production by native *S. cerevisiae* since it is not able to assimilate this kind of carbohydrate. In this case, the hemicellulose fraction, rich in xylose, can be used in other biotechnological processes, and the cellulose fraction will undergo enzymatic hydrolysis for ethanol production.

L. diolivorans is able to use xylose as a carbon source, showing the fastest cell growth rate among the carbohydrates tested (Figure 1). However, for the 1,3-PDO production, this sugar is not one of the most suitable, since it produces less cofactor for the enzymes responsible for converting 3-HPA into 1,3-PDO (Table 1). In this context, the use of other matrices that can provide more NADH is an alternative, especially sucrose, since the results showed that in the presence of xylose, *L. diolivorans* is able to uptake sucrose faster than when just this disaccharide is present in the medium (Tables 1 and 2). For this, an alternative matrix is molasses, rich in sucrose, glucose, and fructose. These carbohydrates, together with xylose, can provide enough carbon for metabolism and provide NADH for the reduction in glycerol into 1,3-PDO.

As a glycerol source, the liquid fraction after the organosolv pretreatment was used. This treatment is used in lignocellulosic biomass to deconstruct the matrix and increase the efficiency of enzymatic hydrolysis. Several substances can be used for this type of pretreatment, but glycerol is shown as a promising agent due to its effect and low cost.

In this case, the use of crude glycerol from biodiesel production was also evaluated and shown as a great replacement for pure glycerol [4]. After this treatment, the liquid fraction still has a high glycerol concentration, which can be used for other purposes. Initially, the glycerol concentration in the liquor was determined by HPLC and revealed a high content of glycerol at about 118.9 ± 6.80 g/L. For 1,3-PDO production, it is necessary to dilute the glycerol to an initial concentration in the medium of 10 g/L. In this context, using this liquor can be an alternative to the use of pure glycerol, even with the presence of possible inhibitors.

3.4. Production of 1,3-PDO Using Waste from the Sugarcane Industry

The results show no significant difference between the media for the main fermentation parameters, including 1,3-PDO production parameters, such as the yield and efficiency show the same level (Table 4). It is important to mention that the crude glycerol used for this organosolv pretreatment came from biodiesel production, that is, it could contain several impurities that can impair 1,3-PDO production [29]. However, the organosolv hydrolysate had no toxic effect on bacterial cells and has great potential to be used as a glycerol source for 1,3-PDO production, showing one more use for this residue in the biorefinery chain.

Table 4. Final parameters of batch fermentation (24 h) in MRS medium (20 g/L glucose) with pure glycerol and organosolv hydrolysate as glycerol source (10 g/L) for 1,3-PDO by the bacterium *L. diolivorans*.

Parameter	Pure Glycerol	Organosolv Hydrolysate
Glucose consumed (g/L)	21.38 ± 0.23 [a]	20.51 ± 0.24 [b]
Acetic acid (g/L)	6.04 ± 0.03 [a]	5.63 ± 0.09 [b]
Lactic acid (g/L)	13.90 ± 0.13 [a]	13.68 ± 0.43 [a]
Ethanol (g/L)	2.78 ± 0.12 [a]	2.84 ± 0.13 [a]
Glycerol consumed (g/L)	10.49 ± 0.42 [a]	9.27 ± 0.35 [b]
1,3-Propanediol (g/L)	8.14 ± 0.06 [a]	7.19 ± 0.05 [b]
$Y_{p/s}$ 1,3-PDO	0.78 ± 0.03 [a]	0.78 ± 0.02 [a]
Efficiency (%)	94.04 ± 3.25 [a]	93.99 ± 2.78 [a]

Average with different letters in the same column indicate a statistical difference ($p < 0.05$).

Following the replacement of substrates with sugarcane byproducts, the hemicellulose fraction of the acid pretreatment of sugarcane bagasse and molasses was characterized by its carbohydrate constitution. As expected, xylose was the highest concentrated sugar in the SBH. In the molasses, sucrose was the most concentrated sugar, followed by fructose and glucose, respectively. In the SBH was quantified 2.6 g/L acetic acid.

