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ARTICLE

CONTROL OF OXYGEN SUPPLY IN BIOCONVERSION OF SUGARCANE TRASH INTO XYLITOL BY *Meyerozyma guilliermondii* InaCCY65

[*Kontrol Suplai Oksigen dalam Biokonversi Daun Tebu Menjadi Xilitol oleh Meyerozyma guilliermondii InaCCY65*]

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ABSRACT

Xylitol is a sugar alcohol used as a sugar substitute for several prevention of health cases such as dental diseases, diabetes, and other health problems. Bioconversion of xylose into xylitol needs an optimum oxygen supply for xylitol synthesis. This research aims to determine the effect of dissolved oxygen on hydrolysate fermentation from sugar cane leaf as a source of xylose to xylitol by *Meyerozyma guilliermondii* InaCCY65. Dissolved oxygen was varied in aeration and fermentation agitation using a 3L scale bioreactor. Analysis of cell growth for several kinetic parameters during fermentation, xylose reductase, and xylitol dehydrogenase activity. Bioconversion of sugarcane trash hydrolysate into xylitol by *M. guilliermondii* InaCCY65 is influenced by the oxygen transfer coefficient (k_La) and aeration conditions. The increase in k_La number showed increased cell growth, xylose consumption, xylitol production, and decreased InaCCY65 cells. The optimum conditions of k_La were obtained at 45/h with 39 g/L xylitol production under the aeration effect. Optimum aeration in the bioconversion of sugarcane trash (SCT) hydrolysate become xylitol by *M. guilliermondii* InaCCY65 is 1.0%; under these conditions, xylitol yield and xylitol productivity are 0.78 g/g and 1.20 g/Lh. The effect of oxygen in the bioconversion of xylose to xylitol also has an impact on xylose reductase (XR) and xylose dehydrogenase (XDH) activities of *M. guiellermondii* InaCCY65. The results showed that the dissolved oxygen concentration must be carefully controlled during xylitol bioconversion to obtain efficient xylitol.

Keywords: lignocellulose, xylitol, oxygen, xylose reductase, xylitol dehydrogenase, fermentation

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ABSTRAK

Xilitol adalah gula alkohol lima karbon yang digunakan dalam industri makanan dan farmasi sebagai pengganti gula untuk pencegahan beberapa penyakit gigi, diabetes, dan manfaat kesehatan lainnya. Dalam biokonversi xilosa menjadi xilitol dibutuhkan pasokan oksigen yang diatur optimum terhadap sintesis xilitol. Penelitian ini bertujuan untuk mengetahui pengaruh oksigen terlarut pada fermentasi hidrolisat daun tebu sebagai sumber xilosa menjadi xilitol oleh Meyerozyma guilliermondii InaCCY65. Oksigen terlarut divariasikan dalam aerasi dan agitasi fermentasi dengan menggunakan bioreaktor skala 3L. Analisa terhadap pertumbuhan sel, beberapa parameter kinetika selama fermentasi, aktivitas xilosa reduktase dan xilitol dehidrogenase. Dari penelitian ini diperoleh kondisi oksigen optimum yang ditandai dengan peningkatan xilitol. Biokonversi hidrolisat daun tebu menjadi xilitol oleh M. guilliermondii InaCCY65 dipengaruhi oleh kondisi kooefisien transfer oksigen (k_La) dan aerasi. Seiring dengan peningkatan kLa terdapat kenaikan pertumbuhan sel, konsumsi xilosa meningkat, dan penurunan produksi xilitol oleh sel InaCCY65. Kondisi k_La optimum diperoleh pada kondisi 45/h dengan produksi xilitol sebesar 39 g/L. Demikian pula aerasi berpengaruh terhadap produksi xilitol. Aerasi optimum dalam biokonversi hidrolisat daun tebu menjadi xilitol oleh M. guilliermondii InaCCY65 adalah 1.0%. Pada kondisi ini hasil xilitol dan produktivitas xilitol masing-masing adalah 0.78 g/g dan 1.20 g/Lh. Efek oksigen dalam biokonversi xilosa menjadi xilitol berdampak juga terhadap aktivitas xilosa reduktase (XR) dan xilosa dehidrogenase (XDH) dari M. guiellermondii InaCCY65. Hasil penelitian menunjukkan bahwa konsentrasi oksigen terlarut harus dikontrol secara hati-hati selama biokonversi xilitol agar diperoleh xilitol yang efisien.

