

Full Length Research Paper

Comparison of different detoxification methods for corn cob hemicellulose hydrolysate to improve ethanol production by *Candida shehatae* ACCC 20335

Jing-Ping Ge, Bai-Yan Cai, Guo-Ming Liu, Hong-Zhi Ling, Bao-Zhu Fang, Gang Song, Xiao-Feng Yang and Wen-Xiang Ping*

No 74, Nangang District, Key Laboratory of Microbiology, College of Life Sciences, Heilongjiang University, Haerbin, 150080 P. R. China,

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Corn cob hydrolysis with 1% (v/v) of sulfuric acid yielded 36.49 total reducing sugars (g/l) along with various fermentation inhibitors such as fufural, phenolics compounds and acetic acid. The acid hydrolysate detoxified with overliming plus activated charcoal brought about a maximum decrease in fufural (100%), acetic acid (62.4%) and phenolics compounds (96.6%). Treatment of hydrolysate with overliming caused 99, 51.4 and 16.7% loss in fufural, acetic acid and phenolics compounds, respectively. Fermentation by *Candida shehatae* ACCC 20335 showed a maximum yield of ethanol (0.31 g/g) and a productivity of 0.152 g/l/h with overliming plus activated charcoal treated hydrolysate.

Key words: Corn cob, detoxification, ethanol, fermentation, hydrolysate.

INTRODUCTION

Fermentation of renewable resources to produce ethanol has attracted great interest because of the energy crisis (Herrera, 2004; Zhang, 2008). Amongst the various agricultural crop residues, corn cob is the most abundant agricultural material in Northeast China. One of main components in corn cob is hemicellulose containing xylose (Tada et al., 2004; Hendriks et al., 2009), as well as glucose and arabinose. Xylose can be fermented to ethanol by several yeast strains, such as *Candida shehatae* (Chandel et al., 2007; Kim et al., 2010), *Pichia stipitis* (Delgenes et al., 1996; Huang et al., 2009; Canilha et al., 2010), *Pachysolen tannophilus* (Cheng et al., 2007; Moya et al., 2008). Among xylose fermenting organisms, *Candida shehatae* is promising for ethanol production using acid hydrolysates. During acid hydrolysis of corn cob, in addition to sugars, fufural, acetic acid and phenolic compounds are produced. These compounds are known to affect ethanol fermentation performance and must be removed. Several detoxification methods

such as overliming (Amartey et al., 1996; Telli-Okur et al., 2008; Chan et al., 2010; Park et al., 2010), activated charcoal (Chandel et al., 2007), electro dialysis (Cheng et al., 2007), biological (Okuda et al., 2008), membrane extraction (Grzenia et al., 2010) are used to remove these inhibitors.

In this paper, acid hydrolysates detoxified by overliming or overliming plus activated charcoal were used to compare their ethanol production capability by *Candida shehatae* ACCC 20335. The ethanol fermentation performance was also compared between concentrated acid hydrolysate as medium and simulated synthetic hydrolysate as medium.

MATERIALS AND METHODS

Bacterial strain and medium

Candida shehatae ACCC 20335 was used in this work. The stock culture of *Candida shehatae* ACCC 20335 were maintained and grown on YPD agar containing (g/l): peptone, 20; yeast extract, 10; D-glucose, 20; agar, 20.1.2 Inoculum preparation and fermentation

The seed cells were prepared in 250 ml flasks containing 100 ml preculture medium (g/l): 5 g KH₂PO₄/l, 2 g (NH₄)₂SO₄/l, 1 g

*Corresponding author. E-mail: gejingping512@yahoo.com.cn.
Fax: +86-451-86609016.

Table 1. Composition of corn cob hemicellulose acid hydrolysate (losses of each composition in brackets).

