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Integrated and partial process of xylitol and bioethanol production from oil palm empty fruit bunches

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KEYWORDS

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OPEFB
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ABSTRACT

Oil palm empty fruit bunches (OPEFBs) are highly abundant in Indonesia and have been highlighted as a potential feedstock for bioethanol and xylitol production. However, the efficacy of the fermentation technology to convert OPEFBs to bioethanol and xylitol, either in partial (i.e. mono-production) or integrated (i.e. co-production) process, still needs further improvement. This study aimed to evaluate the partial and integrated process for xylitol and bioethanol production from OPEFBs. In the integrated process, the remaining solid residues after xylitol extraction are used as feedstock for bioethanol due to their high cellulose compounds. This solid residue is more susceptible to be degraded by cellulase enzymes into glucose and further transformed into bioethanol. In the partial process of xylitol production, xylanase enzyme was used to hydrolyze xylan into xylose, which was then converted into xylitol using *Debaryomyces hansenii*. While in the partial process of bioethanol production, the hydrolysis of cellulose in the OPEFB into glucose was carried out using cellulase enzymes, followed by fermentation using *Saccharomyces cerevisiae*. The results show that the partial process produced xylitol yield ($Y_{p/s}$) of 0.10 g-xylitol/g-xylose, while bioethanol at yield ($Y_{p/s}$) of 0.32 g-bioethanol/g-glucose, respectively. The integrated process generates xylitol yield ($Y_{p/s}$) of 0.298 g-xylitol/g-xylose, with bioethanol yield from the remaining solid at 0.051 g-bioethanol/g-OPEFB (or 0.078 g-bioethanol/g-glucose). These findings, therefore, confirmed that the integrated process of xylitol with bioethanol production might offer higher efficacy of OPEFB utilization into high value-added products.

Introduction

The palm oil production in Indonesia between 2015 and 2019 showed a significant increase from 31,070,015 to 42,869,429 tons (Directorate General of Estate, 2020). A high potential of palm oil is parallel to an increase in waste production, which is estimated to produce 1 ton of oil palm empty fruit bunches (OPEFBs), 0.7 ton of palm fibers, and 0.3 tons of palm shells from 1 ton of palm oil produced (Suhartini et al., 2022). One of the solid wastes highly generated from palm oil plants is oil palm empty fruit bunches (OPEFB), produced from fresh fruit threshing stations (Shinoj et al., 2011). The OPEFB generated is about 30% from fresh fruit bunches. In the palm oil factory, the OPEFB is just burnt (Dishington; 2016); or widely applied for compost; however, it only accounted for 10% of total OPEFB waste potential (Ngadi et al., 2014).

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The OPEFB contains high lignocellulose content; therefore, it can be further used as a raw material (i.e. feedstock) in the manufacture of xylitol and bioethanol (Mardawati et al., 2018). Xylitol is a polyol sugar with 5 carbon chains which has health benefits. Xylitol can prevent tooth decay and is also a suitable sugar for people with diabetes because it does not require insulin to regulate metabolism. As a sugar substitute, xylitol has a sweetness level equivalent to sucrose with a lower calorific value of 2.4 cal/g (Khienpaya et al., 2010).

Many studies have also emphasized the potential use of OPEFBs as feedstock for ethanol or bioethanol production. Ethanol is called ethyl alcohol with the chemical formula of C_2H_5OH or CH_3CH_2OH. Ethanol production, which uses natural raw materials and a biological process, is called bioethanol. Bioethanol has advantages, including its high flammability and good combustion properties, as it contains large amounts of oxygen. Thus, bioethanol, as an alternative to fossil fuels, can reduce environmental pollution due to its lower CO_2 emissions (Mardawati et al., 2014). Bioethanol is a liquid biofuel with the characteristics of flammable, colorless, volatile, easily soluble in water, and has an octane number of 91-105

Lignocellulose is located in plant cell walls, especially in the stem, which consists of lignin, cellulose, and hemicellulose. The lignocellulose of OPEFB contains 43-43.7% of cellulose, 22.93-23.67% of hemicellulose, and 21.28-22.10% of lignin (Mardawati et al., 2014). Since the xylose content in hemicellulose of OPEFB reached 80%, it is highly potential to be converted into xylitol (Foster-Powell et al., 2022). In general, the hemicellulose content in OPEFB can be used as a substrate for xylitol production, while the cellulose content is potential for bioethanol production (Anish and Rao, 2009).

The conversion of lignocellulosic substrates to bioethanol is carried out through several stages, including pretreatment, saccharification (hydrolysis), and fermentation (Anish and Rao, 2009). The hydrolysis process can be carried out enzymatically, in which the cellulose content in a biomass substrate is degraded into D-glucose using cellulase enzymes. Therefore, increasing the substrate concentration can accelerate the hydrolysis rate because more compounds are bound to the enzyme's active site (Mardawati et al., 2014). With a fixed enzyme concentration, a low amount of substrate causes only a small amount of the substrate to bind to the active site of the enzyme. Thus, increasing the substrate concentration can increase product formation. The D-glucose is then converted to ethanol in a fermentation process using yeast. Two fermentation methods that are widely used in bioethanol production include Separated Hydrolysis and Fermentation (SHF) and Saccharification and Simultaneous Fermentation (SSF) (Dahnum et al., 2015). SSF uses one reactor for saccharification and fermentation processes, thus the hydrolysate can be directly converted into ethanol. SSF method allows the formation of a higher ethanol yield than the SHF method. A previous study also found that using the SSF method to produce bioethanol from OPEFB showed a higher bioethanol yield than using the SHF method (Datta et al., 1991). Therefore, this study explored the SSF method using *S. cerevisiae* yeast to convert hydrolysate to bioethanol.

In the production of xylitol, hemicellulose and cellulose that still bind to lignin need to be pretreated to increase the surface area of lignocellulose, thus facilitating a faster hydrolysis process. During the enzymatic hydrolysis, xylan, as the main hemicellulose component, is broken down using xylanase enzyme into monomers consisting of 5 and 6 carbon chain sugars, including xylose. Xylose is then converted into xylitol by yeast in the fermentation process (Datta et al., 1991). According to Khienpanya et al. (2010), *D. hansenii* yeast can ferment OPEFB hydrolysate with more yields.

The hydrolysis process in the xylitol production generated solid residues high in cellulose fiber. These solid residues or waste can be used as raw materials for bioethanol production (Octavia, 2008). According to Octavia (208) and Mardawati et al. (2017), cellulosic material can be used as a carbon source for bioethanol production through hydrolysis and fermentation processes. Producing xylitol and bioethanol from OPEFB can be done in two ways, including partially (i.e. mono-production) and integrated (i.e. co-production) processes. The partial process means that xylitol and bioethanol are directly produced by using OPEFB raw materials. The integrated

process means the remained solid residues from xylitol extraction were further utilized as feedstock for bioethanol production or vice versa. According to Suhartini et al. (2022), three scenarios are potential for valorizing OPEFBs into xylitol and bioethanol, including mono-production of bioethanol or xylitol, co-production of xylitol with bioethanol, and co-production of bioethanol with xylitol. The integrated process of xylitol and bioethanol production is an alternative solution to create a sustainable palm oil industry with a closed-loop system and zero-waste concept integration. Therefore, this study was carried out to investigate the potential of OPEFB as a feedstock for xylitol with bioethanol production. The OPEFB was first hydrolyzed to produce xylose, followed by fermentation into xylitol. The solid residues from xylitol production were used as feedstock for bioethanol production through hydrolysis and fermentation.

This study aimed to evaluate the performance of the integrated process of xylitol with bioethanol production from OPEFB and compare the process's efficacy.

Research Methods

Materials

The raw material used was OPEFB obtained from Incasi Raya Palm Oil Mills, West Sumatra, Indonesia. The cellulase enzyme of Cellic HTec2 and Cellic CTec2 was obtained from Institut Teknologi Bandung (ITB), yeast extract, bacto agar and peptone. The main chemicals used were NH_4OH (Sigma Aldrich), acetate and citric buffers (Sigma Aldrich), 3,5 dinitrosalicylic (DNS) acid reagent (Sigma Aldrich), and H_2SO_4 (Sigma Aldrich). The yeasts used were *Saccharomyces cerevisiae* ITBCC R60 and *Debaryomyces hansenii* ITBCC R85 obtained from culture stock at the Department of Chemical Engineering, ITB, Indonesia.

Preparation of Raw Materials

The OPEFB was prepared by washing with tap water, drying using an oven, reducing the particle size using a disc mill, then sieving with a size of 60 and 80 mesh (Mardawati, 2019). After preparation, the composition of lignocellulose components such as cellulose, hemicellulose, and lignin in OPEFB was analyzed based on the Chesson Method (Octavia, 2008).