***Kata kunci**: xilitol, oksigen, xilosa reduktase, xilitol dehidrogenase, Meyerozyma guiellermondii*

INTRODUCTION

Xylitol is a five-carbon sugar alcohol that has many health benefits (Farias *et al.,* 2022). This sugar has a sweet taste that is almost the same as sucrose, but it has a lower calorie than sucrose (Mathur *et al.,* 2023). In addition, xylitol is an ideal sweetener for diabetes sufferers (Msomi *et al.,* 2023; Maringka *et al.,* 2024), prevents dental caries and ear infections in young children (Nagsuwanchart *et al*. 2021), is used in various food products, oral and personal care, as well as animal nutrition (Kaur *et al.,* 2024).

Currently, xylitol is produced by chemical xylose reduction method originating from biomass sources rich in xylan, such as birch and beech wood (Sugiarto *et al.,* 2022; Mathur *et al.,* 2023). The chemical process is carried out with a nickel catalyst under high pressure and high temperature with a yield of around 50-60% (Akpe *et al.,* 2023). Production of xylitol through bioconversion of xylose with the help of yeast provides an alternative method that is more efficient and environmentally friendly (Farias *et al.,* 2022).

Several yeasts have been reported to be able to produce xylitol, such as *Candida tropicalis* (Bevilaqua *et al.,* 2023), *C. sojae* (Pant *et al.,* 2022), *Wickerhamomyces anomalus* (Deng *et al.,* 2024), *C. guilliermondii* (Estrada-Ávila *et al.,* 2022), *Debaryomyces hansenii* (Jeong *et al.,* 2022), *Kluyveromyces marxianus* (Manaf *et al.,* 2024), *Meyerozyma guilliermondii* (Estrada-Ávila *et al.,* 2022), and *Clavispora lusitaniae* (Ochoa-Chacón *et al.,* 2022). Production of xylitol from agro-industrial bioproducts containing xylose has been reported such as from sugarcane bagasse, rice straw, sugarcane straw, and different lignocellulosic biomass (LCBs) (Hernández-Pérez *et al.,* 2020). Sugarcane trash (SCT) contains cellulose (32–37%), hemicellulose (26–35.5%), and lignin (17.4–21%) (Hermiati *et al.,* 2020; Pramasari *et al.,* 2023). With this content, hemicellulose from SCT can be used in xylitol production (Pramasari *et al.,* 2023).

In this research, *M. guilliermondii* InaCCY65 was used as a bioconversion agent for SCT hydrolysate as a source of xylose into xylitol. The latest study of xylitol production is related to the optimization of temperature, pH, agitation, inoculum concentration, and xylose concentration at the beginning of fermentation using *Meyerozyma* (Singh *et al.,* 2024a). In the bioconversion of xylose into xylitol, an optimum supply of oxygen is needed for xylitol synthesis (Kumar *et al.,* 2022; Singh *et al.,* 2024b). An ideal oxygen supply is required for xylitol synthesis during the bioconversion of xylose into xylitol (Kumar *et al.,* 2022; Singh *et al.,* 2024b). One important factor that controls the effectiveness of oxygen transmission in fermentation systems is the oxygen transfer coefficient (k_La). This parameter's limited solubility in aqueous solutions makes it even more crucial for scale-up. A

lack of oxygen can have detrimental effects on the development of products as well as cell growth, severely impairing overall productivity. Some of the factors that influence it are agitation, aeration, reactor design, and the physical properties of the fermentation medium. The performance of aerobic fermentation processes depends on the precise management and optimization of k_La, which guarantees an adequate supply of oxygen (Jahanian *et al.,* 2024). Information of the oxygen transfer coefficient (k_La) effect on xylitol production by yeast needs to be further analyzed. Understanding the vital role of k_La in ensuring effective oxygen transfer in large-scale bioprocesses (Mathur *et al.,* 2023).

Xylose metabolism is initiated by two enzymatic reactions, namely NAD(P)H-dependent xylose reductase (XR) (Lugani dan Sooch 2020; Yang *et al.,* 2020; Lugani *et al.,* 2021) and NAD^+-dependent xylitol dehydrogenase (XDH) (Ochoa-Chacón *et al.,* 2022). XR catalyzes the reduction of xylose to xylitol, then XDH oxidizes xylitol to xylulose (Bianchini *et al.,* 2022). Furthermore, xylulose is metabolized through the pentose phosphate pathway and the central carbon metabolism to become energy and biomass (Bianchini *et al.,* 2023). NAD^+ and NADH are very sensitive to oxygen tension (Croft *et al.,* 2020). So limiting the amount of oxygen will limit the regeneration of NAD^+ in the respiratory chain and cause a decrease in the NAD^+/NADH ratio. This condition will limit XDH activity and cause xylitol to accumulate as a result of excretion by cells (Felipe Hernández-Pérez *et al.*, 2019). Based on facts, the effect of dissolved oxygen (DO) concentration on XR and XDH activity in *M. guilliermondii* InaCCY65 that produces xylitol from xylose will be interesting to study.

