African Journal of Biotechnology Vol. 11(103), pp. 16772-16776, 25 December, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1057
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Fermentation of pretreated corncob hemicellulose hydrolysate to ethanol by *Candida shehatae* and *Saccharomyces cerevisiae*

Jingping Ge, Tian You, Xiaofeng Yang and Wenxiang Ping*

Key Laboratory of Microbiology, College of Life Science, Heilongjiang University, Harbin 150080, P. R. China.

Accepted 1 August, 2012

To investigate the effect of unknown fermentation inhibitors in corncob hemicellulose acid hydrolysate processed by pretreatment and detoxification on fermentation, corncob hemicellulose acid hydrolysate and artificially prepared hydrolysate were fermented in parallel by *Candida shehatae* YHFK-2. The results show that fermentability of corncob hemicellulose acid hydrolysate was better than that of the artificially prepared hydrolysate and scale-up of *C. shehatae* YHFK-2 fermentation was done, which showed that ethanol production in the fermenter was obviously much more than that in flasks. In addition, *C. shehatae* YHFK-2 and *Saccharomyces cerevisiae* W5 were used for mixed fermentation of corncob hemicellulose acid hydrolysate, which was done for exploring the possibility of efficiently increasing ethanol production.

Key words: Ethanol, corncob hemicellulose acid hydrolysate, fermentation property, stability, mixed fermentation.

INTRODUCTION

Lignocellulose is considered an attractive raw material for the production of fuel ethanol because of its availability in large quantities at low cost (Duff and Murray, 1996). The efficient utilization of the hemicellulose component of lignocellulosic feedstocks offers an opportunity to reduce the cost of fuel ethanol production greatly (Hinman et al., 1989).

Among various agricultural crop residues, corncobs are abundant throughout China. The cellulosic and hemicellulosic fractions in the corncob can be hydrolyzed to sugars that eventually could be fermented to ethanol by microorganisms. The ideal microorganism for ethanol production should be able to utilize pentose and hexose sugars generated by lignocellulose hydrolysis. The best-known alcohol fermenting organism, *Saccharomyces cerevisiae*, is capable of fermenting only hexose sugars to ethanol (Cheng et al., 2007). However, pentose fermenting organisms are scarce. Among various xylose fermenting yeasts, *Candida shehatae* is promising for ethanol production from corncob hemicellulose acid hydrolysate.

The hemicellulose fractions of corncobs contain up to 43% carbohydrates that can be readily hydrolyzed to monomeric sugars by dilute sulfuric acid (Saha et al., 2003; Sun and Cheng, 2002). During acid hydrolysis, large amount of microbial inhibitors are produced that could be removed by pretreatment and detoxification, but a potential drawback of over liming is sugar degradation due to hydroxide-catalyzed degradation reaction (Ali et al., 2006).

In this study, parallel fermentation of corncob hemicellulose acid hydrolysate and artificially prepared hydrolysate were done to investigate the fermentability of corncob hemicellulose acid hydrolysate. To examine the possibility of using corncob hemicellulose acid hydrolysate in industrial ethanol production, scale-up of *C. shehatae* YHFK-2 fermentation was done. Finally, via mixed fermentation of corncob hemicellulose acid hydrolysate by *C. shehatae* YHFK-2 and *S. cerevisiae* W5, the high-producing ethanol method was preliminarily explored.

*Corresponding author. E-mail: gejingping512@126.com. Fax: +86-0451-86609016

MATERIALS AND METHODS

Hemicellulose acid hydrolysate preparation and detoxification

Corncobs from Heilongjiang province in Northeastern China was used as the raw material. Particles ranging between 10 and 20 head mesh size were used in the experiments. The selected particles were washed with warm water (40°C), soaked in 10% (v/v) ammonia for 24 h [particles/ammonia = 1:8 (w/v)); mixed with 1% (v/v) sulfuric acid, and pretreated with 10% (v/v) ammonia in an autoclave at 119°C for 60 min (particles/ammonia = 1:10 (w/v)]. The liquid fraction was separated by filtration and the unhydrolyzed residue was washed with water; the filtrate and washings were pooled together; the prehydrolysate was then over limed with solid CaO; the pH was adjusted to 4.7 with H_3PO_4; the hydrolysate was concentrated under vacuum at 50°C to achieve twice concentration; and 2% activated carbon GH-13 was used to detoxify the concentrated hydrolysate at 40°C and 140 r/min. Finally, the hydrolysate was concentrated under vacuum at 50°C to achieve three times concentration and then it was stored at 4°C.

