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Literature Review

A COMPREHENSIVE REVIEW: INTEGRATED MICROBIAL XYLITOL, BIOETHANOL, AND CELLULASE PRODUCTION FROM OIL PALM EMPTY FRUIT BUNCHES

Budi Mandra Harahap[1], Efri Mardawati[1], Desy Nurliasari[1]

[1] Department of Agroindustrial Technology, Faculty of Agroindustrial Technology, Universitas Padjadjaran,
budi.mandra.harahap@unpad.ac.id

ABSTRACT

Oil palm empty fruit bunch (OPEFB) is one of promising biomass feedstock for green production of biochemical and biofuel. The OPEFB contains valuable sugar polymers such as cellulose and hemicellulose. Enzymatic hydrolysis of both constituents is an eco-friendly conversion process to release monomeric sugars such as glucose and xylose used for fermentation substrates. Xylose, a dominant sugar in hemicellulose, can be biologically converted to xylitol, a low-calorie sugar for food and pharmaceutical applications. Meanwhile, glucose from cellulose hydrolysis can be fermented to bioethanol. Moreover, numerous studies also have reported the use of the pretreated OPEFB as a solid medium for the production of the lignocellulose-degrading enzyme. The current research, however, only focuses on one specific product. An integrated process to produce those products is the best alternative to minimize waste disposal and to increase the value of OPEFB. Thus, this review elaborates the most possible technologies for the integrated production of cellulase, xylitol, and bioethanol as well as the possible challenges in process development.

Kata Kunci: OPEFB, cellulose, xylitol, bioethanol, integrated process

1. INTRODUCTION

The global production of palm oil shows upward trends over the years with a peak of 75.69 million metric tons in 2019/2020 (Shahbandeh, 2020). Indonesia as a leading worldwide exporter of palm oil certainly has numerous palm oil factories that regularly produce both crude palm oil (CPO) and palm kernel oil (PKO) in a huge amount. Environmental issues, however, always raise due to the large-scale production of palm oil(Mahlia, Abdulmuin, Alamsyah, & Mukhlishien, 2001). One such issue is the presence of waste generated from palm oil mills such as oil palm empty fruit bunch (OPEFB). Palm oil mill produces approximately 23 kg OPEFB per 100 kg of fresh fruit. If the production of palm oil in 2018 attained 40 million metric tons (BPS-Statistics Indonesia, 2018), the amount of OPEFB was around 9.2 million metric tons. This shows that OPEFB is abundantly available in Indonesia.

In palm oil mill, the OPEFB is just burnt to generate the steam power plant by incineration or just used for increasing soil nutrient value in palm oil plantation by land application technique (Mahlia et al., 2001). Due to the low value of such products and the availability that is still in large amounts, various studies reported valuable product alternatives (Gupta & Prakash, 2015). The OPEFB can be transformed into various products since it is classified as the lignocellulose feedstock containing 36-43% cellulose, 15-25% hemicellulose, and 22-34% lignin (Ishola, Isroi, & Taherzadeh, 2014; Sudiyani et al., 2013; Triwahyuni, Sudiyani, & Abimanyu, 2015). The OPEFB structure must be degraded to release the fermentable sugars as the monomer of cellulose and hemicellulose polymer (Mardawati et al., 2014). On the other hand, lignin that has a rigid structure hinders the access to this process, so pretreatment is required before the degradation process of cellulose and hemicellulose (Venkateswar Rao, Goli, Gentela, & Koti, 2015).

Hemicellulose, the second major component in the OPEFB, consists of C-5 sugars (xylose and arabinose) and C-6 sugars (mannose, galactose, and glucose) (Saha, 2003). Xylose is a dominant sugar group up to 80% of total hemicellulose in the OPEFB (Harahap & Kresnowati, 2018). A common product from xylose bioconversion is xylitol, one of the sugar alcohols, for diabetics as an artificial sweetener and commonly in toothpaste because of the role in preventing the cavity (Ur-Rehman, Mushtaq, Zahoor, Jamil, & Murtaza, 2015). Green xylitol is produced by the fermentation process. Prior to xylitol fermentation, the adequate xylose amount, however, is needed as a substrate. The stage to recover xylose is initiated by the pretreatment process. Two main phases are formed, viz. residual solid, and spent liquor (Harahap & Kresnowati, 2018). It must be noted that the fundamental consideration in the selection of pretreatment is whether or not the method can maximize the xylose recovery in spent liquor and cellulose content in residual solid, and can minimize sugar dehydration to furan groups. The purpose is that the components in the OPEFB can be nearly wholly recovered.

