[ENV08] Biotechnological production of xylitol from oil palm empty fruit bunch, a lignocellulosic waste

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Introduction

Palm oil mills in Malaysia generate approximately 15 million tons of oil palm empty fruit bunch (OPEFB) annually during processing of palm oil from fresh fruit bunch. This OPEFB fiber is a lignocellulosic waste which is renewable and contain high amount of xylan (approximately 24%), a polymer made of pentose sugar xylose. Hydrolyzing acid such as sulfuric acid, hydrochloric acid or hydrofluoric acid can be used as catalyst for reduction of xylan polymer to monomeric sugar xylose. Xylose is used as the starting material for xylitol production. During hydrolysis process other sugars such as arabinose, galactose, glucose and some unwanted components such as acetic acid and furfural are also released. For optimum production of xylitol it is necessary that the byproducts like acetic acid and furfural concentrations in the hydrolysate are to be kept minimum for efficient bioconversion of xylose to xylitol.

Xylitol is a polyol which is widely used as sweetener in food, beverage, pharmaceutical, and in toothpaste industries. This sugar has drawn people's interest as its sweetness is equivalent to sucrose but lower in calories content. Xylitol can be used as sugar substitute for diabetic patient. It is also used to prevent tooth decay and against acute otitis of children (Bot *et al*., 1995).

Oil palm empty fruit bunch fiber was selected as raw material for xylitol production as it contains high amount of xylan. So far no report is available on production of xylitol from empty fruit bunch fiber. The present work deals with the biotechnolgical production of xylitol from raw and treated hydrolysate using empty fruit bunch fiber as raw material and *Debaryomycess hansenii* NRRL Y-7426 as fermentative organism.

Materials and methods

Raw Material

Empty fruit bunch (EFB) fiber used for experimentation was collected from local palm oil mill (United Oil Palm Industries Sdn. Bhd, Malaysia). The raw material was first sun-dried and grinded into size of less than 1mm and oven dried for overnight. The fiber was screened to get homogenized particle of size <1mm. Analysis of main fraction was done using quantitative acid hydrolysis under standard condition as described by Garrote *et al*. (1999). Xylan content of the sample was found to be 24.01±1.0 weight percent of wood on oven dry basis.

Acid Hydrolysis of EFB Fiber

Hydrolysis of empty fruit bunch fiber was carried out by contacting fiber with 1%-6% sulfuric acid in an autoclave at 120˚C reaction temperature using liquid/wood ratio of 8:1 g/g. These operational conditions were chosen on the basis of previous studies reported by Parajo *et al*. (1995). After hydrolysis reaction, solid part was removed by filtration and the filtrate was neutralized with $CaCO_3$. $CaSO_4$ formed was separated from supernatant by filtration and the filtrate was analysed for xylose, glucose, furfural and acetic acid content.

Charcoal Treatment of Hydrolysate

Charcoal detoxification of hydrolysate was carried out by contacting the hydrolysate and activated powdered charcoal using the mass ratio 10 gg^{-1} at room temperature and was stirred in an orbital shaker for one hour as describe by Rivas *et al*. (2002). The liquid phase was centrifuged with rotation speed 3500 per minutes. The hydrolysate was recovered by filtration.

Microorganism

The bioconversion of xylose to xylitol was performed by yeast *Debaryomycess hansenii* NRRL Y-7426. The yeast was obtained from

American Type of Culture Collection, Virginia USA in inactive type of culture. Freeze dried culture was grown in a culture medium containing 10 g/L of commercial xylose, 5 g/L peptone, 3 g/L yeast extract and 3 g/L malt extract. The microorganism was maintained in agar slant tubes containing a medium formulated same as mentioned above together with 20 g/L agar.

Fermentation Media

The neutralized hydrolysates were supplemented with yeast extract, malt extract and peptone, autoclaved at 121˚C and used as fermentation media for xylitol production.

Fermentation

Culture media made from raw wood hydrolysates were fermented in an orbital shaker with agitation speed 150 rpm, at 30˚C under microanerobic conditions using 50mL Erlenmeyer flask with 20mL of culture. pH of media was set to 5.5 at the beginning of the fermentation.