Pretreatment

The OPEFB was pretreated by using 5% ammonia. First, the OPEFB was soaked in 5% ammonia with a ratio of 1:10 following the procedure of Zulkiple et al. (2016). After the whole part of OPEFB was thoroughly wetted, the sample was heated in an autoclave with the pressure of 1.5 Bar for 15 min. After pretreatment, two fractions were formed, i.e. spent liquor and pretreated solid. Spent liquor was subsequently analyzed to determine the sugars formed (i.e. xylose and glucose).

Integration Production of Xylitol and Ethanol

Hydrolysis

Following pretreatment and to increase the number of sugars, enzymatic hydrolysis was conducted. Hydrolysis of the pretreated OPEFB used the combination of 15 FPU/g Cellic CTec2 and 20% Cellic HTec2. The volume of Cellic CTec2 was calculated by the equation below (Adney and Baker, 2008):

Enzym amount (mL) :

$$\frac{\text{cellulose initial OPEFB (g) x enzym does (FPU/g)}}{\text{enzym activity (U/ml)}} \quad \ldots\ldots\ldots.(1)$$

The measurement of the cellulase activity in Cellic CTec2 was 98.78 FPU/mL following the NREL Method (Adney and Baker, 2008). The spent liquor resulting from the pretreatment step was hydrolyzed using the Cellic HTec2 at a dosage of 684.91 U/mL. The xylanase activity of HTec2 enzyme was measured following the procedure of Bailey et al. (1992). The xylanase activity in Cellic HTec2 was 68,490.65 U/mL, and this enzyme also had high cellulase activity, with a value of 98.78 FPU/mL.

Hydrolysis was conducted at 50 ^oC and pH 5 using a shaker incubator with an agitation of 150 rpm. The citric buffer was added to maintain the stability of pH during hydrolysis. The sugar content in the hydrolysate after the hydrolysis process was determined by the DNS method described in Mardawati et al. (2020).

Preparation of the inoculum of *D. hansenii* and *S.cereviciae*

D. hansenii and *S.cereviciae* were rejuvenated in slant agar. Around 3 loops of the yeast from slant agar were cultivated at the sterile Yeast Extract Peptone Dextrose liquid medium with the composition of 10 g/L peptones, 5% yeast extract, and 20% glucose. The inoculum was incubated at

30 °C with the agitation of 150 rpm for 16-20 h for *S.cereviciae* and 48 h for *D. hansenii*. Before the inoculum was used, the number of the living cell must not be less than 107 cells/mL.

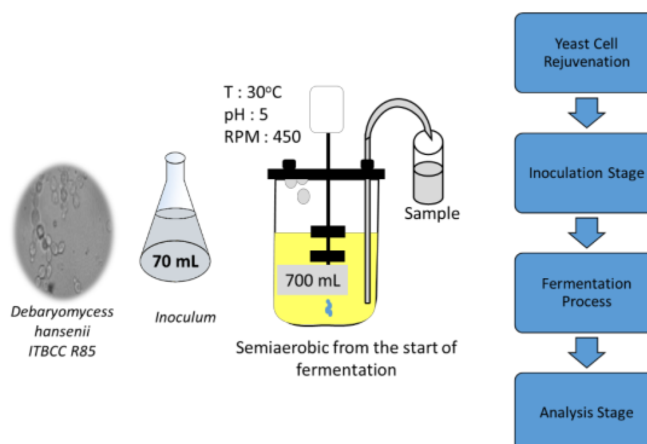

Figure 1. Schematic diagram of the bioreactor in the fermentation process of xylitol

Fermentation

Fermentation was carried out in a 1.3 L fermenter for bioethanol and xylitol production. Bioethanol production was performed at 30 °C, pH 5, the agitation of 300 rpm, for 24 hours without supplying air from outside and with a total working volume of 700 mL. The working volume consists of 500 mL of hydrolysate from solid fraction hydrolysis, 100 mL of inoculum, and 100 mL of other nutrients. The xylitol production was carried out at 30 °C, agitation at 450 rpm, pH 5, and incubated for 144 h. The concentrations of sugars and products were analyzed every 24 h. The total working volume was 750 mL consisting of 400 mL of liquid fraction hydrolysis, 250 mL of distillate bioethanol, and 100 mL inoculum. Meanwhile, evaporation was carried out at a water temperature of 90 °C for 1 h.

Partial Production of Xylitol and Bioethanol

Production of Xylitol

1. Hydrolysis

The hydrolysis was carried out under batch conditions modified from a previous study by Mardawati et al. (2014). OPEFB hydrolysate was prepared with 15 g of OPEFB beaded with 100 mL acetate buffer (pH 5) and thoroughly mixed in an Erlenmeyer flask. Then, 10% xylanase enzyme was added, which had an optimum temperature in the range of 45-50 °C at pH 5. The mixture was incubated in a shaker incubator at 50°C with agitation at 200 rpm for 96 h. The resulting OPEFB hydrolysate was then separated using a centrifuge at 6000 rpm for 20 min to obtain xylose hydrolysate.

2. Fermentation

Fermentation was conducted using xylose hydrolysate with a substrate concentration of 5%. A ratio of xylose substrate and glucose co-substrate (4:1) was used in the fermentation, which refers to the method of Mardawati et al. (2015). Xylose hydrolysate fermentation consists of 3 stages: *D. hansenii* cell inoculation, incubation, and fermentation. The inoculum was prepared by dissolving the glucose yeast extract (GYE) medium with distilled water, then following heating and stirring until the medium turned yellow color. The medium was then poured into a test tube and sterilized by autoclaving at 120 °C for 15 min. The test tube containing sterile GYE was cooled to room temperature in an oblique position until the media had solidified. The inoculated yeast *D. hansenii* cells were then incubated at 30 °C for three days (Khienpanya et al., 2010). During the fermentation process, a sample was observed every few hours over a 96 h period to calculate the number of microorganisms and the formation of xylitol. A schematic diagram of the bioreactor in the fermentation process of xylitol is presented in Figure 1.

Production of Bioethanol

1. Hydrolysis

This study employed enzymatic hydrolysis or saccharification using 5% OPEFB substrate of the buffer volume. The OPEFB substrate was placed

in a shake flask. To maintain the process pH, a citrate buffer with pH 5.0 was added with the same amount of substrate added, following the addition of enzymes (5% of the buffer volume (v/v)). Cellic Htec cellulase enzyme was obtained from ITB. The saccharification process was carried out using an incubator shaker for 48 h. Rotation speed was controlled at 130 rpm and the temperature was set to 50 °C (Octavia, 2008). The sugar content was determined by the DNS method following the procedure of Mardawati et al. (2020).

2. Fermentation

The fermentation of OPEFB for bioethanol production was using SSF methods based on a previous study by Hanidah (2010), with some modifications. The OPEFB substrate used was 5% of the amount of buffer used with *S. cerevisiae* as fermenting microorganism.

Analysis

The parameters analyzed included glucose, xylose, the yield of biomass ($Y_{x/s}$), xylitol, bioethanol, and morphological structures. Cell concentration analysis was performed based on the turbidimetric-gravimetric method using a RH-TECH-752N Model UV-VIS Spectrophotometer (Mardawati et al., 2020). The remaining xylose and glucose in the substrate, as well as the concentration of xylitol and ethanol produced, were determined using chromaster High-Performance Liquid Chromatograph (HPLC) (Waters) with a type BioRad Aminex HPX-87H column and using 5 mM H_2SO_4 as eluent (Mardawati et al., 2017). Morphological analysis of the sample was carried out using Scanning Electron Microscopy (SEM) JEOL JSM-6360LA with a magnification of 500x. The sample was attached using conducting glue to the material. A hand blower was used on the sample to allow it to adhere well to the conducting glue. Then coupling was done to coat the sample with Pt and Au, thus the sample was not damaged during scanning. The samples were stored in a vacuum and then analyzed (Sujatno et al., 2015).

Data Interpretation

The hydrolysis yield measurement was calculated based on the Eq. (2)-(5) (Aliberti et al., 2017; Chen et al., 2008; Zhang et al., 2010)

Glucose hydrolysis yield (%) :

$$\frac{Glu\,(t)}{Glu\,(teo)} \times 100\% \quad (2)$$

Xylose hydrolysis yield (%) :

$$\frac{Xyl\,(t)}{Xyl\,(teo)} \times 100\% \quad (3)$$

Hydrolysis yield (%) in terms of glucose:

Yield of glucose (%) :

$$\frac{Glucose\ release\ x\ 0.90}{Initial\ glucose\ in\ reactor} \times 100 \quad (4)$$

Hydrolysis yield (%) in terms of xylose:

Yield of xylose (%) :

$$\frac{Xylose\ release\ x0.88}{Initial\ xylan\ in\ reactor} \times 100 \quad (5)$$

Where Glu(t) is the glucose concentration (g/L) produced at t time, Glu(teo) is the theoretical glucose from the biomass, Xly(t) is xylose concentration at t time, and Xly(teo) is theoretical xylose.