All these substrates were diluted with distilled water for the fermentation tests. Once again, all media contained sugars at 20 g/L and 10 g/L glycerol from organosolv liquor. Three different media were formulated, combining the MRS in-house medium and the industrial substrates (Figure 2). The results show that the highest sugar consumption was in formulation 2 with molasses and xylose (98%), followed by formulation 1 with SBH and sucrose (78.6%) and formulation 3 with SBH and molasses (66%). Accordingly, the final concentrations of the glycolytic pathway products were similar in formulations 1 and 2. In contrast, while in formulation 3, the acetate and the lactate were about 16% and 27% lower, respectively, and no ethanol was produced. Relative to glycerol consumption and 1,3-PDO production, formulation 3 showed lower results compared to formulations 1 and 2. However, the 1,3-PDO efficiency in formulation 3 was only 10% lower when compared to formulation 2 (Figure 2).

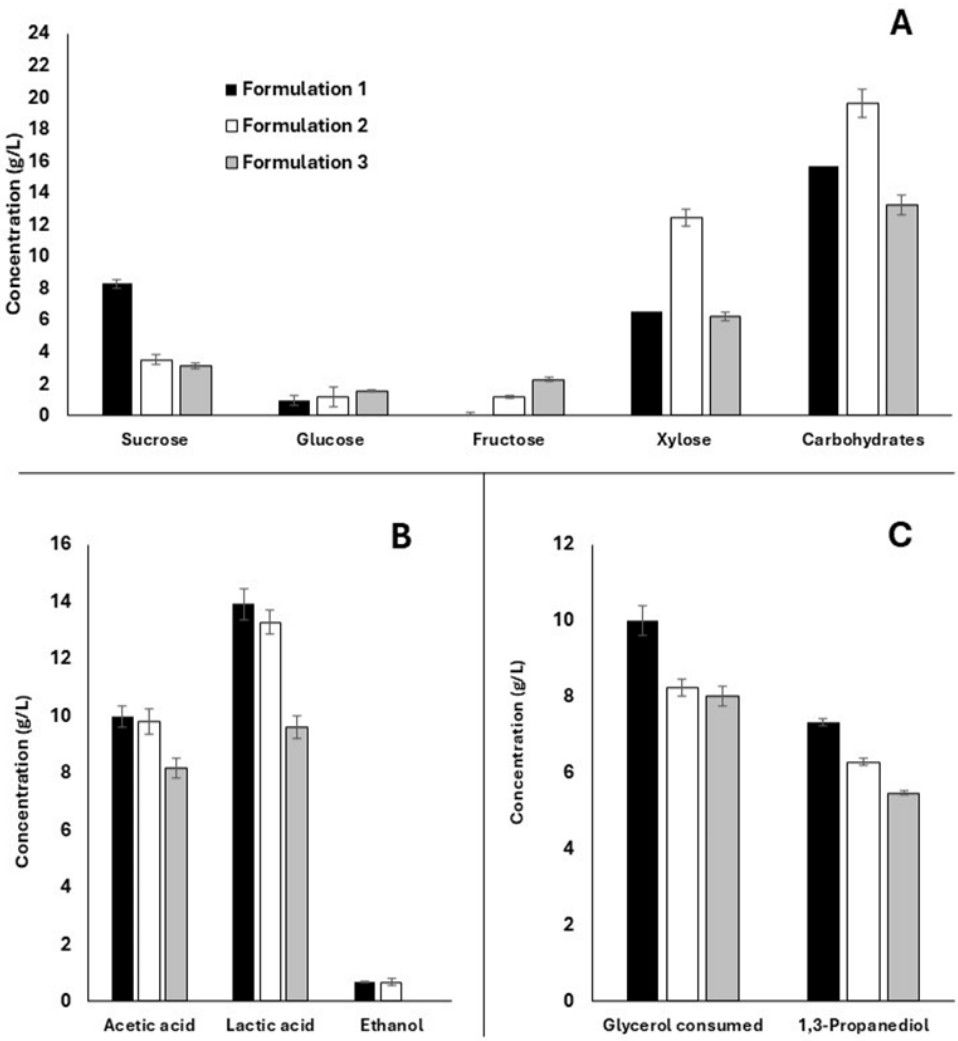

Figure 2. Final parameters of batch fermentation (24 h) with *Lentilactobacillus diolivorans* in MRS in-house medium containing industrial substrates. (**A**) Consumption of carbohydrates in the different formulations; (**B**) Production of the glycolytic metabolites; and (**C**) Glycerol consumption and 1,3-production. MRS In-house medium was supplemented with acid pretreatment of sugarcane bagasse, sucrose, and organosolv liquor (Formulation 1), molasses, xylose, and organosolv liquor (Formulation 2), and acid pretreatment of sugarcane bagasse, molasses, and organosolv liquor (Formulation 3).

Therefore, these results show that the carbohydrate catabolism of the *L. diolivorans* is more affected by the full residue formulation than the glycerol catabolism. It means that for 1,3-PDO bioproduction, the formulation medium with sugarcane bagasse hydrolyzed and molasses can be readily used as carbohydrate and glycerol sources by *L. diolivorans*. Indeed, this bacterium has shown very close yields (0.73, 0.76, and 0.68, respectively, for each formulation to the reference condition (0.75) when using a lignocellulosic matrix) [13]. According to previous work, many species of *Lactobacillus* show the same behavior [30,31].

Then, a Minimum Medium was designed using only some key inorganic elements and supplemented with the residues. In this case, 1,3-PDO production plummeted (1.06 ± 0.11 g/L). It shows that the presence of other nutrient sources, such as nitrogen compounds, is essential for growth and consequently the production of 1,3-PDO. Due to the nutritional requirements, the use of culture media formed only by waste becomes a challenge, even with the *L. diolivorans* resistance to inhibitors. The MRS medium, currently used as a standard medium for the growth of lactobacilli, is highly complex and has several types of nutrients that are not present in the byproducts used, such as vitamins, minerals, peptides, and other essential compounds [32]. Replacing this medium requires sources

