Efficiency of Xylitol Production from _Meyerozyma caribbica_ Y67 with Cell Initiation and Volume Fermentation

H Saputra¹, A Thontowi¹, L N Kholida¹, and A Kanti²

¹Research Center for Biotechnology, Indonesian Institute of Sciences, Jln. Raya Bogor Km 46, Cibinong, West Java 16911, Indonesia
²Research Center for Biology, Indonesian Institute of Sciences, Jl. Raya Bogor km. 46, Cibinong, Bogor, West Java 16911, Indonesia

Email: march4hendra@yahoo.com

Abstract. One of the rare types of pentose sugar is xylitol, which has various benefits in the field of food and medicine. Xylitol is one of the results of xylose fermentation and few microorganisms are able to produce it. _Meyerozyma caribbica_ Y67 is one of the yeast collections of Indonesian Culture Collection (InaCC) which can produce xylitol. The production of xylitol about cell initiation and media volume gave different results for each factor. The fermentation conditions were using erlenmeyer 250 mL, agitation 150 rpm and 30°C temperature. The parameters analyzed were dry cell weight (DCW), xylose, and xylitol. The results of this study showed that cell initiation with an optical density at 600 nm (OD₆₀₀) = 5 (∼1.07x10⁷ CFU or 3.980 g/L) had the highest efficiency in producing xylitol for 24 hours of fermentation, 51,099%; specific growth rate (µ): 0.069. In the media volume variable, for 24 hours fermentation, the high-efficiency value of 20% volume erlenmeyer is 55,708%; (µ): 0.082 and 48 hours fermentation is 40% volume erlenmeyer which is 71,959%; (µ): 0.048. The research is expected to be used as a scale up recommendation for the industry.

1. Introduction
Xylitol is a pentahydroxy sugar-alcohol which exists in a very low quantity in fruits and vegetables and it can be produced by chemical and biotechnological processes [1]. Xylitol has a sweet taste like sucrose but is low in calories and good for dental health. In the industrial scale, xylitol has been applied in food and pharmaceutical, such as candies, chewing gum, and dental care products. Biotechnology is one way to get xylitol when xylose was converted to xylitol with the ability of microorganisms. Some xylitol-producing yeasts, namely _Candida guiliermondii_ [2], _C.tropicalis_[3-4], _Pichiapastoris_ [5], _Saccharomyces cerevisiae_ [6], _Scheffersomycesamazonensis_ [7] and others. Then, xylose as carbon source also can get from biomass, especially lignocellulose, for examples sugarcane straw[2], sugarcane bagasse [6], rice straw [8] and sorghum straw [9].

Indonesia has many diversities, especially yeast and more of them to collect in Indonesian Culture Collection (InaCC) and its chance to explore. The history of research recorded in xylitol production using yeast is still dominated by the _Candida sp_. however, many yeasts are also capable of producing xylitol with its own superiority as the yield of xylitol production is better and resistant to inhibitor compounds than _candida sp _and one of them is _M.carabbbica_. In nature, _M.carabbbica_ has a special ability as a plant biocontrol, for example in mango (_Mangiferaindica_ L.) cv. "Ataulfo" has a pathogen microbe is _Colletotrichum gloeosporioides_ and the yeast _M.carabbbica_ was evaluated for their effectiveness against the pathogen [10].
There are some strategies to get the efficiency of the xylitol fermentation, it can be augmented using high cell concentrations, which can be achieved with varying initial cells. Moreover, it can get yield faster but it also can give a negative effect on production due to by-product [11]. Then, volume media in erlenmeyer also give a variation of xylitol yield. C.guilliermondii FTI 20037 can increase by more than 10 % yields xylitol [2].

In this paper, optimizing fermentation conditions such as initial cell concentrations and volume media is important for obtaining the maximum rate of xylitol production and maximum yield of xylitol from xylose. Kinetic parameters calculation has been correlated in this work.

2. Method

2.1 Microorganism and Media
Yeasts were from the collection of Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences (LIPI). The yeast used M.carabbbica Y67, this strain was maintained by selective growth on yeast peptone (YP) medium (10 g/L yeast extract (Difco, USA) and 20 g/L peptone supplemented with 20 g/L glucose (YPD medium). Xylose was added to YP medium to produce YPX medium and the YPX was used as the fermentation media in this study. YPX composed by yeast extract, 10 g/L, peptone 20 g/L, and n-xylose (Merck, Germany) 20 g/L. All mediums were sterilized using an autoclave (Tomy SX-500, Japan), at the temperature of 121ºC for 15 minutes at 1 atm.