The product yield for the substrate concentration was calculated using Eq. (6) (Mardawati et al., 2015).

$$Y_{P/S} = -\frac{\Delta P}{\Delta S} = \frac{P_t - P_o}{S_o - S_t} \quad (6)$$

Where:
P = xylitol product concentration (g/L)
$Y_{P/S}$ = product-from-substrate yield
($Y_{P/S}$ = g-xylitol/ g-xylose),
($Y_{E/S}$ = g-bioethanol/ g-total substrate)
($Y_{X/S}$ = g-biomass/ g-total substrate)
S = substrate concentration (g/L)

Results and Discussion

Integration Process of Xylitol and Bioethanol Production

Characterization of OPEFB

The results showed that OPEFB was composed of 33.83% ± 1.02% of cellulose, 17.07% ± 0.98% of hemicellulose, and 26.71% ± 0.83% of lignin.

Table 1. Comparison of the characteristics of OPEFB

Lignocellulosic components	% Dry Weight				
	This Study	Abdullah et al. (2011)	Mardawati et al. (2014)	Zulkiple et al. (2016)	Mardawati et al. (2020)
Cellulose	33.83 ± 1.02	57.8	43.32	36.59	39.47 ± 0.74
Hemicellulose	17.07 ± 0.98	21.2	22.10	26.53	17.31 ± 0.56
Lignin	26.71 ± 0.83	22.8	23.67	24.97	23.26 ± 1.00

Table 1 shows that the cellulose yields obtained in this study were lower than that reported in several previous studies by Mardawati et al. (2014) and Abdullah and Sulaiman (2013). This was possibly due to no pre-treatment was subjected to the OPEFB samples used in this study. Lignin has the most complex structure than other components. Thus, pretreatment is needed to open the crystalline structure of lignin and to increase the cellulose conversion rate (Abdullah et al., 2011).

The Effect of Ammonia Pretreatment on the Characteristics of OPEFBs

In the study, the OPEFB sample was pretreated with ammonia solution to degrade and dissolve hemicellulose and lignin into the liquid fraction. The solid fraction is composed of cellulose, residual lignin, and hemicellulose. This solid fraction is easier to be hydrolyzed into glucose with a high yield.

The analysis of the liquid fraction showed that glucose and xylose were found but in a low concentration and yields (Table 2), with the value of 0.376 g/L (0.004 g/g-OPEFB) for glucose and 0.347 g/L (0.004 g / g-OPEFB) for xylose. This result indicates that the liquid fraction produced from the pretreatment process cannot directly be used for fermentation. However, the remaining solid residues contain a higher amount of cellulose and hemicellulose compounds, which is in agreement with other studies. Therefore, the OPEFB hydrolysis process is needed to hydrolyze hemicellulose composed of remaining xylan into sugar monomer (Gigih et al., 2015).

Liu et al. (2013), for instance, reported the pretreatment of miscanthus with ammonia solution showed more than 55% of lignin degradation at a temperature above 120 °C. However, the cellulose was not significantly decreased with increasing temperature. An increase in temperature tends to decrease the amount of hemicellulose contained in the solid fraction and transferred to the liquid phase. However, the hemicellulose was not degraded further. The results from infrared spectra analysis demonstrate that the ester bond after pretreatment disappeared, while the 2D nuclear magnetic resonance (NMR) spectra analysis showed that pretreatment with ammonia was related to xylan deacetylation.

Previous studies by Zang and Wu (2014), using sugarcane baggage and Salvi et al. (2010), using sorghum as raw material also indicated similar results. The optimum condition for the pretreatment of bagasse was obtained at 170 ° C using 15% (w/w) of ammonia solution for 60 min. This pretreatment condition has a delignification of 36.9%, with glucans and xylan retained were 94.7% and 63.1%, respectively. After enzymatic hydrolysis, 34.2 g glucose and 13.2 g xylose per 100 g raw material were obtained. On the other hand, the optimum conditions for sorghum fibers were at 160°C, the pressure of 140-160 psi, sorghum: ammonia: water ratio of 1: 0.14: 8, for 1 hour. A total of 44% lignin and 35% hemicellulose were degraded. After hydrolysis to produce glucose and fermentation to bioethanol, the amount of the products produced after ammonia pretreatment was higher than that of without pretreatment. Based on these two studies, it can be seen that the amount of lignin and hemicellulose in the solid fractions were significantly reduced and transferred to the liquid phase, and the enzymatic digestibility of cellulose was much higher than that without treatment. Therefore, the hydrolysis step is critically required to improve the recovery of sugars (i.e., glucose and xylose) in lignocellulosic biomass.

Hydrolysis of the Solid and Liquid Fractions from the Ammonia Pretreatment

Both solid and liquid fractions from ammonia pretreatment were further hydrolyzed using enzymes Cellic CTec2 and Cellic HTec2. Cellic HTec2 enzyme was formed to support the Cellic CTec2 enzyme in hydrolyzing the cellulose by releasing hemicellulose and increasing its porosity. A previous study by Dahnum et al. (2015), used a combination of Cellic CTec2 and HTec2 enzymes with a dose of 40 FPU/g and 15% of the volume Cellic CTec2 added. Their study showed that glucose and xylose obtained were 10.67% and 1.5% after hydrolysis for 72 h with pretreatment using NaOH. The liquid fractions were hydrolyzed

only using Cellic HTec2 enzyme due to a low amount of dissolved cellulose. This combination is expected to increase the yield of xylose and glucose, thus resulting in higher xylitol and bioethanol production.

Table 2. The amount of sugar, ethanol, and bioethanol, and the utilization of sugar

Pretreatment	
Glucose concentration [g / L]	0.376 ± 0.000
Xylose concentration [g / L]	0.347 ± 0.000
Yglu / OPEFB [g / g]	0.004 ± 0.000
Yxil / OPEFB [g / g]	0.004 ± 0.000
Hydrolysis	
Solids Results of Treatment Introduction	
Glucose concentration [g/L]	14.230 ± 0.669
Xylose concentration [g/L]	5.516 ± 0.193
Yglu/OPEFB [g / g]	0.142 ± 0.007
Yxil / OPEFB [g / g]	0.055 ± 0.002
Fluid Results of Preliminary Treatment	
Glucose concentration [g / L]	1.764 ± 0.068
Xylose concentration [g / L]	0.785 ± 0.036
Yglu / OPEFB [g / g]	0.019 ± 0.001
Yxil / OPEFB [g / g]	0.009 ± 0.000
Fermentation	
Bioethanol Fermentation (48 hours)	
The concentration of remaining glucose [g/L]	0
Residual xylose concentration [g / L]	2.310 ± 0.088
Bioethanol Concentration [g/ L]	4.253 ± 0.131
Yp/s	0.078 ± 0.05
Y EtOH / OPEFB [g / g]	0.051 ± 0.002
% Glucose utilization	100
% Xylose utilization	42
Bioethanol Separation	
Distillate	
Bioethanol Concentration [g / L]	4.250 ± 0.119
Y EtOH / OPEFB [g/g]	0.042 ± 0.001
Concentrate	
Xylose concentration [g / L]	11.471 ± 0.139
Y xil / OPEFB [g /g]	0.026 ± 0.001
Xylitol Fermentation	
The concentration of remaining glucose [g / L]	0.396 ± 0.001
Residual xylose concentration [g / L]	3.400 ± 0.006
Xylitol concentration [g / L]	0.433 ± 0.001
Y x yt / OPEFB [g /g]	0.298 ± 0.000
% Glucose utilization	84%
% Utilization xylose	30%

Based on Table 2, the concentration of and yield of glucose and xylose significantly increased after hydrolysis of both fractions. However, compared to the hydrolysis performance of the solid fractions, the glucose and xylose resulting from the hydrolysis of the liquid fractions were only amounted for 1.764 g/L (0.019 g/g-OPEFB) and 0.785 g/L (0.009 g/g-OPEFB), respectively. These results show that the amount of glucose is higher than the xylose with a ratio of glucose and xylose of 2.2: 1.

Glucose and xylose resulting from the hydrolysis of solid fractions were much higher. This was possibly because ammonia can degrade lignin contained in the solid fractions, thus increasing the accessibility of both the Cellic CTec2 and Cellic HTec2 enzymes. The high amount of glucose found in the hydrolysis of the liquid fractions indicates that cellulose in the form of oligomers was dissolved more following the ammonia pretreatment.