that present the important nutrients to ensure the full functioning of cellular metabolism during the production of 1,3-PDO. Some residues can provide some of these nutrients, such as corn steep liquor, a rich source of nitrogen, amino acids, vitamins, and biotin for microorganisms [33,34].

It is important to mention that to implement a 1,3-PDO production line, it is necessary to increase the titer to justify the costs involved in purifying the compound. Fed-batch trials have been carried out to increase production, reaching concentrations greater than 80 g/L of 1,3-PDO depending on the matrix and methodology used [11,12]. It is necessary to conduct more studies, not just to fully replace the use of commercial culture medium, but to increase production with alternative substrates using a fed-batch approach.

4. Conclusions

L. diolivorans is a strain of bacteria with the potential to produce high concentrations of 1,3-propanediol from various substrates formulated from industrial waste. The application of these byproducts from the sugarcane industry is an interesting alternative for returning matrices to the economic sector, instead of being discarded. Carbohydrate and glycerol sources such as lignocellulosic biomass and crude glycerol can be used in the synthesis of 1,3-PDO efficiently, providing a more sustainable production of this compound. Despite its promise, it is still a challenge to formulate a culture medium using only residues, due to the nutritional requirements of bacteria of the ancient genus *Lactobacillus*. However, the tests using those byproducts from the sugarcane industry show great promise. The next steps of using these byproducts in fed-batch need to be taken to increase the concentration and provide enough material to justify the costs of purification. Also, genetic engineering can be performed to increase 1,3-PDO production and reduce the synthesis of other compounds from bacterial metabolism.

Author Contributions: R.K.S.: Writing—original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. S.B.d.S.: Writing—original draft, Validation, Methodology, Formal Analysis. G.A.d.P.: Validation, Methodology, Investigation. F.G.F.J.: Methodology, Investigation. M.F.A.: Methodology. S.C.R.: Writing—review and editing, Methodology. C.E.d.A.P.: Writing—review and editing; E.D.D.: Writing—review and editing, Methodology, Conceptualization. A.C.d.C.: Funding acquisition, Project administration. M.A.d.M.J.: Writing—review and editing, Funding acquisition, Project administration. R.B.d.S.: Writing—review and editing, Supervision, Methodology, Formal analysis, Conceptualization. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Fundação de Amparo à Pesquisa do Estado de Pernambuco (FACEPE), grant number APQ-0405-2.12/22, and by São Paulo Research Foundation (FAPESP), grant number 2022/07277-7.