2.2. Inoculum Preparation and Fermentation
For inoculum preparation, M.carabbbica was prepared by cultivating the yeasts in the synthetic medium in 250 mL erlenmeyer flask filled with 25 mL of medium. Cultivations were carried out in an orbital shaker at 150 rpm, 30 ºC for 18-24 h. Late exponential-phase cells were collected by centrifugation at 8000 rpm, 4 ºC for 5 min, and the pellet formed was washed with sterile distilled water and resuspended directly into the medium to be used in the fermentation.

2.3. Xylitol Fermentation Conditions
Medium fermentation (xylose concentration of ~20 g/L), the initial cell concentrations employed to start fermentation in YPX medium were to obtain a cell concentration of optical density 1,5 and 10 at 600 nm equal to 1.188, 3.98, and 7.691 g/L respectively. Fermentation in the synthetic medium in 250 mL erlenmeyer flask filled with 25 mL of medium. In vary the volume availability the batch fermentations were carried out in 250 mL Erlenmeyer flasks, containing 50; 100 and 125 mL of the fermentation. The initial cell biomass concentration in all the flasks was to obtain a cell concentration of optical density 1 at 600 nm. The flasks were incubated on a rotary shaker at 30 ºC, 150 rpm for 48 h. The sample of each main culture was collected periodically at 0, 3, 6, 9, 24, 33 and 48 hours of the incubation period. Then, the collected samples were centrifuged and the supernatant was prepared for optical density measurement and HPLC analysis.

2.4. Analytical Methods
Cell dry weight was determined by measuring the optical density at 600 nm (OD600). For cell alive was estimated as viable cells, using CFU (colony forming units) plated on yeast peptone dextrose agar (YPD) medium. Samples were centrifuged for 5 min at 4 ºC and 8000 rpm 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 H2SO4 solution with a flow rate of 0.6 mL/min, oven temperature for column of 60ºC, injection volume of 20µL, and elution time of 30 minutes.
2.5. Kinetic Parameters Calculation

The xylitol conversion yield ($Y_{P/S}$, g g$^{-1}$) was defined as the ratio of the concentration of xylitol produced and xylose consumed when *M. carabica* was used as the sole microorganism.

3. Results and Discussions

*M. caribbica* Y67 was one of many yeasts collection of Indonesian Culture Collection (InaCC). Then it has ability to consume xylose for growing and producing xylitol. Researchers have not yet reported much of their research on xylitol and this makes its novelty value. As seen on the figure 1, the growth rate of *M. caribbica* Y67 in OD$_{600}$ : 10 was faster than another at the same time. Furthermore, the specific growth rate was 0.117 for OD$_{600}$ : 10 (Table 1), its high than another. Increased growth of OD 1, 5, and 10 is fifteen times, five times, and twice respectively. The high increase in OD: 1 growth was due to the constant amount of carbon sources, besides the low number of cell densities compared to the others.

**Figure 1.** Dry cell weight of *M. caribbica* Y67 during the fermentation in the Erlenmeyer flask scale. Initial OD$_{600}$ : 1 ($\approx$2.4 x 10$^6$ cfu) (closed circles), initial OD$_{600}$ : 5 ($\approx$1.07x10$^7$ cfu) (closed squares) and initial OD$_{600}$ : 10 ($\approx$1.1 x 10$^7$ cfu) (closed triangles)

**Figure 2.** DCW, xylitol, and xylose from *M. caribbica* Y67 in variation volume media. Initial OD$_{600}$ : 1 (a), initial OD$_{600}$ : 5 (b) and initial OD$_{600}$ : 10 (c), DCW (solid), xylitol (dash) and xylose (round dot)
Figure 2 shows the xylitol production from *M. caribbica* Y67 in variation initial cell. Until in 24 hours fermentation in 150 rpm and 30 °C, yeast had consume whole xylose in medium and produce xylitol. *M. caribbica* Y67 were the quickest strains to consumed xylose. In initial OD600: 1, xylitol produced was 4.295 g/L and it was the lowest (figure 2a). In others hand, producing xylitol by *M. caribbica* can up to 7 g/L or almost twice, in initial OD600: 5 and 10 were 8.574 g/L and 7.49 g/L respectively (figure 2b and 2c). Xylose consumption is also influenced by the number of cells in the media. The higher the number of cells in the media the faster consumption of xylose. The figure 2 shows OD 5 with a cell count of about $1.07 \times 10^7$ cfu faster than OD 1, nevertheless, OD 10 has different conditions. The possibility that occurs due to the number of cells that experience osmotic stress and cause xylose consumption is not optimal.

