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UTILIZATION OF OIL PALM EMPTY FRUIT BUNCHES ENHANCED WITH MOLASSES FOR XYLITOL PRODUCTION

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ABSTRACT

A minimal oil palm empty fruit bunch (OPEFB) usage encourages increased OPEFB utilization to prevent its loss of economic prospects. The xylose and arabinose constituents of OPEFB's hemicellulose part can be utilized as raw materials for xylitol production. A high diabetes mellitus and periodontal disease prevalence in Indonesia makes xylitol a safe and helpful low-calorie sweetener alternative. This research explores the OPEFB molasses-supplemented media and compares Moniliella pollinis SP5 and other M. pollinis ethyl methanesulfonate (EMS) mutants in xylitol yields. It was found that M. pollinis SP5 accomplished 27% better performance in OPEFB+15%(v/v) molasses than in OPEFB from 0.48 g/g to 0.61 g/g. In addition, M4, M5, and M6 mutants from EMS mutagenesis generated more xylitol concentration in OPEFB + 15%(v/v) molasses compared to the SP5 strain. These findings provide insights into the potential of xylitol manufacture with OPEFB. For future research, it is recommended that xylitol production employing OPEFB be optimized.

Keywords: *Oil palm empty fruit bunches, Xylitol, Moniliella pollinis SP5, Ethyl methanesulfonate, Molasses, Bio-based product*

ABSTRAK

Minimnya penggunaan tandan kosong kelapa sawit (TKKS) mendorong pemanfaatan TKKS untuk menghindari hilangnya prospek ekonomi TKKS. Kandungan xilosa dan arabinosa pada bagian hemiselulosa TKKS dapat digunakan sebagai bahan baku dalam produksi xilitol. Tingginya prevalensi penderita diabetes melitus dan penyakit periodontal di Indonesia menjadikan xilitol sebagai alternatif pemanis rendah kalori yang aman dan bermanfaat. Studi ini bertujuan mengeksplorasi media TKKS dengan suplementasi molase dan membandingkan hasil produksi xilitol dari Moniliella pollinis SP5 dengan beberapa M. pollinis mutan lainnya dari hasil mutagenesis etil metanesulfonat (EMS). Dalam penelitian, ditemukan bahwa M. pollinis SP5 memiliki 27% performa produksi xilitol yang lebih baik, dari 0.48 g/g ke 0.61 g/g, di media TKKS+15%(v/v) molase daripada di media TKKS saja. Selain itu, mutan M4, M5, dan M6 dari mutagenesis menggunakan EMS, menghasilkan konsentrasi xilitol yang lebih tinggi di media TKKS+15%(v/v) molase dibandingkan dengan strain SP5. Hasil ini memberikan informasi mengenai potensial produksi xilitol menggunakan TKKS. Temuan ini memberikan wawasan tentang potensi pembuatan xilitol menggunakan TKKS. Untuk penelitian mendatang, diharapkan bahwa produksi xilitol menggunakan TKKS dapat lebih dioptimasi lebih lanjut.

Kata kunci: *Tandan kosong kelapa sawit, Xilitol, Moniliella pollinis SP5, Ethyl methanesulfonate, Molase, Produk berbasis bio.*

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INTRODUCTION

Indonesia is one of the most significant palm oil producers in the world, contributing approximately 59% of the world's palm oil production (Abidin 2023). According to Indriati et al. (2021), the total oil palm empty fruit bunch (OPEFB) waste reached 51.8 million tonnes in 2019. However, only 10% of the OPEFB waste has been utilized for compost and fuel (Fithri et al. 2020). Therefore, its utilization needs to be maximized to maintain its economic potential in the green and circular economy era. One way is to turn the OPEFB waste into a raw material for sugar alcohol, such as xylitol. Xylitol is a sugar alcohol with characteristics similar to conventional sugar, sucrose, in terms of aroma and taste. However, xylitol has 40% lower calories and is also lower in the glycemic index compared to sucrose (Stylianopoulou 2023). The use of xylitol as a low-calorie sweetener is proven safe for people with diabetes and can prevent *Streptococcus mutans* growth that causes periodontal illness (Salli et al. 2019; Benahmed et al. 2020). The high potential of xylitol utilization and the import-dependent supply of xylitol in Indonesia makes it crucial to explore independent xylitol production using biotechnological approach to support sustainable programs like the green economy and health independence (Arifan and Nuswantari 2020).

OPEFB comprises 46% cellulose, 23% hemicellulose, 16.5% lignin, and other components (protein, oil ashes) (Adiguna and Aryantha 2020). The hemicellulose component of OPEFB can be hydrolyzed through acid hydrolysis into glucose, xylose, and arabinose, which can be used in xylitol production by employing a yeast fermentation process (Mardawati et al. 2020). Xylitol can be made through fermentation techniques employing yeasts with the enzyme xylose reductase, which is capable of catalyzing the reduction of xylose into xylitol in the presence of NADPH as the coenzyme (Zhang et al. 2021). However, the lack of NADPH in the production medium

can hinder the high level of xylitol production, which is often seen as one of the bottlenecks in the xylitol industry that employs yeast as the cell factor (Singh et al. 2023). Thus, to ensure smooth conversion of xylose into xylitol, enhancement of OPEFB hydrolysate can be done by supplying the additional NADPH source like sucrose. The sucrose can be sourced from other agro-industrial wastes such as molasses. Molasses is an industrial cane sugar waste that contains large amounts of sugar like sucrose, making it suitable for energy and NADPH supplies during xylitol production (Karuppiah et al. 2019). The utilization of industrial wastes in xylitol production aims to lower the production cost in the industrial setting as well as serve as the alternative solution to tackle the accumulation of wastes in the environment (Bevilaqua et al. 2023; Liang et al. 2023). However, the usage of industrial wastes as the raw material in xylitol production only focused on one type of industrial waste which is OPEFB as shown in the study by Mardawati et al. (2022), Medina et al. (2018), and Meilany et al. (2020). At present, there are no studies that mention the utilization of OPEFB and molasses together in xylitol production. Hence, this research proposed an exploration study to evaluate the potential of the carbon enhancement in OPEFB hydrolysate through the addition of molasses which provides new insight in the utilization of two types of industrial wastes as the raw material in xylitol production. Moreover, the addition of molasses in xylitol production can also provide micronutrients that can increase the growth of the cell factory as well as buffering agent in the fermentation medium to prevent the fluctuation in pH level (Vučurović et al. 2018).