Analytical Method

At given reaction times, neutralized hydrolysate, charcoal treated hydrolysate and samples from the fermentation media were taken, centrifuged, filtered through 0.45µm membranes and were analyzed using HPLC method for xylose, glucose and xylitol component with LC-NH_2 column (oven temperature: 50˚C, mobile phase: 75 % acetonitrile, flow rate: 1.5mL/min) using RI detector. Acetic acid and furfural were analyzed using GC method. The GC analysis were carried out using 80/120 carbopack with FID detector and nitrogen gas as carrier. The corresponding biomass concentrations were calculated from calibration against dry weight determinations in fermentation media made from standard xylose solutions.

Statistical analysis

All tests were carried out in triplicate and data were expressed as an average values. Non linear regression analysis of experimental data were performed with a commercial optimization routine using Newton's Method (Solver Microsoft Excel 2000, Microsoft Corporation, Redmont, WA, USA) by minimizing the sum of squares of deviations between experimental and calculated data as reported by Aguilar *et al*. (2002).

Kinetic models

It is difficult to find out the strict mechanism of hydrolysis reaction. The first simplified kinetic model for hydrolysis of lignocellulosic material was proposed by Saeman in 1945 which is represented as:

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Decomposition products} \quad (1)$$

This model was designed for hydrolysis of cellulose from fir wood using sulfuric acid as hydrolyzing acid but it can also be applied to hemicellulose hydrolysis (Herrera *et al*., 2004 & Téllez-Luis *et al*., 2002) as follows:

$$\text{Xylan} \xrightarrow{k_1} \text{Xylose} \xrightarrow{k_2} \text{Furfural} \quad (2)$$

where k_1 is the rate of xylose release (min^{-1}) and k_2 is the rate of xylose decomposition (min^{-1}). Based on this reaction model and solving differential equation leads to equation 3 which expresses the concentration of xylan monomer $[X]$ as a function of time (t) :

$$[X] = \frac{k_1[Xn_o]}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + [X_o]e^{-k_2} \quad (3)$$

The initial concentration of xylan and xylose at time 0 are defined as $[Xn_o]$ and $[X_o]$ respectively. Assuming that $[X_o]$ is close to 0 which yields equation 4.

$$[X] = \frac{k_1[Xn_o]}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad (4)$$

Xn_o was calculated by using equation as follows:

$$Xn_0 = \frac{150}{132} \text{x} \frac{CXn_o}{LSR} \text{x10} \quad (5)$$

where CXn_0 represents the initial composition of xylan in EFB fiber which was 24.01g xylan/100g EFB fiber on oven dry basis. LSR is the liquid/solid ratio (8g liquid/g EFB fiber) and 150/132 is the ratio of stoichiometric factors. In this work, equation 4 was used as Saeman's model for hydrolysis of hemicellulose.

Similar model was developed for describing the concentration of glucose in hydrolysate. Again it is to be mentioned that decomposition reaction is negligible and the model can be simplified as follows:

$$\text{Glucan} \xrightarrow{k_3} \text{Glucose} \quad (6)$$

where k_3 is the rate of glucose release (min^{-1}). Solving equation 6 leads to equation 7 which expresses the glucose concentration $[G]$ as a function of time (t):

$$[G] = [G_o]x\left(1 - e^{-k_3 t}\right) \quad (7)$$

where G_o is the potential glucose concentration (g^{-1}) which was estimated by regression.

The byproduct furfural is considered as the main degradation product in hemicellulose hydrolysate. A model was developed for determination of furfural concentration and can be represented by equation 8 where k_4 is the rate of furfural generation (min^{-1}). The furfural concentration $[F]$ represents as function of time (t):

$$[F] = [F_o]x\left(1 - e^{-k_4 t}\right) \quad (8)$$

where $[F_o]$ is potential concentration of furfural (gl^{-1}) which was estimated by regression.

