KKU ENGINEERING JOURNAL 2016;43(S3):474-476 Research Article

KKU Engineering Journal

https://www.tci-thaijo.org/index.php/kkuenj/index

Inhibitor formation during glucose and xylose production from corncob hydrolysate and the effect to bioethanol production

Sompong Sukklang[1)] and Jirawan Apiraksakorn*[2,3)]

[1)]Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand.
[2)]Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand.
[3)]Fermentation Research Center for Value-Added Agricultural Products (FerVAAP), Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand.

Received April 2016
Accepted June 2016

Abstract

Corncob dust was potentially used as an alternative carbon source for ethanol production due to the fermentable sugars derived from acid hydrolysis. Under the optimum condition, glucose and xylose were the major sugars obtained from the hydrolysate with less than 0.4 g/l furfural and 5-hydroxymethyl furfural (5-HMF) also found. The corncob dust hydrolysate was examined for ethanol production by the xylose – fermenting yeast, *Candida shehatae* TISTR5843. The results revealed that the inhibitors might affect cell growth due to the slow change of biomass observed at the early stage cultivation. Glucose was gradually consumed, while xylose was utilized by yeast during the ethanol production. Therefore, the corncob dust hydrolysate was potentially used for cell mass and ethanol production by *Candida shehatae* TISTR5843.

Keywords: Inhibitor, Corncob dust, Glucose, Xylose, Hydrolysis

1. Introduction

Corncob dust is a by-product of the corn feed processing industry. It contains major components of cellulose and xylan which primarily yield glucose and xylose after hydrolysis, respectively [1]. Glucose has been reported as an effective carbon source for ethanol production, but significant amount of xylose released from plant materials degradation should be also considered. The use of ethanol fermentation from mixed - sugars of glucose and xylose has been proposed [2-3].

Acid hydrolysis has been commonly used as one of the effective methods of producing fermentable sugars from lignocellulose. Under high temperature and long period of time, the structural carbohydrate of plant components can be hydrolysed to sugars. But, under these extreme conditions, the sugars are further degraded from pentose and hexose to furfural and 5-hydroxymethyl furfural (5-HMF) [4-5]. Several studies confirm the negative effect of furfural and 5-HMF on ethanol fermentation [6-7]. Sukklang (2013) presented the optimum conditions for xylose production from corncob dust hydrolysis by diluted acid [8]. Therefore, the aim of this research was to examine the potential use of corncob dust hydrolysate for bio-ethanol production by the xylose - fermenting yeast, *Candida shehatae* TISTR5843.

2. Materials and methods

2.1 Corncob dust hydrolysis

Corncob dust was obtained from Yongsuwat Agritrade, Co., Ltd. Nakorn Sawan province, Thailand. The hydrolysis was carried out as described by Sukklang [8].

2.2 Microorganisms and medium

The culture medium was composed of yeast extract 3 g/l, malt extract 3 g/l, peptone 5 g/l and xylose 10 g/l. The fermentation medium was the same composition of culture medium but supplemented with corncob dust hydrolysate instead of xylose addition.

2.3 Fermentation

C. shehatae TISTR5843 was grown in medium at 30°C, 200 rpm for 24 h. Inoculums were re-transferred to fresh medium containing corncob dust hydrolysate and continued grown at 30°C, 200 rpm, 24 h to propagate biomass. The cells were centrifuged (10,000 g, 5 min), washed with 0.85 % (w/v) normal saline and centrifuged again to harvest.

The 2 g of yeast cells were re-suspended in the fermentation medium containing 16 g/l of reducing sugar from corncob dust hydrolysate and fermented at 30°C for 120 h. Samples were taken every 24 h for further analysis.

*Corresponding author. Tel.: +66 4336 2121 ext. 44680
Email address: jirapi@kku.ac.th
doi: 10.14456/kkuenj.2016.159

2.4 Analytical methods

Cell growth was measured at OD_{600}. Glucose, xylose and reducing sugar were analyzed by the peroxidase glucose oxidase assay [9], by *p*-Bromoaniline [10] and by Dinitrosalicylic acid (DNS) method [11], respectively. Ethanol was determined by Gas Chromatography (Shimadzu GC-14B, Kyoto, Japan, Solid phase: polyethylene glycol (PEG-20M), carrier gas: nitrogen, 150 °C isothermal packed column, injection temperature 180 °C, flame ionization detector temperature 250 °C; GC Solution analysis Version 2.30) and 2-propanol was used as an internal standard [12]. Furfural, 5-hydroxymethyl furfural (5-HMF) and acetic acid were determined by HPLC (Aminex HPX-87H column (Bio-Rad, USA) and UV detector (Shimadzu, SPD-20A, Japan). The column temperature was maintained at 50°C. Five mM sulfuric acid was used as the mobile phase at a flow rate of 0.8 ml/min.