Even though the raw materials for xylitol production are available in many ways, efficient production through naturally occurring yeast such as *Candida tropicalis* was hardly achieved due to the lack of robustness, low yield, and cost-ineffective which was mentioned in the study by Bevilaqua et al. (2023). Therefore, this research tried to utilize non-naturally occurring yeast to produce xylitol by modifying the existing gene that is closely related to the required enzyme, xylose reductase, with a cost-effective mutation method such as chemical mutagenesis. *Moniliella pollinis (M. pollinis)* is a yeast that is known for its erythritol production due to the presence of erythrose reductase. However, it is found that erythrose

reductase and xylose reductase have a close phylogenetic relationship with a high similarity in the structure and the amino acid sequences. Therefore, strain optimization to mutate a gene that encodes for erythrose reductase to a gene that encodes for xylose reductase can be done through mutagenesis using mutagens such as ultraviolet light (UV) and ethyl methanesulfonate (EMS) (Cheng et al. 2018). In this research, the target EMS mutagenesis is *M. pollinis* SP5, a mutant type of M. pollinis that has already undergone UV mutagenesis. This approach was done to further randomly mutate the cell factory to improve the robustness and yield cost-effectively and unlock the new possibility of yeast-based xylitol production in the industrial setting. Nonetheless, *M. pollinis* SP5 was also evaluated for its performance before the EMS mutagenesis in xylitol production using the OPEFB hydrolysate that was supplemented with molasses. Hence, to summarize, this research was done to evaluate the potential of OPEFB as the main material and molasses as the supplementary material for the production of xylitol through fermentation methods, explore the performance of *M. pollinis* SP5 in xylitol production, and increase the production of xylitol by mutating *M. pollinis* SP5 using EMS.

MATERIALS AND METHODS

Time and Place

The research is done in the laboratory of the Indonesia International Institute for Life Science (i3L) for four months of intensive research time.

Materials and Equipment

Equipment used included an oven, *SX-700* Autoclave High-Pressure Steam Sterilizer, 1-L *Duran* bottles, pH meter, Biological Safety Cabinet *1300 Series A2-1384-G*, micropipettes, *TOU-50N* Orbital Shaker Incubator, *Thermo Scientific Dionex UltiMate 3000* HPLC, *R-100 Rotavapor* Rotary Evaporator, *B-100* Heating Bath, *V-100* Vacuum Pump, *F-105* Recirculating Chiller, *Pyrex* Erlenmeyer flasks (100 mL & 500 mL), and *Tecan Infinite M200* Multi-Detection Plate Reader. The materials included oil palm empty fruit bunches (OPEFB), xylitol (*Sigma-Aldrich*), xylose, arabinose, Potato Dextrose Agar and Broth (PDA & PDB), Phosphate Saline Buffer (PBS), yeast extract (*Himedia*), H_2SO_4, NH_4Cl, $Ca(OH)_2$, $MgSO_4.7H_2O$, KH_2PO_4, NaOH (*Merck*), deionized water, *M. pollinis* SP5 culture, Polyethylene glycol (PEG) 6000 (*Sigma Aldrich*), ethyl methanesulfonate (EMS) (*Sigma Aldrich*), sodium thiosulfate (*Sigma Aldrich*), micropipette tips, 1.5-mL microcentrifuge tubes, Petri dish, 10-mL serological pipettes (*Biologix*), centrifuge tubes (15 mL and 50 mL), 0.22-µm syringe filter (*Nest*), sterile gauze, 10-mL syringe (*OneMed*), *Whatman* No. 1 filter paper, 96-well microplate (*Iwaki*), and 2-mL HPLC vials (*Shimadzu*).

Methods

Oil Palm Empty Fruit Bunches (OPEFB) Preparation

OPEFB which was used in this research was obtained from an oil palm farm in West Java. It was blended by using a blender and cleanly washed. Furthermore, the pieces of OPEFB were dried overnight by using an oven. Dried OPEFB was then put in a box and stored at room temperature in the Indonesia International Institute for Life Science's (i3L) rooftop storage area.

OPEFB Pretreatment: Acid Hydrolysis and Neutralization

Acid hydrolysis of OPEFB was done by adding the dried OPEFB into a 1-L Duran bottle containing 2% H_2SO_4 with a ratio of 1:10. After that, the mixture-containing Duran bottle was autoclaved for one hour. The OPEFB hydrolysate was then filtered through cotton gauze and filtered with a vacuum pump. Next, the filtrate's pH was neutralized with the addition of $Ca(OH)_2$ until the pH reached 5.5. The neutralized hydrolysate sample was then collected to examine the presence of carbon sources such as glucose, fructose, sucrose, galactose, xylose, and arabinose.