	Concentrated acid hydrolysate (%)	Simulated synthetic hydrolysate	Hydrolysate detoxified by overliming (%)	Hydrolysate detoxified by overliming + activated charcoal (%)
Xylose (g/l)	74.8 ± 0.6 (4.8)	74.8	64.3 ± 0.6 (13.2)	56.3 ± 0.8(28.4)
Glucose(g/l)	20.4 ± 0.4 (4.2)	20.4	19.3 ± 0.3 (9.4)	17.4 ± 0.8(18.3)
Arabinose(g/l)	9.3 ± 0.1 (0)	9.3	8.8 ± 0.2 (5.4)	8.5 ± 0.3(8.6)
Fufural(g/l)	0.013 ± 0.003 (98.6)	0.013	0.010± 0.001 (99.0)	0 (100)
Acetic acid(g/l)	10.1 ± 0.6 (46.6)	10.1	9.2± 0.1 (51.4)	7.1 ± 0.2 (62.4)
Phenolic compounds(R ₂₈₀)	0.985 ± 0.003 (1.5)	0.985	0.833± 0.005 (16.7)	0.034 ± 0.004 (96.6)

The values were the mean of two independent samples, except the simulated synthetic hydrolysate.

peptone/l, 3 g yeast extract/l, 0.2 g MgSO₄·7H₂O/l, 20 g xylose/l. The flasks were incubated at 30°C for 18 h and inoculated into the fermentation medium at 7.5% (v/v), which contains 2.68 g KH₂PO₄/l, 0.36 g (NH₄)₂SO₄/l, 0.25 g MgSO₄·7H₂O/l, 2.59 g yeast extract/l, pH 5.0 and supplemented with concentrated hydrolysate, or detoxified hydrolysate by overliming, or detoxified hydrolysate by overliming plus activated charcoal. Fermentation was carried out at 30°C and a shaking speed of 160 rpm for 50 h.

Hydrolysis of the corn cob

Corn cob was obtained from Wangkui county, Heilongjiang Province, P. R. China. Corn cob was ground, fractioned and milled to obtain particle size lower than 10 mm. Twenty grams of corn cob were hydrolyzed in 1% (v/v) of sulfuric acid at 121°C for 1.5 h at a solid to liquid ratio of 1:10. The liquid phase was separated by centrifugation and the unhydrolyzed residue was washed three times with 40°C warm water. The filtrate and washing solutions were pooled together. The average compositions of the hemicellulose acid hydrolysates were 26.2 g xylose/l, 7.19 g glucose/l, 3.1 g arabinose/l, 6.3 g acetic acid/l, 0.32 g furfural/l. The acid hydrolysates were concentrated to give 6-7% (w/v) xylose.

Detoxification of acid hydrolysates

The acid hydrolysates were detoxified using two methods: overliming and overliming plus activated charcoal. During detoxification with overliming, concentrated hemicellulose acid hydrolysate was heated to 100°C for 15 min, and then overlimed with CaO to pH 7.0, centrifuged to harvest filtrate. The pH was adjusted to 5.0 with sodium sulfite, followed by filtration to remove the insoluble material. The filtrate was concentrated under vacuum at 60°C to give 6-7% (w/v) xylose. When treatment with overliming plus activated charcoal, filtrate obtained from overliming process (without concentrated) was further mixed with 3% of activated charcoal (products of Guanghua Jingke activated charcoal company, China) at 40°C for 1 h at a shaking speed of 200 rpm, then filtrated to remove the insoluble material. The filtrate was concentrated under vacuum at 60°C to give 6-7% (w/v) xylose.

Analytical methods

The hydrolysates were analyzed by HPLC (Shimadzu Corp. Kyoto, Japan). Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) was used with 0.005 M H₂SO₄ as elution solvent at a flow rate of 0.8 ml/min, keeping column oven temperature at 65°C with RI detectors.

Glucose, xylose, arabinose, acetic acid, furfural and ethanol were analyzed by HPLC. Phenolic compounds were analyzed on chromatograms at 280 nm. Cell growth was monitored at 600 nm and converted to cell dry weight (g/l, CDW) by calibration curve ($y = 1.0078x$, $R^2 = 0.9779$).

