Appl Biochem Biotechnol (2015) 177:996–1002
DOI 10.1007/s12010-015-1790-0

Solvents Production from a Mixture of Glucose and Xylose by Mixed Fermentation of *Clostridium acetobutylicum* and *Saccharomyces cerevisiae*

Gao-Xiang Qi[1,3] · Lian Xiong[1,2] · Chao Huang[1,2] · Xue-Fang Chen[1,2] · Xiao-Qing Lin[1,2] · Xin-De Chen[1,2]

Received: 3 May 2015 / Accepted: 29 July 2015 /
Published online: 12 August 2015

Abstract To overcome the xylose utilization defect in ethanol fermentation by wide-type *Saccharomyces cerevisiae* and alleviate the carbon catabolite repression (CCR) in acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum*, a novel mixed fermentation of *S. cerevisiae* and *C. acetobutylicum* was developed. When *S. cerevisiae* was inoculated 24 h earlier than *C. acetobutylicum* CH02, a higher solvents yield was achieved with 0.41 g/g, compared to 0.38 g/g in ABE fermentation, and when *S. cerevisiae* and *C. acetobutylicum* CH02 were inoculated simultaneously, a higher productivity was achieved with 0.32 g/L/h, compared to 0.15 g/L/h in ABE fermentation. The total solvents yield was improved by the high ethanol yield from glucose. The CCR in mixed fermentation was alleviated when glucose was utilized quickly by *S. cerevisiae*, and therefore, the productivity was improved. This study suggests that mixed fermentation is an effective solvents production method from a mixture of glucose and xylose.

Keywords Mixed sugars · Ethanol fermentation · ABE fermentation · Carbon catabolite repression · Mixed fermentation

✉ Xin-De Chen
cxd_cxd@hotmail.com

[1] Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, People's Republic of China

[2] Key Laboratory of Renewable Energy, Chinese Academy of Sciences, Guangzhou 510640, People's Republic of China

[3] Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Introduction

Owing to a limited supply of petroleum oil, increased environmental concerns, and an awareness of global warming, bioproduction of automotive fuels from renewable feedstocks has become a global priority [1]. Lignocellulosic biomass is the most abundant raw material on the planet for biofuel production. The low-carbon alcohols (such as ethanol and butanol) production by biological processes from lignocellulosic biomass has been proven to be technologically feasible.

Lignocellulose is composed of cellulose, hemicellulose, and lignin, which is not available to most of alcohol production microorganisms [2, 3]. Therefore, prior to fermentation, the carbohydrates contained in biomass should be hydrolyzed. Dilute acid hydrolysis is a very effective method, and the hydrolysate contains various sorts of monosaccharides, disaccharides, oligosaccharides, and small amounts of inhibitors [4]. The major monosaccharide components of lignocellulosic hydrolysate are glucose and xylose. *Saccharomyces cerevisiae* is considered to be the most effective ethanol producer with high ethanol yield, tolerance, and productivity. However, substantial amounts of pentose generated by hydrolysis of hemicellulose cannot be metabolized by wild type of *S. cerevisiae* due to the lack of xylose reductase and xylitol dehydrogenase activities, and the economy of ethanol production was affected [5].

Butanol production by acetone-butanol-ethanol (ABE) fermentation is now receiving more and more attention because of the chemical advantages of butanol as a new type of biofuel. First, the calorific value of butanol is higher than ethanol. Second, the noncorrosive property of butanol allows it to be transported with existing equipment for gasoline transportation. Third, butanol can be blended with gasoline at any ratio without modification of engines [6, 7]. Compared with bioethanol and biodiesel, biobutanol can be produced from wider material sources [8]. *Clostridium acetobutylicum*, an important solvents-producing organism, is capable of metabolizing a variety of carbohydrates [9]. Glucose and xylose in the lignocellulosic hydrolysate can be metabolized into acetone, butanol, and ethanol. However, butanol production by ABE fermentation from lignocellulosic hydrolysate is often limited by the poor solvents yield, the low solvent productivity, and the low final solvent titers [10].

In this study, the feasibility of mixed fermentation of *S. cerevisiae* and *C. acetobutylicum* CH02 from a mixture of glucose and xylose for solvents production was investigated. The aim of this work was to overcome the defect of xylose utilization in ethanol fermentation and to improve the solvents yield and productivity in ABE fermentation.

Materials and Methods

Strains, Media, and Seed Culture

Laboratory stocks of *C. acetobutylicum* CH02 spore suspensions were maintained in 5 % corn meal medium at 4 °C. *C. acetobutylicum* CH02 spores were heat-shocked at 100 °C for 90 s followed by cooling in cold water for 5 min. 0.5 mL of heat-shocked spores were inoculated in 10 mL of 5 % corn meal medium and then incubated at 37 °C for 24 h, after which 0.5 mL of actively growing culture was transferred to 10 mL of 5 % corn meal medium for seed culture at 37 °C for 12 h. It should be pointed out that the strain *C. acetobutylicum* CH02 was screened by adaptation which can grow well in the medium without sparging oxygen-free nitrogen gas before inoculation.

S. cerevisiae (purchased from Angel Yeast Co., Ltd.) was maintained on a YPD agar medium (yeast extract 10 g/L, peptone 20 g/L, glucose 20 g/L, and agar 20 g/L) slant in a 20-mL test tube at 4 °C. A loop of slant culture was inoculated into 50 mL of YPD medium (yeast extract 10 g/L, peptone 20 g/L, and glucose 20 g/L); subsequently, the medium was incubated for seed culture at 30 °C and 160 rpm for 24 h.