Carbon Sources Concentration Examination by High-Performance Liquid Chromatography (HPLC)

The concentration of carbon sources (glucose, fructose, sucrose, galactose, xylose, and arabinose) in the hydrolysate was analyzed by using an HPLC (*Thermo Scientific Dionex UltiMate 3000*) with

Shodex SUGAR SP0810 column: ID 8.0 x 300 mm. The HPLC was set with the following settings: column's temperature at 70°C, deionized water for the stationary phase, refractive index detector's temperature at 50°C, water flow at 0.6 mL/minute, and each sample was processed every 45 minutes. The concentration of glucose, fructose, sucrose, xylose, and arabinose could be calculated from the result obtained from HPLC by using the equation of each carbon source's standard curve.

M. pollinis SP5 Mutagenesis with EMS and Sodium Thiosulfate

M. pollinis SP5 was pre-cultured in PDB for two days until the culture concentration reached $1x10^8$ CFU/mL, and the culture was checked with the Miles-Misra method. The preculture was then centrifuged, and the pellet was washed with PBS twice. After that, the washed pellet was resuspended in 1 mL PBS, and 20 µL (2%(v/v)) of sterile EMS was added to the culture. The usage of the 2%(v/v) of sterile EMS was based on the results of the preliminary research. Preliminary research was conducted with different concentrations of EMS: 0 (control),1, 2, and 3%(v/v), and based on the results of the preliminary research, 2%(v/v) EMS led to 99.38% of the death rate of *M. pollinis* SP5, which was sufficient for the mutation of yeast (**Figure 3**). The culture was then incubated at room temperature under a completely dark environment for one hour. After that, 8 mL of 10% sterile sodium thiosulfate was added to the culture, and the sample was incubated for three hours under dark conditions. As a negative control for the experiment, the same procedures were repeated without the addition of EMS. From these samples, 20µL from each microtube of mutation result suspension was then spread on PDA medium and then incubated for 4 days at room temperature. For mutant screening, 20 growing mutant colonies on the PDA medium were selected randomly to be grown in the OHYM medium fortified with 15% (v/v) molasses for 5 days at 28°C and 150 RPM agitation. The control (untreated EMS) colony will also be kept under the same growth condition. The erythritol content in the cell culture supernatant was analyzed using HPLC as the indicator of whether the amount of xylitol produced affects the production of erythritol as the main product of *M. pollinis*.

Xylitol Production by *M. pollinis* Mutants Preculture of *M. pollinis* Mutants

M. pollinis SP5 and 20 colonies of *M. pollinis* SP5 EMS-treated mutants isolated from the random mutation were pre-cultured in PDB for two days. The preculture process was done in the *TOU-50N* Orbital Shaker Incubator at 37°C with 150 RPM agitation.

Xylitol Production by Mutant *M. pollinis* in OPEFB Hydrolysate with Molasses Enhancement

The OPEFB hydrolysate was enriched with yeast extract (10 g/L), NH_4Cl (2 g/L), $MgSO_4.7H_2O$ (1 g/L), KH_2PO_4 (0.2 g/L), $ZnSO_4$ (0.07 g/L), 10%(w/v) PEG 6000, and molasses (0%(v/v) and 15%(v/v), respectively). Preculture of *M. pollinis* SP5 and 20 colonies of EMS-treated mutants of *M. pollinis* SP5 were inoculated into the OPEFB hydrolysate with an inoculum size of 10%(v/v) for seven days. During the fermentation period, the cultures were sampled daily and analyzed for their pH, viable colony count, biomass weight, and HPLC sample. However, the 20 cultures of mutated *M. pollinis* SP5 were only sampled on the fifth day for the screening step using HPLC.

Xylitol Production and Carbon Sources Consumption Quantification

HPLC was used to quantify the production of xylitol and consumption of carbon sources (glucose, fructose, sucrose, xylose, and arabinose). The HPLC was set with the following settings: column's temperature at 70°C, deionized water for the stationary phase, refractive index detector's temperature at 50°C, water flow at 0.6 mL/minute, and each sample was processed every 45 minutes. The HPLC results were used to calculate the xylitol production and carbon sources (glucose, fructose, sucrose, xylose, and arabinose) consumption in the OPEFB hydrolysate media. The parameters of the performance comparison were in the form of xylitol production yield and xylitol production productivity, which was calculated by using the following formulas:

$$Xylitol\ production\ yield = \frac{\text{total of xylitol produced (g)}}{\text{total of all carbons consumed (g)}} \quad (1)$$

$$Xylitol\ production\ productivity = \frac{\text{xylitol concentration (g/L)}}{\text{fermentation time (h)}} \quad (2)$$

Comparison of Xylitol Production by other *M. pollinis* Mutants through Statistical Analysis

Statistical analysis was done using a paired T-test with JASP 0.17.0.0 to compare the xylitol production between 20 *M. pollinis* SP5 mutants with the control being *M. pollinis* SP5. The significance of the difference in xylitol production was assessed through the p-value, where a p-value of less than or equal to 0.05 indicates a significant difference.

