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Sugarcane Bagasse-Based Ethanol Production and Utilization of Its Vinasse for Xylitol Production as an Approach in Integrated Biorefinery

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Abstract: Biorefinery of sugarcane bagasse into ethanol and xylitol was investigated in this study. Ethanol fermentation of sugarcane bagasse hydrolysate was carried out by *Saccharomyces cerevisiae*. After ethanol distillation, the vinasse containing xylose was used to produce xylitol through fermentation by *Candida guilliermondii* TISTR 5068. During the ethanol fermentation, it was not necessary to supplement a nitrogen source to the hydrolysate. Approximately 50 g/L of bioethanol was produced after 36 h of fermentation. The vinasse was successfully used to produce xylitol. Supplementing the vinasse with 1 g/L of yeast extract improved xylitol production 1.4-fold. Cultivating the yeast with 10% controlled dissolved oxygen resulted in the best xylitol production and yields of 10.2 ± 1.12 g/L and 0.74 ± 0.04 g/g after 60 h fermentation. Supplementing the vinasse with low fraction of molasses to improve xylitol production did not yield a positive result. The supplementation caused decreases of up to 34% in xylitol production rate, 24% in concentration, and 24% in yield.

Keywords: yeast; xylitol; bioethanol; sugarcane bagasse; vinasse; biorefinery; value-added products; biofuels

1. Introduction

Lignocellulosic biomass is a very abundant natural resource [1]. A wide range of materials can be categorized as lignocellulosic biomass, including woody materials, grasses, crops, and the associated wastes [2]. The uses of biorefinery have extended from typical heating and steam generation to the production of many high value biochemicals via various conversion processes.

Sugarcane bagasse is an agricultural excess, comprising waste from the sugar industry. Thailand is the world's third-largest sugarcane-based sugar producer [3]. In the 2019/2020 season, sugarcane production in Thailand was 74.9 million tons, from which the bagasse generated was approximately 21 million tons [4]. Although most sugar factories utilize the bagasse to produce steam and generate electricity [5], the bagasse could be used for other value-added purposes via biorefinery.

Bioethanol has been one of the target products in the biorefinery process [6,7]. It is a principal transport fuel, with 102 billion liters produced worldwide in 2021 [8]. Bioethanol from lignocellulosic biomass has immense potential as a transport fuel due to its decarbonization benefits [9]. The two principal sugars in the lignocellulosic biomass structure, glucose and xylose, can be converted to ethanol. However, our previous work has shown that converting the xylose fraction in the mixed sugar hydrolysate into ethanol with the use of wild type yeasts has its limitations. It does not allow high ethanol concentrations



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Copyright: © 2022 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/). from the xylose fraction, and the xylose conversion was limited in a high ethanol environment [10,11]. Therefore, the xylose fraction could be more applicable for other purposes. One option for the xylose fraction of the lignocellulosic biomass is to produce xylitol.

Xylitol is a value-added chemical obtained from biomass, with no petrochemical alternative. Industrial production of xylitol is carried out through catalytic hydrogenation of xylose. Although the chemical conversion process results in high xylitol yields, certain concerns about the process exist. These included the availability of plants with high xylan content, the high energy and pressure used in the process, and the need for pure xylose in the reaction [12].

The biological approach to xylitol production is appealing, as it is more eco-friendly and less costly than the chemical path. The possibility of xylitol production in integrated biorefineries is an added benefit [13]. Xylitol production via microbial bioconversion has often focused on using hemicellulosic hydrolysate prepared from various biomass types, including grasses, agricultural residues, and woods [13,14]. Hydrolysate can be detoxified and concentrated to obtain a higher concentration of xylose [15–17].

In addition to xylitol production using hemicellulosic hydrolysate, integrated xylitol production with ethanol has been used in various operations. A well-studied scheme involved the separate fermentation of ethanol and xylitol from cellulosic and hemicellulosic fractions of biomass [15,17–20]. Two-stage hydrolysis was required for this operation, to obtain hemicellulosic hydrolysate. The remaining cellulignin, when hydrolyzed, would obtain glucose-rich hydrolysate, which could be a substrate in microbial fermentation. Alternatively, cellulignin fraction could be used directly in simultaneous saccharification and fermentation (SSF) for ethanol production. Fermentation using a single strain of yeast, and co-culture of two yeast strains to co-produce ethanol and xylitol were also reported [16,21].

Other proposed operating configurations have included model-based platforms involving separate hydrolysis, fermentation, and SSF for ethanol production, followed by xylitol fermentation. These configurations could be applied as an extension of a lignocellulosic bioethanol plant process, involving single-stage hydrolysis of biomass [22].

In this study, integrated biorefinery of sugarcane bagasse was considered, with enzymatic hydrolysate of the bagasse as the substrate for ethanol production. The vinasse containing unused xylose obtained from simple distillation was investigated for xylitol production as a value-added step in the overall process. Nitrogen supplementation and aeration level in xylitol production were evaluated as prime parameters in xylitol production from vinasse. A study of the addition of molasses to the vinasse to improve the xylitol production was also carried out.

2. Materials & Methods

2.1. Microorganisms

This study used a commercial strain of *Saccharomyces cerevisiae* (Thermal Resistance Yeast, Angel Yeast Co., Ltd., Hubei, China) for ethanol production. The yeast was supplied in powder form and stored in a refrigerator at 4 °C. Xylitol fermentation employed *Candida guilliermondii* TISTR 5068. The stock of *C. guilliermondii* was maintained at -20 °C as cell suspension in 30% (v/v) glycerol. In preparing pre-inoculation cells, the -20 °C yeast stock was streaked on YMX agar plates and incubated at 30 °C for 48 h or until the colonies appeared. Fresh colonies were used to prepare the inoculum. Yeasts on agar plates were refreshed every two weeks to maintain good cell activity.

2.2. Culture Media for Yeast Growth and Fermentation

YM media were used as the inoculum media. One liter of YM-based solution contained 3 g of yeast extract, 3 g of malt extract, and 5 g of peptone. The carbon source was glucose or xylose (designated as YMG or YMX medium) at final concentrations of 20 g/L for *S. cerevisiae* or *C. guilliermondii* cultivation.

Hydrolysate medium, prepared from sugarcane bagasse, was used in all ethanol fermentation. The bagasse properties and the hydrolysate preparation have been described previously [23]. In brief, 15% w/v of sugarcane bagasse was heat-pretreated with 2 M NaOH. After washing and drying, it was then hydrolyzed at a solid loading of 15% w/v with 40 FPU/g of a mixed enzyme, Cellic CTec2 (Novozymes, Bagsvaerd, Denmark), at pH 4.8 and 50 °C for 3 days. The hydrolysate obtained consisted of 120.5 \pm 1.8 g/L of reducing sugar, with 96.52 \pm 9.13 g/L of glucose and 19 \pm 5 g/L of xylose. The hydrolysate and nitrogen source solutions were mixed at the volumetric mixing ratio of 95:5 (hydrolysate: nitrogen source) to make up the medium. Hydrolysate and nitrogen sources were autoclaved separately before they were mixed after cooling to obtain the final concentration of the nitrogen source as planned.