Institutional Review Board Statement: "Not applicable" for studies not involving humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available on request.

Acknowledgments: The authors express their gratitude to all the institutions involved in the development of the present work: the University of Pernambuco, the Federal University of Pernambuco, the University of Campinas, and the São Paulo State University.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

1,3-PDO	1,3-Propanediol
1G	First-generation
2G	Second-generation
3-HPA	3-hydroxypropionaldehyde
ABC	ATP-Binding Cassette
DAD	Diode Array Detector
EMP	Embden–Meyerhof–Parnas pathway
HPLC	High-Performance Liquid Chromatography
LAB	Lactic Acid Bacteria
MRS	Man, Rogosa and Sharpe medium
PLO	Pretreatment Liquor Organosolv
PPP	Pentose Phosphate Pathway
PTS	Phosphotransferase System
RID	Refractive Index Detector
SBH	Sugarcane bagasse acid hydrolyzed

References

1. Freitas, J.V.; Bilatto, S.; Squinca, P.; Pinto, A.S.S.; Brondi, M.G.; Bondancia, T.J.; Batista, G.; Klaic, R.; Farinas, C.S. Sugarcane biorefineries: Potential opportunities towards shifting from wastes to products. *Ind. Crop. Prod.* **2021**, *172*, 114057. [CrossRef]
2. Basso, L.C.; Basso, T.O.; Rocha, S.N. Ethanol production in Brazil: The industrial process and Its impact on yeast fermentation. In *Biofuel Production-Recent Developments and Prospects*; Bernardes, M.A.S., Ed.; Intech: Rijeka, Croatia, 2011; pp. 85–100. [CrossRef]
3. Vaidya, A.A.; Murton, K.D.; Smith, D.A.; Dedual, G. A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose. *Biomass Conv. Bioref.* **2022**, *12*, 5427–5442. [CrossRef]
4. Araújo, M.F.; Volpi, M.P.C.; Mockaitis, G.; Morais Junior, M.A.; Carvalho da Costa, A.; Rabelo, S.C. Crude glycerol organosolv pretreatment: Chain integration for the production of 2G ethanol and biogas. *Fuel* **2025**, *379*, 132984. [CrossRef]
5. Vaz, F.L.; Lins, J.R.; Alencar, B.R.A.; De Abreu, I.B.S.; Vidal, E.E.; Ribeiro, E.; Sampaio, E.V.S.B.; Menezes, R.S.C.; Dutra, E.D. Chemical pretreatment of sugarcane bagasse with liquid fraction recycling. *Renew. Energy* **2021**, *174*, 666–673. [CrossRef]
6. Vandenberghe, L.P.S.; Valladares-Diestra, K.K.; Bittencourt, G.A.; Torres, L.A.Z.; Vieira, S.; Karp, S.G.; Sydney, E.B.; Carvalho, J.C.; Soccol, V.T.; Soccol, C.R. Beyond sugar and ethanol: The future of sugarcane biorefineries in brazil. *Renew. Sustain. Energy Rev.* **2022**, *167*, 112721. [CrossRef]