RESULTS AND DISCUSSION

Carbon Sources from OPEFB Pretreatment and OPEFB Media Profile After Molasses Enhancement

Most studies on xylitol production utilize xylose as the primary carbon source. However, reliance on xylose significantly increases production costs. To address this issue, it is essential to explore alternative, cost-effective carbon sources, such as agroindustrial waste. One promising option is oil palm empty fruit bunches (OPEFB). This biomass requires pretreatment to convert it into fermentable monosaccharides suitable for xylitol production. The pretreatment method for oil palm empty fruit bunches (OPEFB) involved acid hydrolysis with 2% H_2SO_4 followed by neutralization using 2 M $Ca(OH)_2$. This pretreatment process was carried out to recover important carbon sources in this research, specifically xylose as the main target and arabinose as an additional target, which are the primary materials in the production of xylitol. Acid hydrolysis plays a crucial role in breaking down the hemicellulose component of OPEFB, releasing monosaccharides such as xylose and arabinose. The mechanism involves the cleavage of glycosidic linkages in the hemicellulose polymer through the protonation of oxygen atoms in glycosidic bonds, making them more susceptible to nucleophilic attack. This process results in the release of the desired monosaccharides (Becker et al., 2021). After the acid hydrolysis to the OPEFB, the neutralization was done using $Ca(OH)_2$. The reaction between $Ca(OH)_2$ and H_2SO_4 forms neutral salt, resulting in a more neutral media that is more suitable for the growth of the cell factory. In addition, the neutral salt from the neutralization can be easily separated through a filtration process which increases the convenience of the pretreatment process (Unrean and Ketsub 2018). Additionally, the carbon source in the neutral pretreated media was further enriched by the addition of molasses. The results of the HPLC analysis can be seen in **Table 1**.

Table 1. Carbon source percentage after the OPEFB pretreatment and Molasses Enhancement

Carbon Source	OPEFB 0%(v/v) Molasses (g/L)	OPEFB 15%(v/v) Molasses (g/L)
Glucose	1.59	17.39
Fructose	-	10.95
Sucrose	-	47.19
Xylose	11.80	10.07
Arabinose	2.11	4.60
Galactose	0.98	-

Based on the results of the OPEFB pretreatment in **Table 1**, it is evident that xylose obtained the highest percentage among other carbon sources in the recovery process using this pretreatment method, with a concentration of 11.8 g/L, followed by

arabinose at 2.11 g/L after neutralization. These results indicate that this OPEFB pretreatment process is capable of recovering both the primary and additional targets needed in this research. However, the percentage of xylose is still relatively low. According to a study by Manjarres-Pinzon et al. (2017), the xylose concentration recovered from OPEFB reached 32.59 g/L using 2% H_2SO_4 with a 1:8 ratio. This suggests that the pretreatment method in this research is still not effective in recovering the main carbon source contained in OPEFB, and the pretreatment process needs to be optimized to increase the carbon source recovery rate from OPEFB. Due to the low concentration of the carbon source gained from the OPEFB pretreatment, enhancement with molasses was conducted to provide an additional sucrose carbon source that could be focused on as an energy source for growth and NADPH, which is important in xylitol production (Karuppiah et al. 2019; de Souza Queiroz et al. 2021). In **Table 1**, it can be seen that there is an increase in carbon sources in glucose, fructose, sucrose, and arabinose. This can encourage the cell factory to avoid using xylose and arabinose as energy sources, instead leaving them solely as raw materials for xylitol production.

Comparison of *M. pollinis* SP5 Performance in the OPEFB Media with 0%(v/v) and 15%(v/v) Molasses Enhancement

To increase the xylitol production yield, PEG 6000 was added to the media, which aimed for effective cell protection from harmful by-products generated from the acid hydrolysis pretreatment such as furfural and guaiacol that can hinder the growth of the cell factory, preventing cell damage (Pham Le Khanh et al. 2022). To further boost cell productivity, the yeast extract concentration in the media was set to 10 g/L to balance the carbon and nitrogen ratio in the media. This is confirmed by the study of Xie et al. (2022), which stated that the ratio balancing process can increase the growth and cell viability rate. The performance of *M. pollinis* SP5 in the OPEFB-Molasses enhanced media is shown in **Table 2**.

Table 2. The performance of *M. pollinis* SP5 in the OPEFB-Molassed Enhanced media

Parameter	***M. pollinis* SP5 (0%(v/v) Molasses)**	***M. pollinis* SP5 (15%(v/v) Molasses)**
Glucose consumption (g/L)	1.34	17.30
Xylose consumption (g/L)	3.38	5.24
Arabinose consumption (g/L)	1.67	-
Xylitol production (g/L)	2.40	3.19
Xylitol production yield (g/g)	0.48	0.61
Xylitol production productivity (g/L.h)	0.0143	0.0190

As shown in **Table 2**, *M. pollinis* SP5 consumed more xylose and produced more xylitol in the media with 15%(v/v) molasses. This indicates that molasses can supply carbon sources for cells in the bioconversion process of xylitol as it contains sucrose, glucose, and fructose which are essential for the energy source and NADPH supply. Therefore, the cell factory of *M. pollinis* SP5 will not utilize xylose and arabinose as the energy source but rather for xylitol bioconversion only (Karuppiah et al., 2019). On the other side, there were changes in the pH level in the culture media, which shows the slight acidification of the media in the middle of the fermentation process (**Table 3**), an indication of microbes' activity that resulted in an acidic by-product such as acetic acid and citric acid (Deshpande et al. 2022). Even though the pH turned acidic during the fermentation, the medium pH nearing the end of the fermentation process shifted to a more neutral pH. This is evident in **(Table 3)**, which shows that the pH of M. pollinis SP5 cultured in OPEFB with 0% (v/v) and 15% (v/v) molasses increased on days 4 and 5, respectively. This pH rise suggests partial cell death, likely due to the accumulation of metabolites such as ammonia in the medium. This was also potentially caused by the consumption of nitrogen sources from the yeast extract, yielding the basic ammonia and the buffering activity from the molasses (El-Asri and Farag 2023).