The study aimed to determine the effect of dissolved oxygen in the fermentation of sugarcane trash hydrolysate into xylitol. DO was varied in the aeration and agitation of a 3L scale bioreactor fermentation. From this research, optimum oxygen conditions were obtained, characterized by an increase xylitol. From this research, optimum oxygen conditions were obtained, characterized by an increase and efficiency in xylitol. From this research, optimum oxygen conditions were obtained, characterized by an increase and efficiency in xylitol. The hope is that bioconversion of sugarcane trash hydrolysate into xylitol can be developed efficiently

MATERIALS AND METHODS

Sugarcane trash hydrolysate preparation

Sugarcane trash (SCT) was hydrolyzed with 1.8% (w/v) maleic acid and heated using a microwave for 30 minutes (Hermiati *et al.,* 2020). Furthermore, the hydrolysate was sterilized using a 0.22 µm filtration membrane. SCT hydrolysate was vacuum-concentrated at 60ºC and 1 x 104 Pa and stored at 4ºC (Fan *et al.,* 2020). The sterilized hydrolysate was used for xylitol fermentation.

Microorganism and media

M. guilliermondii InaCCY65 from Indonesian Culture Collection (InaCC), National Research and Innovation Agency (BRIN). InaCCY65 strain was preserved on yeast peptone glucose (YPG) medium (10 g/L yeast extract (Difco, USA), 20 g/L peptone, and 20 g/L glucose). YPG medium was sterilized using an autoclave at 121ºC for 15 minutes at 1 atm.

Yeast Peptone Mineral (YPM) medium was used for bioconversion of SCT into xylitol using a bioreactor. Composition YPM medium with 10 g of $(NH_4)_2SO_4$, 2.4 g of KH_2PO_4, 0.2 g of $MgSO_4$, and 0.3 g of $CaCl_2$. YPM medium was sterilized at 121ºC for 15 minutes. SCT hydrolysate was supplemented into the fermentation medium as carbon and nitrogen sources.

Oxygen transfer coefficient and concentration of dissolved oxygen

The k_La was calculated using the dynamic gassing-out approach, which involves calculating the rate of recovery of DO concentration from the air after nitrogen degassing of the liquid phase (Singh *et al.,* 2024). The k_La was investigated at various agitation speeds, the following correlations were found: k_La = 20/h for 1.0 vvm, 100 rpm; k_La = 30/h for 1.0 vvm, 150 rpm; k_La = 45/h for 1.0 vvm, 200 rpm; and k_La = 85/h for 1.0 vvm, 300 rpm. Using an Ingold polarographic electrode, the

DO content in the liquid phase was continually measured and adjusted by varying the agitation speed (100-500 rpm) and aeration rate (0.2-l.0 vvm).

Bioconversion of sugarcane trash into xylitol in bioreactor 3L scale

M. guilliermondii InaCCY65 inoculum was prepared by cultivating the yeast in YPX (yeast extract 10 g/L, peptone 20 g/L, and xylose 20 g/L) in 250 mL flask filled with 50 mL of medium. Cultivations were carried out in an orbital shaker at 150 rpm, 30ºC for 18 h. Late exponential-phase cells were collected by centrifugation at 8000 rpm, 4ºC for 5 minutes, and the pellet formed was washed with sterile distilled water and resuspended directly into the medium to be used in the fermentation. Xylitol fermentation was carried out by adding 10x YPM media and *M. guilliermondii* InaCCY65 preculture (OD_{600} nm= 0.5) until the total culture volume was 2000 mL. Bioreactor Eppendorf New Brunswick Bioflo/celligen 115 type was used as a reactor fermentation. The culture was incubated at 30ºC for 48 hours. All experiments were performed in triplicate. For example, a total of 1 mL of culture sample was taken at fermentation time intervals of 0, 3, 6, 9, 24, 27, 30, 33, and 48 hours after incubation. The parameters analyzed were cell concentration (OD_{600} nm) using a spectrophotometer, as well as fermentation products (xylose and xylitol) using HPLC. The samples were also analyzed for the activity of XR and XDH.

Xylose reductase activity

After being suspended in 7 mL of 50 mM KPi buffer (pH 6.0) containing 7 µL of 100X Halt Protease inhibitor cocktail and 7 µL of 1 M dithiothreitol, the yeast cell around 10 mg/mL was extracted from the culture. The cell suspension was lysed using sonicator. The cell lysates were separated using centrifugation (22.000 × g for 20 minutes at 4°C) (Terebieniec *et al.,* 2021). The supernatant was designated as cell extract. XR activity assay was carried out at 30°C in 50 mM KPi buffer (pH 6.0) containing the appropriate amount of the cell extract, 200 mM D-xylose substrate, and 0.2 mM NADPH. The reaction begins with the addition of NADPH to the reaction mixture. XR activity was determined by monitoring the decrease in absorbance at λ340 nm as NADPH consumption activity (Louie *et al.,* 2021). One unit of XR activity is defined as the consumption of 1 mole of NADPH per minute under specified conditions.