The highest specific growth ($\mu$) of *M. caribbica* Y67 in OD 1 with amount 0.114/ hour (Table 1). This fact due to an amount cell is not numerous and carbon source is stable. In addition, the value corresponds to the value $Y_{x/s}$ (g/g) which shows the highest value compared to the others. However, in xylitol production the number of cells with OD 5 is the highest with the highest $Y_{p/s}$ (g/g) value and it has an efficiency value of 51.099% of theoretical value. Moreover, in 24 hours fermentation OD 5 has xylitol volumetric productivity (g L$^{-1}$ h$^{-1}$) a twice than OD 1. A high initial cell density had no positive effect on the maximum product but it also can the increased amount of by-products [11].

**Table 1.** Kinetic parameters of *M. caribbica* fermentation for each evaluated condition

| Parameters                        | OD 1  | OD 5  | OD 10 |
|-----------------------------------|-------|-------|-------|
| $\mu$ (/hour)                     | 0.114 | 0.069 | 0.044 |
| $Y_{x/s}$ (g g$^{-1}$)            | 0.880 | 0.830 | 0.785 |
| $Y_{p/s}$ (g g$^{-1}$)            | 0.229 | 0.457 | 0.421 |
| Efficiency (%)                    | 25.452| 51.099| 44.876|
| Xylitol volumetric productivity (g L$^{-1}$ h$^{-1}$) | 0.179 | 0.357 | 0.312 |

On variations in the volume of fermentation media, OD 1 was used because of the preparation factor. Figure 3 shows graph of growth of *M. caribbica* Y67 during 48 hours and in conditions are total xylose 20 g/L, agitation 150 rpm and 30 °C. The highest of growth for yeast in these condition was in 20% total medium with seventeen times the beginning of fermentation. In others side, the growth of *M. caribbica* Y67 in 40% and 50% of total media were more slowly with growth 10 times and 6 times respectively.

**Figure 3.** Dry cell weight of *M. caribbica* Y67 during the fermentation in the Erlenmeyer flask scale. 20% medium (closed circles), 40% medium (closed squares) and 50% medium (closed triangles).
Figure 3 shows the xylitol production from *M. caribbica* Y67 in variation total volum media. First, until in 24 hours fermentation only yeast with total volume 20% had almost consume whole xylose in medium. Futhermore, in conditon total 40% and 50% that is more than a third xylose staying in medium. Then, in 20% total media had the highest xylitol that is 9.625 g/L in 24 hours fermentation with efficiency was more than half (Figure 3a). In addition, xylitol volumetric productivity (g L\(^{-1}\) h\(^{-1}\)) of 20% total media was the best for this research (Table 2). Total volume 40% and 50% have slowly consumption xylose and production xylitol (Figure 3b and 3c).

Second, in 48 hours fermentation, yeast is evidently to consume xylitol or we call re-consumed the xylitol product. It show in figure 3a, *M. caribbica* took xylitol to start at the 27th hour. Possible yeast use enzyme, named xylitol dehydrogenase (McXDH) to produce by-product [10]. This happens because there was no xylose in the media for carbon source. Different conditions are the case with figures 3b and 3c where xylose is still available as a carbon source. Finally, the effect of this condition was accumulation xylitol until 48 hours and the highest xylitol is in variation volume 40% total medium for 48 hours fermentation with xylitol an amount is 12.583 g/L.

![Graphs showing xylitol production](image)

**Figure 4.** DCW, xylitol, and xylose from *Meyerozymacaribbica* Y67 in variation volume media. (a) 20% medium, (b) 40% medium, (c) 50% medium, DCW (solid), xylitol (dash) and xylose (round dot)

In 24 hours fermentation, the highest specific growth (μ) of *M. carabbica* Y67 is 20% total media with amount 0.082/ hour and this was related to the value the \(Y_{\mu/s}\) of the treatment. In addition, it has an efficiency value of 55.706% of theoretical value where xylitol volumetric productivity (g L\(^{-1}\) h\(^{-1}\)) is 0.401 and this value is the highest in this research. Fermentation with reduced initial oxygen availability, xylose began to be consumed at a constant rate sooner than in those with the increased initial oxygen availability [2].

