Evaluation of Xylose-Utilising Yeasts for Xylitol Production from Second-Generation Ethanol Vinasse and Effect of Agitation Intensity in Flask-Scale Xylitol Production

(Penilaian Yis mengguna Xilosa untuk Pengeluaran Xilitol daripada Vinasse Etanol Generasi Kedua dan Kesan Keamatan Pergolakan dalam Pengeluaran Xilitol Skala Flask)

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ABSTRACT

This study aimed to select a yeast strain that effectively utilises xylose to produce xylitol from the vinasse of ethanol broth obtained from the fermentation of sugarcane bagasse hydrolysate. Eleven strains of xylose-fermenting yeasts were evaluated for their abilities to utilise xylose and produce xylitol. Two strains that showed outstanding performance in the semi-defined xylose medium were selected for further testing with a vinasse medium. *Candida guilliermondii* TISTR 5068 showed a superior xylitol production of 7.03 ± 0.08 g/L with the xylitol yield of 0.70 g/g_{xylose} when cultured in bagasse-based ethanol vinasse. The strain was further tested for its xylitol production performance when cultured at four different agitation intensities. Excessive agitation resulted in a rapid xylitol production rate but caused xylitol consumption once the xylose was depleted. Moderate agitation resulted in the highest xylitol yield of 0.79 g/g_{xylose}. The results of this study have provided important information for the development of the xylitol production process using waste streams from cellulosic ethanol production.

Keywords: Aeration; biorefineries; fermentation; vinasse; xylitol; yeasts

ABSTRAK

Kajian ini bertujuan untuk memilih strain yis yang menggunakan xilosa secara berkesan untuk menghasilkan xilitol daripada vinasse rebusan etanol yang diperoleh daripada penapaian hidrolisat hampas tebu. Sebelas strain yis difermentasi xilosa dinilai untuk kebolehan mereka untuk menggunakan xilosa dan menghasilkan xilitol. Dua strain yang menunjukkan prestasi cemerlang dalam medium xilosa separa takrif telah dipilih untuk ujian selanjutnya dengan medium vinasse. *Candida guilliermondii* TISTR 5068 menunjukkan pengeluaran unggul xilitol sebanyak 7.03 ± 0.08 g/L dengan hasil xilitol sebanyak 0.70 g/g_{xylose} apabila dikultur dalam vinasse etanol berasaskan hampas. Strain diuji lagi untuk prestasi pengeluaran xilitol apabila dikultur pada empat keamatan pengadukan yang berbeza. Penggoncangan yang berlebihan mengakibatkan kadar pengeluaran xilitol yang cepat tetapi menyebabkan penggunaan xilitol sebaik sahaja xilosa habis. Penggoncangan sederhana menghasilkan hasil xilitol tertinggi iaitu 0.79 g/g_{xylose}. Hasil kajian ini telah memberikan maklumat penting untuk pembangunan proses penghasilan xilitol menggunakan aliran sisa daripada penghasilan etanol selulosa.

Kata kunci: Fermentasi; kilang penapisan bio; pengudaraan; vinasse; xilitol; yis

INTRODUCTION

Xylitol ($C_5H_{12}O_5$ or $HO(CH_2)(CHOH)_3(CH_2)OH$) is a polyalcohol or sugar alcohol. Xylitol occurs naturally in fruits and vegetables such as strawberries, raspberries, mushrooms, and cauliflower (Ahuja et al. 2020). However, its content in fruits and vegetables is low. Xylitol sweetens

with fewer calories (2.4 Cal/g) than table sugar (4 Cal/g) (Pal et al. 2013). It is used in various sectors, including the food and beverage, cosmetics, and pharmaceutical industries. Xylitol is associated with health promotion as it has been reported to prevent tooth decay and ear infections (Rao et al. 2016). It is promoted as a sugar substitute for diabetics because it has insulin-independent metabolism and is anti-carcinogenic (Edelstein et al. 2008; Maguire & Rugg-Gunn 2003; Rafiqul & Mimi Sakinah 2013).

Traditionally and commercially, xylitol is produced chemically by a xylose hydrogenation reaction. This process requires a pure substrate with high reaction temperatures up to 140 °C and pressures up to 50 atm (Dalli, Patel & Rakshit 2017; Dasgupta et al. 2017). For this reason, converting the process to xylitol bioconversion and using xylose from renewable resources as a substrate is an intriguing alternative. In addition, xylitol production by biological means requires milder conditions as it can be carried out at much lower temperatures and pressures.