The residual solid after pretreatment contains a high content of cellulose with high porosity and crystallinity, so it eases the hydrolysis process to produce glucose (K. K. Cheng, Zhang, Chavez, & Li, 2010). Enzymatic hydrolysis by cellulase is a proper technique for glucose recovery from this material. A common product from sugar fermentation that has been numerously studied is bioethanol (Menon & Rao, 2012). Thus, the residual solid after pretreatment is properly transformed into bioethanol. Apart from bioethanol production, this material also is potentially used for a solid medium for cellulase production via solid-state fermentation (Trivedi, Reddy, Radulovich, & Jha, 2015). Solid medium with high moisture level is an appropriate place for filamentous fungi growth.

The current research is only concerned with how to optimize solely single products from OPEFB. The study about the integrated process to produce more than one product is somewhat overlooked. The remained waste is just discarded without any further utilization. After the explanation above, the waste still can be further utilized. Therefore, a comprehensive concept to integrate the production process of xylitol, bioethanol, and the enzyme is addressed in this review. The purpose of the integrated process is to minimize the valuable waste removed and to recover more products, so it can increase the value of the process and economic side.

2. INTEGRATED CONVERSION OF OPEFB

Nowadays, the issue in regards to the depletion of fossil fuel materials as energy sources motivates many researchers to find new energy alternatives. Bioethanol from biomass feedstock is one of the energy alternatives that bring about a lot of benefits as compared to fossil fuel such as a more environmentally friendly process and renewable material (Balat, 2011). However, economic feasibility must be evaluated due to the long process steps. The integrated process with other valuable products is an attractive alternative to minimize the high cost of bioethanol production from lignocellulose feedstock as well as to minimize the amount of waste discarded.

The potential materials for bioethanol production are OPEFB (Kresnowati, Mardawati, & Setiadi, 2015). High cellulose and hemicellulose content in OPEFB can add more value to these materials to not only produce bioethanol, but also xylitol and cellulase. The flow diagram of the integrated production process of those products is shown in Figure 1.

Harahap (2018) reported that OPEFB pretreated with autohydrolysis formed two fractions. The first fraction was spent liquor after pretreatment that composed of high dissolved xylose. The second fraction was

residual solid with a more susceptible structure and contained high cellulose. Hence, according to Figure 1, spent liquor is used for xylitol production while residual solid can be transformed into either bioethanol or solid medium for cellulase production.

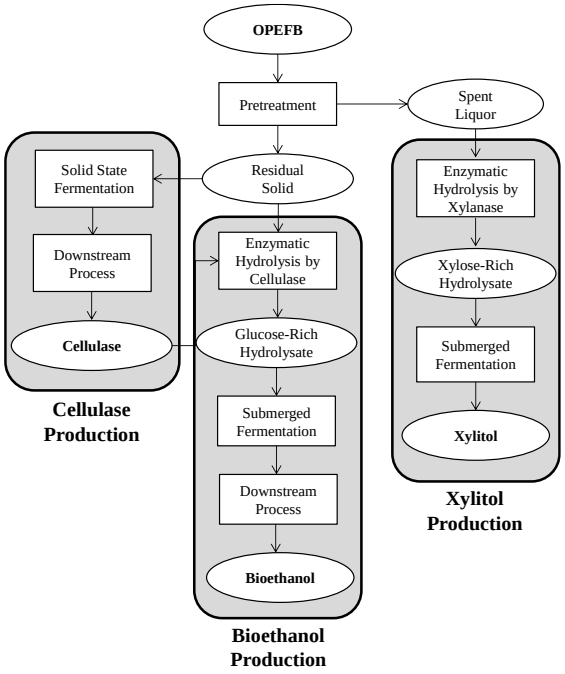

Figure 1. Process Configuration of Integrated Xylitol, Bioethanol, and Cellulase Production from OPEFB

Several studies about the integrated process of xylitol and bioethanol production with various configurations have been reported as shown in Table 1. Xylitol and bioethanol can be simultaneously produced by either a single cell or mixed culture whose function of each cell complements each other. On the other hand, sequential configuration by one or more cells can be one of the alternatives.

The potential yeasts possibly used for simultaneous fermentation of bioethanol and xylitol were *Hansenula polymorpha*, *Candida tropicalis*, *Debaryomyces hansenii*. Such yeasts could metabolize both glucose and xylose in fermentation broth to form such products. Thus, both xylitol and bioethanol can be produced solely by a single cell.