Vinasse medium was used as the main medium for xylitol fermentation. In vinasse preparation, the sugarcane bagasse hydrolysate was prepared and used in ethanol fermentation as described below. After fermentation, the liquid broth was separated from the cells by centrifugation at $11,200 \times g$ for 10 min (Sorvall LYNX 4000/6000, Thermo Scientific, Massachusetts, USA). The broth was subjected to simple distillation to remove ethanol. The vinasse contained $20.00 \pm 1.19 \text{ g/L}$ of xylose, $1.22 \pm 0.08 \text{ g/L}$ of xylitol, $10.00 \pm 1.51 \text{ g/L}$ of glycerol, and $2.40 \pm 0.77 \text{ g/L}$ of ethanol. In medium preparation, the nitrogen source solution was autoclaved and mixed with the vinasse at a volumetric ratio of 95:5.

2.3. Ethanol Fermentation

Ethanol fermentation employed a commercial strain of *Saccharomyces cerevisiae* (Thermal Resistance Yeast, Angel Yeast Co., Ltd., Hubei, China). The inoculum of ethanol fermentation was prepared by adding 0.01 g/L of the yeast powder to the YMG medium and incubated at 30 °C with 200 rpm orbital shaking for 24 h. The inoculum volume was increased to suit the fermentation scale by transferring 10% (v/v) of the seed inoculum into a fresh YMG medium and further incubating under the same condition. All fermentation used 10% inoculum.

Fermentation at flask scale was done in a 250-mL Erlenmeyer flask using 100 mL of hydrolysate media with yeast extract, diammonium phosphate, or YM-based solution as nitrogen sources. The cultures were incubated at 30 $^{\circ}$ C with 100 rpm orbital shaking.

Fermenter-scale fermentation employed a 5-L fermenter with 3.5-L of hydrolysate medium supplemented with the selected nitrogen source. The cultivation was controlled at 30 °C and constantly stirred at 100 rpm. The fermentation ended when there was no change in glucose concentration. The analysis of the final sample was necessary to control the properties of the fermentation broth used in preparing the vinasse. The fermentation broth obtained from the hydrolysate without nitrogen source supplementation contained 43.70 \pm 5.27 g/L of ethanol, 0.65 \pm 0.04 g/L of xylitol, and 17.17 \pm 0.04 g/L of xylose.

2.4. Xylitol Fermentation

Xylitol fermentation employed *C. guilliermondii* TISTR 5068 with cultivation in 250-mL baffled Erlenmeyer flasks or a 2-L fermenter. The inoculum was prepared by transferring a few single colonies from a YMX agar plate to a YMX medium. It was cultivated at 30 °C, 200 rpm for 24 h before inoculation. All cultivations involving xylitol production employed vinasse medium.

Flask-scale fermentation was carried out using 100 mL of the vinasse supplemented with nitrogen sources or mixed with molasses. The cultivation was carried out at 30 $^{\circ}$ C with 200 rpm orbital shaking for 72 h with interval samplings.

Fermenter-scale cultivation was conducted in a 2-L bioreactor with 1 L of working volume. The temperature was controlled at 30 °C. Air was supplied through a ring sparger. The 100% dissolved oxygen (DO) was calibrated using 2 vvm of airstream at a stirring speed of 900 rpm. Under these calibrating conditions, dissolved oxygen was 7.7 mg/L (SensoDirect 150, Lovibond, Dortmund, Germany). The fermentation was carried out for 72 h with interval samplings.

2.5. Analytical Methods

Cell optical density was measured using a spectrophotometer at 600 nm. Cell dry weight (CDW) analysis involved drying the known volume of cell suspension at 105 °C for at least 16 h or until constant weight. The weight of the dried cell was calculated using the known volume of the cell. Reducing sugar was analyzed using 3,5-dinitrosalicylic acid [24]. Total sugar was analyzed using the phenol-sulfuric method.

A high-performance liquid chromatography system (LC-20A, Shimadzu, Kyoto, Japan) equipped with an Aminex HPX-87H column (Bio-Rad, California, USA) was employed to analyze xylose, xylitol, and ethanol. The compounds were detected by a refractive index detector (RID-6A, Shimadzu, Kyoto, Japan). The column's temperature was 40 °C. The injection volume of the sample was 20 μ L. The mobile phase was 5 mM sulfuric acid with a 0.75 mL/min flow rate.

2.6. Statistical Analysis

The SPSS program version 20 was employed for general statistical analysis. One-way ANOVA was used to compare differences between groups and factors. Duncan's test was the post hoc test for mean comparison within the dataset. All tests were carried out at a 95% confidence level.

3. Results and Discussions

3.1. Nitrogen Source Supplementation for Ethanol Fermentation Using Sugarcane Bagasse Hydrolysate

This part of the study aimed to use the vinasse from ethanol fermentation using sugarcane bagasse as the raw material for xylitol production. Because ethanol fermentation from bagasse has not been commercially practiced, we first evaluated the necessity of supplementing nitrogen sources from enzymatic hydrolysate of sugarcane bagasse. The results from this part of the study were applied in the ethanol fermentation. Vinasse was prepared from the ethanol broth and used for xylitol production.

As representatives of organic and inorganic nitrogen sources, yeast extract (YE) and diammonium phosphate (DAP) were compared as potential nitrogen sources for ethanol production. Commercial or industrial grades of these nitrogen sources are available for use on a larger scale if necessary. In the experiments, the amount of each nitrogen source used varied from 1 g/L to 10 g/L. Controls included no nitrogen source supplementation, and supplementation with a YM-based solution.

Nitrogen source supplementation did not have a significant effect on sugar utilization during ethanol fermentation (Figure 1a). Glucose was fully consumed by the yeast, even without any nitrogen source supplementation to the hydrolysate. This result indicated that the sugarcane bagasse hydrolysate prepared in this study contained sufficient nutrients to support the cell activities, which resulted in complete glucose consumption. As well as glucose, xylose consumption was evident during fermentation. A similar amount of xylose was consumed regardless of the amount of nitrogen source supplemented, which resulted in final xylose in the ethanol broth of 14.76 ± 1.96 g/L.

Ethanol production in all the test conditions was similar (Figure 1b). Although there were some fluctuations in the results, these were not significantly different. The ethanol concentrations varied from 43.14 ± 5.46 g/L to 50.80 ± 1.93 g/L (85–96% conversion efficiency). For the diammonium phosphate supplementation, the range of ethanol produced was narrower, from 45.81 ± 2.95 g/L to 48.48 ± 0.89 g/L (84–99% conversion efficiency). Nonetheless, these values were not significantly different, even when compared with the concentration obtained from non-supplemented hydrolysate, which produced 51.15 ± 5.41 g/L of ethanol, or 91% conversion efficiency.



Figure 1. Comparisons of (**a**) sugar utilization (dark bars—glucose, white bars—xylose), (**b**) ethanol, and (**c**) xylitol from ethanol fermentation by *S. cerevisiae* using sugarcane bagasse hydrolysate, supplemented with yeast extract (YE) or diammonium phosphate (DAP), cultivated at 30 °C, 100 rpm for 36 h. Numbers following YE and DAP are concentrations of the respective nitrogen sources in g/L. The same letters above the bars indicated non-significant difference in mean values.