Table 3. Data for pH of OPEFB-Molassed Enhanced media fermented by sp5 *M. pollinis*

Fermentation time (days)	OPEFB + 0%(v/v) molasses	OPEFB + 15%(v/v) molasses
0	5	5
1	5	5
2	5	4.5
3	4	4.5
4	4.5	4
5	5	5
6	5.5	5
7	5.5	5.5

Furthermore, the growth of *M. pollinis* SP5 in OPEFB with 0%(v/v) molasses showed patterns of a log phase until day-2, stationary phase until day-5, and death phase from day-6 whereas the growth of *M. pollinis* SP5 in OPEFB with 15%(v/v) molasses showed a log phase until day-2, stationary phase until day-6 and death phase until day-7 (**Figure 1** and **Figure 2**). These results show that the addition of 15% (v/v) molasses enriches the medium, effectively delaying the progression of cell death. Moreover, it can be seen that *M. pollinis* grown in the 15% (v/v) molasses enhancement had a higher biomass concentration than *M. pollinis* in the 0%(v/v) molasses enhancement. This means that the molasses enhancement in the OPEFB media can encourage the multiplication of the cell factory which leads to higher xylitol production, in which the xylitol product yield increased by 27% with 15%(v/v) molasses (**Table 2**). The xylitol production by *M. pollinis* SP5 in the OPEFB media without the molasses enhancement resulted in 2.40 g/L of xylitol with a product yield of 0.48 g/g and volumetric productivity of the cell factory reached 0.0143 g/L.h. However, when the OPEFB media was enriched with 15%(v/v) molasses, the xylitol production increased to 3.19 g/L of xylitol with a product yield of 0.61 g/g and volumetric productivity of the cell factory reached 0.0190 g/L.h. These results were higher when compared with the previous studies that fermented OPEFB hydrolysate with *Debaryomyces hansenii* with a product yield of 0.201 $g_{xylitol}/g_{xylose}$, and employed the combination of *Saccharomyces cerevisiae* and *D. hansenii*, of which xylitol production of 2.86 g/L was obtained with a product yield of 0.297 $g_{xylitol}/g_{xylose}$ (Mardawati et al. 2018; Mardawati et al. 2022). In another study, fermentation of OPEFB hydrolysate with adapted *Candida tropicalis* resulted in a product yield of 0.44 $g_{xylitol}/g_{xylose}$ (Kim, 2019). The higher xylitol production and yield by *M. pollinis* SP5 indicates that *M. pollinis* SP5 strain has a potential as one of the xylitol producers besides *C. tropicalis* and *D. hansenii*, and the addition of molasses as substrate enhancement in the OPEFB media can positively affect the xylitol production and the performance of the cell factory in producing xylitol.

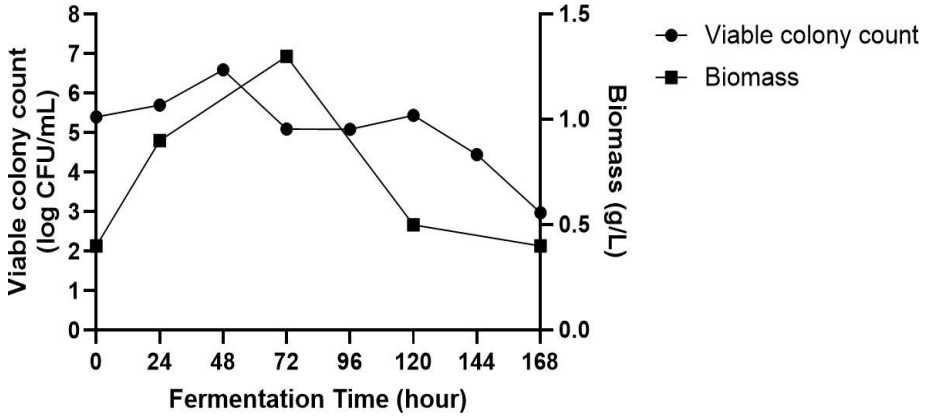

Figure 1. *M. pollinis* SP5 growth curves in OPEFB + 0%(v/v) molasses Media

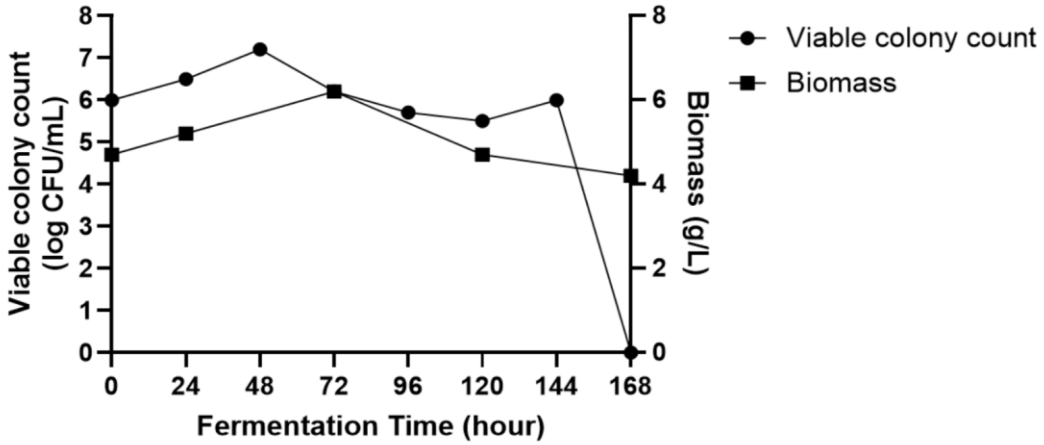

Figure 2. *M. pollinis* SP5 growth curves in OPEFB + 15%(v/v) molasses Media

Comparison of *M. pollinis* EMS Mutants in OPEFB 15%(v/v) Molasses Media

In this research, the potential of *M. pollinis* EMS mutant strains was inspected further since there are no studies that evaluate the effect of EMS on *M. pollinis* in increasing xylitol production. Mutagenesis employing EMS was done to alkylate the guanine (G) base, resulting in a transition of the G/C base pair into A/T that can cause the mutation in the gene that produces the enzyme that can be used for xylitol bioconversion in the cell factory which is xylose reductase from erythrose reductase (Cheng et al. 2018). However, this mutation method is considered unpredictable and creates a diverse mutant population. While it is possible for mutants with desirable traits–such as enhanced bioproduct production and increased enzymatic activity–to occur, there are also possibilities that EMS generates mutants with undesirable characteristics like reduced growth and viability. Therefore, extensive screening is required to select the best strains that can produce xylitol in a higher yield compared to the previous *M. pollinis* UV mutant which is *M. pollinis* SP5. The result from the EMS mutagenesis can be seen in **Figure 3**.