7. Ruy, A.D.S.; Alves, R.M.B.; Hewer, T.L.R.; Pontes, D.A.; Teixeira, L.S.G.; Pontes, L.A.M. Catalysts for glycerol hydrogenolysis to 1,3-propanediol: A review of chemical routes and market. *Catal. Today* **2021**, *381*, 243–253. [CrossRef]
8. Guo, Y.; Dai, L.; Xin, B.; Tao, F.; Tang, H.; Shen, Y.; Xu, P. 1,3-Propanediol production by a newly isolated strain, Clostridium perfringens GYL. *Bioresour. Technol.* **2017**, *233*, 406–412. [CrossRef]
9. Yang, X.; Kim, D.S.; Choi, H.S.; Kim, C.K.; Thapa, L.P.; Park, C.; Kim, S.W. Repeated batch production of 1,3-propanediol from biodiesel derived waste glycerol by Klebsiella pneumoniae. *Chem. Eng. Process.* **2017**, *314*, 660–669. [CrossRef]
10. Pflügl, S.; Marx, H.; Mattanovich, D.; Sauer, M. 1,3-propanediol production from glycerol with lactobacillus diolivorans. *Bioresour. Technol.* **2012**, *119*, 133–140. [CrossRef]
11. Pflügl, S.; Marx, H.; Mattanovich, D.; Sauer, M. Heading for an economic industrial upgrading of crude glycerol from biodiesel production to 1,3-propanediol by lactobacillus diolivorans. *Bioresour. Technol.* **2014**, *152*, 499–504. [CrossRef]
12. Lindlbauer, K.A.; Marx, H.; Sauer, M. Effect of carbon pulsing on the redox household of Lactobacillus diolivorans in order to enhance 1,3-propanediol production. *New Biotechnol.* **2017**, *34*, 32–39. [CrossRef]
13. De Santana, J.S.; Da Silva, J.L.; Dutra, E.D.; Menezes, R.S.C.; De Souza, R.B.; Pinheiro, I.O. Production of 1,3-propanediol by lactobacillus diolivorans from agro-industrial residues and cactus cladode acid hydrolyzate. *Appl. Biochem. Biotechnol.* **2021**, *193*, 1585–1601. [CrossRef]
14. Sun, C.; Ren, H.; Sun, F.; Hu, Y.; Liu, Q.; Song, G.; Abdulkhani, A.; Show, P.L. Glycerol organosolv pretreatment can unlock lignocellulosic biomass for production of fermentable sugars: Present situation and challenges. *Bioresour. Technol.* **2022**, *344*, 126264. [CrossRef]
15. De Souza, R.B.; De Menezes, J.A.S.; De Souza, R.F.R.; Dutra, E.D.; De Morais, M.A., Jr. Mineral Composition of the Sugarcane Juice and Its Influence on the Ethanol Fermentation. *Appl. Biochem. Biotechnol.* **2015**, *175*, 209–222. [CrossRef] [PubMed]