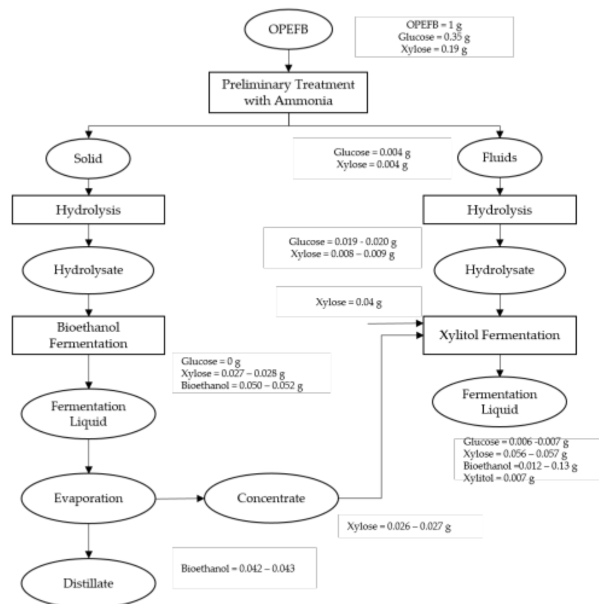

Figure 2. Mass balance from the integrated process of xylitol with bioethanol production

In general, the use of ammonia solution in lignocellulose pretreatment aimed to remove lignin, referred to as the delignification process. According to Salvi et al. (2010), lignin removal is carried out by cutting the lignin C-O-C bonds, as well as the ether and ester bonds in the lignin-carbohydrate complex, releasing cellulose and hemicellulose. Most of the lignin is degraded into dissolved fragments after being pretreated with ammonia (Qin et al., 2013).

The results also show that ammonia pretreatment is not recommended for fractionating the xylose as more cellulose was dissolved into liquid fractions. The increase of glucose content found in the hydrolysate from the hydrolysis of liquid fractions indicates that Cellic HTec2 enzyme contains cellulase with higher activity. The Cellic HTec2 enzyme did not purely contain the xylanase/hemicellulase enzyme.

Glucose content in the hydrolysate from the hydrolysis of the solid fraction was 14.23 g / L and 0.142 g/g-OPEFB, much higher than that of the liquid fractions (1.764 g/L and 0.019 g/g-OPEFB). The xylose recovery of the hydrolysate from the hydrolysis of solid fractions was 5.516 g/L or 0.055 g/g-OPEFB. However, the ratio of glucose and xylose in the liquid hydrolysate was much higher, with a value of 2.6:1. Thus, hydrolysate from the hydrolysis of solid fractions can be used as a substrate for bioethanol production. While hydrolysate from the hydrolysis of liquid fractions is potential as a substrate for xylitol production.

Evaluation of Configuration of Xylitol and Bioethanol Production

Bioethanol Fermentation

The configuration process of xylitol and bioethanol production and the mass balance are shown in Figure 2. Hydrolysate from the hydrolysis of solid fractions was -rich in glucose, with higher glucose and xylose ratio, thus can be used as a substrate in fermentation bioethanol. The glucose was completely consumed after 8 hours (Figure 2), while xylose remained as much as 2.31 g / L with utilization degree of 42%.

The results, shown in Figure 3, demonstrate that *S. cerevisiae* consumed both glucose and xylose. Yeast can consume xylose when glucose has declined, yet the value was not significant. After glucose was fully consumed, xylose was also stopped being consumed, as well as bioethanol production. The volume of bioethanol increases parallel to a decrease in the amount of glucose and xylose. However, when the glucose has been depleted, bioethanol cannot also be produced.

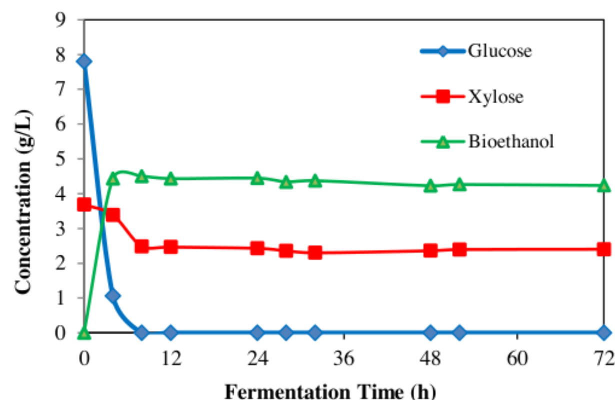

Figure 3. Bioethanol fermentation profile by ***S. cereviciae*** on the integrated process

In this study, the air was not injected into the fermenter. The air was introduced from the headspace of the fermenter. Thus, when the oxygen runs out, anaerobic conditions are formed. Under these conditions, glucose is converted into bioethanol. The resulting bioethanol was 0.051 g/g-OPEFB (4.25 g/L), and the yield per glucose substrate ($Y_{p/s}$) was 0.078 g/measured after 24 h when the glucose was fully consumed. According to Albuquerque et al. (2014), glucose is completely consumed by the *S. cerevisiae* after 2 h fermentation with an initial glucose concentration of 14 g/L, while no significant decrease of xylose and arabinose (18.5 g/L into a 16 g/L) was observed.

Several studies have been carried out to produce bioethanol from OPEFB using *S. cerevisiae*. For example, Kassim et al. (2011) used enzymatic saccharification for producing bioethanol from OPEFB. Their study revealed that OPEFB hydrolysate was fermented with *S. cerevisiae* in batch mode with the highest bioethanol yield of 0.51 g/g- glucose. After glucose was fully consumed and bioethanol was produced, the fermented liquid containing high bioethanol concentration needs to be separated. It is because a high bioethanol concentration can inhibit the cells' growth and viability (D'Amore et al., 1990). The target of bioethanol inhibition in yeast cells is the plasma membrane and other organelle membranes. Damage due to the presence of high ethanol concentration may change the membrane structure and its permeability. *S. cerevisiae* is an ethanol-resistant yeast with a maximum ethanol concentration of about 2.5%. However, *D. hansenii* has a low resistance to ethanol, therefore bioethanol separation is necessary.

The separation of bioethanol was carried out by the evaporation technique. The resulted distillate fraction has a bioethanol yield of 0.042 g/g-OPEFB with a concentration of 4.38 g/L. While the concentrate fraction contained xylose of 0.026 g/g-OPEFB and 11.47 g/L. A decline in bioethanol concentration after the separation process was not significantly different since much higher ethanol was found in the liquid distillate. Furthermore, the heating duration during evaporation at 90°C was for 1 h, causing incomplete decomposition of xylose into furan. This is indicated by a small amount of xylose decreased during evaporation, with values in the range of 0.026-0.027 g (Figure 2). The heating of fermented liquid, however, may allow the formation of other compounds which were not identified in this study. Also, sugar produced could be reacted with compounds containing N-group present in the fermented liquid (reaction Maillard). Thus, inhibition of Enzyme-Substrate complexes' formation may occur, which prevents the catalysis of reactions and decreases (at times to zero) the amount of product produced by a reaction.

Xylitol Fermentation

After the hydrolysate from the hydrolysis of solids fraction was used for bioethanol production, the hydrolysate liquid was used when the production of xylitol and xylose were coupled with the remaining evaporated liquid (concentrated fraction). The combination of the two liquids still contains the glucose, which is carried in the hydrolysate from the hydrolysis of the liquid

fraction. This combination was also able to increase the amount of xylose. After the addition of the inoculum, the initial concentration of xylose in the fermentation liquid was higher than that of glucose. A low ratio of glucose and xylose (glucose per xylose) is expected to enhance the production of xylitol. In this research, xylitol produced was 0.433 g/L or 0.007 g/g-OPEFB. The yield per xylose substrate ($Y_{p/s}$) was 0.298 g/g and the percent utilization was only 30% (Table 2). Compared to other studies, the yield obtained was still low. According to Parajo et al. (1998), fermentation by *D. hansenii* from hydrolysate of sawdust produced approximately 0.5 to 9 g/L of xylitol from 18 g/L of xylose, resulting in a maximum yield of 0.79 g/g and xylitol productivity of 0.03 g/(L h). Other researchers reported that eucalyptus hydrolysates ferment with *C. guilliermondii* yeast produced 19 g/L of xylitol, with a product yield of 0.2 g-xylitol/g-xylose and xylitol productivity of 0.1 g/(L h) (Cannetieri et al., 2001). Xylitol production from hydrolysate obtained from enzymatic hydrolysis of beechwood and walnut shell has a xylitol concentration of 2-3.5 g/L, with the xylitol yield of 0.5 g/g within a 72-h fermentation using *C. tropicalis* yeast (Tran et al., 2004).

Based on Figure 4, xylitol was produced after the start of fermentation, and a significant increase occurred up to 24-h fermentation period. Then, the xylitol production continuously decreased and reached a contact rate after the 72-h fermentation period. This trend in the xylitol formation or production is also associated with a decrease in glucose and xylose.