Lignocellulosic biomass is the most abundant and renewable material. Its sugary components are generating interest in using it for biological conversion into various biofuels and biochemicals such as bioethanol, microbial oil, succinic acid, and fumaric acid (Okolie et al. 2021). The utilisation of biomass via biological conversion usually involves the cellulose fraction which contains only glucose. However, the hemicellulose fraction, which consists mainly of xylose, should not be neglected. Some microorganisms can utilise xylose to produce various biochemicals, including xylitol.

Most studies on xylitol fermentation from lignocellulosic materials have focused on the use of the xylose-rich fraction derived from the solubilisation of hemicellulose by acids (Bedő et al. 2021, 2019; de Souza Queiroz et al. 2021; Du et al. 2020) or xylanase (Xu et al. 2019). However, xylitol production could be an additional step in a second-generation ethanol production process to utilise the xylose remaining in the vinasse. This approach to xylitol production could be integrated into an existing second-generation ethanol production process.

To obtain a suitable yeast strain for xylitol production from vinasse, xylose-utilising yeast strains from a microbial collection were evaluated for their ability to utilise xylose for xylitol production, focusing on the ability to utilise xylose in the vinasse derived from ethanol production from sugarcane bagasse. The selected strain was evaluated for its fermentative characteristics and xylitol production at various agitation intensities on a flask scale. The results of this study served as a preliminary step in the development of a process for the production of using xylose contained in second-generation ethanol vinasse. Xylitol production downstream of second-generation ethanol production could be a value-added step in the overall production process.

MATERIALS AND METHODS

MICROORGANISMS

Eleven yeast strains from the genera *Candida* spp., *Debaryomyces* spp., and *Hansenula* sp. were obtained from MIRCEN, Thailand Institute of Scientific and Technical Research (TISTR) (Table 1). They were chosen based on reports in the literature as xylitol producers. They would be evaluated for their performances in xylitol production in this study. All yeast stocks were stored as a cell suspension in 30% (v/v) glycerol at -20 °C. In preparing the working stock, yeast from the -20 °C stock was streaked on YMX agar plates (see the composition in the section below) and incubated at 30 °C for 48 h or until the colonies appeared.

CULTURE MEDIA FOR YEAST GROWTH AND FERMENTATION

YM medium was used in inoculum preparation and general cultivation in this study. Its nitrogen base solution contains 3 g/L of yeast extract, 3 g/L of malt extract, and 5 g/L of peptone. The YM agar contained 7 g/L of agar in addition to the other components of the YM medium. Xylose was added to make a final concentration of 20 g/L for inoculum preparation and 25 g/L for the cultivation medium (designated as YMX medium).

Vinasse from bagasse-based ethanol production was used in cultivating potential yeast strains for their ability to produce xylitol from xylose in the vinasse. Vinasse was prepared from the fermentation broth of ethanol production using sugarcane bagasse hydrolysate as a substrate. Conditions for ethanol fermentation followed Kongkeitkajorn, Sae-Kuay, and Reungsang (2020) with sugarcane bagasse as the lignocellulosic biomass. The liquid broth was separated from the cells by centrifugation at 11200 g, 25 °C for 10 min (Sorvall LYNX 4000/6000, Thermo Scientific, USA). It was subjected to simple distillation to remove ethanol. The vinasse contained 20.00±1.19 g/L of xylose, 1.22±0.08

TABLE 1. Xylose-utilising yeast strains used in this study

No.	Genus and species	TISTR strain number	Abbreviation
1	*Candida guilliermondii*	5068	CG5068
2	*C. guilliermondii*	5206	CG5206
3	*C. tropicalis*	5045	CT5045
4	*C. parapsilosis*	5315	CP5315
5	*C. magnoliae*	5663	CM5663
6	*C. magnoliae*	5664	CM5664
7	*C. magnoliae*	5165	CM5165
8	*C. pelliculosa*	5809	CP5809
9	*Debaryomyces hansenii*	5155	DH5155
10	*D. hansenii var fabryi*	5265	DH5265
11	*Hansenula polymorpha*	5140	HP5140

g/L of xylitol, 10.00±1.51 g/L of glycerol, and 2.40±0.77 g/L of ethanol.

YEAST CULTIVATION

Yeast cultivation in this study was carried out in a 250-mL Erlenmeyer flask. The inoculum was prepared by transferring a few single colonies of less than a week-old yeast on a YMX agar plate to the YMX medium and incubating at 30 °C with orbital shaking at 200 rpm for 24 h.

The cultivation started by transferring ten percent of the inoculum into a 250-mL flask containing 100 mL of cultivation medium. The culture is incubated at 30 °C at a shaking speed of 200 rpm without any pH control. Samples were taken at intervals and analysed for xylose and xylitol concentration. In addition, the cell optical density was monitored at each sampling point, and the cell dry weight was determined in the final sample.