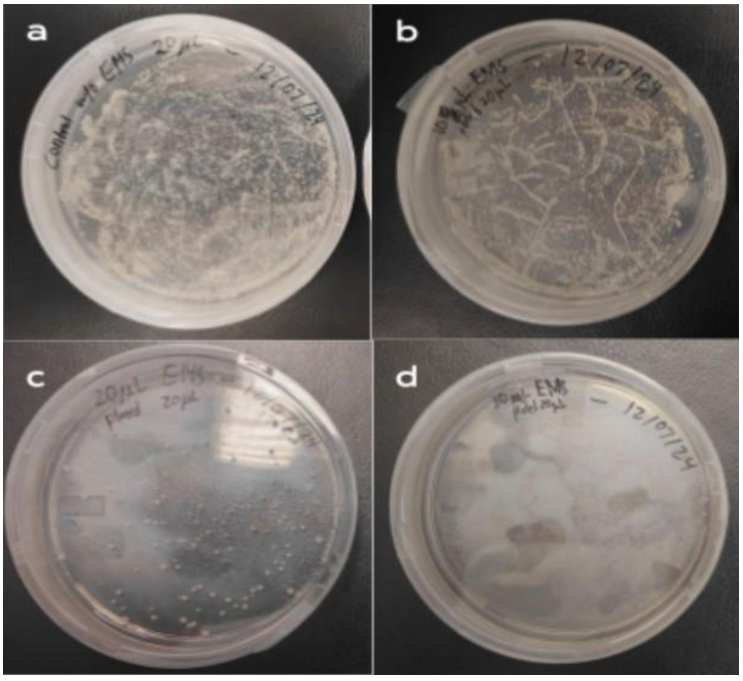

Figure 3. Morphology of mutated SP5 *M. pollinis* before EMS treatment (control) (a), after 10 µL (b), 20 µL (c), and 30 µL EMS treatment (d)

The *M. pollinis* EMS mutants were compared and 20 colonies were chosen randomly. The EMS mutants were named M as mutants, followed by numbers that indicate the order during the random picking. The 20 mutants along with the control which is *M. pollinis* SP5 were inoculated into 15% (v/v) molasses OPEFB media and after 7-day fermentation, the xylitol concentration was checked and statistically analyzed. The performance comparison of the other *M. pollinis* EMS-treated mutants and the control mutant (*M. pollinis* SP5) was revealed through a paired t-test analysis and the p-values can be seen in **Table 4**. Among 20 mutants, 11 mutants were able to produce xylitol and from the 11 mutants, three were capable of producing xylitol significantly more than the control (M4, M5, M6), seven mutants produced xylitol significantly lower than the control (M2, M3, M7, M8, M11, M12, M16), and one mutant was not significantly different than the control (M1).

Table 4. P-values from other *M. pollinis* mutants paired t-tests

Mutants	Xylitol concentration (g/L)	p-value
Control M. SP5	3.19	-
M1	2.70	0.064
M2	2.00*	0.028
M3	1.60*	0.019
M4	4.04*	0,019
M5	8.86*	0,010
M6	10.56*	0,030
M7	0.29*	0.002
M8	0.96*	0.001
M9	ND	-
M10	ND	-
M11	0.21*	0.002
M12	0.25*	0.006
M13	ND	-
M14	ND	-
M15	ND	-
M16	0.33*	0.002
M17	ND	-
M18	ND	-
M19	ND	-
M20	ND	-

Note: *: Significantly different Xylitol production ($p \leq 0.05$), ND: Not Detected.

From the statistical analysis, it can be seen that the mutants (M4, M5, and M6) were capable of producing xylitol with a significant increase in comparison to the control mutant (*M. pollinis* SP5), as much as 0.26 times higher by M4, 1.77 times higher by M5, and 2.31 times higher by M6 than the control mutant (**Table 4**). This shows that mutagenesis using chemical mutagent can improve the performance of the cell factory to produce the desired products which is also aligned with the previous study (Prabhu et al. 2020). Furthermore, this result indicates the possibility of using non-naturally occurring organisms in producing xylitol which gives the xylitol industry more choices in cell factories for production. According to Khatape et al. (2023), for instance, EMS mutagenesis successfully produced a mutant with an increase in the production of another sugar alcohol, erythritol, by 30%. Therefore, this gives an insight into the potential of further optimization for xylitol production using OPEFB and molasses in terms of the cell factory.

CONCLUSION

OPEFB pretreatment methods have successfully retrieved xylose and arabinose as the carbon source of xylitol. In addition, molasses enrichment in OPEFB media and the use of SP5 mutant *M. pollinis* increased the xylitol product yield by 27%, achieving xylitol product yield from 0.48 to 0.61 g/g. Moreover, other *M. pollinis* mutants showed

a higher xylitol production in the OPEFB+15%(v/v) molasses media, where M4, M5, and M6 produced xylitol 0.26, 1.77, and 2.31 times more than the control. In this research, there is still much room for more optimization that can be further inspected in the future, such as the use of other industrial waste for xylitol production and the improvement of purity from the purification. Therefore, such optimizations are expected to be done in the next research to support sustainability in Indonesia.