Each product had different yields, depending on the types of yeast and its raw materials used. For instance, *H. polymorpha* ATCC 34438 showed far higher performance for bioethanol production from sunflower (Martínez, Sánchez, & Bravo, 2012). Likewise, *Candida tropicalis* NBRC 0618 converted hydrolysate from the olive tree to be bioethanol in a larger amount (Mateo, Puentes, Moya, & Sánchez, 2015). On the contrary, Damião Xavier et al. (2018) and Harahap & Kresnowati (2018) reported that higher xylitol yield could be obtained from OPEFB, and sisal fibers by *D. hansenii* R85 and *C. tropicalis* CCT 1516, respectively. The xylitol yield obtained, however, was still low.

The use of mixed culture was able to improve xylitol yield. For example, co-culture use between *C. tropicalis* and *S. cerevisiae* produced higher xylitol, reaching to 0.69 g-xylitol/g-xylose (Latif & Rajoka, 2001). The particular role of *C. tropicalis* was for xylitol production, whereas bioethanol was dominantly formed by *S. cerevisiae*. Other appropriate combinations of yeasts such as *S. arborariae* and *S. cerevisiae* or *W. anomalus* and *S. cerevisiae* in rice hull also particularly showed higher xylitol yield (Hickert, Souza-Cruz, Rosa, & Ayub, 2013; Sehnem, Hickert, da Cunha-Pereira, de Morais, & Ayub, 2017). Nevertheless, the problem of this configuration was that the optimum condition must be proper for both products.

This problem is possible to be coped with the sequential configuration. Xylitol and bioethanol formation used xylose-rich hydrolysate and glucose-rich hydrolysate as substrates, respectively, in separate bioreactors (Kumar, Dheeran, Singh, Mishra, & Adhikari, 2014). A single cell, *Kluyveromyces sp.* IIPE453 could be acted as a biocatalyst for both processes, and its corresponding condition was set according to each optimum condition of products. This technique also was implemented by Swain & Krishnan (2015). According to this study, two-stage sequential *C. tropicalis* gave higher yield than that in a

single batch by *S. cerevisiae* and *C. tropicalis* using rice straw. The same result also was reported by Cheng, Wu, Lin, & Zhang (2014) using corn cob.

Table 1. Previous Studies about The Integrated Xylitol and Bioethanol Production

Raw Materials	Process Configuration	Xylitol Yield [g/g]	Bioethanol Yield [g/g]	Reference
Sun flower stalks	Simultaneous configuration by *Hansenula polymorpha*	0.02	0.14	(Martínez, Sánchez, & Bravo, 2012)
Olive tree	Simultaneous configuration by *Candida Tropicalis*	0.18	0.32	(Mateo, Puentes, Moya, & Sánchez, 2015)
OPEFB	Simultaneous configuration by *Debaryomyces hansenii*	0.40	0.26	(Harahap & Kresnowati, 2018)
Sisal fibres	Simultaneous configuration by *Candida tropicalis*	0.32	0.27	(Damião Xavier et al., 2018)
Corn cob	Simultaneous configuration by *Candida tropicalis* and *Saccharomyces cerevisiae*	0.69	0.32	(Latif & Rajoka, 2001)
Rice hull	Simultaneous configuration by *Spathaspora arborariae* and *Saccharomyces cerevisiae*	0.48	0.39	(Hickert, Souza-Cruz, Rosa, & Ayub, 2013)
Rice hull	Simultaneous configuration by *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*	0.86	0.51	(Sehnem, Hickert, da Cunha-Pereira, de Morais, & Ayub, 2017)
Sugarcane bagasse	Sequential configuration by *Kluyveromyces sp.*	0.61	0.43	(Kumar, Dheeran, Singh, Mishra, & Adhikari, 2014)
Rice straw	Sequential configuration by *Candida tropicalis*	0.74	0.50	(Swain & Krishnan, 2015)
Corn cob	Sequential configuration by *Candida tropicalis*	0.32	0.42	(K. Cheng, Wu, Lin, & Zhang, 2014)
Sugarcane bagasse	Sequential configuration by *Candida tropicalis* and *Saccharomyces cerevisiae*	0.29	0.44	(Arrizon et al., 2012)
Sugarcane bagasse	Sequential configuration by *Candida tropicalis* and *Saccharomyces cerevisiae*	0.50	0.44	(Unrean & Ketsub, 2018)

The last configuration of integrated xylitol and bioethanol is sequential fermentation by mixed culture as reported by Arrizon et al. (2012) and Unrean & Ketsub (2018) using sugarcane bagasse. Two separate bioreactors were used in this configuration. Each product was separately cultivated in each bioreactor. Xylitol and bioethanol were produced by *C. tropicalis*

and *S. cerevisiae*, respectively. Arrizon et al. (2012) reported a higher amount of xylitol, going to 0.44 g-xylitol/g-xylose. Conversely, another study from Unrean & Ketsub, (2018) stated that bioethanol could be recovered in a higher amount than that of xylitol.