In the agitation intensities study, cultivations were carried out in two Erlenmeyer flask types: normal and baffled flasks. The orbital shaking speeds varied at 100 and 200 rpm. The four combinations simulated different aeration levels, from low (normal flask and 100 rpm) to high (baffled flask and 200 rpm).

ANALYTICAL METHODS

In the analysis for cell growth, cell optical density was measured using a spectrophotometer at 600 nm. The cell dry weight analysis employed the weight difference after drying cell pellets at 106 °C until they reached a constant weight.

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

STATISTICAL ANALYSIS

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

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Results

ASSESSING THE XYLOSE-UTILISING YEAST STRAINS
FOR THEIR GROWTH AND POSSIBLE XYLITOL
PRODUCTION

Eleven potential xylose-utilising yeast strains were
tested for their xylose utilisation and xylitol production
ability in a xylose medium with 25 g/L of xylose. The
xylose concentration was approximately equal to the
xylose present in sugarcane bagasse hydrolysate. This

concentration would also be present in the vinasse that
would be used as a primary source for xylitol production
in future process development.

The results in Figure 1(a) showed that most strains
could utilise more than 80% xylose within 48 h. Five
yeast strains showed complete xylose utilisation. Xylose
utilisation was inferior in 3 yeast strains, including
Candida guilliermondii TISTR5260 (CG5206), *C.
parapsilosis* TISTR5315 (CP5315), and *Hansenula
polymorpha* TISTR5140 (HP5140), where only about
50-60% of xylose was utilised.

(a) Xylose consumption
White bars present initial concentration
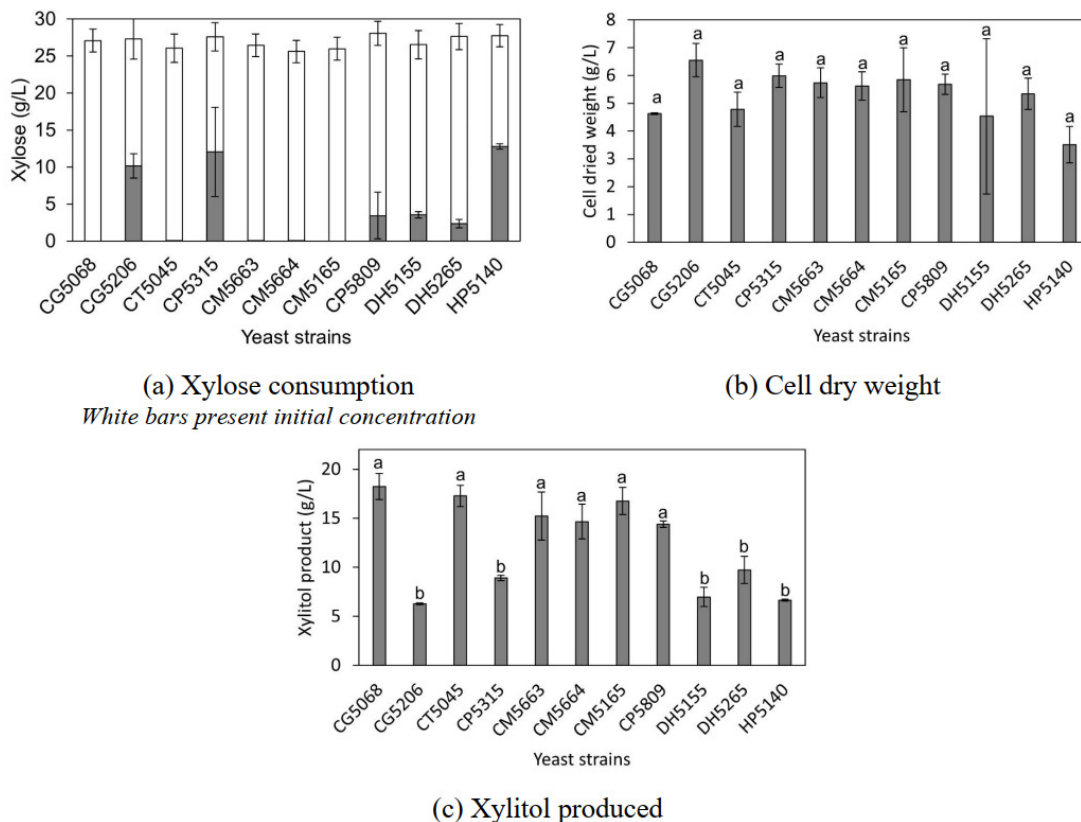
(b) Cell dry weight

(c) Xylitol produced

FIGURE 1. Comparisons of fermentation results between various xylitol-producing yeast
strains when cultivated in 25 g/L xylose medium at 30 °C, and 200 rpm for 48 h

It is worth noting that xylose utilisation in the
two strains of *C. guilliermondii* was different. While
CG5068 could completely utilise xylose within 48 h,
CG5206 could use only 63%. This occurrence appeared
differently in *C. magnoliae* and *Debaryomyces
hansenii*. All strains of the two species demonstrated
similar xylose utilisation abilities. A similar finding was

reported in other works involving strain selection, where
C. tropicalis has been the most studied yeast. Employing
similar aerobic conditions, *C. tropicalis* of different
strains showed various abilities to consume xylose,
ranging from 30% to complete consumption (Martins
et al. 2018; Morais Junior et al. 2019). Therefore, the
assessment for xylose consumption was suggested even
in yeast of the same genus.

