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Downstream Processing of Xylitol from Oil Palm Empty Fruit Bunch Hydrolysate: Effects of Impurities from OPEFB Hydrolysate on Xylitol Crystalls

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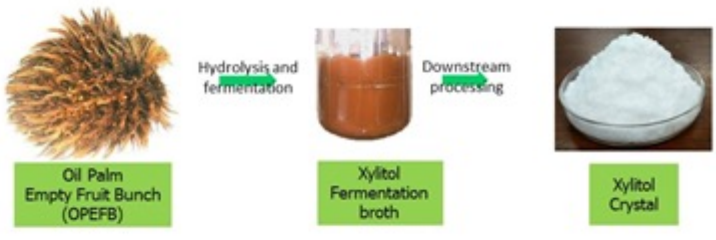
***Abstrak*. Downstream Processing of Xylitol from Oil Palm Empty Fruit Bunch Hydrolysate: Effects of Impurities from OPEFB Hydrolysate on Xylitol Crystalls.** *Tandan kosong kelapa sawit (TKKS) mengandung xilosa yang dapat dimanfaatkan sebagai bahan baku pembuatan xilitol, misalnya melalui fermentasi. Selain mengandung xilitol, kaldu fermentasi yang memanfaatkan hidrolisat TKKS sebagai substrat juga mengandung produk metabolisme lainnya seperti etanol, asam asetat, biomassa sel; dan substrat sisa yaitu: glukosa, xilosa, garam anorganik, dan residu TKKS. Proses hilir kaldu fermentasi ini diperlukan untuk menghasilkan xilitol dengan kemurnian tinggi. Artikel ini menyajikan evaluasi awal proses hilir produksi xilitol, yang meliputi sentrifugasi, adsorpsi dengan karbon aktif, evaporasi, dan kristalisasi. Hasil penelitian menunjukkan bahwa komposisi kaldu fermentasi serta konsentrasi xilitol berpengaruh nyata terhadap rendemen, kemurnian, dan indeks keputihan kristal xilitol yang diperoleh.*

***Kata kunci**: adsorpsi, sentrifugasi, kristalisasi, fermentasi, xilitol.*

***Abstract**. Oil palm empty fruit bunch (OPEFB) contains xylose that can be utilized as the raw material for xylitol production, for example via fermentation. Besides containing xylitol, the fermentation broth using OPEFB hydrolysate as the substrate also contains other metabolic products: ethanol, acetic acid, cell-biomass; left-over substrates: glucose, xylose, inorganic salts, and residue of OPEFB. Downstream processing of the fermentation broth is necessary to produce xylitol with high purity. This article presents the evaluation of downstream processing for xylitol production, which comprises centrifugation, activated carbon adsorption, evaporation, and crystallization. In particular the effects of impurities from OPEFB hydrolysate on xylitol crystal was evaluated. The results show that fermentation broth composition, as well as xylitol concentration, significantly affect the yield, purity, and whiteness index of the obtained xylitol crystal.*

***Keywords**: adsorption, centrifugation, crystallization, fermentation, xylitol.*

Graphical Abstract



1. Introduction

Xylitol is a particular dietary sweetener from polyols family, possesses sweetness power similar to sucrose but the calorific value is only two-thirds of sucrose, reported for preventing ear infections in young children, reduces gingivitis, and control halitosis (Silva and Chandel, 2012). Nowadays, commercial xylitol production is based on the hydrogenation of D-xylose using Raney nickel catalyst at 80 – 130°C and 40 – 70 bar (Mäki-Arvela et.al, 2011). The process consumes much energy which causes the product price to become expensive. An alternative method for producing xylitol is via fermentation of D-xylose using yeast, e.g. *Candida guilliermondii*, *C.tropicalis*, *Pichia anomala*, *C.utilis*, or

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Debaryomyces hansenii (Silva and Chandel, 2012; Chen et al, 2010; Ko et al, 2008). The raw material for both processes, D-xylose, is commonly obtained from acid hydrolysis of lignocellulosic materials which is also known as hemicellulose hydrolysate, e.g. hydrolysate of oil palm empty fruit bunch, corncob, or sugar cane bagasse.

The fermentation broth has a complex composition and low xylitol concentration which makes the separation and purification process of xylitol more challenging (Mussatto et al., 2005). The broth contains impurities such as the matrix of lignocellulose hydrolysate (e.g. metal ions, phenolic compound, furfural, hydroxymethylfurfural), residual of fermentation nutrient (e.g. amino acids, peptide, and inorganic salts), and yeast cell (Sampaio et al, 2006). Some researches have been presented on the purification of xylitol from the fermentation broth, but mostly the fermentation using synthetic media (Desiriani et al, 2017; Misra et al. 2011; Sampaio et al, 2006; De Faveri et al, 2002) or corn cob or corn fiber hydrolysate (Misra et al. 2011; Wei et al, 2010; Mussatto et al, 2006; Rivas et al, 2006; Affleck, 2000).