RESULTS

Detoxification of the corn cob hemicellulose hydrolysate

The detoxification of corn cob acid hydrolysates by overliming and activated charcoal can effectively remove furfural, acetic acid and phenolic compounds. After concentrating the filtrates of acid hydrolysates, 98.6% of furfural, 46.6% of acetic acid and 1.5% of phenolic compounds were removed. The sugar losses were low. Treatment with overliming caused 99, 51.4 and 16.7% decrease in furfural, acetic acid and phenolic compounds, respectively. However, overliming resulted in the loss of 13.2% xylose, 8.4% glucose, 5.4% arabinose (Table 1). Similar results were reported for corn cob and sugarcane bagasse acid hydrolysates by lime (Cheng et al., 2007; Amarty et al., 1996).

When detoxified the concentrated filtrates by overliming plus activated charcoal, furfural could be removed completely, acetic acid and phenolic compounds could be decreased by 62.4 and 96.6%, respectively. The acetic acid losses ability (62.4%) is higher than that reported by Chandel et al. (2007) detoxified with activated charcoal (46.8%). The losses of glucose, xylose and arabinose were higher than those with overliming, which were 28.4, 18.3 and 8.6%, respectively.

Effect of detoxification on ethanol fermentation

The detoxification of corn cob acid hydrolysate by overliming plus activated charcoal resulted in better fermentation ability (Figure 1). Glucose was consumed completely at 10 h and then xylose assimilation began, resulting in 57.3% of xylose consumed in 50 h. However,

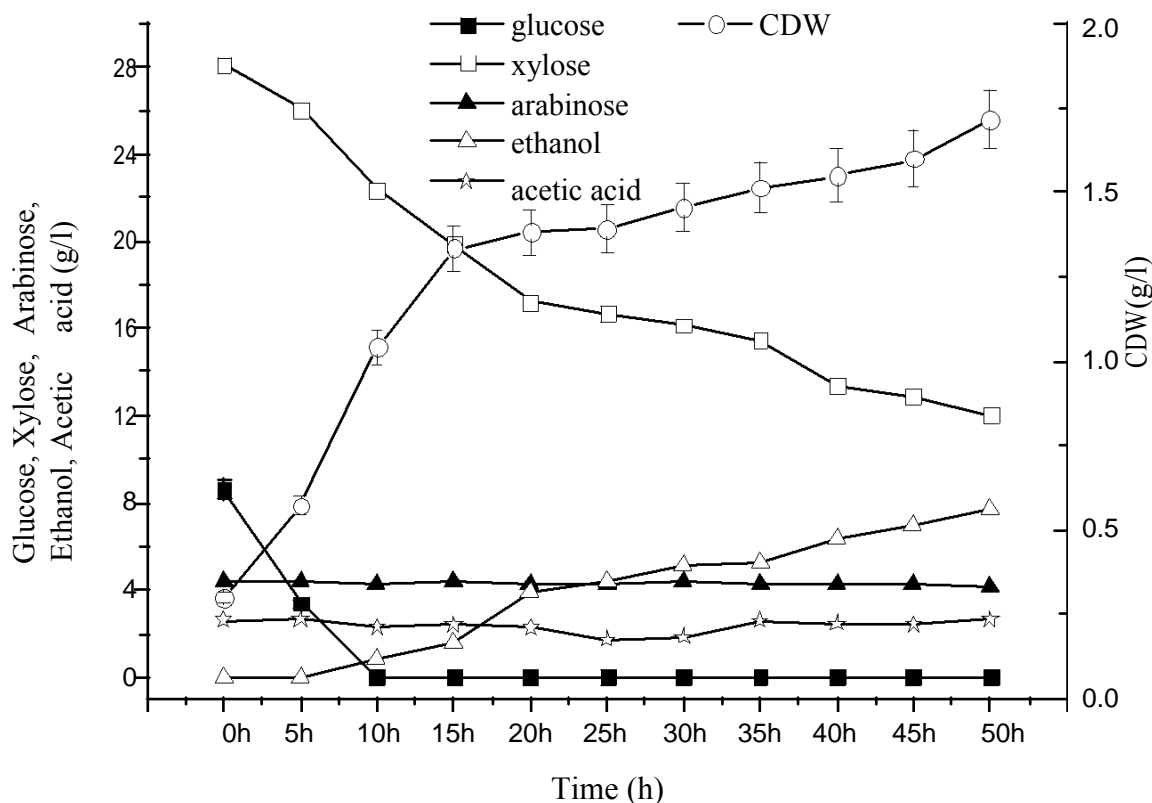


Figure 1. Time course for ethanol production by overliming plus activated charcoal: glucose (■), xylose (□), arabinose (▲), ethanol (△), acetic acid (☆), CDW (○). The values were the mean of two independent samples.