The modelling of another byproduct acetic acid was developed on the basis of the equation 9.

$$\text{Acetyl groups} \xrightarrow{k_5} \text{Acetic acid} \quad (9)$$

where the regression parameter k_5 is the rate of acetic acid generation (min-1). Acetic acid concentration $[A_c]$ can be expressed as a function of time (t) as shown in equation 10:

$$[A_c] = [A_{c_o}]x\left(1 - e^{-k_5 t}\right) \quad (10)$$

where $[A_{c_o}]$ is the potential concentration of acetic acid (gl^{-1}) which was estimated by regression analysis.

A non-linear regression analysis was applied to equation 4, 7, 8 and 10 to obtain the kinetic parameters and constants. The estimation of results was depended on R^2 to establish the reliability of the models.

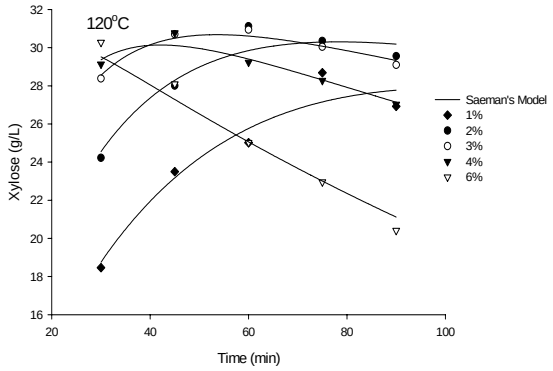
Results and Discussions

Hydrolysis Kinetic

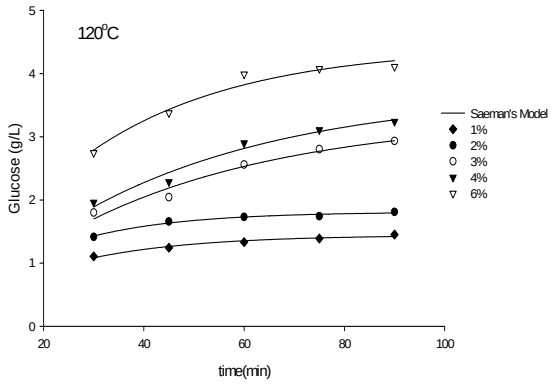
Analysis of empty fruit bunch main fraction showed that this raw material comprised of high content of xylan (24.01±1.0 %) which was adequate for xylitol production. Figures 1 (a-d) show the experimental and predicted data of xylose, glucose, furfural and acetic acid at 120˚C and 1-6% H_2SO_4. Figure 1(a) shows the production of xylose in hydrolysate. Concentration of xylose was increased progressively with increase of H_2SO_4 concentration (1-2%) and reaction time. When higher concentration of H_2SO_4 (3 and 4%) were used, production of xylose was increased till 60 min of reaction time period and then slightly decreased when longer reaction period was used. This decrease may be due to degradation of xylose to furfural. When H_2SO_4 concentration was 6% it ultimately resulted more degradation of xylose and $20.4gl^{-1}$ of xylose was obtained after 90 min reaction time period. Highest concentration of xylose ($31.12gl^{-1}$) was observed at 60 min reaction time period and 2% H_2SO_4 which gave 39% higher compared to those reported by Herrera *et al*. (2004) when sorghum straw was used. The value obtained was close to the potential xylose ($34.1gl^{-1}$) indicating that the conditions applied was suitable for efficient hydrolysis of xylan.

Figure 1(b) explains the production of glucose in hydrolysate. Glucose concentration was progressively increased when higher H_2SO_4 concentration and reaction time was used. Highest concentration of glucose ($4.1gl^{-1}$) was obtained at severe condition (6% H_2SO_4 and 90 min). Furfural generated by xylose degradation was increased when higher concentration of H_2SO_4 and longer reaction time period was used (Figure 1(c)). Highest furfural ($3.34gl^{-1}$) concentration was obtained at 6% H_2SO_4 and 90 min reaction time period. These results suggested that a low H_2SO_4 concentration and low reaction time should be selected to minimize the formation of furfural which is an inhibitor to the growth of microorganism involved in xylitol production.

Figure 1(d) shows the formation of acetic acid in hydrolysate. Acetic acid derived from the hydrolysis of acetyl groups which is bound to the hemicellulose monomer, attained the maximum value ($3.93gl^{-1}$) at highest H_2SO_4 (6%) concentration and longest reaction time period (90min).