Generally, nitrogen sources had a positive effect on the growth and activity of microorganisms [25]. In our study, the addition of a nitrogen source did not influence cell activity, as the sugar consumption and ethanol production remained at similar levels without an added nitrogen source. The results implied that the hydrolysate contained sufficient nitrogen levels to support yeast growth and hence ethanol production. The nitrogen content in the hydrolysate was 0.13 g/100 g or approximately 1.3 g/L. The presence of nitrogen in the hydrolysate was possibly due to a mixed commercial cellulolytic enzyme used in hydrolysate preparation. As the crude hydrolysate was used for ethanol fermentation, the enzyme proteins remained in the hydrolysate and could serve as the nitrogen source in the fermentation. However, supplementation of nitrogen sources has been reported to increase the fermentation rate, thus reducing fermentation time [26]. We did not verify that report, as it was beyond the scope of our current work to consider the necessity of nitrogen source addition when producing ethanol broth. The broth would be used further to prepare the vinasse, which would be the feedstock for the xylitol production.

In addition to glucose consumption for ethanol production, there was an unexpected utilization of xylose, with a small amount of xylitol detected (Figure 1c). *S. cerevisiae* has no specialized xylose transporter; xylose uptake occurs through native hexose transporters [27,28]. This occurrence agreed with a study claiming that enhanced sugar consumption occurred with the addition of nitrogen sources [29]. The uptake was superior as the condition was more aerobic [30], which was not the case in our study as the ethanol fermentation was carried out at 100 rpm, which was not an aerobic condition. Xylitol conversion from xylose in *S. cerevisiae* was due to a nonspecific aldose reductase [31].

Because ethanol production in sugarcane bagasse hydrolysate is not significantly different with or without nitrogen source supplementation, we chose to carry out the ethanol fermentation using sugarcane bagasse hydrolysate without an additional nitrogen source. The ethanol broth was used to prepare the vinasse for xylitol fermentation.

3.2. Evaluating the Supplementation of Nitrogen Sources for Xylitol Production Using Vinasse from Ethanol Fermentation

Following the same investigating principle as in ethanol fermentation, supplementation of yeast extract (YE) and diammonium phosphate (DAP) were applied in xylitol fermentation using vinasse prepared from distillation of ethanol produced from sugarcane bagasse hydrolysate. Nitrogen source supplementation may be necessary as available nitrogen in the hydrolysate could be depleted in the ethanol fermentation stage. The vinasse was used without any treatment or detoxification, although *Candida mogii* fermentation in concentrated stillage (vinasse) after ethanol fermentation from sweet sorghum juice and bagasse showed the necessity of detoxifying the stillage [32].

Supplementation of nitrogen sources into the vinasse improved xylose utilization in the media (Figure 2a). Adding as little as 1 g/L of YE significantly improved xylose utilization in the vinasse media to 95.2 \pm 1.93%, compared with 79.8 \pm 0.5% without added nitrogen source. Adding YE at higher concentrations did not help improve the overall xylose consumption nor the consumption rate, according to the monitoring of reducing sugar during fermentation (profiles not shown). The addition of YE increased the reducing sugar consumption rates from 0.377 g/L.h (no YE addition) to the range of 0.514–0.582 g/L.h (1–10 g/L of YE).

A different response was observed after the addition of DAP. Low concentrations of DAP resulted in a similar xylose consumption of 96.0 \pm 2.6% to 95.6 \pm 4.4%. However, at higher DAP of 7 g/L and 10 g/L, xylose consumption slightly decreased. Regarding the reducing sugar consumption rate (profiles not shown), the addition of DAP at 1 g/L increased the sugar consumption rate to 0.511 g/L.h. The rates continuously decreased as DAP concentration increased.



Figure 2. Comparisons of (**a**) xylose consumption, (**b**) cell dry weight, and (**c**) xylitol from fermentation of *C. guilliermondii* TISTR5068 in vinasse from sugarcane hydrolysate-based ethanol broth supplemented with yeast extract (YE) or diammonium phosphate (DAP), cultivated at 30 °C, 200 rpm for 60 h. Numbers following the abbreviations indicate concentration of the respective nitrogen source in g/L. The same letters above the bars indicated non-significant difference in mean values.

Cell growth in terms of dried weight benefited from nitrogen source addition, especially with yeast extract (Figure 2b). Cell dry weight showed an increasing trend with nitrogen source addition. However, a high concentration of DAP showed an adverse effect on cell growth. A decreasing biomass trend was evident when the DAP concentration was 5 g/L and above. This reduction in cell growth followed decreased xylose consumption at high DAP levels. Regardless of the concentrations, cell yields did not show any significant differences according to the type of nitrogen sources or their concentration (Table 1).

Table 1. Xylitol yields and cell yields obtained from 60-h xylitol fermentation by *C. guilliermondii* TISTR5068 using vinasse supplemented with yeast extract (YE) or diammonium phosphate (DAP).

N Source	Xylitol Y	ield (g/g)	Cell Yield (g/g)		
(g/L)	YE	DAP	YE	DAP	
No N-supp	$0.671~\pm$	0.012 ^{ab}	0.339 ± 0.006 ^a		
1	0.796 ± 0.039 ^ a	$0.645 \pm 0.005~^{ m ab}$	0.315 ± 0.018 $^{\rm a}$	$0.332\pm0.004~^{a}$	
3	$0.732\pm0.065~^{\rm a}$	$0.690 \pm 0.025~^{ m ab}$	0.334 ± 0.015 $^{\rm a}$	$0.341\pm0.055~^{\rm a}$	
5	$0.692 \pm 0.056~^{ m ab}$	0.658 ± 0.008 $^{\mathrm{ab}}$	$0.336 \pm 0.002~^{a}$	$0.291 \pm 0.061~^{a}$	
7	0.704 ± 0.098 $^{\mathrm{ab}}$	0.612 ± 0.011 $^{\mathrm{ab}}$	0.411 ± 0.060 ^ a	0.295 ± 0.116 a	
10	$0.622\pm0.143~^{\mathrm{ab}}$	$0.526 \pm 0.007 \ ^{\rm b}$	0.354 ± 0.038 a	0.261 ± 0.018 a	

Note: Comparisons were made between the same results i.e., within the xylitol yields and the cell yields, regardless of type of nitrogen sources. The same letters indicated non-significant difference in mean values. Theoretical xylitol yield from xylose is 1.013 g/g.

When supplementing the vinasse with nitrogen sources at different concentrations, xylitol production showed an inversing trend with cell dry weight, especially in the case of yeast extract (Figure 2c). Increasing the concentration of YE resulted in a decreasing trend in xylitol production, while the cell dry weights were statistically unchanged. The decrease in xylitol production was evident with increasing DAP concentrations from 5 g/L and above, following drops in xylose consumption and growth. The highest xylitol of 12.6 ± 0.2 g/L was obtained with the addition of 1 g/L of yeast extract. The corresponding yield was also the highest, at 0.796 ± 0.039 g/g_{xylose} (Table 1).

Organic and inorganic nitrogen sources have shown a positive effect on xylitol production by yeasts when used in suitable amounts. Yeast extract has been a popular organic nitrogen source for use in xylitol production. A few studies have reported that excessive levels of yeast extract could be detrimental to xylitol production. Xylitol production from D-xylose by *C. tropicalis* DMS 7524 degenerated when 15 g/L of yeast extract were added to the media [33]; the study recommended adding no more than 1 g/L of yeast extract to the medium for xylitol production. A similar result was also obtained in *C. guilliermondii* FTI 20037, where adding yeast extract of more than 1 g/L resulted in decreasing xylitol concentration, although the cell growth improved [34]. A decreasing trend in xylitol production with increasing yeast extract was reported for *C. tropicalis* NRRLY-12968 growing in the acid hydrolysate of mung bean hull [35].