Table 2. Ethanol production in batch cultures of *Candida shehatae* ACCC 20335 using different medium.

	Concentrated acid hydrolysate	Overliming	Overliming + activated charcoal	Simulated synthetic hydrolysate
Ethanol concentration (g/l)	0.7 ± 0.01	3.8 ± 0.2	7.6 ± 0.1	1.3 ± 0.1
Ethanol yield (g/g)	0.39 ± 0.02	0.30 ± 0.03	0.31 ± 0.01	0.30 ± 0.03
Ethanol productivity (g/l/h)	0.014 ± 0.002	0.076 ± 0.004	0.152 ± 0.001	0.026 ± 0.003
Usage of sugars (%)	4.3 ± 0.1	34.0 ± 0.1	60.1 ± 0.2	8.6 ± 0.1

The values were the mean of two independent samples

there was no consumption of arabinose. The ethanol yield and productivity were 0.31 g/g and 0.152 g/l/h, respectively (Table 2).

The time course for cell growth, sugar utilization and ethanol concentration using the overliming hydrolysate as fermentation medium did not show as good fermentation capability (Figure 2). In 50 h, glucose was utilized completely and only 10.6% of xylose was assimilated. The ethanol yield and productivity were 0.30 g/g and 0.076 g/l/h, respectively (Table 2).

A compared fermentation performance was observed between using undetoxified concentrated acid hydrolysates medium and simulated synthetic

hydrolysate medium (Figures 3 and 4), the composition of latter was designed to simulate the concentrated acid hydrolysates (Table 1). Both of their fermentation capabilities were poor. The one using concentrated acid hydrolysate medium was much worse indicating other inhibitors in the hydrolysates, except fufural, acetic acid and phenolic compounds.

DISCUSSION

The results showed that treatment of hydrolysate with overliming plus activated charcoal brought about the