Microorganism and culture medium

C. shehatae YHFK-2 (strain number ACCC20335) was grown in yeast extract peptone xylose broth and yeast extract peptone xylose solid medium and were incubated at 30°C. *S. cerevisiae* W5 (strain number ACCC20251) was grown in yeast extract peptone dextrose broth and yeast extract peptone dextrose solid medium and was incubated at 30°C.

The artificially prepared hydrolysate fermentation medium consisted of 5.12 g glucose/L, 68.74 g xylose/L, 10.19 g arabinose/L, 0.51 g acetic acid/L, 0.36 g ammonium sulfate/L, 2.68 g potassium dihydrogen phosphate/L, 2.59 g yeast extract/L, and 0.1 g magnesium/L, while the corncob hemicellulose acid hydrolysate fermentation medium was the same except for the presence of other unidentified components intrinsic in the corncob hemicelluloses acid hydrolysate.

Fermentation of corncob hemicellulose acid hydrolysate and the artificially prepared hydrolysate by *C. shehatae* YHFK-2

Activated *C. shehatae* YHFK-2 was inoculated into YPX broth at 5% (v/v), and the flasks were incubated at 30°C and 140 r/min for 24 h (OD_{600nm} = 2). Each sample was subsequently inoculated into corncob hemicellulose acid hydrolysate fermentation medium and artificially prepared hydrolysate fermentation medium at 10% (v/v), respectively, and the flasks were incubated at 30°C and 140 r/min for 64 h. The liquid volume in each 250 ml flask was 80 ml and each sample was repeated in parallel. After 64 h, the concentrations of residual xylose, acetic acid, ethanol, and furfural were determined by high performance liquid chromatography (HPLC).

Fermentation scale-up by *C. shehatae* YHFK-2

Activated *C. shehatae* YHFK-2 was inoculated into YPX broth and the flasks were incubated at 30°C and 140 r/min for 24 h (OD_{600nm} = 2). *C. shehatae* YHFK-2 was subsequently inoculated into a sterilized 5 L fermenter containing 2.76 L of corncob hemicellulose acid hydrolysate fermentation medium (the corncob hemicellulose acid hydrolysate fermentation medium and 5 L fermenter were sterilized together) at 8% (v/v) and was incubated at 30°C and 140 r/min for 82 h. The oxygen input quantity was 0.1 vvm. During fermentation, a 5 ml sample was taken out every 5 h. A 4 ml sample was used to measure the OD value of the fermentation broth at 600 nm, and growth curves were drawn. A 1 ml sample was used to determine concentrations of ethanol and xylose in the fermentation broth by HPLC and then the fermentation results were analyzed.

Mixed fermentation of corncob hemicellulose acid hydrolysate by *C. shehatae* YHFK-2 and *S. cerevisiae* W5

Activated *C. shehatae* YHFK-2 and *S. cerevisiae* W5 were inoculated into YPX broth, respectively, and the flasks were incubated at 30°C and 140 r/min for 24 h (OD_{600nm} = 2). Both *C. shehatae* YHFK-2 and *S. cerevisiae* W5 were subsequently inoculated into the corncob hemicellulose acid hydrolysate fermentation medium and artificially prepared hydrolysate fermentation medium at 5% (v/v), respectively, and the flasks were incubated at 30°C and 140 r/min for 64 h. The liquid volume in each 250 ml flask was 80 ml and each sample was repeated in parallel. After 36, 48, and 64 h, concentrations of residual sugars, acetic acid, ethanol, and furfural were determined by HPLC, respectively.

Analytical methods

The liquid samples were analyzed by HPLC (Shimadzu LC-10ATvp), equipped with UV and RI detectors. Glucose, xylose, arabinose, acetic acid and ethanol were analyzed by a refractive index detector (RID-10A) and Aminex HPX-87H column (300 x 7.8 mm Aminex HPX-87H Ion Exclusion column) at 65°C with 5 mmol/L H_2SO_4 as mobile phase at 0.8 ml/min. Furfural was detected on UV chromatograms at 250 nm and phenolic compounds at 761 nm. (Cheng et al., 2006, 2007).

RESULTS

Hemicellulose acid hydrolysate preparation and detoxification

After acid hydrolysis, the reducing sugar content in the hydrolysate was somewhat improved by reusing the washing fluid and almost 100% of furfural, 93.4% of acetic acid and 32.3% of phenolic compounds in the hydrolysate were removed by a series of detoxification steps and activated carbon absorption.