A significant reduction of glucose occurred starting from 0-h to 24-h fermentation. Then, from the 24-h fermentation period, glucose concentration slightly decreased and remained stable from 48-h to 72-h, indicating that its consumption process was stopped. A slight difference was observed in the xylose consumption profile, where a gradual decline occurred up to 48-h. This was followed by a constant xylose concentration, showing the yeast's loss of xylose consumption rate. It is probably due to a substrate limitation and the accumulation of side products (toxic), causing the cell to enter the death phase. As a result, xylitol was no longer produced. Xylitol is obtained during the logarithmic and stationary phases, highlighting that the cell's growth is associated with product formation. According to Sampaio et al. (2005), the cell growth in *D. hansenii* UFV-170 was divided into three phases. First is a rapid cell growth rate (μ = 0.24 /h) and second is a slow cell growth rate with a low xylitol formation. Finally, a xylose consumption rate where xylitol production was higher but the cell growth rate was very low.

The results of this study were lower than previous studies by Mardawati et al. (2015) (Table 8). The fermentation of OPEFB hydrolysates by *D. hansenii,* which contains enough glucose and xylose, generated the average xylitol of 3.088 g/L with xylose utilization of 66% during 96-h (Mardawati et al., 2015). However, in Mardawati's study (Mardawati et al., 2015), synthetic xylose was used as an additional substrate apart from hydrolysate, which may contribute to a high xylitol yield.

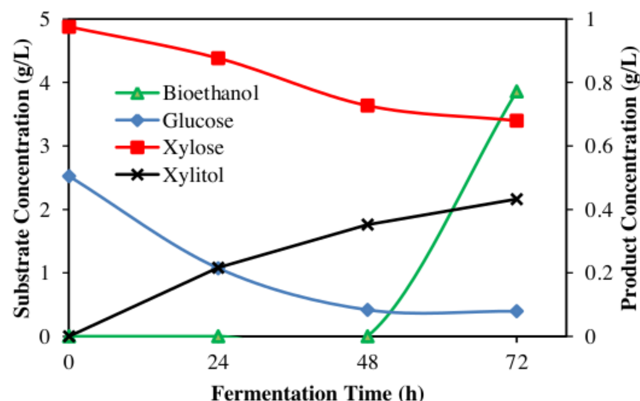

Figure 4. Xylitol fermentation profile by *D. hansenii* in the integrated process

Apart from producing xylitol, *D. hansenii* also has the ability to produce bioethanol (Kassim et al., 2011). In this study, bioethanol was first produced at 48–hour fermentation when the substrate started to run out or was unconsumed. Yet, the formation of bioethanol is not associated with a decrease in the amount of substrate. The results show that bioethanol produced reached the value of 0.772 g/L or 0.013 g/g-OPEFB.

Studies on xylitol and bioethanol production have been extensively investigated in various configurations. A study by Cheng et al. (2014) (Table 8), for instance, investigated the use of *Candida tropicalis* W103 for xylitol and bioethanol production from corn cob. In their study, corn cob was hydrolyzed using an acid solution resulting in two factions of solid and liquid. Both fractions were fermented under aerobic conditions to form xylitol, with the maximum concentration of xylitol generated was 17.1 g/L with a yield of 0.3 g/g xylose. The remained solids were separated and hydrolyzed using cellulase enzymes to produce glucose. After that, the glucose was fermented using the same yeast strain under anaerobic conditions, which resulted in 25.3 g/L of bioethanol after 72-h fermentation. Under anaerobic conditions, *C. tropicalis* consumed glucose in the first stage, followed by the consumption of xylose. Under the aerobic condition, however, the yeast was able to consume both glucose and xylose at the same time. However, the glucose consumption rate was higher than that of the xylose.

Another study by Hickert et al. (2013) (Table 8) used two types of yeasts, including *S. cerevisiae* ICV D254 and *Spathaspora arborariae* NRRL Y-48658, for the production of bioethanol and xylitol from rice husk. The first method used in this study was the hydrolysis of rice husks using 1% sulfuric acid in an autoclave (at 121 ° C for 1 h). The liquid fraction was separated from the solid fraction using filtration. Then, the samples were vacuumed at 70 °C to reach a concentration of glucose, xylose, arabinose, and protein of 27 g/L, 13 g/L, 5 g/L and 5 g/L, respectively. The fermentation was then performed by using a mixture of both yeasts. The alternative method was to simultaneously hydrolyze and ferment liquid and solid fractions using Power cell Enzyme (15 FPU/g) and the addition of both yeasts' mixture. The first method produced bioethanol and xylitol with the value of 11.9 g/L and 3 g/L, while the second method produced higher bioethanol (14.5 g/L) and the same amount of xylitol (3 g/L).

Therefore, to improve the efficacy of xylitol and bioethanol production and yields, a further in-depth study is essential, such as the evaluation of the process configuration for the xylitol and bioethanol production. Also, the investigation of the pretreatment methods which can produce a high amount of substrates (i.e. xylose and glucose) is critically needed.

Partial Process Production

Xylitol Production

1. Hydrolysis

The results from the OPEFB hydrolysis were glucose and xylose. The main product in the hydrolysis of hemicellulose by the xylanase enzyme is xylose. Glucose is expected to be present in the OPEFB hydrolysate, which can further be used as a substrate in xylitol fermentation (Mardawati et al., 2014). The presence of glucose in the hydrolysate is beneficial because it can reduce production costs by reducing the addition of glucose as a nutrient for the growth of *D. hasenii* ITB CCR85 in the xylitol fermentation process.

The study indicates that both glucose and xylose showed an increase in concentration with increasing the hydrolysis time. At the 96-h hydrolysis time, the highest concentrations of glucose and xylose were 5.20 g/L and 3.02 g/L, respectively (Table 3). Furthermore, glucose and xylose concentrations were also influenced by substrate concentration. Nascimento et al. (2012) reported that at a substrate concentration of 20%, enzymatic hydrolysis of *Propis juliflora* batch produced monosaccharides at the value of 80.78 g/L. He et al. (2017) reported that at a substrate concentration of more than 20%, more xylose could be produced than using a substrate concentration below 15%.

Table 3. Concentration and yields of glucose and xylose

Hydrolysis Time (h)	Concentration (g/L)		Hydrolysis Yields (%)	
	Glucose	Xylose	Glucose	Xylose
0	0	0	0	0
12	2.26	1.80	15.17	23.05
48	3.50	2.51	23.49	32.07
72	4.01	2.59	26.92	33.23
96	5.20	3.03	34.90	38.80

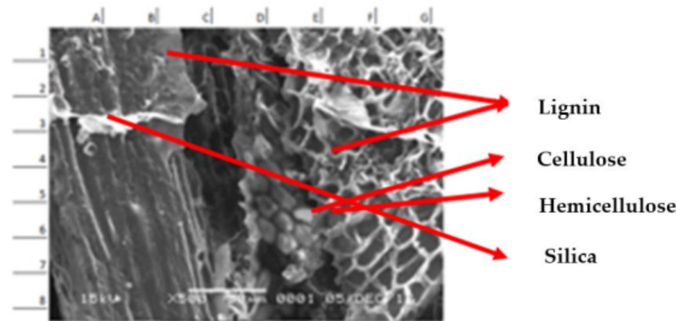

Figure 5. Microstructure of the OPEFB after hydrolysis by SEM image with a magnification of 500x

Table 4. Yield of xylitol and glycerol products with pure and OPEFB hydrolysate substrates

Substrate	Fermentation Time (h)	Xylose (g/L)	Glucose (g/L)	Biomass (g/L)	$Y_{x/s}$ (g/g)	Xylitol (g/L)	$Y_{px/s}$ (g/g)	Gliserol (g/L)	$Y_{pg/s}$ (g/g)
OPEFB Hydrolysate	0	42.44	1.29	0.5	0	0	0	0	0
	48	1.87	6.43	10.4	0.2	0	0	0	0
	72	0	1.13	59.7	1.4	2.61	0.06	0.02	0
	96	0	0.25	35.4	0.8	4.12	0.1	0.01	0.0002
Pure	0	20.27	5.7	0.5	0	0	0	0	0
	48	0	14.52	3.8	0.2	0.39	0.02	0.2	0.01
	72	0	10.46	13.4	0.6	0.25	0.01	0	0
	96	0	4.82	16.4	0.8	1.82	0.1	0	0

Note: *$Y_{x/s}$: concentration of biomass against xylose substrate, $Y_{px/s}$: Xylitol product yield against the substrate, $Y_{pg/s}$: Glycerol product yield against substrate

In the hydrolysis step, an increase in both glucose and xylose yield was proportional to an increase in the hydrolysis time. During 96-h of hydrolysis at 45-50 ℃ and pH 5, the xylose and glucose yield was 38.80% and 34.90%, respectively. These results are consistent with the study of Brienzo et al. (2010), where the xylose yield was 22.48% at 45-60 °C and pH 5.