(a)



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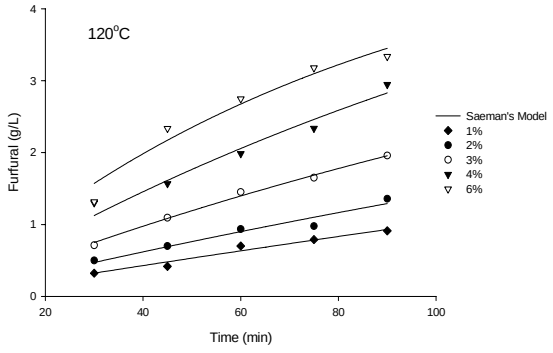

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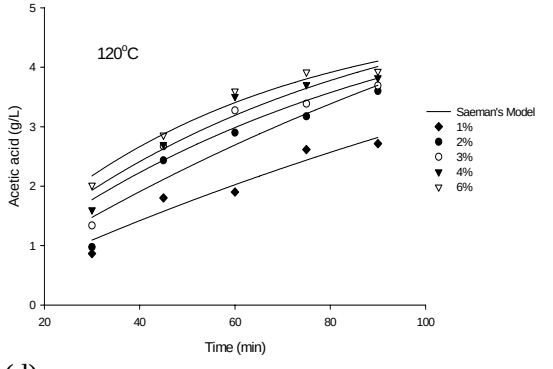


(d)

FIGURE 1 Experimental and predicted data of (a) xylose, (b) glucose, (c) furfural and (d) acetic acid in hydrolysate using 1-6% H_2SO_4 at 120˚C.

Kinetic and statistical parameters obtained for xylose, glucose, furfural and acetic acid released during H_2SO_4 hydrolysis of empty fruit bunch fiber at different sulfuric acid concentration is shown in table 1.

Concentration of initial xylan $[Xn_o]$ was fixed at 34.1gl^{-1}, taking into account the solid/liquid ratio and stoichiometric ratios. The rate of xylose production (k_1) was found to be higher compared to degradation rate (k_2) value which described the formation of xylose was faster than the generation of furfural byproduct. The determination coefficient (R^2) showed a good agreement between experimental and predicted data. The values of k_1 and k_2 were increased with H_2SO_4 concentration which predicted that the maximum value of xylose should be obtained with higher H_2SO_4 concentration and less reaction time period.

Glucose is a by-product obtained during the hydrolysis of EFB biomass. In the model of glucose, potential glucose $[G_o]$ was introduced as a new regression parameter as it could not be obtained experimentally. The rate of glucose production (k_3) varied between 0.024-0.046 min^{-1} while the potential glucose $[G_o]$ value varied between 1.44-4.4gl^{-1}. These values were considered as susceptible values for glucan released (Herrera *et al*., 2004 & Tellez-Luis *et al*., 2002). The high value of determination coefficient (R^2) confirmed that the models did fit well (R^2>0.97).

In the model of furfural, it was observed that the rate of furfural formation (k_4) was increased with increase in H_2SO_4 concentration which was in agreement with the rate of xylose degradation to furfural (k_2). Potential furfural concentration $[F_o]$ varied from 5.3-7.8gl^{-1} for each regression. These values were higher than the values reported using sugar cane bagasse which gave 2-4gl^{-1} (Aguilar *et al*., 2002)

The behavior of acetic acid formation was expected similar to xylan as the acetyl groups contained in the hemicellulose. Acetic acid concentration was increased with increase of H_2SO_4 concentration and the value of $[Ac_o]$varied from 5-7gl^{-1}. The value of determination coefficient (R^2) showed a good

agreement between experimental and predicted data (R^2>0.9).

TABLE 1 Kinetic and statistical parameters of product released during hydrolysis of empty fruit bunch at 120˚C.