Fermentation of corncob hemicellulose acid hydrolysate and the artificially prepared hydrolysate by C. shehatae YHFK-2

The effect of unknown fermentation inhibitors on the fermentability of the hydrolysate was analyzed and the results are shown in Table 1. *C. shehatae* YHFK-2 first consumed glucose. After glucose was exhausted, xylose assimilation began. Therefore, after 64 h, glucose concentration was 0 g/L. Ethanol production in corncob hemicellulose acid hydrolysate reached 27.01 g/L, which was 14.62% higher than that in artificially prepared hydrolysate. The utilization ratio of the reducing sugars in corncob hemicellulose acid hydrolysate was approximately equivalent to that in artificially prepared hydrolysate. Ethanol yield in corncob hemicellulose acid hydrolysate was 16.99% higher than that in artificially prepared hydrolysate. The results show that ferment-

Table 1. Fermentation of artificially prepared hydrolysate and corncob hemicellulose acid hydrolysate using *Candida shehatae* YHFK-2.

YHFK-2	Reducing sugars utilization ratio (%)	Ethanol yield (g/g)	Ethanol production (g/L)
Artificially prepared hydrolysate	70.86	0.3889	23.06
Corncob hemicellulose acid hydrolysate	68.89	0.4685	27.01

Table 2. Fermentation scale-up by *Candida shehatae* YHFK-2.

Time (h)	Xylose concentration (g/L)	Xylose utilization amount (g/L)	Ethanol production (g/L)	Ethanol yield (g/g)	pH
0	87.49	0	0	0	--
12	77.62	9.87	1.9	0.193	4.59
17	72.36	15.13	4.27	0.282	4.57
27	72.05	15.44	4.66	0.302	4.55
32	71.26	16.23	5.27	0.325	4.52
37	66.51	20.98	6.82	0.352	4.5
42	61.83	25.66	9.98	0.389	4.45
47	53.48	34.01	14.39	0.423	4.44
52	43.61	43.88	19.22	0.438	4.4
57	32.37	55.12	25.85	0.469	4.37
62	22.35	65.14	33.16	0.509	4.33
67	20.6	66.89	34.18	0.511	4.33
72	16.92	70.57	34.09	0.483	4.31
77	13.01	74.48	32.88	0.441	4.28
82	9.7	77.79	29.17	0.375	4.26

ability of corncob hemicellulose acid hydrolysate was better than that of the artificially prepared hydrolysate.

Fermentation scale-up by C. shehatae YHFK-2

The fermentation of corncob hemicellulose acid hydrolysate was scaled-up by *C. shehatae* YHFK-2 and the results are shown in Table 2. The ethanol production reached the highest point (34.18 g/L) at 67 h and at 67 h, the ethanol production, the xylose utilization ratio, and the ethanol yield in the fermenter was 20.98, 7.56 and 8.32% higher than that in the flasks (Table 1), respectively.

Fermentation of corncob hemicellulose acid hydrolysate by C. shehatae YHFK-2 and S. cerevisiae W5

Fermentation of corncob hemicellulose acid hydrolysate by *C. shehatae* YHFK-2 and *S. cerevisiae* W5 separately and together was performed and the results are shown in Table 3 and Figure 1. The fermentation of corncob hemicellulose acid hydrolysate by only *S. cerevisiae* W5 showed that *S. cerevisiae* W5 could only ferment glucose to ethanol and could consume trace xylose to grow, but could not ferment xylose to ethanol (Figure 1a). By only *C. shehatae* YHFK-2, xylose concentration gradually decreased from 68.75 to 4.39 g/L at 64 h; glucose was completely consumed in 36 h; and ethanol concentration gradually increased from 15.1 g/L at 36 h to 27.7 g/L at 64 h (Figure 1b). Mixed fermentation by both *C. shehatae* YHFK-2 and *S. cerevisiae* W5 (Figure 1c) showed similar results as by *C. shehatae* YHFK-2 alone, yielding 15.8 g of xylose/l and 23.7 g of ethanol/l after 64 h. Ethanol production and xylose utilization ratio by both *C. shehatae* YHFK-2 and *S. cerevisiae* W5 were slightly lower than that of *C. shehatae* YHFK-2 alone.

DISCUSSION

In this work, the corncob hemicellulose acid hydrolysate pretreatment and detoxification process successfully improved reducing sugar content in the hydrolysate and removed many fermentation inhibitors, which provided the great basis for subsequent fermentation. In order to be appled to industrial production, the corncob hemicellulose acid hydrolysate pretreatment and detoxification process needed to be further optimized in

Table 3. Fermentation of corncob hemicellulose acid hydrolysate by *Saccharomyces cerevisiae* W5 and *Candida shehatae* YHFK-2.