2. Morphological Characteristics of OPEFB after hydrolysis

The morphological structures of OPEFB after hydrolysis were observed using SEM. From Figure 5, it can be seen that the surface morphology of OPEFB was damaged due to hydrolysis. The surface of the OPEFB after the hydrolysis was covered by silica. In the lignocellulose structure, silica is formed because of the interaction between the siliceous pathway and the surface matrix. During the plant's growth period, the basic elements of silica are transported through the silica pathway to the craters on the surface of the plant's membrane cell (Omar et al., 2014). Silica contributes to the strength and stiffness of OPEFB (Halbon et al., 1994). Loss of silica may open the silica pathway and produce more amorphous areas in the OPEFB. Thus, it can enhance the performance of the enzymatic hydrolysis process (Omar et al., 2014)

3. Fermentation

The fermentation was conducted in batch condition with xylose substrate and glucose co-substrate addition. Table 4 shows the yield for xylitol and glycerol products with pure and OPEFB hydrolysate substrates. At 96-h fermentation, the xylitol obtained was 4.12 g/L from 42.44 g/L xylose, with the xylitol yield of 0.1 g/g. Compared to the integrated process, the xylitol yield was much lower. Integrated hydrolysis produces monomer sugars (i.e., xylose and glucose) in one bioreactor. In this condition, *D. hansenii* can convert both xylose and glucose simultaneously, thus producing a higher yield.

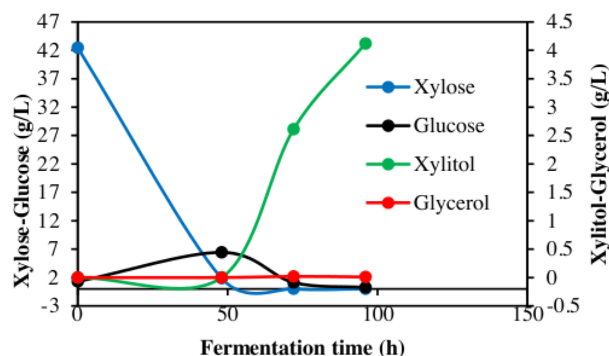

Figure 6. Xylitol fermentation profile by *D. hansenii* in the partial process

Table 5. Growth phases of *D. hansenii* ITB CC R85 cells

Growth phase (h)	Substrates	
	OPEFB hydrolysate	Pure xylose
Lag	0-4	0-8
Logarithmic	4-72	8-72
Stationer	72-96	72-96
Dead	Undefined	Undefined

Using OPEFB hydrolysate as substrate, the highest amount of biomass was 59.7 g/L with 4.12 g/L of xylitol from 42.44 g/L of xylose. The concentration of glucose was 1.29 g/ L (Table 4). This study's results were higher than that of the results from Mardawati et al. (2017), which was 2.1 g/L of xylitol and 2.9 g/ L of biomass from 20 g/L of xylose at 96-h fermentation. The comparison of this work with previous studies can be seen in Table 8.

The increase in xylitol concentration was proportional to a decrease in the xylose concentration. Xylose is used as a substrate and a food supply for microorganisms (Figure 6). Besides xylitol, glycerol was also produced as one of the derivative products from xylose fermentation. In this study, a low amount of glycerol was produced after 72-hour fermentation, with a value of 0.02 g/L. Fermentation was assisted by *D.hansenii* ITB CCR85. The growth phases of *D.hansenii* ITB CCR85 cells are presented in Table 5.

The differences in lag phase duration between the use of pure and OPEFB hydrolysate substrate were possibly due to the variation in its environmental conditions. Increasing the substrate concentration is concurrent with a faster adaptation ability of the cells (Omar et al., 2014). The logarithmic phase is the stage where the cell metabolism is most active and produces optimum enzymes. The cells could synthesize products much faster with a constant amount until the nutrient content in the substrate are depleted. However, any accumulation of products can inhibit the cell's growth.

The maximum value of the cell growth rate in the OPEFB hydrolysate substrate was higher than that of the counterpart. This result indicates that the OPEFB hydrolysate has the potential as a raw material for xylitol production by using the partial process (Table 5).

- **Bioethanol Production**

1. Hydrolysis

Enzymatic hydrolysis is a fundamental stage in the SSF method. In this process, the initial glucose for bioethanol conversion is produced.

Table 6. Glucose concentration and yields of hydrolysis

Hydrolysis Time (h)	Glucose Concentration (g/L)	Hydrolysis Yields (%)
0	0	0
24	4.90	32.88
48	5.56	37.32
72	6.22	41.74
96	6.50	43.62

Table 7. Concentration of and yield ($Y_{p/s}$) of ethanol during fermentation

Fermentation Time (h)	Glucose Concentration (g/L)	Cell Density (10^6CFU/mL)	Bioethanol Concentration (g/L)	$Y_{p/s}$ (g/g)
0	6.5	0	0	0.00
3	3.85	0.57	1.59	0.24
6	3.8	0.83	1.77	0.27
9	3.8	2.6	1.67	0.26
12	2.41	6.5	1.76	0.27
24	0	7.2	1.95	0.30
48	0	5.2	1.98	0.30
72	0	3.3	1.94	0.30

Table 6 shows the glucose concentration from the OPEFB hydrolysis. The results demonstrate that an increase in the hydrolysis time contributes to increasing the glucose concentration. After 96-h hydrolysis, the glucose yield was 43.62% from 6.50 g/L glucose. The substrate concentration affects the amount of glucose produced. In this study, the OPEFB substrate with a concentration of 5% was used. Increasing the viscosity of the material can reduce the enzyme's mobility. Furthermore, there was a potential inhibition from the end product, which may prevent hydrolysis (Anish et al., 2009). These results are consistent with previous studies, that increasing the substrate loading and the reaction time is parallel to an increase in the amount of glucose released (Suhartini et al., 2022; Aliberti et al., 2017).

2. Fermentation

The product yield per substrate ($Y_{p/s}$) describes the amount of bioethanol produced per glucose substrate used. The bioethanol yield is presented in Table 7. It is shown that the $Y_{p/s}$ at 24-h to 96-h fermentation was 0.30 g/g. At 96-h fermentation, the highest bioethanol concentration was obtained with the value of 1.94 g / L.

Compared to the integrated process, the yield obtained from the partial process was much higher. This is related to the glucose concentration used as a substrate in bioethanol fermentation. The integrated hydrolysis process resulted in a lower glucose concentration (5.2 g/L) than the partial process (6.5 g/L). This result may indicate that a high substrate concentration contributes to increase the product yield. The OPEFB substrates contain high cellulose, hemicellulose, and lignin components. Cellulose is a polysaccharide with complex bonds which can be broken down into a simpler form (i.e., monosaccharides) through hydrolysis. Cellulose components are rich in glucose; therefore, it plays an important role in the formation of bioethanol. The resulting hydrolysate then becomes a substrate to provide nutrients for the growth of microorganisms during the fermentation process (Azizah et al., 2012). Several previous researchers also reported studies of bioethanol production from biomass (Table 8).

Figure 7 shows an increase in bioethanol concentration corresponding to a decrease in glucose concentration. Glucose was completely consumed at 12-h fermentation. The bioethanol concentration increased over 48-h fermentation and remained stable over time. This might be caused by the formation of inhibitory compounds, such as furfural, acetic acid, and phenolic. Phenolic compounds are produced from partial lignin damage, which could occur during a pretreatment process (Mardawati et al., 2019).

Table 8. Summary table of comparison of ethanol and xylitol contents between this study and previous studies

Process of production	Xylitol			Bioethanol			
	Yield (Yp/s) concentration (g/L)	**Microorganism**	**Biomass**	**Yield- (Yp/s) concentration (g/L)**	**Microorganism**	**Biomass**	**Ref.**
	0.298 g/g 4.433 g/L	*D. hansenii*	OPEFB	0.078 g/g 4.253 g/L	*S. cereviciae*	OPEFB	This work
	0.3 g/g	*Candida tropicalis W103*	Corncob	25.3 g/L	*Candida tropicalis W103*	Corncob	(Cheng et al., 2014)
Integrated	17.1 g/L						
	3 g/L	*Spathaspora arborariae* NRRL Y-48658 *S. cerevisiae* ICV D254	Rice hull	14.5 g/l	*Spathaspora arborariae* NRRL Y-48658 *S. cerevisiae* ICV D254	Rice hull	(Hickert et al., 2013)
	0.10 g/g 4.12 g/L	*D. hansenii*		0.30 g/g 1.95 g / L.	*S. cereviciae*	OPEFB	This work
	3.088 g/L	*D. hansenii*	OPEFB	-	-	-	(Mardawati et al., 2015)
	2.1 g/L	*D. hansenii*	OPEFB	-	-	-	(Mardawati et al., 2017)
	0.79 g/g 0.5 to 9 g/L	*D. hansenii*	Sawdust	-	-	-	(Parajo et al., 1998)
Partial	19 g/L 0.2	*C. guilliermondii*	Eucalyptus	-	-	-	(Canettieri et al., 2001)
	2-3.5 g/L 0.5 g/g	*C. tropicalis*	Beechwood and walnut shell	-	-	-	(Tran et al., 2004)
	-	-	-	0.51 g/g	*S. cereviciae*	OPEFB	Kasim et al., 2011)
	-	-	-	28.6 g/L	*S. cerevisiae, Candida tropicalis, S. stipitis*	Rice straw	(Suriyachai et al., 2013)
				~42 g/L	*S. cerevisiae IR2T9-a*	Eucalyptus globulus wood	(Jin et al., 2013)
				45.5 g/L	Genetically engineered *S. cerevisiae Y35*	Corn stover	(Yanase et al, 2010)