Among inorganic nitrogen sources, urea and ammonium salts are the most commonly used in xylitol production studies. Any form of ammonium salts, including the dihydrogen phosphate form, resulted in similar xylitol production, except for the chloride form, which showed an inferior result when used at the same concentration [35,36]. In our results, high concentrations of diammonium phosphate reduced the fermentation performance, especially the xylitol production. A study of xylitol production from corncob hemicellulose hydrolysate indicated the significant influence of (NH₄)₂SO₄, KH₂PO₄, and yeast extract [37]. Upon optimizing those components, the optimal concentrations of KH₂PO₄ and yeast in the higher level of the varied range, whereas the optimal concentrations of KH₂PO₄ and yeast effect of high phosphate concentration on xylitol production, which could explain the lower xylitol production observed in this study when supplementing the vinasse with high concentrations of diammonium phosphate.

In a study employing a high concentration of phosphate buffer, it was indicated that reduced xylose consumption and xylitol yield could be caused by high osmotic pressure due to high ionic concentrations in the media [38]. In addition, high salt concentrations could reduce the oxygen solubility in the media. Both of these reasons could apply in our study. Although the phosphate concentrations we used were much lower than that in that previously reported study, the vinasse used as the media for xylitol production could fit the category of high salt concentration, even at the maximum of 10 g/L (75.7 mM) DAP added into the media.

The results from this section indicate that nitrogen sources, especially yeast extract (as an organic nitrogen source), improved sugar consumption rates and hence fermentation rates. High xylose consumption, accompanied by high cell growth due to excess nitrogen source supply, did not favor xylitol production. Based on the above observations, the vinasse was supplemented with 1 g/L of yeast extract.

3.3. Effect of Dissolved Oxygen (DO) on Xylitol Production Using Vinasse from Ethanol Fermentation of Sugarcane Bagasse Hydrolysate

This part of the study involved supplying different levels of dissolved oxygen (DO) to the fermentation medium. The fermentation was carried out in a fermenter system. The vinasse supplemented with 1 g/L of yeast extract was used for xylitol production by *C. guilliermondii* TISTR5068. Two patterns of aeration were investigated in the study. Controlled dissolved oxygen levels at 5%, 10%, and 15% were achieved through cascade control (DO/stirrer speed) with a constant airflow of 2 vvm. An uncontrolled dissolved oxygen pattern employed a constant stirring speed at 100 rpm with 1 vvm airflow. A negative control experiment with 100 rpm stirring speed and no aeration was also carried out for comparison.

The results in Figure 3 and Table 2 demonstrate the importance of aeration on xylitol fermentation. The fermentation profiles indicate that the dissolved oxygen level affected fermentation rates, such that the higher DO resulted in faster xylose consumption (Figure 3a). The xylose profiles demonstrated that the xylose consumption rate at 5% DO was slower than those at 10% and 15%, at which similar rates were evident for each. In the uncontrolled aeration using a constant stirring speed at 100 rpm with 1 vvm airflow, the dissolved oxygen was high at the beginning of the fermentation, dropped to 0% and remained at that level throughout the fermentation. Note that in this case the DO of 0% implied that the yeast used the dissolved oxygen supply as in the negative control run (100 rpm with no aeration). Xylose consumption was similar at approximately 90%. Regardless of the incomplete xylose consumption, the residual concentrations were low, ranging from 1–2 g/L.

Aeration	Xylose	Xylitol *	Qp*	$Y_{p/s} * (g/g_{xylose})$	CDW	$Y_{x/s}$
Conditions	Used (%)	(g/L)	(g/L.h)		(g/L)	(g/g _{xylose})
100 rpm 1 vvm & 100 rpm 5% DO 10% DO	$\begin{array}{c} 28.1 \pm 0.0 \ ^{\rm b} \\ 90.9 \pm 1.5 \ ^{\rm a} \\ 88.3 \pm 4.7 \ ^{\rm a} \\ 91.9 \pm 2.1 \ ^{\rm a} \\ \end{array}$	$\begin{array}{c} 0.04\pm 0.00\ ^{\rm b}\\ 9.67\pm 0.10\ ^{\rm a}\\ 9.54\pm 0.53\ ^{\rm a}\\ 9.96\pm 0.55\ ^{\rm a}\end{array}$	$\begin{array}{c} 0.001 \pm 0.000 \ d \\ 0.134 \pm 0.001 \ c \\ 0.159 \pm 0.009 \ b \\ 0.186 \pm 0.010 \ a \\ 0.187 \pm 0.010 \ a \end{array}$	$\begin{array}{c} 0.010 \pm 0.001 \ ^{c} \\ 0.610 \pm 0.014 \ ^{b} \\ 0.656 \pm 0.022 \ ^{b} \\ 0.685 \pm 0.029 \ ^{a} \\ 0.685 \pm 0.029 \ ^{a} \end{array}$	$\begin{array}{c} 1.3 \pm 0.8 \ ^{\rm c} \\ 5.4 \pm 0.8 \ ^{\rm b} \\ 6.0 \pm 0.8 \ ^{\rm b} \\ 11.0 \pm 0.5 \ ^{\rm a} \end{array}$	$\begin{array}{c} 0.308 \pm 0.202 \ ^{\rm c} \\ 0.342 \pm 0.064 \ ^{\rm c} \\ 0.408 \pm 0.048 \ ^{\rm bc} \\ 0.765 \pm 0.106 \ ^{\rm ab} \\ 0.226 \pm 0.044 \ ^{\rm ab} \end{array}$

Table 2. Parameters from xylitol fermentation by *C. guilliermondii* using vinasse supplement by 1 g/L of yeast extract cultivated at different dissolved oxygen levels for 72 h.

Note: $Y_{p/s} = xy$ litol yield, $Q_p * = xy$ litol productivity, CDW = cell dry weight and $Y_{x/s} =$ biomass yield; * Values are reported for the times of maximum concentration. Comparisons of mean values were made within the same column. The same letters indicated non-significant difference in mean values.

Cell growth followed the xylose consumption rates (Figure 3b). Faster growth was evident with higher dissolved oxygen levels. In high DO conditions, yeast growth continued even when xylose consumption almost stopped, as observed after 48 h and 60 h of

fermentation. A significant increase in the cell dry weight resulted from aeration at any level (Table 2). Increasing the dissolved oxygen level to 10% significantly increased the cell biomass. Further increase in DO to 15% did not significantly promote further cell growth. Nonetheless, cell yield had an increasing trend with higher dissolved oxygen levels in the range investigated.



Figure 3. Profiles of (**a**) xylose, (**b**) cell optical density, and (**c**) xylitol for cultivated *C. guilliermondii* TISTR5068 in vinasse medium at 30 °C and various aeration patterns for 72 h. □—no aeration, 100 rpm; ▲—1 vvm, 100 rpm, ●—5% DO; v—10% DO; ■—15% DO.

