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Utilization of Sugars in Hydrolysate from Oil Palm Empty Fruit Bunches for Ethanol Fermentation Using *Pichia stipitis* CBS 5773

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The utilization of pentose derived from hydrolysis of oil palm empty fruit bunches (EFB) were studied using (a) acid hydrolysis at a mild condition, (b) detoxification of hydrolysate containing pentose, (c) cell growth, and (d) fermentation to produce ethanol. Although *Saccharomyces cerevisiae* and *Pichia stipitis* were observed to be able to metabolize pentose in the hydrolysate derived from EFB, only *Pichia stipitis* was able to produce ethanol. When xylose was used as a model compound for pentose, the optimum xylose concentration for fermentation was 30 g/L with the ethanol concentration in fermentation broth upto 13 g/L. Fermentation with higher xylose concentrations produced ethanol with a concentration of about 6 g/L. Fermentation using pentose derived from detoxified hydrolysate could produce ethanol with a concentration in fermentation broth of about 3 g/L.

Keywords: Acid hydrolysis, ethanol fermentation, oil palm empty fruit bunches (EFB), pentose, *and Pichia stipitis* CBS 5773.

INTRODUCTION

The generation of oil palm empty fruit bunches (EFB) is approximately as much as the production of crude palm oil (CPO). EFB is readily collected in a CPO mill with typical generation rates in the range of 8–15 ton/hour. This lignocellulose solid waste contains three major constituents—*cellulose, hemicellulose,* and *lignin*—which may be converted into useful products such as pulp, furfural, fermentable sugars, and technical lignin (Rusmanto and Susanto 2000).

The present researchers' previous study showed that the hydrolysis of EFB under dilute sulfuric acid solution produced hydrolysate containing pentose (C_5-sugars) with a concentration of up to 30 g/L and furfural with a concentration of up to 25 g/L. Sugars and furfural yields were found to depend on the temperature and time of hydrolysis.

The present study aims to utilize pentose for ethanol-fermentation with emphases on the:

1. application of some methods for detoxification of hydrolysate obtained from hydrolysis of EFB, and
2. use of *Pichia stipitis* with pentose as a main carbon source.

This research is, therefore, an extension of the researchers' previous study on the utilization of EFB as a source of chemicals (see Figure 1). It is hoped that the methods developed in this work will also be applicable for other abundant

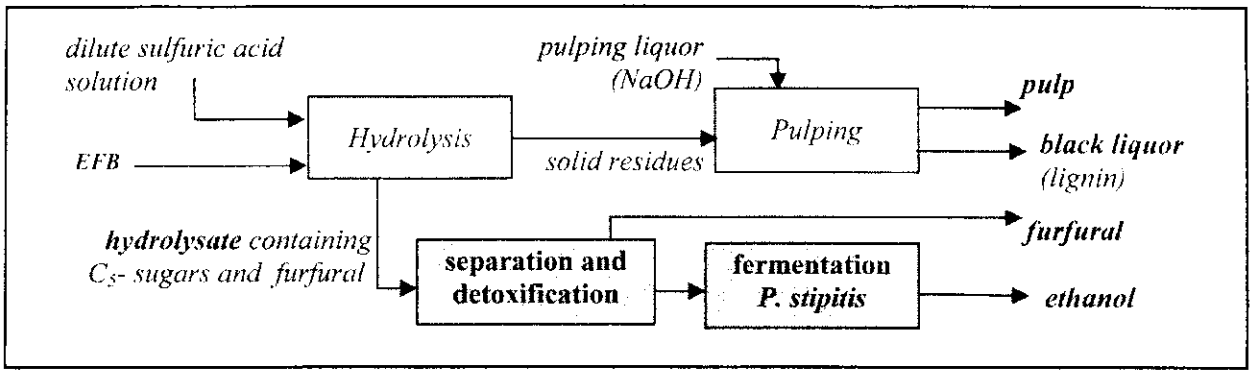


Figure 1. Proposed Process for the Utilization of EFB

agricultural wastes that contain pentosan, such as corn cobs, rice straw, and bagasse.