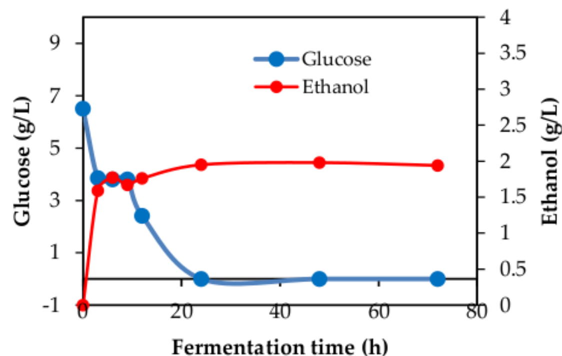

Figure 7. Bioethanol fermentation profile by *S. cerevisiae* on the partial process

This corresponds to the low delignification degree, hence the remains of the pretreatment process caused inhibition. During fermentation, a decrease in ethanol could also be caused by the composition of the substrate, reduction of the enzyme's active sides, and the inefficiency of mass transfer (Mardawati et al., 2019).

Conclusion

In this study, the production of xylitol and ethanol has been explored using two process configurations. The results showed that the xylitol yield was higher in the integrated process than in the partial process. Meanwhile, the ethanol yield is lower in the integrated process compared to the partial. OPEFBs have good potential to be converted into derivative products such as bioethanol and xylitol, even at a large scale. The findings demonstrated that the integrated process of transforming OPEFBs into xylitol and bioethanol has superior performance to the partial processes. In the partial process, the fermentation generated xylitol yield ($Y_{p/s}$) of 0.10 g-xylitol/g-xylose and bioethanol yield of 0.32 g-bioethanol/g-glucose. The xylitol and bioethanol concentrations obtained were 4.12 g/L and 1.94 g/L, respectively. While in the integrated process, the fermentation of hydrolysate from the hydrolysis of the solid fraction produced 0.078 g-bioethanol/g-glucose and $Y_{p/s}$ of 0.298 g-xylitol/g-xylose.

Declarations

Conflict of interests The authors declare no competing interests.

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References

Abdullah, M. A., Nazir, M. S., and Wahjoedi, B. A. (2011) 'Development of value added biomaterials from oil palm agro-wastes', *2nd International Conference on Biotechnology and Food science*, 7, pp. 32-35

Abdullah, N., and Sulaiman, F. (2013) 'The properties of the washed empty fruit bunches of oil palm', *Journal of Physical Science*, 24(2), pp. 117–137

Adney, B., and Baker, J. (2008) *Measurement of cellulase activities*. United States: NREL

Albuquerque, T. L. D., Silva, I. J. D., Macedo, G. R. D., Rocha, M. V. P. (2014) 'Biotechnological production of xylitol from lignocellulosic wastes: A review', *Process Biochemistry*, 49(11), pp. 1779-1789

Aliberti, A., Ventorino, V., Robertiello, A., Galasso, M., Blaiotta, G., Comite, E., Faraco, V., and Pepe, O. (2017) 'Effect of cellulase, substrate concentrations, and configuration processes on cellulosic ethanol production from pretreated arundo donax', *Bioresource*, 12(3), pp. 5321–5342

Anish, R., and Rao, M. (2009) 'Bioethanol from lignocellulosic biomass' in Pandey, A. (eds.) *Handbook of Plant-Based Biofuels*. Boca Raton: CRC Press.

Azizah, N., Al-Baarri, A. N., and Mulyani, S. (2012) 'Pengaruh lama fermentasi terhadap kadar alkohol, pH, dan produksi gas pada proses fermentasi bioetanol dari whey dengan substitusi kulit nanas (Effect of long fermentation on alcohol content, ph, and gas production in the fermentation process of bioethanol from whey

with pineapple skin substitution)', *Jurnal Aplikasi Teknologi Pangan*, 1(3), pp. 72–77 [In Indonesian]

Bailey, M. J., Biely, P., and Poutanen, K. (1992) 'Interlaboratory testing of methods for assay of xylanase activity', *Journal of Biotechnology*, 23(3), pp. 257 – 270

Brienzo, M., Carvalho, W., and Milagres, A. M. F. (2010) 'Xylooligosaccharides production from alkali-pretreated sugarcane bagasse using xylanases from *Thermoascus aurantiacus*', *Applied Biochemistry and Biotechnology*, 162, pp. 1195-1205

Canettieri, E. V., Silva, J. B. A., and Felipe, M. G. (2001) 'Application of factorial design to the study of xylitol production from eucalyptus hemicellulosic hydrolysate', *Applied Biochemistry and Biotechnology*, 94(2), pp. 159-168

Chen, M., Zhao, J., and Xia, L. (2008) 'Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars', *Carbohydrate Polymers*, 71(3), pp. 411-415

Cheng, K., Wu,, J. Lin, Z., and Zhang J. (2014) 'Aerobic and sequential anaerobic fermentation to produce xylitol and ethanol using non-detoxified acid pretreated corncob', *Biotechnology for Biofuels*, 7, pp. 1-9

D'Amore, T., Panchal, C. J., Russel, I., and Stewart, G. G. (1990) 'A study of ethanol tolerance in yeast', *Critical Reviews in Biotechnology*, 9(4), pp. 287 – 304

Dahnum, D., Tasum, S. O., Triwahyuni, E., Nurdin, M., and Abimanyu, H. (2015) 'Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch', *Energy Procedia*, 68, pp. 107–116

Datta, A., Betterman, A., and Kirk, T. K. (1991) 'Identification of spesific manganese peroxidase among lignolitic enzym secreted by Phanerochaete chrysosporium during wood decay', *Applied and Environmental Microbiology*, 57(5), pp. 1453–1460

Directorate General of Estate (2020) *Palm oil production by province (2016-2020)* [Online]. Available at: https://ditjenbun.pertanian.go.id/2020 [In Indonesian]

Dishington, J. M. (2016) *The oil palm agribusiness has much to contribute to the colombian strategy to face the challenges of climate change*. Available at: https://web.fedepalma.org/informes-de-gestion-fedepalma

Foster-Powell, K., Holt, S. H. A., and Brand-Miller, J. C. (2002) 'International table of glycemic index and glycemic load values: 2022', *The American Journal of Clinical Nutrition*, 76(1), pp. 5–56

Gigih, T. P., Novia., and Khairunnas. (2015) 'Pengaruh konsentrasi natrium hidroksida saat pretreatment dan waktu fermentasi terhadap kadar bioetanol dari daun nanas (Effect of sodium hydroxide concentration during pretreatment and fermentation time on bioethanol levels from pineapple leaves)', *Jurnal Teknik Kimia*, 21(3), pp. 16–25 [In Indonesian]

Halbon, J., Gorwa, M. F., Meinander, N., Penttila, M., Keranen, S., Hahn-hagerdal, B. (1994) 'The influence of cosubstrate and aeration on xylitol formation on recombinant *Saccharomyces cerevisiae* expressing the XYL I gene', *Applied Microbiology and Biotechnology*, 42(3), pp. 326–333

Hanidah, I. I. (2010) 'Hidrolisis bagas tebu (*Saccharum officinarum L.*) secara asam - enzim dan fermentasi hidrolisat oleh *Pichia stiptis*, *Saccharomyces cerevisiae*, dan *Zymomonas mobilis* (Hydrolysis of sugar cane bagasse (*Saccharum officinarum* L.) by acid - enzymes and hydrolyzate fermentation by *Pichia stiptis, Saccharomyces cerevisiae,* and *Zymomonas mobilis*)', International Seminar Biotechnology [In Indonesian]

Harijono, A., Hertomo, B., and Kasijanto. (2018) 'Penggunaan bioetanol sebagai alternatif campuran bahan bakar pada mesin otto (Bioethanol utilization as an alternative fuel in otto machine)', *Jurnal Rekayasa Energi dan Mekanika*, 1(2), pp. 54-64 [In Indonesian]

He, L., Han, Q., Jameel, H., Chang, H., Philipps, R., and Wang, Z. (2017) 'Comparison of one stage batch and fed batch enzymatics hydrolisis of pretreated hardwood for teh production of biosugar', *Applied Biochemistry and Biotechnology*, 184, pp. 1441–1452

