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Fermentation of xylose, arabinose, glucose, their mixtures and sugarcane bagasse hydrolyzate by yeast *Pichia stipitis* for ethanol production

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Abstract

Xylose, arabinose and glucose were studied their fermentabilities to ethanol by yeast *Pichia stipitis*. The experiments were conducted by using 1.0, 5.0, 10.0 and 20.0 g/l of mono-saccharide solutions in a close fermentation system at 30 ° C with 100-rpm shaking rate for 120 h. Fermentabilities of mono-saccharides appeared to produce high ethanol yield when a low concentration of xylose and arabinose was applied. Glucose fermentability was, however, found to be preferable at high sugar concentration. The highest ethanol yields could be achieved at 106.27, 86.25, and 73.10%, reported as a relative % to its theoretical ethanol yield, by using 1.0 g/l of xylose, 1.0 g/l of arabinose, and 20.0 g/l of glucose solutions, respectively. The empirical equations were then established based on the fementabilities obtained to predict ethanol yield for a given concentration of mono-saccharides. A comparative study on fermentation of the mono-saccharide mixture and sugarcane bagasse hydrolyzate, of which xylose, arabinose and glucose concentrations were similar, were moreover conducted. It was revealed that the empirical equations provided an excellent estimation of ethanol concentration when the mono-saccharide mixture was used. The presence of furans and other compounds in sugarcane bagasse hydrolyzate besides the mono-saccharides, however, resulted in a lower ethanol fermentability compared with that calculated by the empirical equations. This is due apparently to an inhibition effect of these additional components to *Pichia stipitis*.

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Keywords: Arabinose; Bioethanol; Glucose; Hydrolyzate; *Pichia stipitis*; Sugarcane bagasse; Xylose

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Nomenclature

$Y_{EtOH,Fermentation}$:	Ethanol yield from fermentation (wt%)
$Y_{EtOH,Theoretical}$:	Theoretical ethanol yield (wt%)
$Y_{EtOH,Empirical}$:	Ethanol yield estimated by empirical equation, reported as relative percentage to its theoretical value (%)
$C_{EtOH,Fermentation}$:	Ethanol concentration from fermentation (g/l)
$C_{EtOH,Empirical}$:	Ethanol concentration, estimated from empirical equation (g/l)
C_{Xyl}:	Concentration of xylose (g/l)
C_{Ara}:	Concentration of arabinose (g/l)
C_{Glu}:	Concentration of glucose (g/l)

1. Introduction

Thailand's agriculture is diversified and competitive. Its great variety of products are exported widely to international countries. There are large amounts of agricultural residues and wastes that can be available as a lignocellulosic biomass. They are composed mainly of cellulose, hemicelluloses and lignin. By applying appropriate liquefaction techniques, lignocelluloses can be decomposed to obtain various mono-saccharides. Cellulose will be converted to glucose, C6 sugar, while hemicellulose from agricultural residues that mostly belong to monocot plant species will give C5 sugars such as xylose and arabinose [1]. It was reported that rice husk hemicellulose could be hydrolyzed to xylo-saccharides and other saccharides as treated by hot-compressed water at 230 °C/10 MPa, but cellulose required a higher temperature (270 °C) to be hydrolyzable because of its crystalline structure [2]. These saccharides would be used as substrates in ethanol fermentation. *Saccharomyces cerevisiae*, known as a bakery yeast, is the most employed yeast for ethanol production at industrial level [3]. This yeast can actually ferment only C6 sugar. In view of getting more advantage from lignocelluloses, hemicellulosic C5 sugars should also be converted to ethanol. *Pichia stipitis* appears to be an interesting alternative. It can ferment both of C5 and C6 sugars to ethanol at high efficiency [4,5]. More technical information regarding its fermentability by using hemicellulosic sugars and hydrolyzates from various lignocelluloses as substrates would provide more insights into the advanced technology in bioethanol production from lignocelluloses. In this current study, therefore, the effects of types and concentrations of mono-saccharides, i.e. xylose, arabinose and glucose on ethanol fermentation by using *Pichia stipitis* were explored. A comparative study on fermentation of the mono-saccharide mixture and sugarcane bagasse hydrolyzate were moreover conducted.