THEORETICAL BACKGROUND

Acid hydrolysis of hemicellulose

The *acid hydrolysis of hemicellulose* is a well-known process used in the past to obtain pentose and furfural. Corn cobs and oat hulls were usually used as raw materials in this conventional way of producing furfural. The hydrolysis of hemicellulose, particularly of *pentosan*, may be represented in the following reactions:

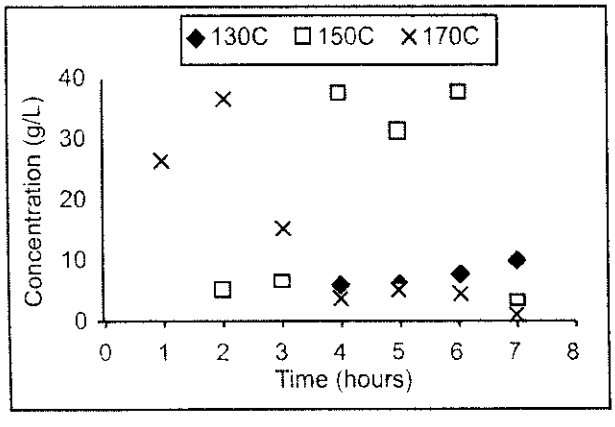
pentosan → pentose (C5-sugars)
pentose → furfural
furfural → smaller molecules

The aforementioned series of reaction are greatly affected by the hydrolysis temperature and time. The typical hydrolysis condition for the production of pentose is a temperature of about 200°C for a few seconds. For the production of furfural, however, hydrolysis may be carried out at about 150°C for a longer period.

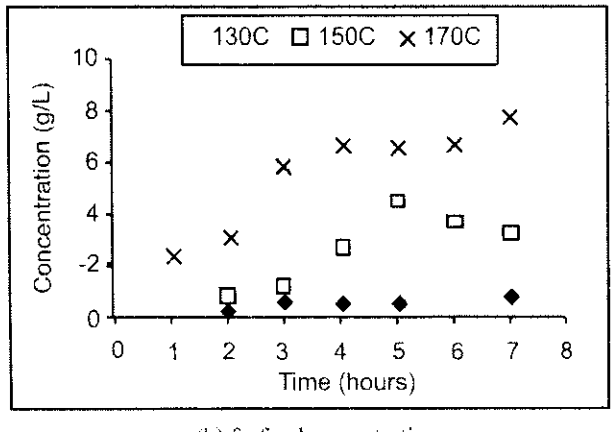
The typical progression in the sugar and furfural contents in a hydrolysate are presented in Figure 2 (Fadjarwaty, Rusmanto, and Susanto 2000). At a hydrolysis temperature of 170°C, the concentrations of sugars in hydrolysate attained a maximum value after 2 h, or 4 h at 150°C. Experiments also indicated that hydrolysis at high temperatures gave a better yield for sugar production. To avoid the need for a high-pressure vessel, hydrolysis is usually carried out at a temperature of about 170°C for 2 h. Therefore, hydrolysate may contain a significant amount of furfural along with the desired amount of pentose (refer to Figure 2b).

Detoxification

Dilute acid hydrolysis of biomass naturally produces hydrolysate that contain various degradation products, such as aliphatic acids,



(a) sugars concentration



(b) furfuralconcentration

Figure 2. Typical Progress in Hydrolysis

Table 1. Example of Microbial Growth in Xylose (McMillan 1996)

	Microorganism	Substrate	Max. Ethanol Conc. *(% w/v)*	Yield *(g/g)*	Productivity *($g \cdot L^{-1} \cdot h^{-1}$)*
1	Wild type yeast *with limited oxygen*	pure xylose	3–5	0.40–0.48	0.30–0.90
2	Recombinant bacteria *anaerobic*	pure xylose	2–4	0.43–0.48	0.30–1.30
	Detoxified Hydrolysate				
3	*P. stipitis CBS 5773*	sugarcane bagasse	24.0	0.35	0.48
4	*P. stipitis CBS 5776*	red oak	9.9	0.46	–
5	*P. stipitis CBS 5776*	aspen	10.9	0.47	0.20

furan derivates, and phenolic compounds. These compounds inhibit the metabolism of microorganisms so they must be removed from hydrolysate before fermentation. Furfural at a concentration of 1.3 g/L in fermentation media has been identified as an inhibitor for microbial growth (Palmqvist et al. 1997).

Thus, several detoxification methods have been proposed, namely: alkaline treatment (pH adjustment), sulfite treatment, anion exchange, carbon adsorption, and vacuum evaporation (Larsson et al. 1999, Palmqvist et al. 1997). The effectiveness and economy of a hydrolysate treatment both depend on the nature of hydrolysate and the characteristics of the microorganisms.