Parameter	Reducing sugars utilization ratio (%)	Ethanol yield (g/g)	Ethanol productivity [g/(l·h)]
W5 (36 h)	17.97	0.1077	0.045
W5 (48 h)	12.74	0.0949	0.021
W5 (64 h)	21.85	0.0217	0.0062
YHFK2 (36 h)	38.54	0.467	0.4185
YHK2 (48 h)	61.44	0.4272	0.4577
YHFK2 (64 h)	85.29	0.3878	0.4326
Mixed fermentation (36 h)	25.11	0.3252	0.1899
Mixed fermentation (48 h)	42.61	0.3157	0.3157
Mixed fermentation (64 h)	71	0.3991	0.3706

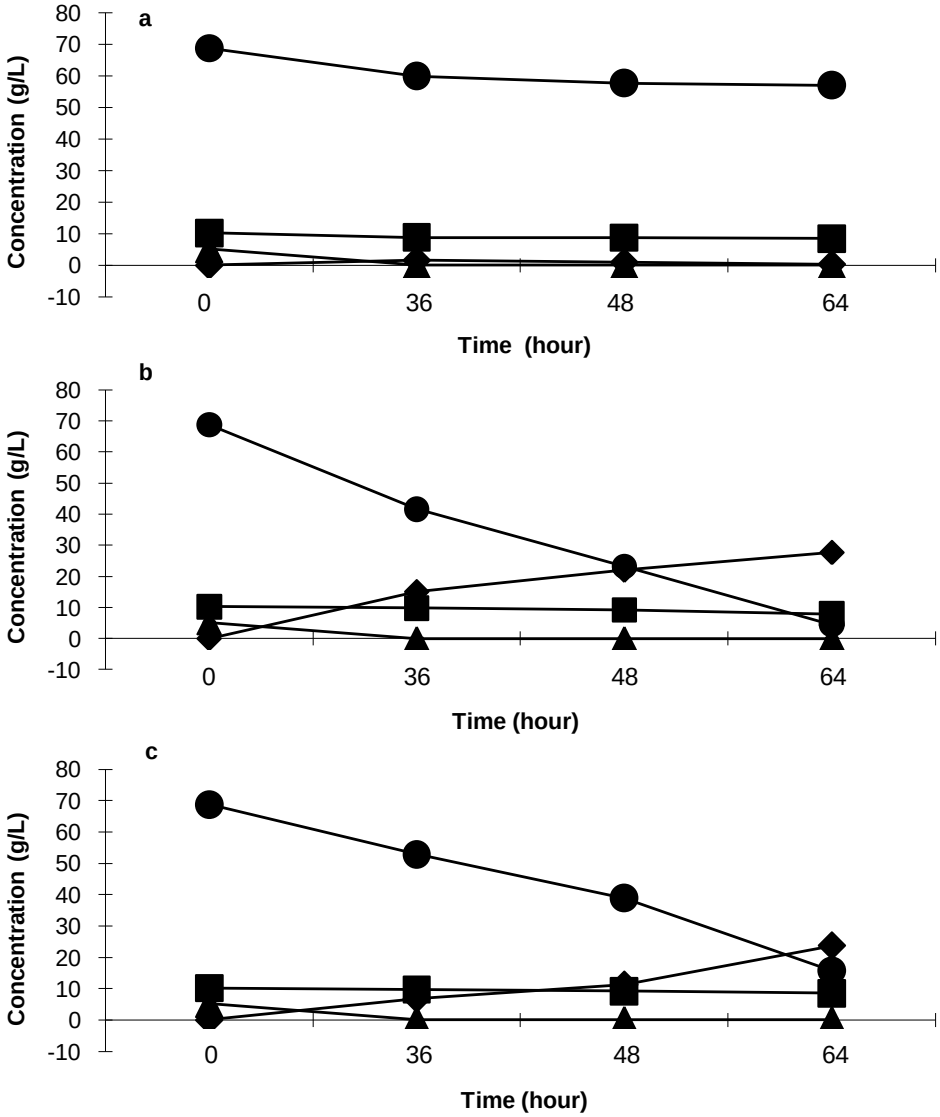

Figure 1. Time course of corncob hemicellulose acid hydrolysate fermentation by a *Saccharomyces cerevisiae* W5, b *Candida shehatae* YHFK-2, and c both *Saccharomyces cerevisiae* W5 and *Candida shehatae* YHFK-2: xylose (●), arabinose (■), ethanol (◆), glucose (▲).

two aspects: (1) 10% ammonia-pretreatment time needed to be reduced which increased production cycle time and production cost; (2) detoxification effectively removed many fermentation inhibitors, but it also caused the loss of fermentative sugars.