3. PRETREATMENT

The most crucial step in the integrated process of xylitol and bioethanol production is the pretreatment. In bioethanol production, the objective of pretreatment was to degrade the lignin, to release hemicellulose, to improve the porosity, and to reduce the crystallinity of cellulose (Das, Mondal, & Roy, 2015). The successful pretreatment of bioethanol production is when the lignin and hemicellulose were wholly secreted, and the pretreated solid retained cellulose in high quantity. On the contrary, the pretreatment in xylitol production was aimed to recover xylose and xylooligosaccharides in liquid fraction (Michelin, Romaní, Salgado, Domingues, & Teixeira, 2017). Furthermore, when the pretreated solid still left high hemicellulose content, to maximize the recovery, chemical or enzymatic hydrolysis by xylanase was required.

Several considerations of this pretreatment must be highlighted. Besides, the process must recover high xylose in spent liquor, due to severe condition, further decomposition of sugars formed must be avoided. This was because the yield of xylose automatically would decline. In addition, furan as one of the inhibitor groups of both hydrolysis and xylose fermentation would be formed from this sugar decomposition (Wannawilai, Chisti, & Sirisansaneeyakul, 2017). Figure 2 showed the possible derivative by-products formed during pretreatment (Palmqvist, 2000). To sum up, The technique of pretreatment for bioethanol and xylitol production was slightly different.

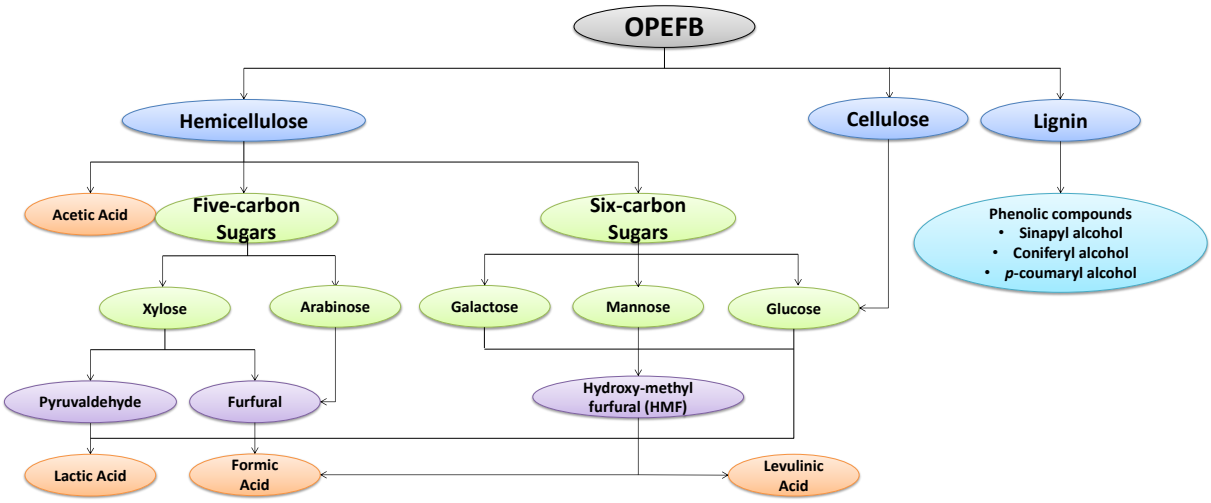

Figure 2. Possible derivative products formed during OPEFB pretreatment

According to Figure 2, several organic acids formed during pretreatment. As we know, organic acid function to catalyze the hydrolysis process. The more hydrogen ion groups are present in the liquor, the more rapid of catalytic degradation occurs (Vanderghem, Richel, Jacquet, Blecker, & Paquot, 2011). Thus, the conventional pretreatment method to recover high xylose amount is by acid hydrolysis. However, it also must be noticed that the concentration, temperature, pressure, and pretreatment time must be controlled so that no furan is formed.