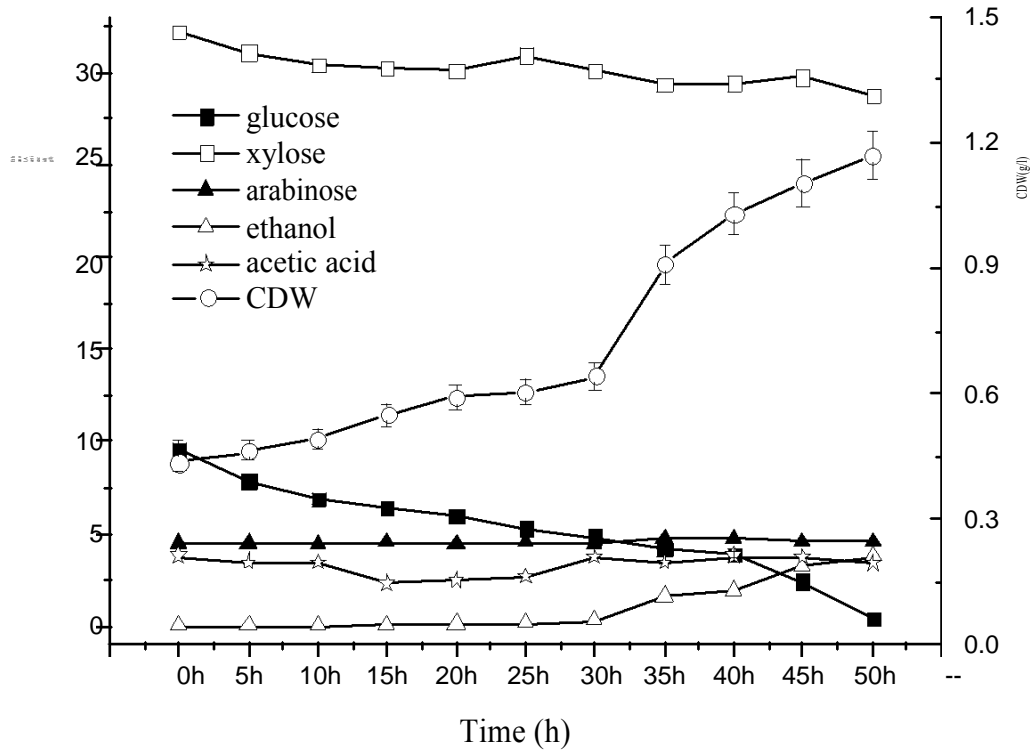


Figure 2. Time course for ethanol production by overliming: : glucose (), xylose (), arabinose (), ethanol (), acetic acid (), CDW (O). The values were the mean of two independent samples.

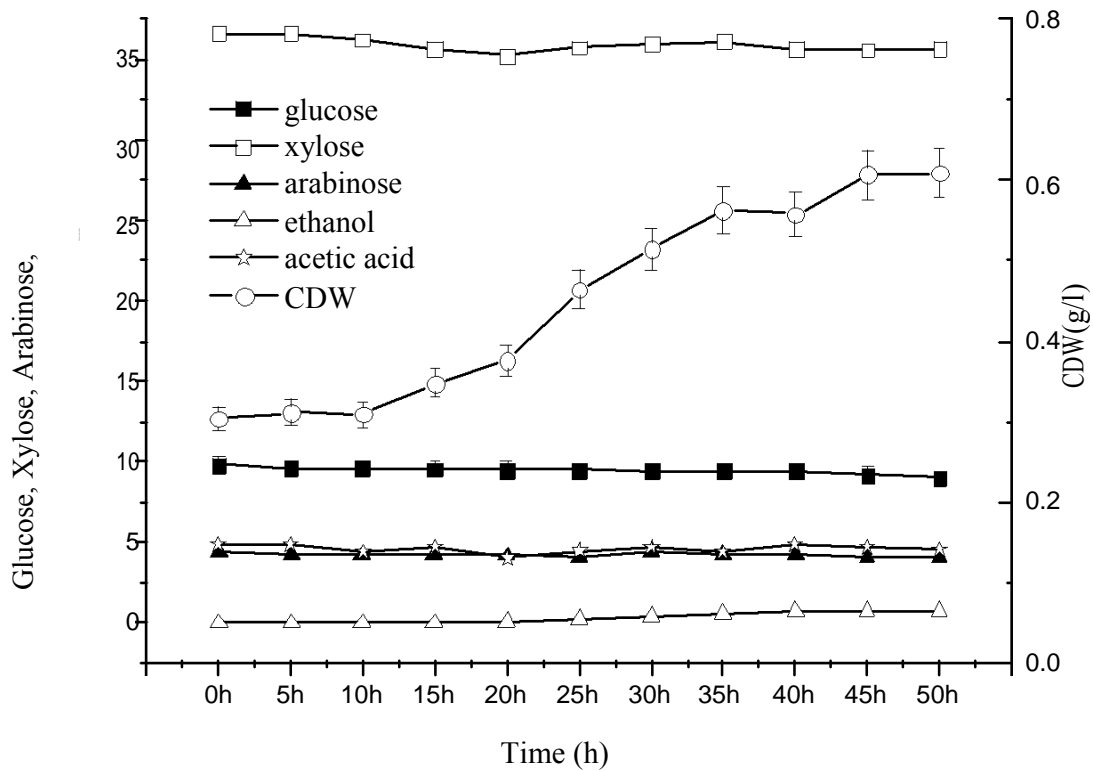


Figure 3. Time course for ethanol production by concentrated acid hydrolysate: glucose (), xylose (), arabinose (), ethanol (), acetic acid (), CDW (O). The values were the mean of two independent samples.