The growth of the yeast strains (Figure 1(b)) mostly followed how the xylose was utilised. The more xylose was utilised, the higher the cell mass was produced. However, an exception existed in CG5206 and CP5315, where high cell mass was achieved regardless of the low xylose utilisation. The high cell yield could be strain specific. In addition, since the selection study was carried out for 48 h, it was not conclusive that high cell yield would still maintain for these two strains if the fermentation was prolonged to allow for more xylose utilization. However, prolonged fermentation was not a choice in the selection step as fast xylose utilization and high xylitol production were the main criteria for the selection.

Most of the yeast strains in the test produced high xylitol following high xylose utilisation. Low xylitol production in CG5206 could be that it used xylose more efficiently for growth. On the other hand, CP5315 seemed to produce xylitol more efficiently when compared with CG5206, judging from the xylitol yield shown in Table 2.

TABLE 2. Xylitol yields and cell yields obtained from xylose-utilising yeast strains in a xylose medium

Xylitol producing strains	Xylose utilised (%)	Xylitol produced (g/L)	Xylitol yield (g/g)	Cell yield (g/g)
C. guilliermondii TISTR 5068	100 ±0.00 [a]	18.24±1.87 [a]	0.67±0.01 [a]	0.17±0.02 [b]
C. guilliermondii TISTR 5206	63.05±3.31 [b]	6.24±0.13 [b]	0.37±0.01 [d]	0.38±0.02 [ab]
C. tropicalis TISTR 5045	99.94±0.09 [a]	17.29±1.54 [a]	0.66±0.01 [a]	0.18±0.01 [b]
C. parapsilosis TISTR 5315	57.62±26.73 [b]	8.92±0.38 [b]	0.62±0.26 [ab]	0.42±0.20 [a]
C. magnoliae TISTR 5663	99.86±0.09 [a]	15.23±3.47 [a]	0.57±0.08 [abc]	0.22±0.01 [ab]
C. magnoliae TISTR 5664	99.92±0.12 [a]	14.65±2.50 [a]	0.57±0.05 [abc]	0.22±0.01 [ab]
C. magnoliae TISTR 5165	100±0.00 [a]	16.77±1.95 [a]	0.64±0.02 [ab]	0.22±0.04 [ab]
C. pelliculosa TISTR 5809	88.40±14.97 [a]	14.41±0.48 [a]	0.59±0.03 [abc]	0.23±0.00 [ab]
D. hansenii TISTR 5155	86.72±0.87 [a]	6.98±1.40 [b]	0.30±0.03 [d]	0.21±0.19 [b]
D. hansenii var fabryi TISTR 5265	91.63±2.23 [a]	9.73±0.98 [b]	0.38±0.05 [cd]	0.21±0.02 [b]
H. polymorpha TISTR 5140	53.86±1.76 [b]	6.65±2.18 [b]	0.45±0.04 [bcd]	0.23±0.0 [ab]

From the results of xylitol production (Figure 1(c)), six strains showed similar xylitol production performance. They included CG5068, CT5045, CP5809, and the three strains of *C. magnoliae*. However, when considering the xylitol yields (Table 2), only CG5068 and CT5045 would be chosen for further selection as they were the two strains that produced the highest xylitol concentration with a distinct difference in xylitol yields compared to the others. *C. guilliermondii* TISTR 5068 (CG5068) could produce 18.24 g/L of xylitol with a yield of 0.67 g/g. *C. tropicalis* TISTR 5045 (CT5045) could produce 17.28 g/L of xylitol with a similar xylitol yield.

REFINING THE SELECTION OF THE REPRESENTATIVE STRAINS IN THE VINASSE MEDIUM

The two strains (CG5068 and CT5045), which showed similar xylitol production in the xylose medium, were further assessed for their xylitol production in a vinasse medium prepared from the distillation of bioethanol produced from sugarcane bagasse. It contained xylose as the primary component, as indicated in the Materials and Methods section.

The fermentation results in Figure 2 showed that both strains could use xylose and produce xylitol from the vinasse. They used xylose to the same extent but at different rates, judging from the slopes of the profiles.