Dilute acid pretreatment for xylose recovery has been considerably reported (Table 2). For instance, the pretreatment of corn stover used dilute sulfuric acid performed at moderate temperature (120°C) with a longer duration to 1.5 h. This pretreatment succeeds to recover

xylose up to 83% with low glucose (approximately 7.5 g/L) (Hong et al., 2016).

Common pretreatment for bioethanol production, steam explosion, also could be used for xylose production under the controlled condition as reported by Duangwang, Ruengpeerakul, Cheirsilp, & Yamsaengsung, (2016). Unfortunately, the steam explosion pretreatment of OPEFB only could obtain the low yield because of further degradation of sugars.

Table 2. Pretreatment for Xylose Recovery from Various Raw Materials

Biomass Feedstocks	Pretreatment types	Pretreatment Condition	Xylose Yield	Reference
Corn stover	Sulfuric acid	9% (w/v) solid loading, 2.5% sulphuric acid at 120 °C for 1.5 h	83%	(Hong et al., 2016)
Oil palm empty fruit bunch	Steam explosion	At 160-200 °C with pressure of 0.6-1 MPa for 5 min	0.09 g/g-OPEFB	(Duangwang et al., 2016)
Oil palm fronds	Inorganic salt and oxidative agent	10% (w/v) solid loading, 0.2 mol/L of $FeCl_3$ reaction at 120 °C for 30 min	72%	(Loow et al., 2017)
Corn cob	Carbon-based solid acid	10% (w/v) solid loading, 0.25 g a new carbon-based solid (C-SO_3H) acid catalyst at 140 °C for 6 h	78 %	(Deshavath, Dasu, Goud, & Rao, 2017)
Oil palm empty fruit bunch	Autohydrolysis	10% solid loading treated by autoclave with water as solution at 127.9 °C for 60 min	0.08 g/g-OPEFB	(Harahap & Kresnowati, 2018)
Corn cob	Oxalic acid	5% (w/v) solid loading, 0.15 mol/L oxalic acid at 140 °C for 2.5 h	85 %	(B. Cheng et al., 2018)
Sugarcane bagasse	Microwave-assisted oxalic acid	2% (w/v) solid loading, 0.4 mol/L oxalic acid at 120 °C for 10 min	96 %	(Yan et al., 2018)
Sweet sorghum bagasse	Self-produced organic acids	5% (w/v) solid loading, 1-1.1% (w/v) self-produced organic acids at 178-182 °C for 40-45 min	84 %	(Lyu et al., 2019)
Oil palm fronds	Sequential ultrasonication and deep eutectic solvent	10% (w/v) solid loading treated by ultrasonication with water as solution at 70% amplitude for 30 min and followed by Choline chloride and urea with 1 : 2 ratio at 120 °C for 4 h	58 %	(Ong et al., 2019)

Another interesting pretreatment technique such as the combination of inorganic salt and oxidative agent pretreatment was able to give 72% xylose recovery with low glucose (less

than 4 g/L) using oil palm fronds (Loow et al., 2017). The use of inorganic salts brought about several advantages such as less corrosive chemicals, relatively cheaper equipment use, recyclable chemicals by ultrafiltration, and lower temperature requirement. Moreover, the use of a new carbon-based solid (C-SO3H) acid catalyst could be used for pretreatment. This catalyst could escalate the xylose recovery by up to 78%.

Oxalic acid, a stronger weak acid than other similar types, could improve the performance of pretreatment (B. Cheng et al., 2018). The yield obtained reached 85% under the mild condition with only 0.032 g glucose/g biomass produced. Oxalic acid was also recyclable, so the chemical reuse could reduce the cost of pretreatment. The assistance of microwave in oxalic acid pretreatment could significantly elevate the xylose recovery up to 96% with such a low glucose content (0.02 g/g). Furthermore, this pretreatment type was also performed under mild condition (120 °C for 10 min).

Apart from dilute acid and inorganic salt, more eco-friendly pretreatment that can be selected as another alternative was the autohydrolysis of Liquid Hot Water (LHW). Theoretically, the concept of lignocellulose degradation is rather similar to dilute acid pretreatment. The difference is the self-produced organic acid. The autohydrolysis was initiated with water dissociation into hydrogen ion and hydroxyl. Hydrogen ion cleaved hemicellulose linkage including acetyl group. The reaction eventually released monomeric and oligomeric sugars and acetic acid. As shown in Figure 2, the other organic acids such as lactic, formic, and levulinic acid are also formed. Autohydrolysis under mild condition just recovered xylose up to 0.08 g/g-OPEFB, and the more glucose was formed (0.09 g/g-OPEFB) (Harahap & Kresnowati, 2018).