Xylitol purification from fermented corncob hydrolysate can be performed by silica gel adsorption, continued by evaporation to obtain 60% yield of crystallization and 33% total xylitol recovery from the fermented broth (Mussatto et al, 2006). On the other research, the evaporation of synthetic xylitol-xylose solution continued by crystallization gave 56% crystal yield from 730 g/l concentrated solutions (De Faveri et al, 2002). Sampaio et al. (2006) reported xylitol purification from fermentation on synthetic media by centrifugation followed by microfiltration, treatment with activated carbon, evaporation, and crystallization. The treatment with 20 g/l activated carbon at 25°C for 1 h was able to remove nearly 79% colored contaminants, 94% amino acids, and 69% total proteins, while xylitol recovery was almost complete.

Misra et al (2011) carried out the xylitol separation and purification from the fermentation of synthetic xylose and corncob hemicellulosic hydrolysate via liquid-liquid extraction continued by precipitation. This process configuration was able to recover 67.44% xylitol. However, this method is difficult to apply and expensive. Another process configuration evaluated was absorption by 15.0 g/l of activated carbon followed by vacuum concentration to reach 10 times supersaturation of initial concentration and crystallization at -20°C for initiation and the at 8°C. Overall this process gave 43.97% xylitol recovery. Rivas et al. (2006) reported the use of 1 kg of charcoal/15 kg of fermented corncob hydrolysates increased in xylitol content of up to 0.6873 kg/kg of nonvolatile components, almost achieved complete decoloration, 81.9% proteins removal, and 66.7 % other nonvolatile compounds removal, keeping xylitol loss to an acceptable level (3.2% of the initial amount).

Wei et al. (2010) suggested decoloration of fermentation broth from corncob hydrolysate with activated carbon, followed by a desalting process with a combination of two ion-exchange resins (732 and D301) and UBK-555(Ca^{2+}) to separate residual sugars. The process was continued vacuum-concentration and crystallization produced 95% purity of regular tetrahedral crystals. Another method to recover xylitol from fermentation broth is membrane separation. Affleck (2000) separated xylitol from fermented corn fiber hydrolysate by 10,000 molecular weight cutoff (MWCO) polysulfone membrane, allowing 82.2–90.3 % of xylitol contained in the broth to pass through and retained 49.2–53.6 % of the Lowry's method positive material, i.e. oligopeptides and peptides. Crystallization of permeate produced 90.3% crystal purity.

Not many references discussed the downstream processing of xylitol from fermentation broth using OPEFB hydrolysate as the substrate. The color of fermentation broth using OPEFB hydrolysate as the substrate also presents another challenge for xylitol purification. Kresnowati et al (2015) suggested alternative process configurations for the downstream processing of microbial xylitol to consist of 4 steps: biomass separation, removal of protein and other macromolecules, xylitol concentration, and purification. This paper presents preliminary results on xylitol downstream processing from the fermentation of OPEFB hydrolysate using the previously suggested process configuration. The four steps of downstream processing were translated as centrifugation, carbon active adsorption, evaporation, and crystallization. As a comparison, a similar process configuration was also applied to xylitol fermentation broth using synthetic media as substrate. The evaluation was focused on the xylitol yield, xylitol crystal purity, and the whiteness index of the crystal.

2. Methodology

2.1. Preparation of xylitol-containing fermentation broth

Fermentation was conducted using either OPEFB hydrolysate or synthetic media as the substrate. OPEFB hydrolysate was obtained by hydrolyzing a pre-sterilized mixture of 73 g OPEFB in 730 ml buffer citrate solution at pH 5 using Cellic Htec2 (Novozyme) at 50°C for 120 h and mixed by 150 rpm shaker speed. The obtained hydrolysate was filtered before further use. Fermentation was conducted by mixing the pre-sterilized 1,100 ml nutrient solution (Mardawati et al, 2015), OPEFB filtrate, and 250 ml *Debaryomyces hansenii* inoculum. The fermentation condition was set at 30°C for 120 h and agitated at 150 rpm, using Bioflow 115 fermentation system (New Brunswick). For fermentation on synthetic media, the OPEFB filtrate was substituted with a pre-sterilized concentrated sugar solution, giving final xylose concentration at the beginning of fermentation subsequently 22 g/L.

2.2. Adsorption using activated carbon

The fermentation broth was centrifuged at 6000 rpm and 5°C for 15 minutes. Further separation was conducted by adsorption of the obtained supernatant by 30 g/l activated carbon at room temperature for 1 h.

Subsequently, the solid was separated from the liquid by vacuum filtration.