In the mixed fermentation of glucose and xylose, *C. shehatae* started to ferment xylose to ethanol only after glucose was exhausted, which occurs after all of the glucose is converted to xylose and is known as the “glucose effect” (Ji et al., 2008).

Though the mixed fermentation led to a delay in xylose fermentation, and the secondary growth of strains, etc., the ethanol production ability of corncob hemicellulose acid hydrolysate was better than that of the fermentation medium using an equivalent amount of xylose as the single carbon source because the ethanol conversion of glucose was higher than that of xylose.

Using parallel fermentation of corncob hemicellulose acid hydrolysate and the artificially prepared hydrolysate, it was found that complex components in the corncob hemicellulose acid hydrolysate probably promoted ethanol production to some degree. Perhaps buffer salts could balance the fermentation broth pH, allowing the fermentation broth pH to be maintained at a higher level. Because higher pH is more suitable for strain growth, the consumption and the utilization ratio of the reducing sugars were greater. Thus, the fermentation properties of the corncob hemicellulose acid hydrolysate were more stable than those of the artificially prepared hydrolysate.

Mixed fermentation using *C. shehatae* and *S. cerevisiae* could make the xylose-fermentation strain and the glucose-fermentation strain simultaneously ferment xylose and glucose to ethanol. Though *C. shehatae* could also consume glucose, its glucose utilization ratio was lower than that of *S. cerevisiae* W5. Meanwhile, the fermentation period of *C. shehatae* was longer and its adaptability was generally weaker than those of *S. cerevisiae* W5. Therefore, mixed fermentation using *C. shehatae* and *S. cerevisiae* could not only increase the glucose utilization ratio of corncob hemicellulose acid hydrolysate, but also shortened the lag phase, which shortened the fermentation period. At the same time, in the mixed fermentation, W5 had stronger fermentation properties so as to more quickly remove the presence of glucose repressed transcriptions of xylose metabolic key genes in *C. shehatae*. However, the glucose content in corncob hemicellulose acid hydrolysate was low (about 4 to 6 g/L) and after exhausting glucose, W5 could consume ethanol and xylose leading to decreased ethanol production. In addition, interspecies competition might exist between YHFK-2 and W5 causing decreased ethanol production. Therefore, further study is necessary to coordinate the activities of both of the strains.

Finally, fermentation scale-up using *C. shehatae* YHFK-2 was done and it was found that compared with fermentation processes in flasks, the ethanol production, the xylose utilization ratio and the ethanol yield increased, because several main fermentation conditions in the fermenter were better than those in the flask, such as oxygen input quantity and temperature adjustment.

ACKNOWLEDGEMENTS

This study was supported by High-level talents (innovation team) Projects of Heilongjiang University (Hdtd2010-17), Educational Commission of Heilongjiang Province of China (11551z011), the Special Fund for Scientific and Technological Innovative Talents in Harbin (RC2010XK002028), the National Natural Science Foundation of China (Grant No. 31070446), and National High Technology Research and Development Program of China (863 Program) (No. 2007AA 100702-6).

REFERENCES

Ali M, Mark R, Daniel JS (2006). Conditioning hemicellulose hydrolysates for fermentation: Effects of overliming pH on sugar and ethanol yields. Process Biochem. 41:1806-1811.

Cheng KK, Zhang JA, Liu DH, Sun Y, Yang MD, Xu JM (2006). Production of 1,3-propanediol by Klebsiella pneumoniae from glycerol broth. Biotechnol. Lett. 28(22):1817-1821.

Cheng KK, Ge JP, Zhang JA, Ling HZ, Yang MD, Xu JM (2007). Fermentation of pretreated sugarcane bagasse hemicelluloses hydrolysate to ethanol by *Pachysolen tannophilus*. Biotechnol. Lett. 29:1051-1055.

Duff SJB, Murray WD (1996). Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresour. Technol. 55:1-33.

Hinman ND, Wright JD, Hoagland W, Wyman CE (1989). Xylose fermentation, an economic analysis. Appl. Biochem. Biotechnol. 20/21:391-402.

Saha BC (2003). Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30:279-291.

Sun Y, Cheng JY (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol. 83:1-11.

Ji GS, Wang XW, Yong Q, Yu SY (2008). Ethanol fermentation with xylose and glucose mixtures and *Pichia stipitis*. Zhejiang Forest. College 25:148-152.