3. Results

3.1 Inhibitor formation under the optimum condition for corncob dust hydrolysis

Under optimum conditions xylose production from corncob dust hydrolysis [8], yielded not only xylose and glucose, but furfural, 5-HMF and acetic acid (Table 1).

Table 1 Corncob dust hydrolysate composition

Sugars (g/l)			Inhibitiors (g/l)		
Xylose	Glucose	Reducing	Furfural	5-HMF	Acetic acid
9.14	7.90	17.04	0.39	<0.1	0.5

3.2 Bioethanol production from corncob dust hydrolysate

C. shehatae TISTR 5843 was grown in corncob dust hydrolysate. Cell growth corresponded to reducing sugar consumption which reached the maximum after 18 h cultivation (Figure 1). Then, the biomass was transferred to a production medium for ethanol fermentation.

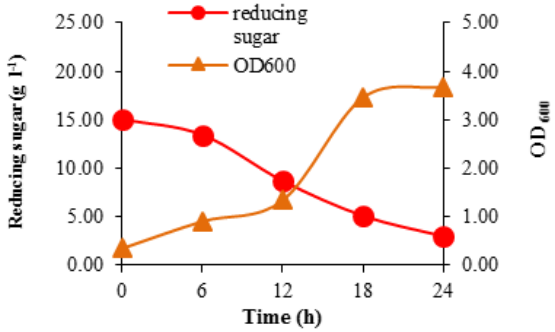

Figure 1 Production of *C. shehatae* TISTR 5843 biomass in corncob dust hydrolysate at 30˚C, 200 rpm for 24 h

To produce ethanol from corncob dust hydrolysate, *C. shehatae* TISTR 5843 biomass was further fermented under static condition at 30˚C. Figure 2 shows that the ethanol increased up to 2.55 g/l at 120 h by using both glucose and xylose. The ethanol yield and sugars consumption are presented in Table 2.

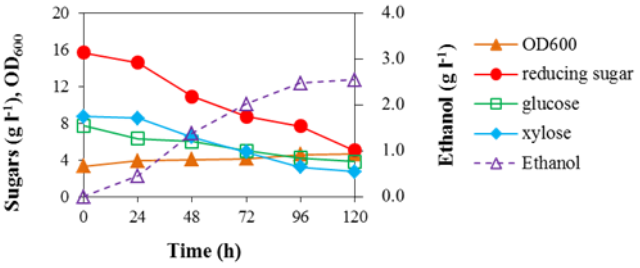

Figure 2 The ethanol production from corncob dust hydrolysate by *C. shehatae* TISTR 5843

Table 2 Ethanol production and yield from corncob dust hydrolysate fermentation by *C. shehatae* TISTR 5843

	Value
Ethanol (g/l)	2.55
Ethanol yield (g/g)	0.24
Reducing sugar consumption (g/l/)	10.65
Glucose consumption (g/l)	3.87
Xylose consumption (g/l)	6.01

4. Discussion

The fermentation–inhibiting substances were derived from sugar degradation under the extreme conditions with high temperature and a long period of time [4-5]. The pentose sugars such as xylose degraded to furfural, while the hexose sugars, which are more difficult to degrade became 5-hydroxymethyl furfural (5-HMF). Although Kahar et al. (2010) performed the experiment at even more extreme conditions by using 5 % sulfuric acid at 128°C for 120 min, the 5-HMF results were the same [5].

To produce ethanol, *C. shehatae* TISTR 5843 was grown and further fermented in corncob dust hydrolysate. The biomass production pattern shows that cell growth slowly changed at an early stage. The yeast cells might have had to adapt to the hydrolysate before increasing rapidly and reaching the maximum after 18 h cultivation. Then, the biomass was transferred to a new hydrolysate for ethanol fermentation. The sugar utilized pattern in figure 2 confirmed the ability of both glucose and xylose consumption of *C. shehatae* TISTR 5843. Glucose was gradually consumed, while xylose was utilized later and delayed for 24 h. Glucose is the first priority carbon source for microorganism due to its being simple and easy utilized. After 120 h fermentation, there were some sugars left. By acid hydrolysis, structural carbohydrates randomly degraded to sugars and other incomplete hydrolyzed products such as oligosaccharides which could not be used by the microorganism [13].