Utilization of pentose

Among the prospective renewable biofuels, ethanol is probably one of the more economical to produce since it can be made from cellulosic wastes instead of conventional feedstocks such as mollase and starch (McMillan 1996). Unfortunately, the ability of conventional yeasts to metabolize pentose is low. Thus, some recombinant microorganisms have been developed for the utilization of pentose. Some examples of microbial growth in pentose are presented in Table 1.

In the researchers' previous study, xylose as a model compound was first used to test the ability of *Saccharomyces cerevisiae* and *Pichia stipitis* to metabolize pentose (Cundasari, Syamsuriputra, and Susanto 2000). Both

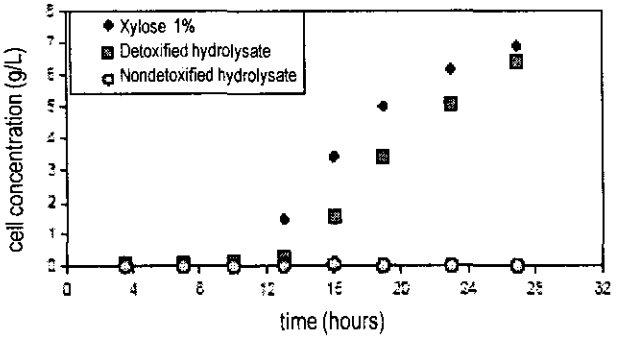


Figure 3. Growth of S. cerevisiae in C5-sugars

microbes are able to growth in media with xylose or pentose derived from the hydrolysis of EFB as a carbon source (see Figure 3). Unfortunately, *S. cerevisiae* did not produce ethanol, while *P. stipitis* produced ethanol with a concentration of about 3 g/L in the fermentation broth.

MATERIALS AND METHODS

Raw material

EFB was obtained from a CPO mill in West Java. A pile of feedstock was prepared by cutting EFB into coarse fibers with about 1-cm long. The raw material was airdried and then stored in a plastic bag. The chemical composition of EFB (ovendry basis) were as follows: α-cellulose, 40.91%; β,γ-cellulose, 20.63%; pentosan (hemicellulose), 22.13%; and lignin, 18.90%.

Acid hydrolysis

The hydrolyses of EFB were carried out in a 20-L digester. Concentrated sulfuric acid of 98%

was used as a catalyst with a charge of 5% mass to EFB based on the researchers' previous experiments. The ratio of cooking liquor to EFB for the process was about 8–10 L/kg. Hydrolysis was conducted at a temperature of about 170°C for 2 h (refer to Figure 2).

Shortly, a draft tube was installed inside the digester to generate effective internal circulation of liquor. This modification was introduced to obtain a more intimate contact at a lower ratio of cooking liquor to EFB and, therefore, to increase the concentration of C5-sugars in the hydrolysate.

Detoxification

In the present study, the hydrolysate obtained from the hydrolysis of EFB was treated as follows. The hydrolysate was evaporated using a rotary vacuum evaporator at about 0.3 bar abs. Theoretically, furfural would evaporate first with its azeotropic mixture with water. Meanwhile, the bottom product would contain a more concentrated pentose solution in water.

The bottom product of evaporation was treated with NaOH solution (10% w/v) for pH 10. Precipitated matters were then removed by filtration. Instead of alkaline treatment, adsorption using active carbon was applied in some experiments to remove furfural and other organic compounds from the bottom product of the evaporation.

Finally, the clear hydrolysate was adjusted to pH 4.5 using sulfuric acid. This detoxified hydrolysate was then used for the subsequent experiments on fermentation.

Ethanol fermentation

Fermentation was carried out using *P. stipitis* CBS 5773 obtained from the Central Bureau voor Schimmelculture, Delft, The Netherlands.

Glucose was initially used as carbon source during the incubation of the strain. Glucose was then substituted step by step with xylose as a model compound for pentose and, eventually, with pentose in the detoxified hydrolysate. The details regarding these experimental works were reported by Susanto and Syamsuriputra (2003).

Inoculum was prepared by planting *P. stipitis* strain on glucose yeast extract agar (GYA). After growing the inoculum for 48 h, it was transferred to another solid medium called *YMPX*, which contains: Yeast 3 g/L; Malt-extract 3 g/L; Peptone 5 g/L; and Xylose 30 g/L.