Xylose consumption and hence fermentation rate had been reported to increase with an increase in oxygen transfer coefficient in *Candida boidinii* NRRL Y-17213 [39]. This report explains our results, as higher dissolved oxygen levels allowed higher oxygen transfer. Our results also suggested a limit on the xylose consumption rate, as increasing the DO level from 10% to 15% did not further increase the xylose consumption rate.

The xylitol profiles showed dependency on the level of dissolved oxygen. Notwithstanding the distinct effect of DO level on xylose consumption and cell growth, the xylitol production rate was similar for controlled DO of 10% and 15% (Figure 3c and Table 2). A slower production rate was observable in the uncontrolled DO conditions. However, the final xylitol concentrations produced at all the aerated conditions were insignificantly different (Table 2). The dissolved oxygen level showed a slight but significant effect on xylitol yield, where the yield increased with an increase in DO level, up to DO of 10%. A further increase to 15% DO did not significantly improve the xylitol yield.

In the controlled DO conditions, xylitol profiles showed a drop in concentration after 48 h for fermentation at 10% and 15% DO and after 60 h at 5% DO. These decreases in xylitol occurred after the xylose consumption had ended. The reductions shared a similar rate, regardless of the dissolved oxygen levels.

Controlling dissolved oxygen to a certain level, as we applied in this study, can help with continuous and complete consumption of xylose. In studies of *Candida* sp. strains under constant agitation, incomplete utilization of xylose was evident at low aeration rates or low k_La [39,40]. A similar result was observed in another study of *C. guilliermondii* at a constant aeration rate, where a low agitation rate resulted in incomplete xylose consumption [41]. A common characteristic of incomplete xylose consumption was slow or no cell growth following faster growth early in the fermentation. Controlling the dissolved oxygen promoted complete xylose consumption, even at a low level of 0.5%, although with a slower fermentation rate [42].

The metabolisms of xylose and xylitol depend on NAD(P)+/NAD(P)H redox balance [43]. Oxygen-limited conditions result in the ineffective regeneration of NAD(P)+, causing intracellular NAD(P)H levels to increase. High NAD(P)H represses the activity of xylitol dehydrogenase, causing xylitol to accumulate. Our results fit this scenario. The range of dissolved oxygen covered in this study gave rise to oxygen-limited conditions, where the imbalance of the cofactors occurred with a fast fermentation rate, as demonstrated in Figure 3. We also investigated xylitol production at a higher dissolved oxygen level of 20%, and found that the yeast grew to a much higher cell density with a fast xylose consumption rate, but with no xylitol production (results not shown). Proper aeration led to high cell growth and no xylitol production [42].

In our study, a drop in xylitol concentration occurred after 48 h when xylose levels were low. Xylitol consumption or reassimilation have been reported in xylitol-producing yeasts such as *Starmerella meliponinorum* and a strain of *C. guilliermondii* [44,45]. More aerobic conditions caused faster xylitol consumption in a recombinant *Saccharomyces cerevisiae* [43]. In our study, small but noticeably faster xylitol assimilation could be observed when the dissolved oxygen level increased from 5% to 15%.

From the results, we selected controlled dissolved oxygen at 10% as the condition for xylitol production from the vinasse medium. A higher DO level was not required as it did not improve the production in terms of xylitol concentration, yield, or productivity. The results also suggested that xylitol production performance was not particularly sensitive to changes in dissolved oxygen ranging from 5% to 15%. In addition, close attention should be paid to the harvesting time, as the yeast could reassimilate the produced xylitol during prolonged fermentation.

3.4. Effect of Molasses Addition to the Vinasse Medium on Xylitol Production

Investigation of molasses addition to the xylitol fermentation followed the claims made by several studies that the small addition of carbon sources such as glucose, maltose, or sucrose could improve xylitol production [16,46–48]. We were interested in supplemen-

tation with molasses, a by-product of the sugar crystallization process that contains sucrose and other invert sugars that could support the yeast function. Molasses was added to the vinasse medium on a weight basis, from 0.5% w/v to 3.5% w/v of molasses. At these amounts, the total sugar corresponding to the molasses in the medium would range from 11 g/L to 16 g/L, which accounts for 0.59:1 to 0.94:1 of the xylose contained in the vinasse medium. Two control runs were also carried out, using only the vinasse (V-only) and only the molasses at 3.5 g/L (M-only).

For sugar consumption performance, only the xylose profiles are shown (Figure 4a). However, the yeast was able to utilize the sugar in molasses, which was evident in the M-only control run (results not shown). The results demonstrate that supplementing the molasses to the vinasse did not promote xylose utilization rates (Figure 4a and Table 3). It prolonged the fermentation time such that supplementing molasses from 2 g/L and above increased the fermentation time to 72 h, instead of 60 h when supplementing at lower amounts.

Table 3. Parameters from xylitol fermentation by *C. guilliermondii* supplemented by various molasse concentrations into the vinasse medium.

Molasses Supp.	Q _{xylose} (g/L.h)	Xylitol ** (g/L)	$Y_{p/s}$ (g/g _{xylose})	Qp (g/L.h)	CDW (g/L)	Y _{x/s} (g/g _{total sugar})
V-only	0.357 ± 0.029 $^{\rm a}$	9.27 ± 0.05 $^{\rm a}$	$0.499\pm0.004~^{a}$	0.150 ± 0.001 $^{\rm a}$	$5.82\pm0.05~^{\rm c}$	$0.193 \pm 0.002^{\text{ b}}$
M-only	-	-	-	-	6.57 ± 0.27 a	0.349 ± 0.013 $^{\rm a}$
0.5 g/L	0.239 ± 0.038 ^b	$8.19\pm0.09~^{\rm b}$	0.443 ± 0.005 ^b	0.136 ± 0.002 ^b	5.25 ± 0.08 ^d	0.176 ± 0.020 ^b
1 g/L	0.299 ± 0.000 $^{\mathrm{ab}}$	$8.33\pm0.04~^{b}$	0.471 ± 0.002 ^b	$0.139 \pm 0.001 \ ^{\mathrm{b}}$	5.80 ± 0.03 ^c	$0.174 \pm 0.001 \ ^{\mathrm{b}}$
2 g/L	$0.247 \pm 0.003 \ ^{\mathrm{b}}$	$7.70\pm0.05~^{\rm c}$	0.454 ± 0.003 ^b	$0.107 \pm 0.001 \ ^{\rm c}$	6.03 ± 0.03 ^{bc}	0.168 ± 0.002 ^b
3 g/L	0.263 ± 0.006 ^b	$6.87\pm0.14~^{\rm e}$	0.360 ± 0.006 ^d	0.096 ± 0.003 ^d	6.42 ± 0.02 $^{\mathrm{ab}}$	0.176 ± 0.001 ^b
3.5 g/L	0.242 ± 0.009 ^b	$7.26\pm0.01~^{\rm d}$	$0.394 \pm 0.020 \ ^{\rm d}$	0.101 ± 0.000 ^{cd}	6.47 ± 0.20 $^{\rm ab}$	$0.185 \pm 0.006 \ ^{\rm b}$

 $Q_{xylose} = xylose$ utilization rates calculated from the slope of the xylose profiles, $Q_p = xylitol$ productivity calculated when xylose was depleted, $Y_{p/s} = xylitol$ yield based on xylose, $Y_{x/s} =$ biomass yield based on total sugar; ** Xylitol was reported as the difference between the value for depleted xylose and the initial value. Comparisons of mean values were made within the same column. The same letters indicated non-significant difference in mean values.