	1%	2%	3%	4%	6%
Xylose					
k_1 (min^{-1})	0.028	0.045	0.068	0.082	0.169
k_2 (min^{-1})	0.002	0.002	0.002	0.003	0.006
R^2	0.94	0.93	0.95	0.93	0.97
Glucose					
k_3 (min^{-1})	0.046	0.052	0.024	0.024	0.033
G_o (gl^{-1})	1.444	1.810	3.328	3.702	4.428
R^2	0.97	0.98	0.97	0.97	0.97
Furfural					
k_4 (min^{-1})	0.001	0.003	0.005	0.006	0.012
F_o (gl^{-1})	7.766	5.251	5.510	6.567	5.283
R^2	0.96	0.95	0.99	0.96	0.96
Acetic Acid					
k_5 (min^{-1})	0.005	0.006	0.013	0.014	0.019
Ac_o (gl^{-1})	7.492	8.460	5.631	5.560	5.020
R^2	0.93	0.90	0.90	0.93	0.96

Detoxification of hydrolysate

Presence of byproduct components in hydrolysate such as acetic acid and furfural created problems during fermentation especially for the growth of microorganism. Detoxification of hydrolysate with activated charcoal was employed since it is lower in cost and an efficient method for removal of byproduct components (Parajo *et al*., 1996). Concentration of byproduct components decreased more than 50% from their initial concentration while concentration of xylose and glucose decreased less than 10% when activated charcoal treatment was applied. Results which obtained demonstrated that treatment methods were effective in removal of inhibitory components from hydrolysate.

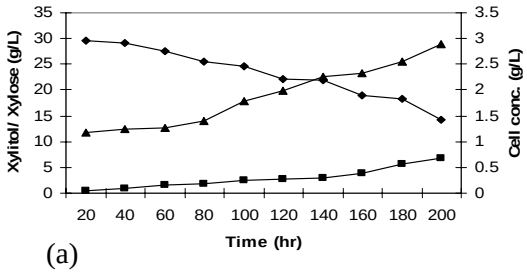
Fermentation

Bioconversion of xylose to xylitol was performed with hydrolysate containing optimum concentration of xylose for both raw wood hydrolysate (RW) and charcoal treated hydrolysate (CTH). Fermentation behavior for both hydrolysates was verified (Figure 2).

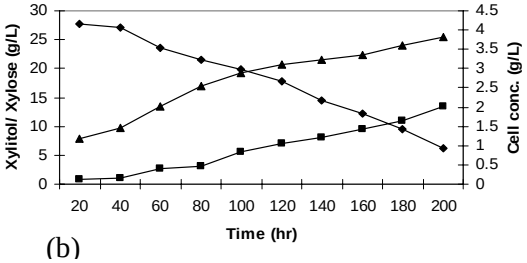
Figure 2 represents the fermentation activity when (a) raw wood hydrolysate and (b) charcoal treated media were used. The productivity of xylitol was increased with time. Higher productivity (Q_p=0.06$gl^{-1}hr^{-1}$) was found with charcoal treated hydrolysate media compared to raw wood hydrolysate (Q_p=0.03$gl^{-1}hr^{-1}$). The results explained the ability of growth of microorganism in the media with less concentration of inhibitory components.

The product yield over substrate consumption ($Y_{p/s}$) for both media described that CTH media allowed higher product yield compared to RW media. Highest yield ($Y_{p/s}$=0.61gg^{-1}) obtained with CTH media while $Y_{p/s}$=0.29gg^{-1} observed with RW media after 120 hrs. The generations of cell increase with fermentation time indicated that growth performance of CTH media allowed better growth compared to that of RW media.

It was observed that 79% of initial xylose was consumed at the end of the runs with CTH hydrolysate while only 55% with RW hydrolysate. These results demonstrated that charcoal treated hydrolysate was more suitable as fermentative media for xylitol production compared to raw wood hydrolysate. In CTH hydrolysate the inhibitor components like acetic acid and furfural were low compared to RW hydrolysate which favored proper environment for growth of microorganism and effective utilization of substrate and hence higher yield of xylitol in the resulting medium.



(a)



(b)

FIGURE 2 Fermentation behavior by *Debaryomyces hansenii* NRRL Y-7426 in (a) RW media and (b) CTH media with ⊣■⊢ :xylitol, ⊣◆⊢ :xylose and ⊣▲⊢ :cell.

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