The adaptation of the strain to the medium which contained xylose was carried out for 48 h. A single viable colony of *P. stipitis* was then inoculated into a 20-mL liquid YMPX medium and was left to grow for the next 24 h. Finally, the viable microbe in the 20-mL liquid YMPX was inoculated into a similar 150-mL liquid YMPX medium.

Erlenmeyer flasks containing various formulation of YMPX media were shaked on a rotary shaker at 120 rpm. The growth of *P. stipitis* in the 150-mL liquid YMPX medium was observed. Samples were taken every 3 or 4 hours to measure cell concentration during the exponential growth phase. This aerobic growth was found to be about 24 h. When the growth reached the stationary phase, anaerobic fermentation was started. The weight losses of the flasks were measured as an indication both of CO_2 release and ethanol formation.

Analysis

The concentration of sugars in the hydrolysate was analyzed as total sugar reduction using spectrophotometry. Furfural in the hydrolysate was analyzed using a high performance liquid chromatography (HPLC) with carbowax separation column and flame ionization detector. This separation column can also be used for analyzing alcohols and organic acids. Cell concentration was measured using the counting chamber or turbidity meter.

RESULTS AND DISCUSSION

Detoxification

As expected, evaporation reduced the furfural content in the hydrolysate to as low as 0.79 g/L (in the bottom product of the evaporator) as can be seen in Table 2. When 75% of the hydrolysate had been evaporated, the concentration of sugars at the bottom of the evaporator was as high as 96.6 g/L, which was high enough for fermentation. Unfortunately, furfural content also increased, due probably to a slipping back during the evaporation.

Table 2. Vacuum Evaporation of Hydrolysate

Expt. No.	Evaporated Volume %	Original Hydrolysate		Distillate	Bottom Product		Reduction of Furfural %
		Furfural g/L	Sugars g/L	Furfural g/L	Furfural g/L	Sugars g/L	
1	30	11.28	9.00	14.28	7.20	10.14	54.0
2	40	3.83	28.90	8.18	0.79	42.59	87.1
3	55	5.34	19.86	79.32	1.03	12.65	91.8
4	60	11.28	9.00	19.54	1.08	45.20	95.9
5	60	3.83	28.90	6.62	1.77	23.66	82.7
6	70	3.83	28.90	5.79	1.51	34.54	88.2
7	75	3.83	28.90	6.02	1.75	96.60	88.6
8	80	9.54	16.59	9.56	1.75	85.50	96.3
9	85	5.34	19.86	5.40	5.22	69.97	86.3

Table 3. Alkaline Treatment

Expt. No.	Furfural (g/L)		Reduction of Furfural (%)	Expt. No.	Furfural (g/L)		Reduction of Furfural (%)
	Before Alkalination	After Alkalination			Before Alkalination	After Alkalination	
2	0.79	0.17	78.7	10	5.54	2.37	57.3
2	0.79	0.27	65.9	11	5.54	2.80	49.5
4	1.08	0.29	72.5	12	5.54	2.86	48.4
6	1.75	0.83	52.6	13	5.54	2.68	51.6

Note: *Experiment numbers 2, 4, and 6 are the same as those in Table 2.*

The use of a fractionating column instead of a simple evaporator may improve the removal of furfural from the bottom of the evaporator. In addition, it may also increase the furfural concentration in the distillate over the liquid–liquid equillibrium composition of about 85 g/L. In this case, a furfural-rich layer with a concentration of about 94% will be obtained.

Further reduction of the furfural content in the bottom product of the evaporator may be obtained with the addition of NaOH solution. However, this is not as effective as when using vacuum evaporation at a ratio of 50 to 95 % effectivity as can be seen in tables 2 and 3. Moreover, the alkaline treatment would result in the addition of chemicals into the hydrolysate. Thus, the addition of NaOH was not considered suitable for the detoxification of the hydrolysate.

The adsorption using activated carbon also reduced the furfural content at the bottom product of the evaporator from 3.12 to 0.88 g/L or by 71.7%. Unfortunately, because some sugars were likewise adsorbed by the activated carbon, the results showed too the significant reduction in sugar content from 70 to 16 g/L. Hence, selecting the right type of activated carbon would perhaps exhibit more effective adsorption of furfural from the sugar solution.

Fermentation

A temperature of about 27°C and a pH of 4.5 were found to compose the best condition for the growth of *P. stipitis* using xylose as carbon source. In these conditions, cell concentration went up to 0.9 g/L, which was more or less the same as that reported by McMillan (1996). The exponential phase took place for about 20–30 h depending on the initial concentration of pentose.