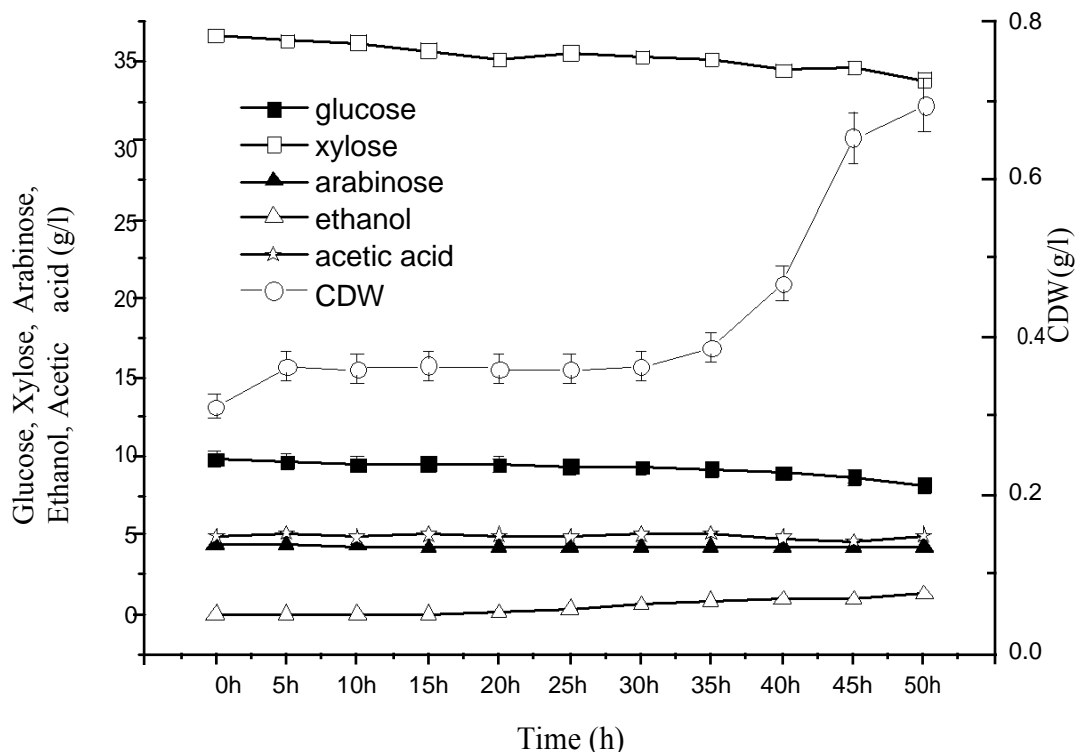


Figure 4. Time course for ethanol production by simulated synthetic hydrolysate: : glucose (), xylose (), arabinose (), ethanol (), acetic acid (), CDW (O). The values were the mean of two independent samples.

maximum removal of fufural, acetic acid and phenolic compounds, which was also reflected in the production of higher amount of ethanol. Fufural could be removed completely after detoxified the acid hydrolysate with overliming plus activated charcoal. The removal of acetic acid was 51.4% by overliming, which was higher than those reported by Cheng et al. (2007) and Amartey and Jeffries (1996). When using overliming plus activated charcoal as detoxified method, the removal of acetic acid was 62.4% and this was higher than that reported by Chandel et al. (2007) using the same method. The highest removal of acetic acid was reported by Cheng et al. (2007) with electrodialysis, which was 99%.

The concentration of acetic acid in the hydrolysate will largely affect the fermentation results. Generally, acetic acid is inhibitory to yeast at a level of about 2.0 -5.0 g/l (Van et al., 1988). However, the amount of acetic acid is 7.1 g/l in this paper. The higher amount of acetic acid will inhibit the fermentation performance. More efficient detoxification methods will be developed to decrease the acetic acid or raise the pH value to alleviate the effect caused by acetic acid.

However, the loss of sugar will also decrease the ethanol production. Using trialkylamine extraction method (Zhu et al., 2011) can effectively remove some of the inhibitory compounds, without loss of sugar. Therefore, if we utilize of various methods of detoxification, the

ethanol production will greatly improve.

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