2.3. Vacuum concentration and crystallization

To increase the xylitol concentration in the solution, commercial xylitol was added to the solution before vacuum evaporation and crystallization. Except indicated otherwise 52 g of commercial xylitol was added to the solution. The previously carbon adsorbed solution was concentrated by evaporation at 90°C and 0.43 bar using Rotavapor (Labconco). The process was stopped when no more liquid droplet was observed at the receiving flask. Crystallization of concentrate of xylitol solution was undertaken at 50°C and 80 rpm for 48 h. Nucleation of xylitol crystal was induced by adding 0.01% commercial xylitol to overcome the crystallization inhibition (Canilha et al, 2008). The obtained crystals were washed using 80 ml ethanol and filtered by a vacuum filter and then dried at 50°C for 24 h.

2.4. Analysis

The sugar concentration in the fermentation broth was determined by HPLC using HPX-87H column, operated at 60°C, refractive index detector, operated at 40°C, 0.01 N sulfuric acid as mobile phase at a flow rate of 0.6 mL/minute.

The yield of xylitol crystal was calculated as the ratio between the mass of the obtained xylitol crystal and the initial xylitol mass in the broth, as is described in equation (1).

$$yield = \frac{total\ mass\ of\ dried\ xylitol\ crystall}{initial\ mass\ of\ xylitol\ in\ the\ solution} \qquad (1)$$

The purity of crystal was determined by thermal analysis using STA PT1600 TGA–DSC (Linseis) at a heating rate of 4°C/minute. Thermal analysis using DSC provides melting point, T1, and melting enthalpy, ΔHm. The impurities fraction, x, was evaluated by the van't Hoff equation as presented by equation (2) (Tong et al, 2007).

$$x = \frac{\Delta H_m (T_0 - T_1)}{R T_0^2} \qquad (2)$$

where R is gas law constant and T0 is the melting point of pure substance.

The crystal color parameters such as L, lightness (0 scale for black and 100 scale for pure white); a, red to green parameter; and b, blue to yellow parameter, were obtained by 3nh colorimeter (Shenzhen Threenh Technology Co.). The crystal whiteness index is evaluated by equation (3).

$$\text{Whiteness index} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \qquad (3)$$

3. Results and discussion

Overall downstream processes that were conducted for the xylitol-containing-fermentation broth were as followed: centrifugation of fermentation broth, activated carbon adsorption, vacuum concentration, and crystallization. The quality of the produced xylitol crystals, i.e. yield, purity, and whiteness index which were obtained from both fermentation on synthetic media and fermentation on OPEFB hydrolysate are presented in Table 1.

Table 1. Comparison of yield, purity, and whiteness index of obtained crystals from fermented synthetic media and OPEFB hydrolysate

	OPEFB hydrolysate	Synthetic medium	Xylitol commercial
Purity	95.5 ± 0.6	95.2 ± 4.6	94.43
Whiteness index	64.6 ± 4.4	71.3 ± 3.3	90.7 ± 0.0
Yield	55.2% ± 3.5%	49.3% ± 10.7%	

Interestingly, no significant differences were observed in the purity of the produced xylitol crystal from fermentation on synthetic media and fermentation on OPEFB hydrolysate. The yield of xylitol crystal from synthetic media observed in this research was 49.32%. This number is higher than Misra et al. who reported a yield of xylitol crystal of 39.33% for crystallization at 30°C for 3-4 days (Misra et al, 2011). But this number is lower than Wei et al. (2010) who reported a yield of xylitol crystal of 60.2% for crystallization at -20°C for 48 h.

The color of the xylitol crystals obtained from fermentation on synthetic medium, however, was whiter than the ones obtained from fermentation on OPEFB hydrolysate. The crystal color was influenced by the presence of impurities such as pigments from lignin derivate and caramelized residual sugar in the mother liquor. Although a preceeding active carbon adsorption process has been conducted before the evaporation and crystallization, visual observation indivated that this colour removal process has not been successfully removed all the colour and impurities from the OPEFB hydrolysate fermentation broth. Besides, the presence of impurities also increased the viscosity of the mother liquor (Misra et al, 2011; Wei et al., 2010) and hinder xylitol crystallization (Canilha et al, 2008). The existence of other residual sugars in the solution did not only influence the purity and yield of xylitol crystal, but also the morphology of the obtained crystal, which is in agreement with phenomena reported in literature, for example by (McLachlan and Ni, 2016). On the other hand, the presence of pigments and inorganic salts in the mother liquor had little impact on crystal shape (Wei et al, 2010). The incorporation of impurities on crystal occurred when the desorption of impurity molecules from the interface is not fast enough so that the impurity molecules become kinetically incorporated (Beckmann, 2013). Pigments that are the derivatives of lignin have a higher molecular mass than xylitol. The molecular mass influenced the velocity of the molecule, that is molecule velocity in liquid state inversely to the square root of molecular mass (Montimer, 2008). Therefore, the velocity of pigments is lower than xylitol in concentrate, thereby the pigments were trapped on the crystal structure.