C. tropicalis TISTR 5045 (CT5045) used xylose slightly faster than *C. guilliermondii* TISTR 5068 (CG5068) during the early stage of fermentation. The growth profiles demonstrated a faster growth rate in CG5068. It resulted in a higher final cell concentration of 6.90 g/L when compared with CT5045 which produced 6.38 g/L of cell concentration.
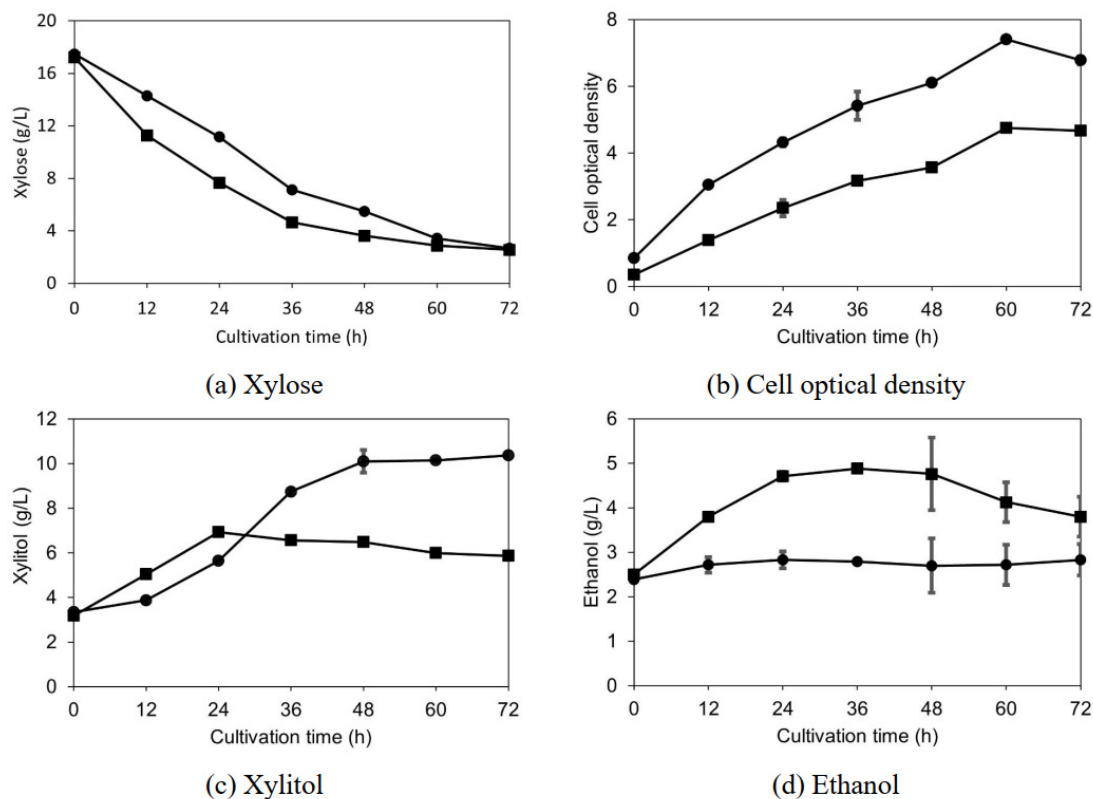

FIGURE 2. Fermentation profiles of *C. guilliermondii* TISTR 5068 and *C. tropicalis* TISTR 5045 in vinasse medium at 30 °C, 200 rpm for 72 h. ● – CG5068, ■ – CT5045

C. guilliermondii TISTR 5068 produced significantly higher xylitol from xylose in the vinasse than *C. tropicalis* TISTR 5045, regardless of faster xylitol production by CT5045 during the first 24 h of the cultivation. CG5068 produced xylitol continuously throughout the fermentation period, while CT5045 stopped the xylitol production after 24 h, although it continued to grow and consume xylose. CG5068 could achieve xylitol production of up to 7.03 g/L (discounting the initial xylitol presented in the vinasse). The yield was 0.70 g/g, which was 1.75 times higher than that obtained from CT5045.

Apart from xylitol, ethanol was detected in the fermentation by CT5045, while essentially no ethanol was produced by CG5068. The ethanol profiles in Figure 2(d) demonstrate that CT5045 produced ethanol simultaneously with xylitol during the first 24 h of the fermentation, with a concentration up to 4.8 g/L. The ethanol slowly decreased after 48 h, and the final concentration was 3.8 g/L.

The results of this selection step using a vinasse medium showed that *C. guilliermondii* TISTR 5068 (CG5068) was the suitable xylitol-producing yeast strain. It not only produced a high concentration of xylitol with a reasonable yield but also utilised our target raw material for xylitol production (the vinasse) without any undesirable by-products such as ethanol.