Yeast growth profiles, according to cell optical density, showed that molasses at 3.5 g/L (M-only control) resulted in the fastest growth rate during the first 12 h of fermentation (Figure 4b) and the highest cell dry weight (Table 3). The addition of molasses into the vinasse medium slightly increased cell growth with increased molasses supplementation. However, the cell yields ($Y_{x/s}$) did not change. It should be emphasized that the control with molasses medium contained the lowest total sugar concentration at approximately 21 g/L, while vinasse media with and without molasses supplementation contained total sugar in the range of 44.7 to 61.3 g/L. Low sugar concentration would benefit cell growth, as the effect of osmotic pressure was less than that in the higher sugar concentrations.

Supplementation with molasses did not improve the xylitol production rate or the concentrations. The xylitol profiles in Figure 4c indicate that by using the vinasse medium without molasses supplementation, the xylitol production rate was faster and resulted in the highest xylitol concentration. The more molasses, the lower the xylitol obtained (Table 3). This trend was also true for xylitol yields and productivities.

In addition to xylitol, ethanol was another product of fermentation. In the M-only control, ethanol concentration increased until 24 h, reaching a concentration of 5.17 g/L. It consistently dropped after prolonged fermentation (Figure 4d). The drop in ethanol matched the increase in cell optical density, implying that the yeast cells utilized ethanol as a carbon source to support their growth. In the media supplemented with molasses, the yeast produced ethanol early in the fermentation process. The amount of ethanol corresponded to the amount of molasses supplemented. Supplementing 0.5 g/L of molasses resulted in 2.40 \pm 0.01 g/L of ethanol, while supplementing 3 g/L and 3.5 g/L of molasses resulted in 6.13 \pm 0.02 g/L and 5.89 \pm 0.07 g/L of ethanol, respectively.



Figure 4. Profiles of (a) xylose, (b) cell optical density, (c) xylitol, and (d) ethanol when cultivating *C. guilliermondii* TISTR5068 in vinasse medium supplemented with various molasses concentrations at 30 °C, 200 rpm for 72 h. —vinasse medium, •—only molasses at 3.5 g/L, \blacksquare —0.5 g/L molasses supplemented, \square —1 g/L molasses supplemented, \blacktriangle —2 g/L molasses supplemented, \bigtriangleup —3 g/L molasses supplemented, and \diamond —3.5 g/L molasses supplemented.

The results obtained in our study did not suggest a positive effect of molasses supplementation on xylitol production by C. guillermondii TISTR5068 in a vinasse medium. The results contradicted claims that supplementation of other carbon sources helps improve various aspects of xylitol production including xylose consumption rate, xylitol production rate, xylitol concentration, and yield [16,46–48]. Supplementation with molasses appeared to deteriorate xylitol production while improving yeast growth.

Several studies into carbon source supplementation to improve xylitol production involved supplementing carbon sources in their pure forms. These studies did not report any ethanol production by yeast, even with distinctively high concentrations of the supplementary glucose and sucrose [47,48]. However, other studies used complex carbon sources, such as sugarcane syrup and molasses, as supplements to xylitol production using sugarcane bagasse and straw hydrolysates, and reported the production of ethanol [16,47]. The amount of ethanol depended on the amount of sugar supplemented; over 20 g/L was reported. Our results showed that maximum ethanol of ~5 g/L was produced from the supplementation of sugar via molasses addition. Ethanol has been reported to harm the cell viability of xylose-utilizing yeast, with viability dropping to 50% at high ethanol concentrations [49]. Ethanol tolerance in xylose-utilizing yeast was also strain-dependent [50]. Exposure to ethanol during the first 12 h to 24 h could affect the yeast performance, especially in conversion of xylose to xylitol, causing inferior xylitol production when supplementing with molasses.

Although slight improvement in xylitol production, especially in xylose uptake rate, when supplemented with sugar syrup or molasses was reported in other studies [16,47], our results did not follow those earlier findings. A significant decrease in the xylose utilization rate was evident in our case. Nonetheless, the reduction in xylitol yield that we observed concurred with a study that used molasses as a supplement [16].

Based on our results and those reported, we inferred that the differences in various aspects of xylitol production performance would depend on substrate type and characteristics. The amount and type of supplementary carbon did not necessarily guarantee improved xylitol production. In our study, we employed vinasse of ethanol broth obtained from the fermentation of sugarcane bagasse hydrolysate. It contained mainly xylose with other sugars and oligosaccharides, and a low level of ethanol remaining after the distillation.

3.5. Proposed Process for Ethanol and Xylitol Production from Sugarcane Bagasse

By integrating the results obtained in this study, each step in the proposed process was combined, and the flow chart of the process is demonstrated in Figure 5. In this process, separate streams of ethanol and xylitol resulted. Ethanol obtained in this study was within the same range as a previous study with a similar design using sweet sorghum juice and bagasse [32]. Increased quantities of xylitol were obtained in this study, compared with less than 5 g/L obtained from the stillage (vinasse) of the study employing *Candida mogii*.

Other studies have reported co-production of ethanol and xylitol with separate streams of hydrolysate. A similar range of ethanol concentrations resulted, with values between 48–56 g/L [16–18]. These values were comparable to results from this study at approximately 50 g/L. The higher xylitol obtained in those studies, ranging from 24.0 g/L to 34.5 g/L, was the result of high xylose concentration after concentrating the hemicellulosic hydrolysate, or from high solid loading in hydrolysis by xylanase. Regardless of the lower xylitol concentration in this study (~10 g/L) due to the use of non-concentrated vinasse, the conversion yield was comparable.

From the diagram (Figure 5), it should be noted that some xylose was converted to xylitol during the ethanol fermentation, due to the activities of native hexose transporters and a nonspecific aldose reductase, as discussed earlier. Regarding the separation of ethanol by simple distillation, ethanol cannot be fully separated from the aqueous solution so a small amount of ethanol was present in the vinasse. The mass balance showed a 15% loss from distillation. This loss could be due to evaporation and residual liquid holdup in the instrument, because a simple laboratory glass distillation set was used. In addition, a

noticeably large loss of bagasse after pretreatment was due to lignin loss and small residue loss during the washing steps after pretreatment, when mesh cloth was used for draining the liquid.



Figure 5. Diagram of the proposed ethanol and xylitol production process, illustrating important substrates and products at each stage of the operation.

4. Conclusions

This study demonstrated the integrated biorefinery of sugarcane bagasse for ethanol and xylitol production. The results showed the possibility of integrating xylitol production into the lignocellulosic-based ethanol process by utilizing vinasse from the distillation stage containing remaining xylitol. A small supplementation with nitrogen sources was necessary to improve xylitol production from the vinasse. Limited oxygen conditions promoted xylitol production, whereas excessive aeration was observed to result in xylitol consumption in the later stages of fermentation, resulting in a low xylitol yield. It was not necessary to supplement a carbon source to improve the xylitol production when using vinasse as the substrate. Using a waste stream from cellulosic ethanol production, as demonstrated in this study, represents waste utilization through biological processes and serves as a part of the circular bioeconomy.