The basic concept of acid formation during autohydrolysis was applied by (Lyu et al., 2019). The presence of acids that their ratio had been previously adjusted in spent liquor was used for pretreatment. The yield obtained in this study was 84% using sweet sorghum biomass.

The emerging technology for pretreatment was ultrasonication (Ong et al., 2019). This pretreatment was combined with deep eutectic solvent (DES) that could improve delignification performance and xylose recovery. The use of DES is typically similar to solvent pretreatment. However, DES chemicals were degradable, and this technique could reduce cost production.

4. XYLITOL PRODUCTION

The spent liquor from pretreatment containing high xylose content was appropriately used as a substrate for xylitol fermentation. However, when xylan conversion to xylose from pretreatment is still low, enzymatic hydrolysis is needed.

Hydrolysis xylan to xylose is catalyzed by xylanase. This enzyme is commercially produced by filamentous fungi. The xylanase types acted as hydrolytic agents were Endo-1.4-β-xylanase and β-xylosidase (Polizeli, 2005). Endo-1.4- β-xylanase (1.4- β-D xylan xylanohydrolase; EC 3.2.1.8) randomly cleaved the main chain of inner xylan. As a consequence, the xylooligosaccharide mixture was formed. On the other hand, β-xylosidase (EC 3.2.1.37) could hydrolyze a short chain of xylooligosaccharide to xylose. Besides that, some enzymes were able to cleaved the part of xylan branches bound with xylose such as α-L- arabinofuranosidase (EC 3.2.1.55), α-L-glucuronidase (EC 3.2.1.139), acetyl xylan esterase (EC 3.1.1.72), and coumaroyl esterase (EC 3.1.1.73).

Several factors such as process condition (pH and temperature) and the inhibitor presence influence the hydrolysis performance. Besides, more glucose and xylose as a substrate could inhibit the enzyme. The more sugars in

hydrolysate, the less percentage of hemicellulose hydrolyzed.

In general, xylose catabolism by yeast was initiated by gradual redox reactions, and this process involved xylose reductase (XR) enzyme at the first step and xylitol-dehydrogenase (XDH) enzyme at the following step. XR enzyme was active when NADH and/or NADPH were available, and subsequently this transformed xylose to xylitol as an intermediate product. On the other hand, the XDH enzyme converted xylitol to D-xylulose with the existence of NAD+ (Parajo, Domínguez, & Domíngues, 1998).

Table 3 Previous Studies about Xylitol Production from Various Biomass Feddstock

Raw Materials	Pretreatment/ Detoxification	Microorganism Types	Xylitol Yield	Xylitol Volumetric Productivity	Reference
Sugarcane straw	Dilute sulfuric acid/activated charcoal	*Candida guilliermondii* FTI 20037	0.67 g/g	0.34 g/L/h	(Hernández-Pérez, de Arruda, & Felipe, 2016)
Corn Stover	Dilute sulfuric acid/ No	*Candida tropicalis* XK12K	0.98 g/g	0.57 g/L/h	(Hong et al., 2016)
Corncob	Tetrabutylammonium hydroxide (TBAH) extraction and dilute sulphuric acid/ activated charcoal	*Candida tropicalis* CICC1779	0.77 g/g	2.45 g/L/h	(Jia et al., 2016)
Cotton stalk	Dilute sulfuric acid/activated charcoal	*Candida tropicalis* KUEN 1022	0.36 g/g	0.06 g/L/h	(Sapcı, Akpinar, Bolukbasi, & Yilmaz, 2016)
Corn cob	Dilute acid/no	*Candida tropicalis*NCIM 3123	0.73	0.43 g/L/h	(Yewale, Panchwagh, Rajagopalan, Dhamole, & Jain, 2016)
Sugarcane bagasse	Autohydrolysis followed by sulfuric acid/no	*Candida tropicalis* MTCC 184	0.66	0.80 g/L/h	(Tizazu, Roy, & Moholkar, 2018)
Sugarcane bagasse	Steam explosion followed by dilute sulfuric acid/no	*Candida tropicalis* JA2	0.86	2.81 g/L/h	(Morais Junior, Pacheco, Trichez, Almeida, & Gonçalves, 2019)

Xylitol was optimally formed when the oxygen availability in the bioreactor was limited. Redox imbalance restricted the oxidation rate, so xylitol was accumulated under semi aerobic (Van Zyl, Prior, Kilian, & Kock, 2009). However, when fermentation was performed

under aerobic conditions, the metabolism pathway leads to biomass production (Maris et al., 2006). Several yeasts were able to produce bioethanol, apart from xylitol, when the process condition was set under anaerobic conditions.