16. Chen, X.; Li, H.; Ji, X.; Shen, Z.; Guo, H.; Yao, S.; Wang, M.; Xiong, L.; Chen, X. Preparation, separation and purification of 5-hydroxymethylfurfural from sugarcane molasses by a self-synthesized hyper-cross-linked resin. *Sep. Purif. Technol.* **2023**, *315*, 123661. [CrossRef]
17. Biebl, H.; Menzel, K.; Zeng, A.P.; Deckwer, W.D. Microbial production of 1,3-propanediol. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 289–297. [CrossRef] [PubMed]
18. Doleyres, Y.; Beck, P.; Vollenweider, S.; Lacroix, C. Production of 3-hydroxypropionaldehyde using a two-step process with Lactobacillus reuteri. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 467–474. [CrossRef]
19. Wisselink, H.W.; Weusthuis, R.A.; Eggink, G.; Hugenholtz, J.; Grobben, G.J. Mannitol production by lactic acid bacteria: A review. *Int. Dairy J.* **2002**, *12*, 151–161. [CrossRef]
20. Schoch, C.L.; Ciufo, S.; Domrachev, M.; Hotton, C.L.; Kanna, S.; Khovanskaya, R.; Leipe, D.; Mcveigh, R.; O'Neill, K.; Robbertse, B.; et al. NCBI Taxonomy: A comprehensive update on curation, resources and tools. *Database* **2020**, *2020*, baaa062. [CrossRef]
21. Kowalcyk, M.; Mayo, B.; Fernandez, M.; Aleksandrzak-Piekarczyk, T. Updates on Metabolism in Lactic Acid Bacteria in Light of Omic Technologie. In *Biotechnology of Lactic Acid Bacteria: Novel Applications*, 2nd ed.; Mozzi, F., Raya, R.R., Vignolo, G.M., Eds.; Wiley-Blackwell: West Sussex, UK, 2016; p. 124. [CrossRef]
22. Cheng, K.K.; Zhang, J.A.; Liu, D.H.; Sun, Y.; Liu, H.J.; Yang, M.D.; Xu, J.M. Pilot-scale production of 1,3-propanediol using Klebsiella pneumoniae. *Process Biochem.* **2007**, *42*, 740–744. [CrossRef]
23. Maervoet, V.E.; De Maeseneire, S.L.; Avci, F.G.; Beauprez, J.; Soetaert, W.K.; De Mey, M. 1,3-propanediol production with Citrobacter werkmanii DSM17579: Effect of a dhaD knock-out. *Microb. Cell Fact.* **2014**, *13*, 70. [CrossRef]
24. Jolly, J.; Hitzmann, B.; Ramalingam, S.; Ramachandran, K.B. Biosynthesis of 1,3-propanediol from glycerol with Lactobacillus reuteri: Effect of operating variables. *J. Biosci. Bioeng.* **2014**, *118*, 188–194. [CrossRef]
25. Eiteman, M.A.; Lee, S.A.; Altman, E. A co-fermentation strategy to consume sugar mixtures effectively. *J. Biol. Eng.* **2008**, *2*, 3. [CrossRef]
26. Wisedchaisri, G.; Park, M.S.; Iadanza, M.G.; Zheng, H.; Gonen, T. Proton-coupled sugar transport in the prototypical major facilitator superfamily protein XylE. *Nat. Commun.* **2014**, *5*, 4521. [CrossRef]
27. Miwa, T.; Esaka, H.; Umemori, J.; Hino, T. Activity of H+-ATPase in ruminal bacteria with special reference to acid tolerance. *Appl. Environ. Microbiol.* **1997**, *63*, 2155–2158. [CrossRef] [PubMed]
28. Kandler, O. Carbohydrate metabolism in lactic acid bacteria. *Antonie Van Leeuwenhoek* **1983**, *49*, 209–224. [CrossRef] [PubMed]
29. Chatzifragkou, A.; Dietz, D.; Komaitis, M.; Zeng, A.P.; Papanikolaou, S. Effect of biodiesel-derived waste glycerol impurities on biomass and 1,3-propanediol production of Clostridium butyricum VPI 1718. *Biotechnol. Bioeng.* **2010**, *107*, 76–84. [CrossRef] [PubMed]
30. Guo, W.; Jia, W.; Li, Y.; Chen, S. Performances of Lactobacillus brevis for producing lactic acid from hydrolysate of lignocellulosics. *Appl. Biochem. Biotechnol.* **2010**, *161*, 124–136. [CrossRef]
31. Niel, E.W.J.; Larsson, C.U.; Lohmeier-Vogel, E.M.; Rådström, P. The potential of biodetoxification activity as a probiotic property of Lactobacillus reuteri. *Int. J. Food Microbiol.* **2012**, *152*, 206–210. [CrossRef]
32. Da Silva, P.K.N.; Mendonça, A.A.; De Miranda, A.R.; Calazans, T.L.S.; De Souza, R.B.; De Morais, M.A., Jr. Nutritional requirements for Lactobacillus vini growth in sugarcane derivative substrate of ethanol fermentation. *FEMS Microbiol. Lett.* **2019**, *366*, 16. [CrossRef]
33. Sharma, N.; Prasad, G.S.; Choudhury, A.R. Utilization of corn steep liquor for biosynthesis of pullulan, an important exopolysaccharide. *Carbohydr. Polym.* **2013**, *93*, 95–101. [CrossRef]
34. Khan, I.; Nazir, K.; Wang, Z.P.; Liu, G.L.; Chi, Z.M. Calcium malate overproduction by Penicillium viticola 152 using the medium containing corn steep liquor. *Appl. Biochem. Biotechnol.* **2014**, *98*, 1539–1546. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.