Xylitol dehydrogenase activity

XDH activity was measured by the reduction of NAD^+ as measured by increase in absorbance at 340 nm upon the addition of xylitol (Li *et al.,* 2021). The activity reaction included in 50 mM xylitol, 1 mM NAD^+, 10 mM $MgCl_2$, 50 mM glycine-sodium hydroxide buffer at pH 9.0, and enzyme solution in a final volume of 1 mL. After 15 minutes of incubation at 45°C, the solution was brought to a boil for 10 minutes. One unit of the recombinant XDH activity was defined as the amount of enzyme required to liberate one µmol of NADH per minute under assay conditions (Regmi *et al.,* 2024).

Products fermentation analysis

Dry cell weight was determined by measuring the optical density at 600 nm (OD600). Active cell was estimated as viable cells using colony forming units (CFU) plated on yeast peptone dextrose agar (YPD) medium. A standard curve was created between cell concentration and cell dry weight and fermentation products were analyzed by HPLC. Samples were centrifuged at 8000 rpm agitation for 5 minutes at 4ºC to remove the cells for extracellular metabolite analysis. Xylose and xylitol produced in the culture supernatant were quantified using a HPLC system (Shimadzu LC-20AB, Japan) equipped with detection of the compound used refractive index detector (RID) and Aminex column 87 HPX Biorad. Xylose and xylitol were eluted using 5 mM H_2SO_4 solution with a flow rate of 0.6 mL/min, oven temperature of column was 60ºC, injection volume of sample is 20 µL, and elution time of sample is 30 minutes.

The kinetic parameters calculation

The calculation of the fermentation kinetic parameters was done using the formulae listed as follows:

$$\text{Consumption rate of xylose, XCR } \left(\frac{\text{g}}{\text{Lh}}\right) = \frac{\text{Xylose}_{\text{initial}} \left(\frac{\text{g}}{\text{L}}\right) - \text{Xylose}_{\text{residual}} \left(\frac{\text{g}}{\text{L}}\right)}{\text{Fermentation time (h)}} \quad (1)$$

$$\text{Xylitol conversion yield, Yp/s } \left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{Xylitol } \left(\frac{\text{g}}{\text{L}}\right)}{\text{Xylitol}_{\text{initial}} \left(\frac{\text{g}}{\text{L}}\right)} \quad (2)$$

$$\text{Xylitol productivity } \left(\frac{\text{g}}{\text{Lh}}\right) = \frac{\text{Xylitol}_{\text{final}} \left(\frac{\text{g}}{\text{L}}\right) - \text{Xylitol}_{\text{residual}} \left(\frac{\text{g}}{\text{L}}\right)}{\text{Fermentation time (h)}} \quad (3)$$

$$\text{Xylitol yield } \left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{Xylitol}_{\text{final}} \left(\frac{\text{g}}{\text{L}}\right) - \text{Xylitol}_{\text{initial}} \left(\frac{\text{g}}{\text{L}}\right)}{\text{Xylose}_{\text{initial}} \left(\frac{\text{g}}{\text{L}}\right) - \text{Xylose}_{\text{residual}} \left(\frac{\text{g}}{\text{L}}\right)} \quad (4)$$

The amounts of each media component in the fermentation medium at the time of fermentation are indicated by the word's residual and final. The xylitol titer (g/L) reaches its peak during the fermentation period.

RESULTS

Xylitol production under variation of oxygen transfer coefficient

The effect of k_La on xylitol production by *M. guilliermondii* InaCCY65 was carried out in a 3L scale bioreactor. Xylitol fermentation conditions were varied in several k_La conditions. InaCCY65 yeast cell growth increased at k_La 85/h. and the growth cell of InaCCY65 was decreased under k_La conditions below 30/h (Figure 1A). As the k_La number is increases the growth cell of InaCCY65 become higher.

The activities of *M. guilliermondii* InaCCY65 cells grew under several k_La conditions, xylose consumption decreased at k_La 30/h and k_La 20/h (Figure 1B). Xylose at k_La 45/h and 80/h decreased relatively faster than at 20/h and 30/h. Under these conditions, no xylose remains (Figure 1B). Meanwhile, xylose decreased more slowly than in the two previous conditions at k_La 20/h and 30/h. After forty-eight hours of incubation, xylose remained 12 g/L and 9 g/L, respectively. These results show that the higher the k_La, the higher the xylose consumption.