The research on xylitol fermentation has been reported as shown in Table 3. According to this table, most of the yeast type used for xylitol fermentation are *C. tropicalis*. Nevertheless, *D. hansenii* was also potentially used for xylitol production (Mardawati, Wira, Kresnowati, Purwadi, & Setiadi, 2015). Various raw materials, strains, pretreatment types, and detoxification effect to the xylitol yield as well as xylitol volumetric productivity. Xylitol produced by *C. tropicalis* JA2 from sugarcane bagasse with a steam explosion followed by sulfuric acid pretreatment had high xylitol yield and volumetric productivity, which were 0.86 g/g-xylose and 2.81 g/L/h, respectively.

5. BIOETHANOL PRODUCTION

Bioethanol is commercially produced by yeast (mostly *S. cerev*isiae) through the fermentation route. The process of bioethanol production was conventionally derived from feedstock such as sugarcane or starch materials. The utilization of these materials causes competition between energy and food needs. The most possible materials for bioethanol production is from biomass feedstock. This inconsumable material type contains sugar polymer such as cellulose and hemicellulose in a rigid structure because of lignin presence. Accordingly, a series of bioethanol production stages from this material is slightly different.

In general, the stages involve pretreatment to wreck and dissolve lignin, hydrolysis to depolymerize cellulose and hemicellulose into monomeric sugars, fermentation by yeast to form bioethanol, and lastly, downstream process step depending on final product desired. The pretreatment for integrated xylitol and bioethanol production is different from the sole bioethanol production that has addressed above. Hydrolysis for glucose recovery includes either acid and enzymatic hydrolysis. The drawbacks of acid hydrolysis are that it performs under harsh conditions, most possibly forms inhibitors such as furan groups due to sugar dehydration, is more corrosive and damages the equipment, and is not green technology (Taherzadeh & Karimi, 2007). The advantages of enzymatic hydrolysis can tackle these drawbacks.

The use of cellulose brings about some benefits such as mild hydrolysis condition, high yield and selectivity, no sugar reduction, and a more eco-friendly process (Taherzadeh & Karimi, 2007). The specific part of lignocellulose, cellulose, is the hydrolytic target of this enzyme. The types of cellulase acted as degrading celluloses are endoglucanase or endo-1.4-β-glucanase (EC 3.2.1.4), cellobiohydrolase EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21).

Glucose produced from the hydrolysis step is fermented by ethanol-producing yeast. Considerable research on bioethanol production from OPEFB has been reported. One of the main factors influenced is the pretreatment type. Fatriasari, Raniya, Oktaviani, & Hermiati (2018) fermented OPEFB by *S. cerevisiae* InaCCY93 to produce bioethanol with microwave-assisted acid pretreatment. The yield obtained was 0.43 g-bioethanol/g-glucose. Another study reported by Kim & Ho (2013) stated that 0.45 g-bioethanol/g-glucose was obtained by *S. cerevisiae* W 303-1A with sulfuric acid followed by NaOH pretreatment.

The higher yield of bioethanol, around 0.46 and 0.48 g-bioethanol/g-glucose, was produced by *S. cerevisiae* ATCC 96581 and *S. cerevisiae* ATCC 26602, respectively (Millati et al., 2011; Sukhang, Choojit, & Reungpeerakul, 2019). Both used heat-assisted sulfuric acid pretreatment. The use of different yeast types, *Kluyveromyces marxiamus* TISTRS5116, could also produce high bioethanol using this raw material, approximately 0.3 g-bioethanol/g-OPEFB.

6. CELLULASE PRODUCTION

The pretreated solid as a residue of xylose production is also possible to be used for a solid medium of cellulose fermentation. Solid medium with sufficient moisture content facilitates filamentous fungi to optimally grow. The water consumption is also far lower than conventional fermentation (Submerged fermentation). Consequently, SSF can overcome the environmental problem because of the huge amount of liquid waste discarded.