2. Material and methods

Pure chemical substances of xylose, arabinose and glucose were used without purification. Sugarcane bagasse hydrolyzate was obtained by hot-compressed water treatment at 210 °C for 0 min using 600-ml parr reactor. Fermentation experiments were conducted by using 1.0, 5.0, 10.0 and 20.0 g/l of xylose, arabinose and glucose solutions. A mixture of these mono-saccharides and sugarcane bagasse hydrolyzate, of which xylose, arabinose and glucose concentrations were similar, were moreover used as the substrates for fermentation. *Pichia stipitis* (ATC.58376) was purchased as a dry yeast. It was cultured on a YMP agar and subsequently inoculated into YMP broth. This inoculation was maintained at 30 °C at 100 rpm of shaking rate for 24 h prior to use in the experiments. The obtained yeast broth and fermentation substrates were mixed to obtain the desire mono-saccharide concentration with 0.01 g/l of dry yeast cell and 150 ml of the total volume. The batch fermentation was performed in a close system using 500-ml Erlenmeyer flask at 30 °C and 100 rpm of shaking rate for 120 h. The cell growth of yeast was determined by using spectrometer photometer at a wavelength of 600 nm. Fermented samples were recovered by 0.2-μm nylon filter, and subsequently subjected to a high performance liquid chromatography (Sugar KS-801 column, Shodex) for compositional analysis in terms of sugars, furans and ethanol [1,2]. The mobile phase was deionized water at the flow-rate of 1.0 mL/min and the column temperature was set at 80 °C.

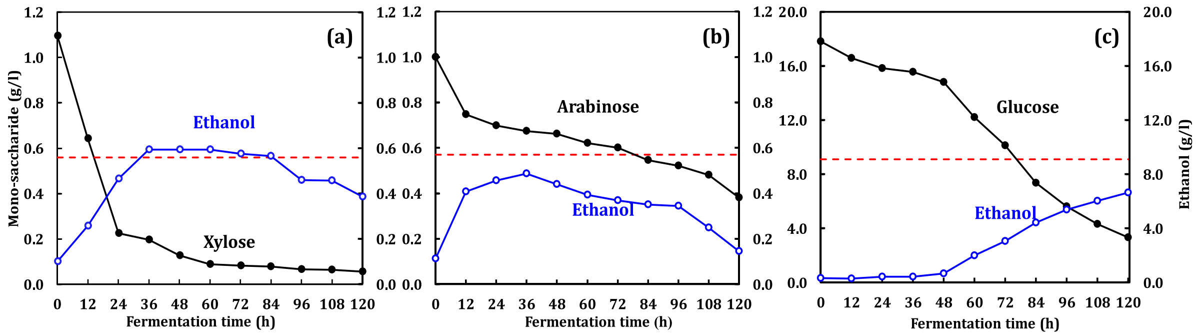

Fig. 1. Fermentation of mono-saccharide solutions, (a) 1.0 g/l of xylose, (b) 1.0 g/l of arabinose, and (c) 20.0 g/l of glucose. Dash line shows theoretical yield of ethanol.

3. Results and discussion

3.1. Fermentation of mono-saccharide solutions

The effects of mono-saccharides, i.e. xylose, arabinose and glucose, and their concentrations on ethanol fermentabilities of *Pichia stipitis* were investigated. It was revealed that types and concentrations of mono-saccharides played an important role in fermentation. Fermentabilities of mono-saccharides appeared to produce high ethanol yield when a low concentration of xylose and arabinose was used. Glucose fermentability was, however, found to be better at high sugar concentration. Fig. 1 illustrates fermentation data of the studied mono-saccharides. It was revealed that *Pichia stipitis* could convert 1.0 g/l of xylose to ethanol efficiently (Fig. 1a). As seen, 54.2 wt% of ethanol yield could be achieved within only 36 h of fermentation. This ethanol yield was slightly over the theoretical value of 51.11 wt% (indicated by dash line) due possibly to an inclusion of a small amount of glucose in fermentation broth during inoculation step of yeast and/or error in the HPLC analysis. For arabinose fermentation at 1.0 g/l (Fig. 1b), a similar trend of ethanol production which could obtain the highest yield (44.1 wt%) within 36 h was observed. It was noted that, however, ethanol reduced in later hours. Such consumption of ethanol was also observed after 84 h in fermentation of 1.0 g/l xylose. The ethanol reassimilation after xylose utilization in fermentation by *Pichia stipitis*, reported previously [6], agrees well with our findings. This study, however, have added more information regarding such phenomenon in arabinose fermentation. As for glucose fermentation at 20.0 g/l (Fig. 1c), production of ethanol appeared to be slower than the fermentation of C5 sugars at 1.0 g/l. At 120 h, ethanol yield was found to be reached at 37.4 wt%. If we prolong a fermentation time, a higher ethanol yield might be expected. It is commonly known that yeast cannot produce ethanol efficiently under a high sugar concentration. In fact, at 1.0 g/l of glucose, *Pichia stipitis* also quickly converted sugar to ethanol, similarly to xylose and arabinose fermentation described above. The maximum value of ethanol yield (20.4 wt%) could be obtained within 72 h, but it was lower than that obtained by 20.0 g/l of glucose (detailed data are given elsewhere). In conclusion, the highest ethanol yields could be achieved at 106.27, 86.25, and 73.10%, reported as a relative % to its theoretical ethanol yield, by using 1.0 g/l of xylose, 1.0 g/l of arabinose, and 20.0 g/l of glucose solutions, respectively.