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REFERENCES

Abidin JZ (2023) Tata kelola industri kelapa sawit berkelanjutan dalam mendukung ketahanan energi nasional. J Agrosociology Sustain 1:59–74 . doi: 10.61511/jassu.v1i1.2023.136

Adiguna GS, Aryantha INP (2020) Aplikasi fungi rizosfer sebagai pupuk hayati pada bibit kelapa sawit dengan memanfaatkan limbah tandan kosong kelapa sawit

sebagai media pertumbuhan. MANFISH J 1:32–42. doi: 10.31573/manfish.v1i01.43

Arifan F, Nuswantari SR (2020) The xylitol production efficiency from corn cob waste by using stirred tank bioreactor-tubular loop liquid emulsion membrane (LEM). IOP Conf Ser Earth Environ Sci 448:012023. doi: 10.1088/1755-1315/448/1/012023

Becker M, Ahn K, Bacher M, Xu C, Sundberg A, Willför S, Rosenau T, Potthast A (2021) Comparative hydrolysis analysis of cellulose samples and aspects of its application in conservation science. Cellulose 28:8719–8734. doi: 10.1007/s10570-021-04048-6

Belivaqua GC, Filho FM, Forte MBS (2023) Simultaneous production of xylitol and arabitol by *Candida tropicalis* fermentation improving agro-industrial wastes valorization. Food and Bioproducts Processing 140:29-45 . doi: 10.1016/j.fbp.2023.04.006

Benahmed AG, Gasmi A, Arshad M, Shanaida M, Lysiuk R, Peana M, Pshyk-Titko I, Adamiv S, Shanaida Y, Bjørklund G (2020) Health benefits of xylitol. Appl Microbiol Biotechnol 104:7225–7237 . doi: 10.1007/s00253-020-10708-7

Cheng H, Wang S, Bilal M, Ge X, Zhang C, Fickers P, Cheng H (2018) Identification, characterization of two NADPH-dependent erythrose reductases in the yeast *Yarrowia lipolytica* and improvement of erythritol productivity using metabolic engineering. Microb Cell Fact 17:133 . doi: 10.1186/s12934-018-0982-z

Dasgupta D, Bandhu S, Adhikari DK, Ghosh D (2017) Challenges and prospects of xylitol production with whole cell biocatalysis: A review. Microbiol Res 197:9–21 . doi: 10.1016/j.micres.2016.12.012

Deshpande MS, Kulkarni PP, Kumbhar PS, Ghosalkar AR (2022) Erythritol production from sugar based feedstocks by *Moniliella pollinis* using lysate of recycled cells as nutrients source. Process Biochemistry 112:45–52 . doi: 10.1016/j.procbio.2021.11.020

de Souza Queiroz S, Jofre FM, dos Santos HA, Hernández-Pérez AF, Felipe M das G de A (2023) Xylitol and ethanol co-production from sugarcane bagasse and straw hemicellulosic hydrolysate supplemented with molasses. Biomass Convers Biorefinery 13:3143–3152 . doi: 10.1007/s13399-021-01493-y

El Asri O, Farag MA (2023) The potential of molasses from different dietary sources in industrial applications: A source of functional compounds and

health attributes, a comprehensive review. Food Biosci 56:103263 . doi: 10.1016/j.fbio.2023.103263

Fithri L, Puspaningsih NNT, Asmarani O, Ni'matuzahroh, Fitrah Dewi GD, Arizandy RY (2020) Characterization of fungal laccase isolated from oil palm empty fruit bunches (OPEFB) and its degradation from the agriculture waste. Biocatal Agric Biotechnol 27:101676 . doi: 10.1016/j.bcab.2020.101676

Harahap BM (2020) Degradation techniques of hemicellulose fraction from biomass feedstock for optimum xylose production: A review. J Keteknikan Pertan Trop dan Biosist 8:107–124 . doi: 10.21776/ub.jkptb.2020.008.02.01

Hidayat MS, Hasibuan A, Harahap B, Nasution SP (2022) Pemanfaatan tandan kosong kelapa sawit sebagai bahan pupuk di PT Karya Hevea Indonesia. Fact J Ind Manaj dan Rekayasa Sist Ind 1:52–58 . doi: 10.56211/factory.v1i2.172

Indriati L, Elyani N, Dina SF (2020) Empty fruit bunches, potential fiber source for Indonesian pulp and paper industry. IOP Conf Ser Mater Sci Eng 980:012045 . doi: 10.1088/1757-899X/980/1/012045

Karuppiah V, Sun J, Li T, Vallikkannu M, Chen J (2019) Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity. Front Microbiol 10 . doi: 10.3389/fmicb.2019.01068

Khatape AB, Rangaswamy V, Dastager SG (2023) Strain improvement for enhanced erythritol production by *Moniliella pollinis* Mutant-58 using jaggery as a cost-effective substrate. Int Microbiol 27:581–596 . doi: 10.1007/s10123-023-00411-8

Kim, SH (2019) Xylitol production from byproducts generated during sequential acid-/alkali-pretreatment of empty palm fruit bunch fiber by an adapted *Candida tropicalis*. Frontiers in Energy Research 7 . doi: 10.3389/fenrg.2019.00072

Kumar V, Yadav SK, Kumar J, Ahluwalia V (2020) A critical review on current strategies and trends employed for removal of inhibitors and toxic materials generated during biomass pretreatment. Bioresour Technol 299:122633 . doi: 10.1016/j.biortech.2019.122633