Table 4 Solid-state fermentation (SSF) by Filamentous Fungi for cellulase production

Solid state medium	Filamentous fungi	Fermentation Scale	Maximum enzyme activity	Reference
Green seaweed Ulva fasciata	*Cladosporium sphaerospermum*	Flask	Endoglucanase = 10.20 U/g FPase = 9.60 U/g	(Trivedi et al., 2015)
Wheat bran	*Trichoderma reesei*	Rotary tank bioreactor	Endoglucanase = 13.5 U/g FPase = 8.2 U/g	(Ortiz et al., 2015)
Wheat bran	*Aspergillus oryzae*	Lab-scale bioreactor	Endoglucanase = 123.64 U/g FPase = 0.40 U/g β-glucosidase = 18.32 U/g	(Pirota et al., 2016)
Vegetable waste	*Trichoderma sp.*	Flask	FPase = 16.1 FPU/g	(Lah et al., 2016)
Wheat bran + Avicel	*Penicillium oxalicum*	Flask	FPase 34 U/g	(Su et al., 2017)
Agave atrovirens	*Trichoderma asperellum*	Tray bioreactor	Endoglucanase = 12,860 U/g Exoglucanase = 3144,4 U/g β-glucosidase = 384.4 U/g	(Nava-Cruz et al., 2016)
Carnauba straw residue	*Trichoderma reesei*	Flask	Xylanase = 99.5 U/g Endoglucanase = 13 U/g FPase = 0.9 U/g	(da Silva et al., 2018)
Wheat bran	Trichoderma reesei	Tray bioreactor	457 U/g	(Idris, Pandey, Rao, & Sukumaran, 2017)
Banana leaves	Aspergillus sp.	Flask	Endoglucanase = 2.2 U/g FPase = 0.94 U/g	(Kulkarni et al., 2018)
Wheat bran	Inonotus obliquus	Flask	Endoglucanase = 27.15 U/g FPase = 3.16 U/g β-glucosidase = 2.53 U/g	(Xu, Lin, Zang, & Shi, 2018)

Several cellulase-secreting fungi mostly reported are Trichoderma, Aspergillus, Penicillium, and Cladosporium. Different fungi and solid medium types give different cellulase activity (Table 4). The maximum enzyme activity is achieved when the fermentation is conducted under favorable conditions. The fermentation temperature of fungi mentioned in

Table 4 is in the range between 25 and 37 °C. The lowest and highest temperature at that range was *C. sphaerospermum* (Trivedi et al., 2015) and *Aspergillus sp*, respectively (Kulkarni, Vaidya, & Rathi, 2018).

The moisture content used was various from 60% to 80% with additional growth nutrition and appropriate pH. For instance, *A. oryzae* grew on wheat bran moistened with Mendel's medium until the moisture content reached 70% (Pirota et al., 2016). Another example was *Aspergillus sp.* grown on banana leaves moistened with sterile salt solution (pH 5) to reach 67% moisture content (Kulkarni et al., 2018).

The fermentation duration affects enzyme activity. For example, *T. asperellum* peaks the highest endocellulase, exoglucanase, and β-glucosidase when the fermentation time was 216, 240, and 312 h, respectively (Nava-Cruz et al., 2016). Meanwhile*, I. obliquus* showed maximum endoglucanase, FPase activity at the time of 10 and longer duration (12 h) to achieve maximum β-glucosidase activity (Xu et al., 2018).

The fermentation could be conducted in either static or agitated reactor. *T. reesei* was fermented on a static tray bioreactor, giving the activity of 457 U/g (Idris et al., 2017). Differently, the same fungi, *T. reesei* resulted in 13.5 U/g endoglucanase activity and 8.2 U/g FPase activity using a rotary tank bioreactor with 2 rpm for 2 minutes of each direction, clockwise and counter-clockwise (Ortiz et al., 2015).

7. CONCLUSION

The integrated bioconversion of OPEFB into xylitol, bioethanol, and cellulase is an attractive design as a strategy to minimize the amount of waste and to add the value of OPEFB. The possible configurations of the integrated xylitol and bioethanol are the simultaneous and separate production of both products by using either a single or mixed culture. The attentive step to recover in a huge amount of each product is the pretreatment process. The main objective of this pretreatment is to recover xylose in spent liquor after pretreatment and to retain high cellulose in residual solid. Thus, the proper pretreatment method is required. After pretreatment, both residual solid and spent liquor are hydrolyzed to recover the sugar, and the sugar is further fermented by particular yeast. Cellulase, a hydrolytic enzyme, also can be produced from residual solid by filamentous fungi via solid-state fermentation and used for cellulose hydrolysis step.

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