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References

- Zoghlami, A.; Paës, G. Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. *Front. Chem.* 2019, 7,874. [CrossRef] [PubMed]
- Ullah, K.; Kumar Sharma, V.; Dhingra, S.; Braccio, G.; Ahmad, M.; Sofia, S. Assessing the Lignocellulosic Biomass Resources Potential in Developing Countries: A Critical Review. *Renew. Sustain. Energy Rev.* 2015, *51*, 682–698. [CrossRef]
- 3. OECD/FAO. Sugar Production Classified by Traditional Crops. In OECD-FAO Agricultural Outlook 2021–2030; OECD Publishing: Paris, France, 2021. [CrossRef]
- 4. Office of the Cane and Sugar Board. Report on Sugarcane Plantation, Production Year 2019/20. Available online: http://www.ocsb. go.th/upload/journal/fileupload/923-1854.pdf (accessed on 6 September 2021).
- Office of the Cane and Sugarcane Board. Electricity and Steam Production of the Sugar Cane and Sugar Industry. Available online: http://www.ocsb.go.th/upload/bioindustry/fileupload/10169-3442.pdf (accessed on 17 May 2021).
- Cheng, H.H.; Whang, L.M. Resource Recovery from Lignocellulosic Wastes via Biological Technologies: Advancements and Prospects. *Bioresour. Technol.* 2022, 343, 126097. [CrossRef]
- Velvizhi, G.; Balakumar, K.; Shetti, N.P.; Ahmad, E.; Kishore Pant, K.; Aminabhavi, T.M. Integrated Biorefinery Processes for Conversion of Lignocellulosic Biomass to Value Added Materials: Paving a Path towards Circular Economy. *Bioresour. Technol.* 2022, 343, 126151. [CrossRef]
- Renewable Fuels Association. 2022 Ethanol Industry Outlook. Available online: https://ethanolrfa.org/media-and-news/ category/news-releases/article/2022/02/rfa-s-2022-ethanol-industry-outlook-zeroes-in-on-new-opportunities (accessed on 13 May 2022).
- Raj, T.; Chandrasekhar, K.; Naresh Kumar, A.; Rajesh Banu, J.; Yoon, J.J.; Kant Bhatia, S.; Yang, Y.H.; Varjani, S.; Kim, S.H. Recent Advances in Commercial Biorefineries for Lignocellulosic Ethanol Production: Current Status, Challenges and Future Perspectives. *Bioresour. Technol.* 2022, 344, 126292. [CrossRef] [PubMed]
- 10. Yuvadetkun, P.; Boonmee, M. Ethanol Production Capability of *Candida shehatae* in Mixed Sugars and Rice Straw Hydrolysate. *Sains Malays.* **2016**, *45*, 581–587.
- 11. Kongkeitkajorn, M.B.; Sae-Kuay, C.; Reungsang, A. Evaluation of Napier Grass for Bioethanol Production through a Fermentation Process. *Processes* **2020**, *8*, 567. [CrossRef]
- 12. Delgado Arcaño, Y.; Valmaña García, O.D.; Mandelli, D.; Carvalho, W.A.; Magalhães Pontes, L.A. Xylitol: A Review on the Progress and Challenges of Its Production by Chemical Route. *Catal. Today* **2020**, *344*, 2–14. [CrossRef]
- Felipe Hernández-Pérez, A.; de Arruda, P.V.; Sene, L.; da Silva, S.S.; Kumar Chandel, A.; de Almeida Felipe, M.D.G. Xylitol Bioproduction: State-of-the-Art, Industrial Paradigm Shift, and Opportunities for Integrated Biorefineries. *Crit. Rev. Biotechnol.* 2019, 39, 924–943. [CrossRef]
- West, T.P. Xylitol Production by *Candida* Species from Hydrolysates of Agricultural Residues and Grasses. *Fermentation* 2021, 7, 243. [CrossRef]
- Antunes, F.A.F.; Thomé, L.C.; Santos, J.C.; Ingle, A.P.; Costa, C.B.; Dos Anjos, V.; Bell, M.J.V.; Rosa, C.A.; Silva, S.S.D. Multi-Scale Study of the Integrated Use of the Carbohydrate Fractions of Sugarcane Bagasse for Ethanol and Xylitol Production. *Renew. Energy* 2021, 163, 1343–1355. [CrossRef]
- De Souza Queiroz, S.; Jofre, F.M.; dos Santos, H.A.; Hernández-Pérez, A.F.; das Graças de Almeida Felipe, M. Xylitol and Ethanol Co-Production from Sugarcane Bagasse and Straw Hemicellulosic Hydrolysate Supplemented with Molasses. *Biomass Convers. Biorefinery* 2021, 1–10. [CrossRef]
- 17. Unrean, P.; Ketsub, N. Integrated Lignocellulosic Bioprocess for Co-Production of Ethanol and Xylitol from Sugarcane Bagasse. *Ind. Crops Prod.* **2018**, *123*, 238–246. [CrossRef]
- Raj, K.; Krishnan, C. Improved Co-Production of Ethanol and Xylitol from Low-Temperature Aqueous Ammonia Pretreated Sugarcane Bagasse Using Two-Stage High Solids Enzymatic Hydrolysis and *Candida tropicalis*. *Renew. Energy* 2020, 153, 392–403. [CrossRef]
- Shankar, K.; Kulkarni, N.S.; Sajjanshetty, R.; Jayalakshmi, S.K.; Sreeramulu, K. Co-Production of Xylitol and Ethanol by the Fermentation of the Lignocellulosic Hydrolysates of Banana and Water Hyacinth Leaves by Individual Yeast Strains. *Ind. Crops Prod.* 2020, 155, 112809. [CrossRef]
- Dasgupta, D.; Ghosh, D.; Bandhu, S.; Adhikari, D.K. Lignocellulosic Sugar Management for Xylitol and Ethanol Fermentation with Multiple Cell Recycling by *Kluyveromyces marxianus* IIPE453. *Microbiol. Res.* 2017, 200, 64–72. [CrossRef]
- 21. Zahed, O.; Jouzani, G.S.; Abbasalizadeh, S.; Khodaiyan, F.; Tabatabaei, M. Continuous Co-Production of Ethanol and Xylitol from Rice Straw Hydrolysate in a Membrane Bioreactor. *Folia Microbiol.* **2016**, *61*, 179–189. [CrossRef]