Based on the fementabilities of xylose, arabinose and glucose at various concentrations obtained, the empirical equations were then established to predict ethanol yield and/or concentration for a given figure of mono-saccharides. The empirical equations that can predict a relative ethanol yield to its theoretical value (Y-variable) for a given concentration of mono-saccharides (X-variable); xylose (Eq. (1)), arabinose (Eqs. (2) and (3)), and glucose (Eq. (4)), are as follows:

$$Y_{EtOH,Empirical} = 0.0207{C_{Xyl}}^3 + 0.7798{C_{Xyl}}^2 - 10.309C_{Xyl} + 115.82 \quad when\ C_{Xyl}\ is\ 1.0\ to\ 20.0\ \text{g/l} \tag{1}$$

$$Y_{EtOH,Empirical} = 1.3457{C_{Ara}}^2 - 22.489C_{Ara} + 107.39 \quad when\ C_{Ara}\ is\ 1.0\ to\ 10.0\ \text{g/l} \tag{2}$$

$$Y_{EtOH,Empirical} = -0.6464C_{Ara} + 26.464 \quad when\ C_{Ara}\ is\ 10.0\ to\ 20.0\ \text{g/l} \tag{3}$$

$$Y_{EtOH,Empirical} = -0.0954{C_{Glu}}^2 + 3.4797C_{Glu} + 41.879 \quad when\ C_{Glu}\ is\ 1.0\ to\ 20.0\ \text{g/l} \tag{4}$$

Table 1. Ethanol concentration from fermentation compared with the figure that is estimated by empirical equations.

Carbon source of fermentation	$C_{EtOH,Fermentation}$ (1)	$C_{EtOH,Empirical}$ (2)	Relative % [(1)/(2) × 100]
Mono-saccharide mixture	1.2676	1.3250	95.67
Sugarcane bagasse hydrolyzate	1.1354	1.7594	64.54

3.2. Fermentation of mono-saccharide mixture and sugarcane bagasse hydrolyzate

Chemical composition of hydrolyzate obtained from sugarcane bagasse as treated by hot-compressed water at 210 °C for 0 min was xylose 2.3860 g/l, arabinose 0.6862 g/l, glucose 1.1710 g/l, xylo-oligosaccharides 3.5667 g/l, cello-oligosaccharide 0.0950 g/l, furfural 0.0144 g/l, 5-HMF (5-hydroxymethylfurfural) 0.0323 g/l, and others. A comparative study on fermentation of the mono-saccharide mixture and sugarcane bagasse hydrolyzate, of which xylose, arabinose and glucose concentrations were similar, were then conducted. Table 1 shows the ethanol concentrations obtained from fermentation experiments compared with the figures estimated from empirical equations. As a result, the relative percentages of ethanol concentration indicate that the empirical equations provided an excellent estimation of ethanol concentration when the mono-saccharide mixture was used. The relative percentage of ethanol concentration could be achieved at 95.67%. However, that of sugarcane bagasse hydrolyzate was lesser (64.54%). The presence of other compounds such as oligosaccharides and furans in the hydrolyzate resulted in a significant difference in ethanol concentration. This lower ethanol fermentability is due apparently to an inhibitor effect of furan compounds, i.e. furfural and 5-HMF. In addition, yeast cell growth, sugar consumption and ethanol production rates were retarded in the fermentation batch that used hydrolyzate as a substrate. There might be also other factors that would possibly effect to ethanol fermentability of the hydrolyzate, for example, pH in the fermentation broth, etc.

4. Conclusion

The ethanol fermentability of *Pichia stipitis* was altered by the effects of types and concentrations of mono-saccharides. It was remarkably noted that the empirical equations developed in this study could satisfactorily predict ethanol concentration for a given figure of mono-saccharides. It was successful for the fermentation system that used mono-saccharide mixture as a substrate, but not for the fermentation experiment of sugarcane bagasse hydrolyzate. Furan compounds and/or others unidentified products in hydrolyzate significantly caused an inferior effect to ethanol production. To increase ethanol yield from lignocellulosic hydrolyzate, however, further studies on elimination of inhibitors and optimization of fermentation conditions must be considered. Based on these findings, it would elucidate the feasibility to utilize agricultural residues as a potential resource for renewable energy and value-added biochemical.

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