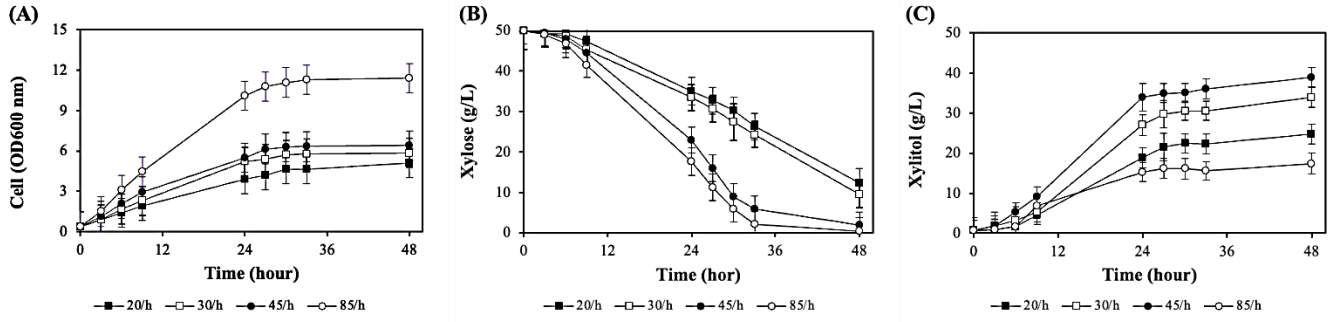

Figure 1. Response of cell growth (A), xylose consumption (B), and xylitol production of *M. guiellermondi* InaCCY65 in variation of k_La. *(Respon pertumbuhan sel (A), konsumsi xilosa (B), dan produksi xilitol (C) oleh M. guiellermondi InaCCY65 dalam variasi k_La).*

Simultaneously with increased cell growth and xylose consumption at k_La 85/h, the opposite condition occurred with low xylitol formation (Figure 1C). At k_La 85/h there was a decrease in xylitol formation compared to k_La conditions. The highest xylitol was obtained at k_La 45/h conditions forty-eight hours after incubation with concentration 39 g/L. Lower xylitol production occurred in other k_La conditions, the higher k_La make decreasing in xylitol production. The k_La variation conditions above made the conversion of xylose to xylitol by *M. guilliermondii* InaCCY65 is influenced by k_La number. The resume that as k_La increases, InaCCY65 cell growth increases, xylose consumption increases, and xylitol production decreases.

Xylitol production under variation of aeration

The aeration effect on xylitol production by *M. guiellermondi* InaCCY65 was analyzed at variations of 0, 0.7, 1.0, and 1.5%. Figure 2A-C showed the cell growth, xylose consumption, and xylitol production by *M. guiellermondii* InaCCY65 under different aeration conditions. The highest cell growth of *M. guiellermondii* InaCCY65 occurred at aeration 1.5%, especially in the 24 h after incubation. The smaller the aeration (0, 0.7, and 1%), the lower the cell growth (Figure 2A). The specific condition under these aeration conditions there was a phenomenon of slowing down the maximum growth of InaCCY65 cells at the 33 h after incubation.

The growth cell can make xylose as a substrate decreases in concentration. The decreasing of xylose concentration is means that substrate consumption occurs by InaCCY65 cells. The fastest rate of xylose consumption occurred at 1% aeration of 3.16 g/Lh which occurred at the 9 h after incubation. The lowest level of xylose consumption occurred at 1.5% aeration of 0.82 g/Lh are in 9 h after incubation.

The increasing of xylose consumption due to the highest xylitol production by the InaCCY65 strain was obtained at 1% aeration after 33 h of incubation with a concentration of 39 g/L. In aeration condition of 0.7%, 0%, and 1.5% there were a decrease value in xylitol production such as 34, 25, and 18 g/L respectively (Figure 2B). Thus, xylose consuming by *M. guiellermondii* InaCCY65 and it will be converted into xylitol. The xylitol yield during fermentation at 1.0% aeration at 33 h was 0.78 g/g. The xylitol yield was higher than during fermentation under other aeration conditions such as 0.50, 0.68, and 0.39 g/g at 0, 0.7, and 1.5% aeration respectively (Figure 2C).