Hickert, L. R., Souza-Cruz, P. B., Rosa, C. A., Ayub, M. A. Z. (2013) 'Simultaneous saccharification and co-fermentation of un-detoxified rice hull hydrolysate by *Saccharomyces cerevisiae* ICV D254 and *Spathaspora arborariae* NRRL Y-48658 for the production of ethanol and xylitol. *Bioresource Technology,* 143, pp. 112 – 116

Jin, M., Sarks, C., Gunawan, C., Bice, B. D., Simonett, S. P., Narasimhan, R. A., Willis, L. B., Dale, B. E., Balan, V., and Sato, T. K. (2013) 'Phenotypic selection of a wild *Saccharomyces cerevisiae* strain for simultaneous saccharification and co-fermentation of AFEX™ pretreated corn stover', *Biotechnology and Biofuels,* 6, pp. 1-14

Kassim, M. A., Kheang, L. S., Bakar, N. A., Aziz, A. A., and Som, R. M. (2011) 'Bioethanol production from enzymatically saccharified empty fruit bunches hydrolysate using *Saccharomyces cerevisiae*', *Research Journal of Environmental Sciences*. 5(6), pp. 573 – 586

Khienpanya, N., Laemsak, N., Sirisansaneeyakul, S., Vanichsriratana, W., Sultan, I. N., Tareen, A. K., and Parakulsuksatid, P. (2010) 'Influence of particle size of pretreatment oil palm trunk fibers from simultaneous saccharification and fermentation on ethanol production', *Thai Society*

for Biotechnology and International Conference, pp. 21-28

Liu, Z., Padmanabhan, S., Cheng, K., Schwyter, P., Pauly, M., Bell, A. T., Prausnitz, J. M. (2013) 'Aqueous-ammonia delignification of miscanthus followed by enzymatic hydrolysis to sugars', *Bioresource Technology*, 135, pp. 23 – 29

Mardawati, E., Andoyo, R., Muhaemin, M., Nurjanah, S., Natawigena, D., Herwanto, T., Handarto., Utama, G. L., Rosalinda., Poppy and Kramadibrata, A. M. (2019) 'Fermentation process of glycerol to arabitol from byproducts of *Reutalis trisperma* biodiesel using yeast *of Debaryomyces hansenii*', *IOP Conference Series: Earth and Environmental Science,* 347(1), pp. 1-8

Mardawati, E., Kresnowati, M., Purwadi, R., Bindar, Y., and Setiadi, T. (2018) 'Fungal production of xylanase from oil palm empty fruit bunches via solid state cultivation', *International Journal on Advanced Science Engineering and Information Technology*, 8(6), pp. 2539–2546

Mardawati, E., Maharani, N., Wira, D.W., Harahap B. M., Yuliana, T., and Sukarminah, E. (2020) 'Xylitol production from oil palm empty fruit bunches (OPEFB) via simultaneous enzymatic hydrolysis and fermentation process', *Journal of Industrial Information Technology in Agriculture*, 2(1), pp. 29–36

Mardawati, E., Purwadi, R., Kresnowati, M., and Setiadi, T. (2017) 'Evaluation of the enzymatic hydrolysis process of oil palm empty fruit bunch using crude fungal xylanase', *ARPN Journal of Engineering and Applied Sciences,* 12(18), pp. 5286-5296

Mardawati, E., Trirakhmadi, A., and Kresnowati, M., and Setiadi, T. (2017) 'Kinetic study on fermentation of xylose for the xylitol production', *Journal of Industrial Information Technology in Agriculture*, 1(1), pp. 1–6

Mardawati, E., Werner, A., Bley, T., Kresnowati, M., and Setiadi, T. (2014) 'The enzymatic hydrolysis of oil palm empty fruit bunches to xylose', *Journal of the Japan Institute of Energy*, 93(10), pp. 973–978

Mardawati, E., Wira, D. W., Kresnowati, M., Purwadi, R., and Setiadi, T. (2015) 'Microbial production of xylitol from oil palm empty fruit bunches hydrolysate : The effect of glucose concentration', *Journal of the Japan Institute of Energy,* 94(8), pp. 769-774

Nascimento, D. C. O., Ferreira, A. S., Monteiro, S. N., Aquino, R. C. M. P., and Kestur, S. G. (2012) 'Studies on the characterization of piassava fibers and their epoxy composites', *Composites Part A: Applied Science and Manufacturing*, 43(3), pp. 353-362

Ngadi, N., and Lani, N. S. (2014) 'Extraction and characterization of cellulose acetate from empty friut bunch (EFB) fiber', *Jurnal Teknologi*, 68(5), pp. 35–36

Octavia, S (2008) Efektivitas Kombinasi Proses Perendaman dengan Amoniak dan Asam pada Pengolahan Awal Biomassa Sebagai Bahan Baku Pembuatan Bioetanol (The Effectiveness of Combination Between Acidic and Ammonia Soaking Process in the Pretreatment of Biomass as a Raw Material in Bioethanol Processes). Thesis. Intitut Teknologi Bandung. Bandung [In Indonesian]

Omar, F. N., Mohammed, M. A. P., and Baharuddin, A. S. (2014) 'Microstructure modelling of silica bodies from oil palm empty fruit bunch (OPEFB) fibres', *Bioresources*, 9(1), pp. 938-951

Parajo, J. C., Dominguez, H., and Dominguez, J. M. (1998) 'Biotechnological production of xylitol. Part 1: Interest of xylitol and fundamentals of its biosynthesis', *Bioresource Technology*, 65(3), pp. 191-201

Qin, L., Liu, Z., Jin, M., Li, B., and Yuan, Y. (2013) 'High temperature aqueous ammonia pretreatment and post-washing enhance the high solids enzymatic hydrolysis of corn stover', *Bioresource Technology,* 146, pp. 504–511

Salvi, D. A., Aita, G. M., Robert, D., and Bazan, V. (2010) 'Dilute ammonia pretreatment of sorghum and its effectiveness on enzyme hydrolysis and ethanol fermentation', *Applied Biochemistry and Biotechnology*, 161(1), pp. 67 – 74

Sampaio, F. C., Mantovani, H. C., Passos, F. J. V., de Moaraes C. A., Converti, A., and Passos, F. M. L. (2005) 'Bioconversion of D-xylose to xylitol by *Debaryomyces hansenii* UFV-170: Product formation versus growth', *Process Biochemistry*, 40(11), pp. 3600-3606

Shinoj, S., Visvanathan, R., Psanigrahi, S., and Kochubabu, M. (2011) 'Oil palm fiber (OPF) and its composites: A review', *Industrial Corps and Products*, 33 (1), 7-22

Suhartini, S., Rohma, N. A., Mardawati, E., Kasbawati., Hidayat, N., and Melville, L. (2022) 'Bioreferining of oil palm empty fruit bunches for bioethanol and xylitol production in Indonesia: A review', *Renewable and Sustainable Energy Reviews,*154, pp. 1-25

Sujatno, A., Salam, R., Bandriyana and Dimyati, A. (2015) 'Studi scanning electron microscopy (SEM) untuk karakterisasi proses oxidasi paduan zirkonium (Study of scanning electron microscopy (SEM) for characterization of oxidation processes of zirconium alloys)', *Jurnal Forum Nuklir*, 9(1), pp. 44–50 [In Indonesian]

Suriyachai, N., Weerasaia, K., Laosiripojana, N., Champreda, V., and Unrean, P. (2013) 'Optimized simultaneous saccharification and co-fermentation of rice straw for ethanol production by *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* co-culture using design of experiments', *Bioresource Technology*, 142, pp. 171–178

Tran, L. H., Yogo, M., Ojima, H., Idota, O., Kawai, K., Suzuki, T., and Takamizawa, K. (2004) 'The

production of xylitol by enzymatic hydrolysis of agricultural wastes', *Biotechnology and Bioprocess Engineering*, 9(3), pp. 223-228

Yanase, S., Hasunuma, T., Yamada, R., Tanaka, T., Ogino, C., Fukuda, H., and Kondo, A. (2010) 'Direct ethanol production from cellulosic materials at high temperature using the thermotolerant yeast *Kluyveromyces marxianus* displaying cellulolytic enzymes', *Applied Microbiology and Biotechnology*, 88(1), pp. 381–388

Zhang, H., and Wu, S. (2014) 'Dilute ammonia pretreatment of sugarcane bagasse followed by enzymatic hydrolysis to sugars', *Cellulose*, 21, pp. 1341 – 1349

Zhang, M., Su, R., Oi, W., and He, Z. (2010) 'Enhanced enzymatic hydrolysis of lignocellulose by optimizing enzyme complexes', *Applied Biochemistry and Biotechnology*, 160(5), 1407-1414

Zulkiple, N., Maskat, M. Y., and Hassan, O. (2016) 'Pretreatment of oil palm empty fruit fiber (OPEFB) with aqueous ammonia for high production of sugar', *Procedia Chemistry*, 18, pp. 155–161

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