Liang P, Cao M, Li J, Wang Q, Dai Z (2023) Expanding sugar alcohol industry: Microbial production of sugar alcohols and associated chemocatalytic derivatives. Biotechnology Advances 64:108105 . doi: 10.1016/j.biotechadv.2023.108105

Malekmahmoodi M, Shamsi M, Roozbahani N, Moradzadeh R (2020) A randomized controlled trial of an educational intervention to promote oral and dental health of patients with type 2 diabetes mellitus. BMC Public Health 20:287 . doi: 10.1186/s12889-020-8395-4

Manjarres-Pinzón K, Arias-Zabala M, Correa-Londono G, Rodriguez-Sandoval E (2017) Xylose recovery from dilute-acid hydrolysis of oil palm (*Elaeis guineensis*) empty fruit bunches for xylitol production. African J Biotechnol 16:1997–2008 . doi: 10.5897/AJB2017.16214

Manjarres-Pinzón K, Mendoza-Meza D, Arias-Zabala M, Correa-Londoño G, Rodriguez-Sandoval E (2022) Effects of agitation rate and dissolved oxygen on xylose reductase activity during xylitol production at bioreactor scale. Food Sci Technol 42 . doi: 10.1590/fst.04221

Mardawati E, Febrianti EA, Fitriana HN, Yuliana T, Putriana NA, Suhartini S, Kasbawati (2022) An integrated process for the xylitol and ethanol production from oil palm empty fruit bunch (OPEFB) using *Debaryomyces hansenii* and *Saccharomyces cerevisiae*. Microorganisms 10:2036 . doi: 10.3390/microorganisms10102036

Mardawati E, Maharani N, Wira DW, Harahap BM, Yuliana T, Sukarminah E (2018) Xylitol production from oil palm empty fruit bunches (OPEFB) via simultaneous enzymatic hydrolysis and fermentation process. J Ind Inf

Technol Agric 2. doi: 10.24198/jiita.v2i1.25064

Medina JDC, Woiciechowski AL, Filho AZ, Brar SK, JúniorAIM, Soccol CR (2018) Energetic and economic analysis of ethanol, xylitol and lignin production using oil palm empty fruit bunches from a brazilian factory. Journal of Cleaner Production 195:44-45 . doi: 10.1016/j.jclepro.2018.05.189

Meilany D, Kresnowati MTAP, Setiadi T, Boopathy R (2020) Optimization of Xylose Recovery in Oil Palm Empty Fruit Bunches for Xylitol Production. Applied Sciences 10:1391 . doi: 10.3390/app10041391

Pham Le Khanh H, Nemes D, Rusznyák Á, Ujhelyi Z, Fehér P, Fenyvesi F, Váradi J, Vecsernyés M, Bácskay I (2022) Comparative investigation of cellular effects of polyethylene glycol (PEG) derivatives. Polymers (Basel) 14:279 . doi: 10.3390/polym14020279

Prabhu AA, Bosakornranut E, Amraoui Y, Agrawal D, Coulon F, Vivekanand V, Thakur VK, Kumar V (2020) Enhanced xylitol production using non-detoxified xylose rich pre-hydrolysate from sugarcane bagasse by newly isolated *Pichia fermentans*. Biotechnology for Biofuels 13 . doi: 10.1186/s13068-020-01845-2

Salli K, Lehtinen MJ, Tiihonen K, Ouwehand AC (2019) Xylitol's health benefits beyond dental health: A comprehensive review. Nutrients 11:1813 . doi: 10.3390/nu11081813

Singh AK, Deeba F, Kumar M, Kumari S, Wani SA, Paul T, Gaur NA (2023) Development of engineered *Candida tropicalis* strain for efficient corncob-based xylitol-ethanol biorefinery. Microbial Cell Factories 22 . doi: 10.1186/s12934-023-02190-3

Stylianopoulou C (2023) Carbohydrates: Chemistry and classification. In: Encyclopedia of Human Nutrition. Elsevier, pp 114–125

Sutomo S, Purwanto NH (2022) Pengaruh konsumsi tisane daun belimbing wuluh terhadap perubahan kadar gula dalam darah pada penderita diabetes mellitus tipe 2. J Keperawatan 16:1–15

Uhliariková I, Matulová M, Capek P (2021) Optimizing acid hydrolysis for monosaccharide compositional analysis of *Nostoc cf. linckia* acidic exopolysaccharide. Carbohydr Res 508:108400 . doi: 10.1016/j.carres.2021.108400

Unrean P, Ketsub N (2018) Integrated lignocellulosic bioprocess for co-production of ethanol and xylitol from sugarcane bagasse. Industrial Crops and Products 123:238–246 . doi: 10.1016/j.indcrop.2018.06.071

Vučurović VM, Puškaš VS, Miljić UD (2018) Bioethanol production from sugar beet molasses and thick juice by free and immobilised *Saccharomyces cerevisiae*. Journal of the Institute of Brewing 125:134-142 . doi: 10.1002/jib.536

Xie Y, Zhou L, Dai J, Chen J, Yang X, Wang X, Wang Z, Feng L (2022) Effects of the C/N ratio on the microbial community and lignocellulose degradation, during branch waste composting. Bioprocess Biosyst Eng 45:1163–1174 . doi: 10.1007/s00449-022-02732-w

Zhang L, Chen Z, Wang J, Shen W, Li Q, Chen X (2021) Stepwise metabolic engineering of *Candida tropicalis* for efficient xylitol production from xylose mother liquor. Microb Cell Fact 20:105. doi: 10.1186/s12934-021-01596-1