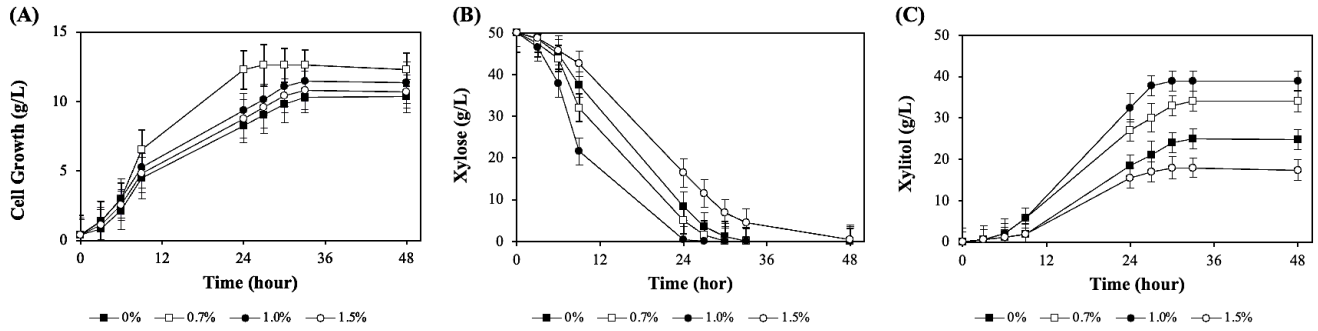

Figure 2. Response of cell growth (A), xylose consumption (B), and xylitol production of *M. guiellermondii* InaCCY65 in variation of dissolved oxygen concentration. *(Respon pertumbuhan sel (A), konsumsi xilosa (B), dan produksi xilitol (C) oleh M. guiellermondi InaCCY65 pada variasi konsentrasi oksigen terlarut).*

Based on aeration conditions variations on above the conversion of xylose become xylitol by *M. guilliermondii* InaCCY65 is influenced by aeration conditions. Existing data shows that increasing aeration relate to the increasing of InaCCY65 cell growth (1.5% aeration). This condition causes an increase in xylose consumption and xylitol production, the highest xylose consumption rate was obtained at 1.0% aeration conditions, and the highest xylitol production was obtained at 1.0% aeration.

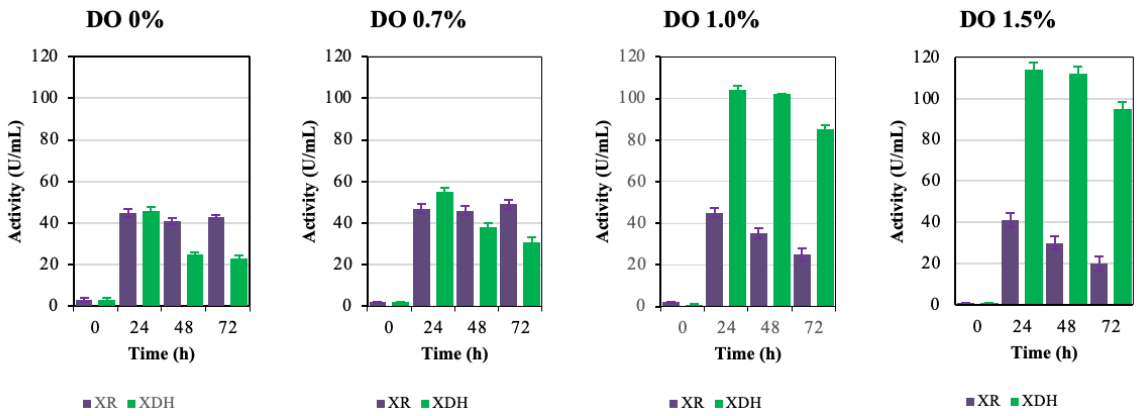

Figure 3. Xylose reductase (XR) and xylitol dehydrogenase (XDH) activities of *M. guilliermondii* InaCCY65 under varying dissolved oxygen concentration. *(Aktivitas xilosa reduktase (XR) dan xilitol dehidrogenase (XDH) dari M. guiellermondi InaCCY65 dalam variasi konsentrasi oksigen terlarut).*

The effect of DO levels on xylitol production by *M. guiellermondii* InaCCY65 can be explained by knowing the activities of XR and XDH (Fig 3). The XR activity (xylose reduction to xylitol) was found to be maximum at a DO concentration of 0 and 0.7% and the XR activity of both conditions was relatively stable from 24 h to 72 h after incubation due to DO 1.0 and 1.5% there was a decrease in XR activity after 48 hours of incubation.

At DO levels of 0% and 0.7%, the highest XDH activity was obtained at 24 h after incubation. After that, XDH activity decreased by around 54% and 69%, respectively at DO 0% and 0.7%. Otherwise at DO levels of 1.0% and 1.5% there was an increase in XDH activity in both conditions compared to DO 0% and 0.7% up to 189-300%. The highest XDH activity was obtained at 24 hours after incubation and decreased after 48 hours of incubation. This condition occurs at DO levels of 1.0% and 1.5%. The activity values of the two enzymes was mentioned previously XR and XDH are influenced by DO levels during the fermentation of xylose to xylitol by *M. guiellermondii* InaCCY65.