EFFECT OF AGITATION INTENSITY ON FLASK-SCALE
CULTIVATION OF *C. guilliermondii* TISTR 5068

Flask-scale cultivation was often employed in the early stages of fermentation studies, including those that required aeration. Choosing an appropriate agitation during the early stage would provide more pragmatic results before cultivation in an up-scale bioreactor. In this part of the study, *C. guilliermondii* TISTR 5068 was tested for growth and xylitol production in flask-scale experiments under different agitation intensities to determine its effect on xylitol production.

Different agitation intensities were created by using combinations of flask types (baffled and normal Erlenmeyer flasks) and orbital shaking speeds; as alternatives to varying shaking speeds alone. Using a baffled flask allowed for higher turbulence, dictating a

higher oxygen transfer rate at a lower orbital shaking speed. Oxygen supplied to the fermentation medium played an important role in cell growth and xylitol production as it affected the activities of xylose reductase and xylitol dehydrogenase and determined the fate of xylitol (Ding & Xia 2006; Pal et al. 2013).

Fermentation profiles in Figure 3 defined three distinct agitation intensities generated by using the combinations of two types of flasks and two orbital shaking speeds. The highest agitation intensity was obtained from using a baffled flask and shaking at 200 rpm (B-200). Using a normal flask shaking at 200 rpm (N-200) was the moderate agitation intensity. The other two combinations at 100 rpm (B-100 and N-100) represented low agitation intensity. The profiles also implied different aeration levels caused by the intensity of agitation.

(a) Xylose

(b) Cell optical density

(c) Xylitol

♦ Normal flask, 100 rpm
■ Normal flask, 200 rpm
▲ Baffled flask, 100 rpm
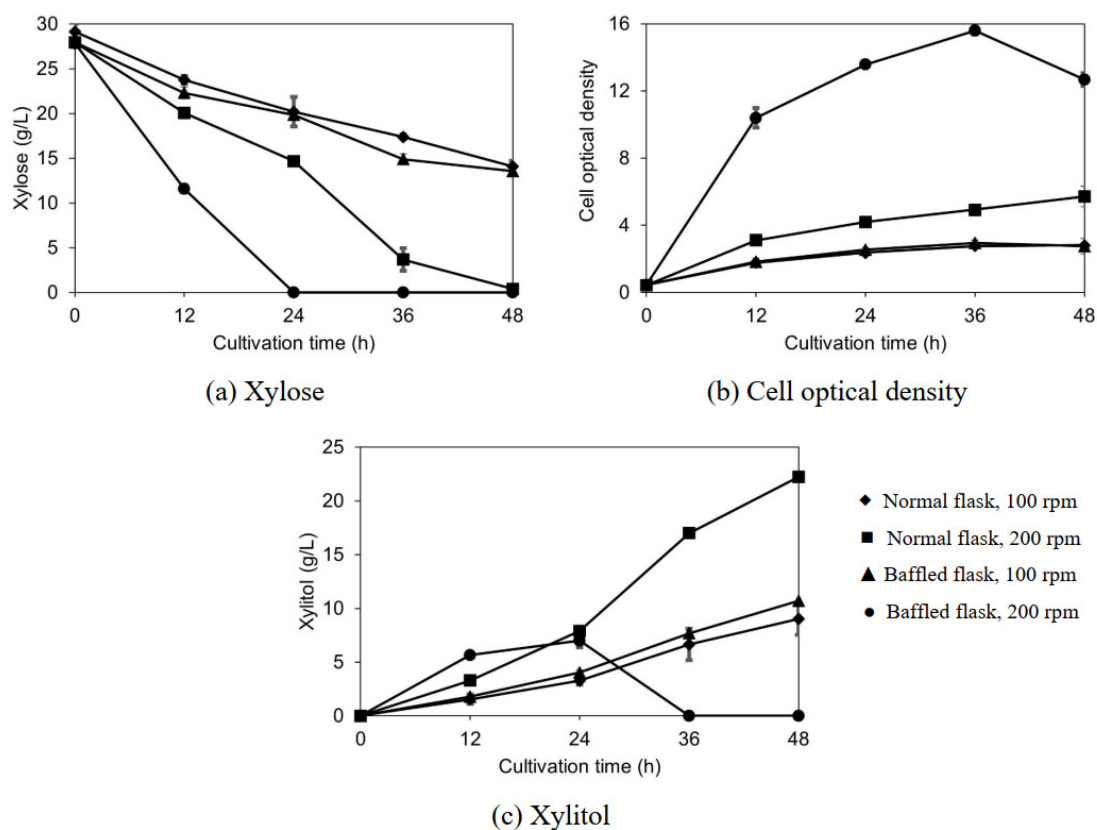● Baffled flask, 200 rpm

FIGURE 3. Cultivation profiles of *C. guilliermondii* TISTR 5068 during the cultivation using 25 g/L xylose medium in different agitation intensities at 30 °C, for 48 h

Higher agitation intensities resulted in faster xylose consumption, as observed from the slopes in Figure 3(a). At the highest agitation intensity, xylose was fully consumed after 24 h, while it took 48 h at the moderate agitation intensity. The xylose consumption was much slower, and the yeast could utilise approximately 50% of the initial xylose in the cultivation with the low agitation intensity (Table 3).