The existence of impurities on the crystal structure led to weaker crystals. Belhamri and Mathlouthi (2004) explained that the lower the molecular weight of impurity, the higher its adsorption on crystal faces and the higher its retarding effect on growth. While, high molecular weight (HMW) impurities such as caramel, although having less blocking effect are generally implied in the mechanism of inclusion of color inside the crystal which imprisons the droplets of HMW colorants remaining at the surface of growing crystals without changing the crystal shape.

The effects of impurities on xylitol crystal quality were studied further by varying the xylitol concentration in the solution. Two experiments were performed. In the first series of experiments, the volume of process solution was increased from 150 mL to 450 mL whereas the same amount of xylitol was added to both solutions, that was 52 g. In the second series of experiments, the volume of process solution was set to be 150 mL whereas the amount of xylitol added was varied, 17.3 g and 52 g, respectively. The quality of the produced xylitol crystals was presented in Figure 1-2.

Lower crystal quality, i.e. whiteness index, purity, and yield, was observed at higher volume (Figure 1). This confirmed previous observations (Misra et al, 2011; Wei et al, 2010; Canilha et al, 2008) that the increasing impurities of the mother liquor correlated with the decreasing crystal quality. On the other hand, an increase in xylitol concentration in the process solution increased the crystal quality (Figure 2). De Faveri et al. (2002) reported increasing xylitol concentration improves xylitol recovery through crystallization. The obtained results showed that the increase in xylitol concentration did not only increase xylitol recovery, which was indicated by the yield of xylitol crystal but also increased in purity and color of the produced crystals.

Xylitol down processing processes configuration that consisted of centrifugation, carbon active adsorption, evaporation, and crystallization has been evaluated. These four consecutive steps represented biomass separation, removal of protein and other macromolecules, xylitol concentration, and purification process. Overall, this downstream process configuration was shown to give a considerable yield of xylitol crystal with relatively high purity (> 90%). Although no significant difference was observed in the final xylitol crystal purity produced from the synthetic media and OPEFB hydrolysate fermentation broth, a significant difference in the crystal color was observed. This indicated the need for a more robust and efficient method for color removal. The different scales of experiments strongly suggested the need for higher xylitol concentration in the fermentation broth.

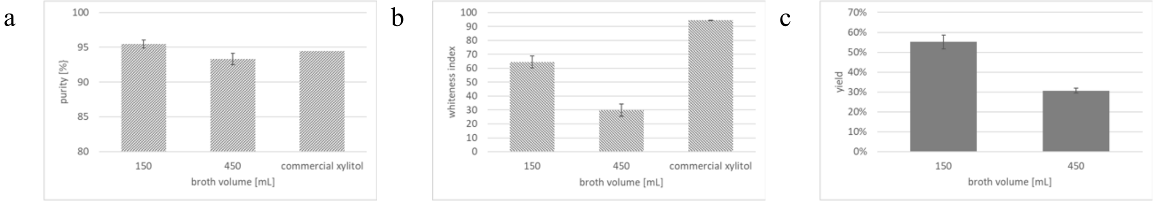

Figure 1. Comparison of purity (a), whiteness index (b), and yield (c) of the obtained crystals from two scales experiment 52 g xylitol in 150 ml and 450 ml broth volume

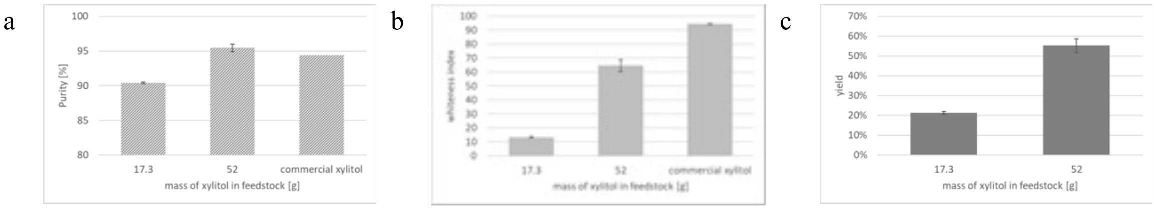

Figure 2. Comparison of purity (a), whiteness index (b), and yield (c) of the obtained crystals from two scales experiment 17.3 g and 52 g xylitol in 150 ml broth volume

4. Conclusions

The use of OPEFB hydrolysate, instead of pure xylose, as fermentation media affects the quality of final products xylitol crystal, in particular in terms of crystal color. Xylitol concentration in the fermentation broth also affects the downstream processing of xylitol, in particular, it influences the yield, purity, and color of obtained xylitol crystals. Prior separation of nonvolatile impurities in the broth, such as inorganic salts and reducing sugars, may enhance the yield, purity, and whiteness index of obtained crystals.

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