DISCUSSION

M. guilliermondii is an unconventional yeast that naturally assimilates xylose. Yan *et al.*, (2021) reported that this yeast is a cell factory for xylitol production. In the metabolism of M. guilliermondii, the enzyme XR catalyzes the conversion of xylose to xylitol. Where the function of this enzyme depends on nicotinamide adenine dinucleotide (NAD) hydrogen (H) (Atzmüller *et al.*, 2020). Furthermore, XDH-dependent NAD^+ converts xylitol to xylulose. Low oxygen supply results a low NAD^+/NADH ratio, which promotes xylitol buildup (Figure 4). Otherwise, this condition can reduce the carbon flow through the pentose phosphate phatway (PPP), which is primarily required for NADH renewal. It can be concluded that regulated oxygen supply contributes significantly to xylitol synthesis (Kumar *et al.*, 2022).

The results of the bioconversion of xylose from sugar cane leaf hydrolysate into xylitol by *M. guilliermondii* InaCCY65 are influenced by k_La conditions. Existing data showed k_La increases, cell growth increases, xylose consumption increases, and xylitol production decreases by InaCCY65 cells. The growth of *M. guilliermondii* InaCCY65 cells implies good metabolic activity and membrane integrity during fermentation (Yan *et al.*, 2021). The existence of metabolic activity is known by the consumption of xylose during fermentation and production of xylitol by InaCCY65 cells. In micro-aerobic conditions (below 45 h k_La), cell growth decreased and xylitol productivity was reduced. When k_La increased from 20/h to 85/h, cell growth showed an increase from 4.98 to 13.1 g/g. The maximum xylitol production was 35.8 g/l at k_La 30/h, while the maximum xylitol productivity was 0.667 g/Lh at k_La 45/h. When k_La was increased from 45/h to 85/h, there was a

marked increase in cell growth and there are increasing number of xylitol production. The xylose consumption showed a difference relate with k_La range and changes in oxygen supply and xylose carbon flux from xylitol production to growing cells (Veras *et al.,* 2019; Prabhu *et al.,* 2020).

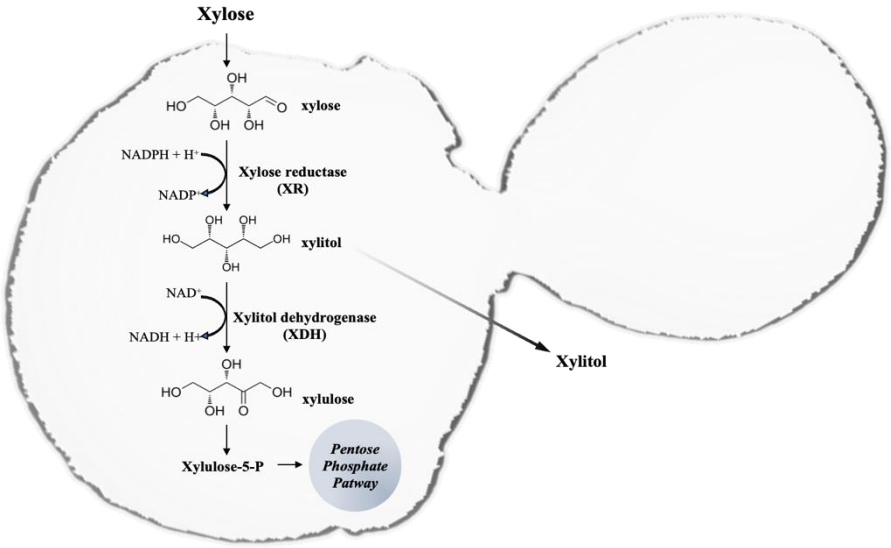

Figure 4. The metabolic pathways for xylose metabolism in yeast. *(Jalur metabolisme xilosa pada khamir).*

The results of aeration optimization show that along with increasing aeration there is an increase in InaCCY65 cell growth (1.5% aeration). However, this condition does not necessarily cause an increase in xylose consumption and xylitol production. The highest xylose consumption rate was obtained at 1.0% aeration conditions, and the highest xylitol production was obtained at 1% aeration. Under micro-aerobic conditions (DO below 1.5%), cell growth decreases and xylitol productivity also decrease. The condition under aerobic conditions (DO concentration above 1.5%), cell growth was markedly increased, and xylitol production was suppressed. The optimal xylitol production within DO must be maintained at a low level. The maximum xylitol concentration from 50 g/l xylose is 39 g/L at a DO concentration of 1.0%, while the maximum xylitol productivity is 0.78 g/L at a DO concentration of 1.0%. In this DO range, changes occur in cell growth, xylose consumption and xylitol production. This is related to the oxygen content which changes the xylose carbon flux into xylitol. The results of this study indicate that DO concentrations must be carefully controlled for efficient xylitol production, and low DO levels (in the range of approximately 0-1.0%) are best for xylitol production by *M. guiellermondii* InaCCY65.