TABLE 3. Summary of fermentation by *C. guilliermondii* TISTR 5068 in various agitation intensities when using 25 g/L xylose medium and cultivated at 30 °C for 48 h

Conditions*	Xylose consumption (%)	Final biomass (g/L)	Cell yield (g/g)	Xylitol (g/L)	Xylitol yield (g/g)
N-100	46.3 ± 12.4[b]	1.27 ± 0.03 [b]	0.10 ± 0.02 [b]	8.97 ± 0.00[b]	0.31 ± 0.06 [b]
N-200	98.6 ± 2.0 [a]	2.03 ± 0.80 [b]	0.07 ± 0.02 [b]	22.22 ± 0.63[a]	0.79 ± 0.03 [a]
B-100	51.5 ± 1.2 [b]	1.03 ± 0.03 [b]	0.07 ± 0.00 [b]	10.69 ± 0.00[b]	0.38 ± 0.01 [b]
B-200	100.0 ± 0.0 [a]	13.83 ± 1.61 [a]	0.50 ± 0.04 [a]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]

*N = normal Erlenmeyer flask; B = baffled Erlenmeyer flask; 100 and 200 = 100 and 200 rpm

The yeast's growth pattern followed the trend in xylose consumption (Figure 3(b)). The most agitated/aerated condition (B-200) resulted in the highest increase in cell density. However, much slower growth was observed in the moderate agitated/aerated condition (N-200). The growth rate and final cell dry weight obtained were closer to the least agitated/aerated conditions (Figure 3(b) and Table 3). High agitation promoted oxygen transfer to the liquid fermentation medium and hence the yeast growth. The final cell dry weights in Table 3 clearly showed a positive effect of oxygen levels from rigorous agitation on the yeast growth. Approximately 7-fold higher biomass resulted when cultured at the highest agitation intensity. Final biomass at a moderate level of agitation (N-200) was not significantly different from cultures at low agitation intensities (N-100 and B-100). The cell yields from those conditions were also statistically indifferent.

Xylitol production depends, to a great extent, on agitation intensity. Diverse profiles were evident depending on the agitation intensity (Figure 3(c)). At low agitation intensities (N-100 and B-100), the overall xylitol production rate was slow, as observed from the shallow slopes of the profiles, and reflected the xylose consumption. As the agitation intensities increased, the xylitol production rates also increased. However, excessive aeration provided at the high agitation intensity caused xylitol to decrease after the steep increase during the first 12 h. The profiles in Figure 3 also showed that when xylose was depleted after 24 h, the yeast continued to grow. This relationship implied that xylitol could be reassimilated and used for cell growth. The xylitol profile also suggested that xylitol consumption could start as early as 12 h of fermentation. It should be noted that there was no ethanol detected in the fermentation, which was consistent with the results in previous section that CG5068 did not produce ethanol (Figure 2(d)).

The results from this part suggest that the level of aeration in the cultivation would be a critical success factor in scaling up xylitol fermentation. Appropriate agitation intensity during flask-scale studies offered an overview of the results for further up-scaling to a bioreactor scale. In addition, as xylitol could serve as the substrate for yeast cell growth, fermentation time would also be critical. Harvesting or ceasing the aeration based on xylose depletion would reduce the risk of xylitol loss due to cells' consumption.

DISCUSSION

Numerous xylose-utilisation yeasts produce xylitol, although at different capacities and even among the same genera. The initial selection resulted in *C. guilliermondii* (CG5068) and *C. tropicalis* (CT5045) with xylitol yields of 0.66-0.67 g/g. These values concurred

with the range obtained from the other studies that specifically employed these two yeasts. *C. guilliermondii* grown in semi-defined media with xylose has been reported with xylitol yields of 0.68 g/g (Mussatto, Silva, and Roberto 2006), 0.69 g/g (Thancharoen, Deeseenthum & Vichitphan 2016), and 0.74 g/g (Arruda & Felipe 2009). More variance in xylitol yields was reported in studies using *C. tropicalis*. A xylitol yield of 0.47 g/g was reported in the KS 10-3 strain (Thancharoen, Deeseenthum & Vichitphan 2016), while 0.86 g/g was reported in the strain JA2 (Morais Junior et al. 2019).