In 48 hours fermentation, the highest specific growth (μ) of *M. carabbica* Y67 is 20% total media with amount 0.060/hour and it also releated to \(Y_{\mu/s}\) value. However, for xylitol production the highest
was xylitol volumetric productivity at 0.262 g L\(^{-1}\) h\(^{-1}\). Besides, it can get highest efficiency at 71.959% of theoretical value and \(Y_{ps}\) as 0.660 g/g. \textit{M. carabbica} Y67 was evaluated and compared with \textit{Meyerozyma carabibica} JA9 and it was higher than \textit{M. carabibica} JA9 with reaching product yield and productivity as 0.54 (g/g) [13]. However, it was little lower with \textit{Candida tropicalis} DMKU-XE235 as 0.69 g/g [14]. This initial cell and volume fermentation report provides suggestions the next study toward according to needs what the efficiency production or xylitol volumetric productivity. In the future, to develop xylitol for dental health with xylitol produced in addition to \textit{candida} sp [15].

**Table 2.** Kinetic parameters of \textit{M. carabibica} fermentation for each evaluated condition

| Parameters                          | 24 hours          | 48 hours          |
|-------------------------------------|-------------------|-------------------|
|                                     | 20%   | 40%   | 50%   | 20%   | 40%   | 50%   |
| \(\mu\) (hour)                      | 0.082 | 0.056 | 0.052 | 0.060 | 0.048 | 0.037 |
| \(Y_{xs}\) (g g\(^{-1}\))         | 0.434 | 0.305 | 0.301 | 1.093 | 0.597 | 0.340 |
| \(Y_{ps}\) (g g\(^{-1}\))         | 0.562 | 0.563 | 0.491 | 0.343 | 0.660 | 0.660 |
| Efficiency (%)                      | 55.706| 37.029| 27.621| 37.365| 71.959| 65.361|
| Xylitol volumetric productivity (g L\(^{-1}\) h\(^{-1}\)) | 0.401 | 0.270 | 0.192 | 0.135 | 0.262 | 0.227 |

4. Conclusion

The ability of \textit{M. carabibica} Y67 to partially consumed xylose and produce xylitol in the synthetic medium can be considered a starting point for the development of technologies for xylitol production. Initial cell affects xylitol's production, a cell concentration of optical density 5 at 600 nm has the highest efficiency (%). This study suggested that the production of xylitol with volume media of 40% for 48 hours of fermentation for maximum yield.

5. Acknowledgement

This research was funded from the research fund by Insentif Riset Sistem Inovasi Nasional (INSINAS) project “Produksi Xylitol dari Biomassa Lignoselulosa sebagai Pemanis Alami Rendah Kalori Pencegah Obesitas” in the fiscal year 2019.

6. References

[1] Rehman U, Mushtaq Z, Zahoor T, Jamil A, Murtaza M A 2015 Crit Rev. Food Sci. Nutr. 55 (11) 1514-28
[2] Pérez H, Arruda P V, Felipe M G A 2016 Braz. J. Microbiol. 47(2) 489-96
[3] Kim S, Lee J, Sung B H 2019 Front. Bioeng. Biotechnol. 71-12
[4] Tizazu B Z, Roy K, Moholkar V S 2018 Bioresour. Technol. 268 247-258
[5] Cheng H, Lv J, Wang H, Wang B, Li Z, Deng Z 2014 Appl.Microbiol Biotechnol. 98 (8) 3539-52
[6] Baptista S L, Cunha J T, Romani A, Domingues L 2018 Bioresource Technology. 267 481-491
[7] Marton J M, Felipe M G A, Silva J B A, Pessoa A J 2006 Braz. J. Chem. Eng. 23 (9) 21-26
[8] Cadete R M, Cheab M A M, Viana A L, Oliveira E S, Fonseca C, Rosa C A 2016 World J Microbiol Biotechnol 32 207
[9] Guirimand G, Sasaki K, Inokuma K, Bamba T, Hasunuma T, Kondo A 2016 Appl. Microbiol. Biotechnol. 100 (8) 3477-87
[10] Sene L, Arruda P V, Oliveira S M M, Felipe M G A 2011 Braz. J. Microbiol. 42 (3) 1141-6
[11] Sundararam A, Krishna Murthy T P 2014 J. Appl. Environ. Microbiol. 2(4) 166–175
[12] Akinori M, Shigeaki S 2010 Appl. Biochem. Biotechnol. 162 1952–1960
[13] Wiphat S, Hidenobu K Poonsuk P, Yasuhisa A 2016 J Biosci. Bioeng. 123 (1) 20-27
[14] Trichez D, Steindorff, A S, Soares C E V F, Formighieri E F, Almeida J R M 2019 FEMS Yeast Research 19 (4) 1-15
[15] Junyapate K, Jindamorakot S, Limtong S 2014. Antonie van Leeuwenhoek 105 471–480
[16] Janakiram C, Kumar C V D, Joseph J 2017 Journal of Natural Science, Biology and Medicine 8 (1) 16-21