At the end of the exponential phase, the anaerobic condition was applied and the production of ethanol was observed through the evolution of CO_2 bubbles. The ethanol production, as calculated from the weight loss in the fermentation flasks, are given in Figure 4. Note that fermentation using standard xylose resulted

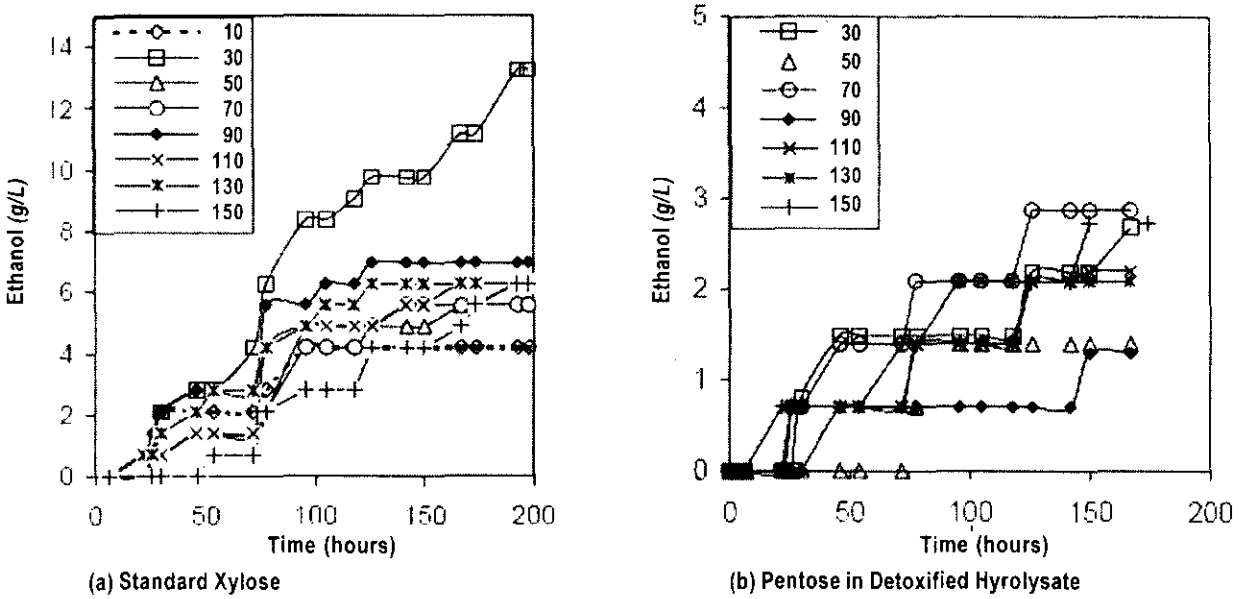


Figure 4. Progress in Ethanol Production With Pentose Concentration as Parameter, g/L