In the refining selection step, ethanol was detected during the fermentation of the vinasse by *C. tropicalis* TISTR 5045, while no ethanol production was evident in *C. guilliermondii* TISTR 5068. *C. tropicalis* has been reported to produce ethanol from xylose, especially under aerobic conditions but not anaerobically (Jeffries 1981; Veras, Parachin & Almeida 2017). Research that attempted to isolate and select pentose-fermenting yeasts for ethanol production has reported the dual production of ethanol and xylitol in several *C. tropicalis* strains (Martins et al. 2018). In addition, the drop in ethanol concentration produced by *C. tropicalis* was also evident when xylose concentration in the medium started to deplete (Jeffries 1981). Our results on CT5045 concurred with those reports.

Regarding *C. guilliermondii*, there is no supporting evidence that the yeast could produce ethanol from xylose. A study that attempted to use a strain of *C. guilliermondii* in ethanol production from mixed glucose and xylose medium prepared from soybean hull hydrolysate showed that it produced ethanol from the glucose but later was consumed once the glucose was depleted. No ethanol was produced during the xylose consumption phase (da Cunha-Pereira et al. 2017). The result could imply a normal ethanol fermentation ability of *C. guilliermondii* only in glucose or hexose medium. Another study that investigated the effect of biotin on ethanol fermentation from xylose reported that *C. guilliermondii* preferred xylitol production over ethanol, which was the opposite of *Pachysolen tannophilus* (Lee et al. 1988). Therefore, under limited oxygen conditions, *C. guilliermondii* could have a metabolic preference for converting xylose to growth with xylitol accumulation rather than converting to ethanol through the action of pyruvate decarboxylase.

Our results on the effect of agitation intensity concurred with most studies that employed various xylitol-producing yeast strains. Although there could be some variation in results, they were mainly due to the

agitation ranges or robustness used in a particular study. A study on the effect of agitation rate on xylitol production by *Candida magnoliae* TISTR 5663 claimed that a higher xylitol yield was achieved when cultivating the yeast at a higher agitation rate (Wannawilai et al. 2017). However, in the most vigorous agitation in our study, the result showed no xylitol detected at the end of the fermentation due to xylitol reassimilation and consumption. The reassimilation of xylitol has been reported elsewhere, especially when the xylose level is low or depleted. *Debaryomyces hansenii* (isolated from sugarcane) could consume xylitol when xylose in the medium was less than 3 g/L to support its metabolic functions (Gírio et al. 2000; López-Linares et al. 2018). In addition, *Starmerella meliponinorum* FRP.09 reassimilated xylitol once xylose was depleted (da Silva, do Nascimento Serpa & Brod 2020). A report on xylitol consumption at low xylose concentration also existed for *C. guilliermondii* (Schirmer-Michel et al. 2008).

In addition to low xylose in the fermentation, the level of aeration could also affect the reassimilation of xylitol. A study employing a recombinant *Saccharomyces cerevisiae* showed that more xylitol was consumed when the condition was more aerobic (Tani, Taguchi & Akamatsu 2017). High oxygen levels increased the activity of NAD^+/NADH-dependent xylitol dehydrogenase, which favoured the conversion of xylitol to xylulose (Kumar et al. 2018). Xylulose then follows the pentose phosphate pathway, enters glycolysis, and further stages for energy and biomass production. As a result, higher growth was observed in our results and other xylose-utilising yeasts such as *Starmerella meliponinorum* FRP.09 (da Silva, do Nascimento Serpa & Brod 2020).

Therefore, less growth would also be necessary for successful xylitol production (Lu et al. 1995). Moderate aeration would allow for yeast growth and, at the same time, cause xylitol to accumulate following a limited reduction of NAD^+ to NADH, which causes a decrease in the activity of xylitol dehydrogenase (Martínez-Corona et al. 2016). This result concurred with our findings that the moderate level of agitation (N-200) resulted in the highest xylitol production.

Conclusions

Although xylose-utilising yeasts generally produce xylitol, their performance varies even within the same genus. The selection of potential strains should occur early in the study, especially when a new substrate

is introduced. In this study, *C. guilliermondii* and *C. tropicalis*, members of *Candida* spp., performed differently in xylitol production from the vinasse obtained from ethanol broth of sugarcane bagasse hydrolysate. In addition, appropriate agitation intensity in flask-scale studies should be chosen to correctly assess the fermentation performance of the yeast, especially in xylitol production. Moderate agitation intensity in flask-scale cultivation would give a more pragmatic assessment of xylitol production before conducting more definitive studies in larger and more precisely controlled conditions.

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