From this study, ethanol yield was 0.24 (based on reducing sugar consumption); 47 % of the theoretical yield. Lower ethanol production might have been caused by the change of xylose to xylitol instead of ethanol because of inappropriate fermentation [14]. Another possible reason could be the inhibiting effect of furfural and HMF on alcohol dehydrogenase (ADH) [6]. Due to some ethanol fermentation obstacles found from this study, the alternative ethanol production from corncob dust by the xylose - fermenting yeast

could be improved by increasing initial sugars provided and limiting fermentation–inhibiting substances.

5. Conclusions

The results indicate that *C. shehatae* TISTR 5843 could produce ethanol by using both glucose and xylose from corncob dust hydrolysate. The potent of ethanol production from lignocellulosic materials depend on an efficient hydrolysis method. Not only are high initial sugars for fermentation required, but the inhibitor formation should be suppressed.

6. Acknowledgements

This work was supported by by National Research Council of Thailand (NRCT), Cultivating a Research Project to Build an International Research, Khon Kaen University, Thailand and Center for Alternative Energy Research and Development, Khon Kaen University, Thailand. Appreciation is expressed to Yongsuwat Agritrade Co. Ltd. for supplying corncob dust through all for this research. We would like to thank Mrs. Janice Loewen Hamman for English proof reading.

7. References

[1] Fang X, Shen Y, Zhao J, Bao XN, Qu YB. Status and prospect of lignocellulosic bioethanol production in china. Bioresource Tech 2000;101:4814-4819

[2] Chen Y, Dong B, Qin W, Xiao D. Xylose and cellulose fractionation from corncob with three different strategies and separate fermentation of them to bio-ethanol. Bioresource Tech 2010;101:6994-6999.

[3] Agbogbo FK, Coward-Kelly G, Torry-Smith M, Wenger KS. Fermentation of glucose/xylose mixtures using *Pichia stipites*. Process Biochem 2006;41:2333-2336.

[4] Gong CS, Cao NU, Du J, Tsao GT. Ethanol production by renewable sources. Adv Biochem Eng Biotech 1999;65:207-241.

[5] Kahar P, Taku K, Tanaka S. Enzymatic digestion of corncob pretreated with low strength of sulfuric acid for bioethanol production. J Biosci Bioeng 2010;11:453-458.

[6] Modig T. Kinetics and inhibition effects of furfural and hydroxymethyl furfural on enzymes in yeast [Internet]. 2015 [cited 2015 Aug 20]. Available from: http://www.chemeng.lth.se/exjobb/012.pdf

[7] Lu P, Chen LJ, Li GX, Shen SH, Wang LL, Jiang QY, Zhang JF. Influence of furfural concentration on growth and ethanol yield of *Saccharomyces kluyveri*. J Environ Sci (China) 2007;19:1528-32.

[8] Sukklang S. The optimum condition and estimation of the consumption energy for dust hydrolysis [Thesis]. Khon Kaen : Graduate School, Khon Kaen University; 2013. [InThai].

[9] Huggett AS, Nixon DA. Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. Lancet 1957;2:368-70.

[10] Deschatelets L, Yu EKC. A simple pentose assay for biomass conversion studies. Appl Microb Biotech 1986;24:379-385.

[11] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426-428.

[12] Deesuth O, Laopaiboon P, Jaisil P, Laopaiboon L. Optimization of Nitrogen and Metal Ions Supplementation for Very High Gravity Bioethanol Fermentation from Sweet Sorghum Juice Using an Orthogonal Array Design. Energies 2012;5:3178-3197.

[13] Jeong TS, Um BH, Kim JS, Oh KK. Optimizing dilute-acid pretreatment of rapeseed straw for extraction of hemicellulose. Appl Biochem Biotech 2010;161:22-33.

[14] Winkelhausen E, Kuzmanova S. Review: Microbial conversion of d-xylose to xylitol. J Fermentation Bioengineering 1998;86:1-14.