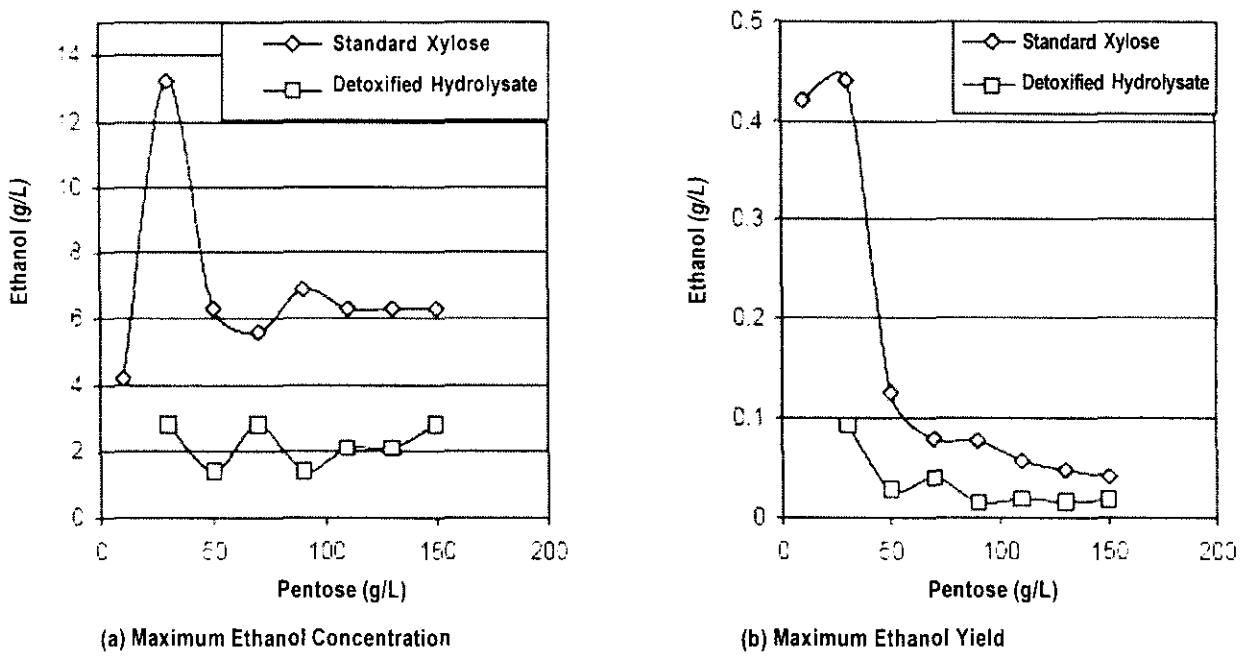


Figure 5. Performance of Fermentation at Various Pentose Concentrations

in higher concentrations of ethanol than those that use pentose in the detoxified hydrolysate. These observations were understandable since the hydrolysate still contained many organic compounds and various types of sugars.

A maximum ethanol concentration of about 13 g/L was obtained from fermentation using standard xylose at a concentration of 30 g/L (see Figure 5). Higher xylose concentrations gave significantly lower ethanol concentrations. This phenomenon was also reported by other researchers such as McMillan (1996).

The conversion of pentose to ethanol in these experiments, however, were very low, falling less than 0.10 g/g compared to the theoretical yield of 0.51 g/g. This could be due to the difficulty of transporting xylose across the cell membrane (McMillan 1996). The researchers, however, reported exceptions in their experiments on fermentation using standard xylose at low concentrations of 10 and 30 g/L.

Tabel 6. Ethanol Fermentation and Subsequent Distillation

Fermentation in 1 L Fermentor *Pichia stipitis CBS 5773; Xylose as a Model for C_5-Sugars*				
	Experiment A		Experiment B	
Initial Xylose Concentration (g/L)	60.00		80.00	
Final Cell Concentration (g/L)	24.40		23.40	
Final Ethanol Concentration (g//L)	26.00		29.00	
Ethanol/Sugars Yield (g/g)	0.43		0.36	
Batch Distillation				
Fraction of Distillate Number	Feed = 865 mL Ethanol Concentration = 26 g/L		Feed = 450 mL Ethanol Concentration = 29 g/L	
	Distillate *(mL)*	Ethanol Conc. in Distillate *(%-mass)*	Distillate *(mL)*	Ethanol Conc. in Distillate *(%-mass)*
1	13	83.60	7	79.70
2	13	65.20	7	84.60
3	13	8.80	7	39.80
4	13	3.10	7	5.20
5	13	1.10	7	0.43
6	13	0.50	5	0.04

Ethanol recovery

Ethanol was recovered easily from the fermentation broth by means of batchwise distillation. Ethanol with a concentration of up to 80% could be obtained as the first fractions of the distillate (about 15% to initial distillation charge). The typical results in the batch distillation of the fermentation broth are itemized in Table 6 (Cundasari, Syamsuriputra, and Susanto 2000).

CONCLUSIONS

The utilization of pentose derived from the hydrolysis of oil palm empty fruit bunches for ethanol fermentation has been studied successfully to be able to draw the following conclusions:

1. Vacuum evaporation significantly reduced the furfural content in the hydrolysate and increased the sugar concentration up to 96.6 g/L.
2. The condensate from the evaporation, which contained a significant amount of furfural, might be treated further for furfural recovery.
3. Alkaline treatment and adsorption onto activated carbon were considered ineffective methods for the detoxification of hydrolysate from oil palm EFB.
4. The optimum condition for fermentation using standard xylose should be as follows: pH of 4.5, temperature of 27°C, and xylose concentration of 30 g/L. In this condition, fermentation produced ethanol with a concentration of up to 13 g/L in the broth.
5. At a pH of 4.5 and a temperature of 27°C, the optimum concentration of pentose derived from the detoxified hydrolysate was 10 g/L. Hovewer, the ethanol concentration in the fermentation broth was only 3 g/L.

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