The effect of oxygen in the bioconversion of xylose to xylitol also has an impact on the XR and XDH activities of *M. guiellermondii* InaCCY65. The high level of xylose reductase activity resulted in xylitol production of up to 39 g/L at aeration 0.7% (Figure 2C). The XDH connected to NAD^+, xylitol is removed from cells or oxidized to xylulose. XDH activity increased to 189-300% with increasing DO concentration, namely at 1.0% and 1.5%. In both conditions, high levels of XDH activity cause most of the xylitol present to be converted into xylulose, which is then metabolized into cell material (Figure 4). Thus, less xylitol production and more cells accumulate. These findings indicate that oxygen limitation is the main factor in the formation of xylitol in accordance with the results of several previous studies (Kim and Kim 1997; Singh *et al.,* 2024b).

High DO concentrations cause NADH to be oxidized to NAD^+, and high NAD^+/NADH ratios cause the oxidation of xylitol to xylulose. This condition causes xylitol to be further metabolized into cell material. Thus, the xylitol product becomes less with more cells accumulate (Yan *et al.,* 2021). The best aeration conditions for xylitol production by *M. guilliermondii* InaCCY65 are 0.7% as a comparison of xylitol productivity from several yeasts using different hydrolysates is shown in Table 1. Xylitol yield from *M. guilliermondii* InaCCY65 was higher than other yeast strains. This means the yeast strain is more efficient in converting xylose from SCT into xylitol compared to

other microorganisms in the table. However, the xylitol productivity of strain InaCCY65 has a value below that of *C. tropicalis* ATCC13804 in wheat straw. Xylitol productivity is defined as the highest xylitol concentration at that time. The difference in xylitol productivity values is thought to be due to the use of different substrates and fermenter vessel volumes.

Table 1. Comparative reports of xylitol productivity from various hydrolysates (*Laporan komparatif produktivitas xylitol dari berbagai hidrolisat*)

Microorganism *(Mikroorganisme)*	**Hydrolisate** (*Hidrolisat*)	**Vessel** (*Kontainer*)	**Xylitol Yield** (*Hasil Xylitol*) **(g/g)**	**Xylitol Productivity** (*Produktivitas Xylitol*) **(g/Lh)**	**Reference** (*Referensi*)
M. guilliermondii InaCC Y65	Sugarcane trash	3 L bench fermentor	0.78	1.20	Present study
C. tropicalis ATCC 13804	Wheat straw	1 L bioeractor	0.58	1.49	(Singh *et al.,* 2024c)
C. tropicalis ATCC201935	Sugarcane bagasse	1 L bench fermenter	0.60	0.33	(Hernández-Pérez *et al.,* 2022)
C. guilliermondii FTI 20037	Sugarcane bagasse	2.4 L bioreactor	0.65	0.28	(Vaz de Arruda *et al.,* 2017)
C. tropicalis MTCC 6192	Rice straw	14 L bioreactor	0.60	0.26	(Singh *et al.,* 2021)

CONCLUSION

Bioconversion of sugarcane trash hydrolysate into xylitol by *M. guilliermondii* InaCCY65 is influenced by k_La and aeration conditions. As k_La increases, cell growth increases, xylose consumption increases, and xylitol production by InaCCY65 cells decreases. Optimum k_La conditions were obtained at 45/h with xylitol production of 39 g/L. The optimum aeration in the bioconversion of sugarcane trash hydrolysate into xylitol by *M. guilliermondii* InaCCY65 is 1.0%. The optimum aeration condition such as xylitol yield and xylitol productivity are 0.78 g/g and 1.20 g/Lh. The effect of oxygen in the bioconversion of xylose to xylitol also has an impact on the XR and XDH activities of *M. guiellermondii* InaCCY065. The results showed the dissolved oxygen concentration must be controlled during xylitol bioconversion to obtain efficient xylitol production.

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AUTHOR CONTRIBUTIONS

AT: conceptualization, research performing, writing – original draft and editing, visualization, methodology, formal analysis, and data curation. AK: conceptualization, validation, supervision, data curation, and review. SL: validation, supervision, and review. AM: conceptualization, validation, supervision, and review.

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