- Morales-Rodriguez, R.; Perez-Cisneros, E.S.; de Los Reyes-Heredia, J.A.; Rodriguez-Gomez, D. Evaluation of Biorefinery Configurations through a Dynamic Model-Based Platform: Integrated Operation for Bioethanol and Xylitol Co-Production from Lignocellulose. *Renew. Energy* 2016, 89, 135–143. [CrossRef]
- Ngamsirisomsakul, M.; Reungsang, A.; Kongkeitkajorn, M.B. Assessing Oleaginous Yeasts for Their Potentials on Microbial Lipid Production from Sugarcane Bagasse and the Effects of Physical Changes on Lipid Production. *Bioresour. Technol. Rep.* 2021, 14, 100650. [CrossRef]
- 24. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem. 1959, 31, 426–428. [CrossRef]
- 25. Martínez-Moreno, R.; Morales, P.; Gonzalez, R.; Mas, A.; Beltran, G. Biomass Production and Alcoholic Fermentation Performance of *Saccharomyces cerevisiae* as a Function of Nitrogen Source. *FEMS Yeast Res.* **2012**, *12*, 477–485. [CrossRef] [PubMed]
- Li, Z.; Wang, D.; Shi, Y.-C. Effects of Nitrogen Source on Ethanol Production in Very High Gravity Fermentation of Corn Starch. J. Taiwan Inst. Chem. Eng. 2017, 70, 229–235. [CrossRef]
- Zhao, Z.; Xian, M.; Liu, M.; Zhao, G. Biochemical Routes for Uptake and Conversion of Xylose by Microorganisms. *Biotechnol. Biofuels* 2020, 13, 21. [CrossRef] [PubMed]
- 28. Subtil, T.; Boles, E. Competition between Pentoses and Glucose during Uptake and Catabolism in Recombinant *Saccharomyces cerevisiae*. *Biotechnol. Biofuels* **2012**, *5*, 14. [CrossRef]
- 29. Salmon, J.M.; Vincent, O.; Mauricio, J.C.; Bely, M.; Barre, P. Sugar Transport Inhibition and Apparent Loss of Activity in *Saccharomyces cerevisiae* as a Major Limiting Factor of Enological Fermentations. *Am. J. Enol. Vitic.* **1993**, *44*, 56–64.
- Batt, C.A.; Caryallo, S.; Easson, D.D.; Akedo, M.; Sinskey, A.J. Direct Evidence for a Xylose Metabolic Pathway In Saccharomyces cerevisiae. Biotechnol. Bioeng. 1986, 28, 549–553. [CrossRef]
- Toivari, M.H.; Salusjärvi, L.; Ruohonen, L.; Penttilä, M. Endogenous Xylose Pathway in Saccharomyces cerevisiae. Appl. Environ. Microbiol. 2004, 70, 3681–3686. [CrossRef]
- 32. Stoklosa, R.J.; Nghiem, N.P.; Latona, R.J. Xylose-Enriched Ethanol Fermentation Stillage from Sweet Sorghum for Xylitol and Astaxanthin Production. *Fermentation* **2019**, *5*, 84. [CrossRef]
- Silva, S.S.; Afschar, A.S. Microbial Production of Xylitol from D-Xylose Using Candida tropicalis. *Bioprocess Eng.* 1994, 11, 129–134. [CrossRef]
- Silva, S.S.; Quesada-Chanto, A.; Vitolo, M. Upstream Parameters Affecting the Cell Growth and Xylitol Production by Candida guilliermondii FTI 20037. Zeitschrift Für Naturforsch. C 1997, 52, 359–363. [CrossRef]
- Mushtaq, Z.; Imran, M.; Zahoor, T.; Ahmad, R.S.; Arshad, M.U. Biochemical Perspectives of Xylitol Extracted from Indigenous Agricultural By-Product Mung Bean (Vigna Radiata) Hulls in a Rat Model. J. Sci. Food Agric. 2014, 94, 969–974. [CrossRef] [PubMed]
- Vongsuvanlert, V.; Tani, Y. Xylitol Production by a Methanol Yeast, *Candida boidinii* (*Kloeckera* Sp.) No. 2201. J. Ferment. Bioeng. 1989, 67, 35–39. [CrossRef]
- Ling, H.; Cheng, K.; Ge, J.; Ping, W. Statistical Optimization of Xylitol Production from Corncob Hemicellulose Hydrolysate by Candida tropicalis HDY-02. New Biotechnol. 2011, 28, 673–678. [CrossRef] [PubMed]
- Cortez, D.V.; Roberto, I.C. Effect of Phosphate Buffer Concentration on the Batch Xylitol Production by *Candida guilliermondii*. Lett. Appl. Microbiol. 2006, 42, 321–325. [CrossRef] [PubMed]
- Winkelhausen, E.; Amartey, S.A.; Kuzmanova, S. Xylitol Production from D-Xylose at Different Oxygen Transfer Coefficients in a Batch Bioreactor. Eng. Life Sci. 2004, 4, 150–154. [CrossRef]
- Ding, X.; Xia, L. Effect of Aeration Rate on Production of Xylitol from Corncob Hemicellulose Hydrolysate. *Appl. Biochem. Biotechnol.* 2006, 133, 263–270. [CrossRef]
- Mussatto, S.I.; Roberto, I.C. Xylitol Production from High Xylose Concentration: Evaluation of the Fermentation in Bioreactor under Different Stirring Rates. J. Appl. Microbiol. 2003, 95, 331–337. [CrossRef]
- 42. Faria, L.F.F.; Gimenes, M.A.P.; Nobrega, R.; Pereira, N. Influence of Oxygen Availability on Cell Growth and Xylitol Production by *Candida guilliermondii*. In *Biotechnology for Fuels and Chemicals*; Humana Press: Totowa, NJ, USA, 2002; pp. 449–458. [CrossRef]
- 43. Tani, T.; Taguchi, H.; Akamatsu, T. Analysis of Metabolisms and Transports of Xylitol Using Xylose- and Xylitol-Assimilating *Saccharomyces cerevisiae*. J. Biosci. Bioeng. 2017, 123, 613–620. [CrossRef]
- Schirmer-Michel, Â.C.; Flôres, S.H.; Hertz, P.F.; Matos, G.S.; Ayub, M.A.Z. Production of Ethanol from Soybean Hull Hydrolysate by Osmotolerant *Candida guilliermondii* NRRL Y-2075. *Bioresour. Technol.* 2008, 99, 2898–2904. [CrossRef]
- Da Silva, R.O.; Serpa, M.D.N.; Brod, F.C.A. Influence of Agitation and Aeration on Xylitol Production by the Yeast Starmerella meliponinorum. Quim. Nova 2020, 43, 705–710. [CrossRef]
- Wannawilai, S.; Lee, W.C.; Chisti, Y.; Sirisansaneeyakul, S. Furfural and Glucose Can Enhance Conversion of Xylose to Xylitol by Candida magnoliae TISTR 5663. J. Biotechnol. 2017, 241, 147–157. [CrossRef] [PubMed]
- Hernández-Pérez, A.F.; de Arruda, P.V.; Felipe, M.D.G.D.A. Sugarcane Straw as a Feedstock for Xylitol Production by *Candida* guilliermondii FTI 20037. Braz. J. Microbiol. 2016, 47, 489–496. [CrossRef] [PubMed]
- 48. Tamburini, E.; Bianchini, E.; Bruni, A.; Forlani, G. Cosubstrate Effect on Xylose Reductase and Xylitol Dehydrogenase Activity Levels, and Its Consequence on Xylitol Production by *Candida tropicalis*. *Enzyme Microb. Technol.* **2010**, *46*, 352–359. [CrossRef]

- Castañón-Rodríguez, J.F.; Domínguez-González, J.M.; Ortíz-Muñiz, B.; Torrestiana-Sanchez, B.; de León, J.A.R.; Aguilar-Uscanga, M.G. Continuous Multistep versus Fed-Batch Production of Ethanol and Xylitol in a Simulated Medium of Sugarcane Bagasse Hydrolyzates. *Eng. Life Sci.* 2015, *15*, 96–107. [CrossRef]
- 50. Snoek, T.; Verstrepen, K.J.; Voordeckers, K. How Do Yeast Cells Become Tolerant to High Ethanol Concentrations? *Curr. Genet.* **2016**, *62*, 475–480. [CrossRef]