The media for ABE fermentation, ethanol fermentation, and mixed fermentation were the same, which composed of 25 g/L glucose and 25 g/L xylose. Wheat bran, which contains an adequate amount of nutrients, was found to be an ideal source of nitrogen and phosphorus in ABE, ethanol, and mixed fermentation. Wheat bran was used as nitrogen and phosphate sources in all batch fermentations without mineral supplement, and the supplementation level of wheat bran was based on ABE fermentation.

Batch Fermentation

Batch fermentations were carried out in 250-mL conical flasks filled with 200-mL medium incubated in a static incubator. The inoculation levels of *C. acetobutylicum* CH02 and *S. cerevisiae* were controlled at 5 % for batch ethanol, ABE, and mixed fermentations, and the temperature was controlled at 37 °C.

Analytical Methods

Solvents (acetone, butanol, and ethanol) were analyzed using gas chromatograph (Agilent 7890A GC), and sugars (xylose and glucose) were analyzed using high-performance liquid chromatography (Waters 2685 HPLC) according to our previous study [11].

Results and Discussion

Results of ABE Fermentation Under Different Wheat Bran Supplementation Conditions

Wheat bran is an available side product of the wheat milling industry. Its major constituents are nonstarch polysaccharides (46 %), starch (10–20 %), protein (15–22 %), lignin (4–8 %), and minor constituents [12]. Wheat bran has been used mainly as animal feed stock until now. There are reports about butanol production from wheat bran by ABE fermentation [13]. In this work, wheat bran was found to be an ideal crude nitrogen and phosphorus source for ABE fermentation. In the media optimization, a series of batch fermentations with the media supplemented with wheat bran ranging from 5.50 to 19.25 g/L was conducted, and the results are shown in Table 1.

As can been observed in Table 1, ABE titers reached the highest level of 18.45 g/L when the addition of wheat bran was 11.00 g/L. When the addition of wheat bran was lower than 11.00 g/L, the residual sugars were high, which means the nitrogen and phosphorus source is not sufficient. ABE titers declined when the addition of wheat bran was higher than 11.00 g/L, indicating an effect of inhibitory on ABE fermentation. Under the best wheat bran supplementation condition, the ABE yield of 0.38 g/g was achieved.

Table 1 Results of ABE fermentation under different wheat bran supplementation conditions

Wheat bran (g/L)	Products (g/L)				Residual sugars (g/L)	Yield (g-ABE /g-sugars)
	Acetone	Butanol	Ethanol	ABE		
5.50	2.96 ± 0.04	6.19 ± 0.06	1.47 ± 0.03	10.61 ± 0.12	22.08 ± 0.46	0.38 ± 0.01
8.25	4.46 ± 0.34	9.63 ± 0.52	1.67 ± 0.16	15.76 ± 0.76	7.54 ± 0.43	0.37 ± 0.02
11.00	5.62 ± 0.12	11.22 ± 0.20	1.61 ± 0.13	18.45 ± 0.42	1.56 ± 0.25	0.38 ± 0.01
13.75	5.14 ± 0.07	10.90 ± 0.09	1.64 ± 0.08	17.68 ± 0.08	2.50 ± 0.33	0.37 ± 0.00
16.50	5.23 ± 0.15	10.48 ± 0.14	1.63 ± 0.12	17.34 ± 0.22	2.86 ± 0.09	0.37 ± 0.01
19.25	5.22 ± 0.12	10.54 ± 0.10	1.68 ± 0.16	17.44 ± 0.11	2.61 ± 0.41	0.37 ± 0.01

Solvents Production and Sugars Utilization During ABE and Ethanol Fermentation

Profiles of sugars utilization and solvents production by ABE fermentation were illustrated in Fig. 1a; glucose was consumed quickly within 60 h, during which no xylose uptake was detected. Xylose uptake began at 60 h, which suggested the effect of CCR, and the effect has been reported by other investigators [14]. The solvents were not detected at 12 h, which suggested the transition from acidogenesis to solventogenesis occurred between 12 and 24 h. The ABE titers reached 18.45 g/L at the end of the fermentation at 120 h.

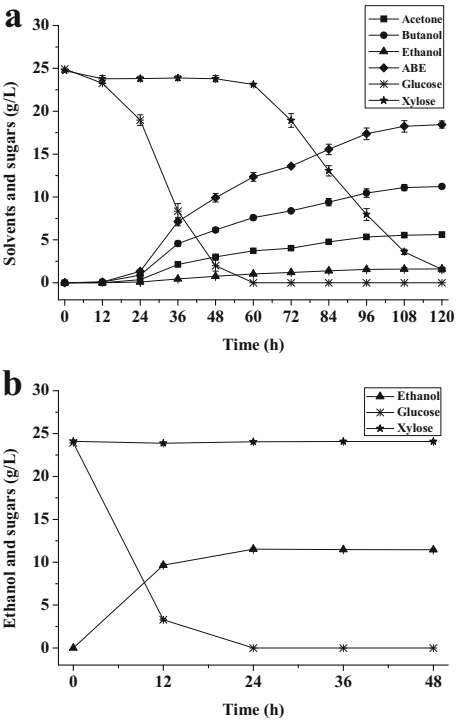

Fig. 1 Sugars utilization and solvents production during ABE and ethanol fermentation (**a**, ABE fermentation; **b**, ethanol fermentation)

Profiles of sugars utilization and ethanol production during ethanol fermentation were illustrated in Fig. 1b; glucose was metabolized and ethanol was produced quickly within 24 h, which suggested the metabolic rate of glucose in *S. cerevisiae* was much higher than that in *C. acetobutylicum* CH02. Xylose uptake was not observed during the whole fermentation process, which suggested that *S. cerevisiae* was unable to utilize xylose. A final ethanol titer of 11.46 g/L was obtained at 24 h.

Solvents Production and Sugars Utilization During Mixed Fermentation

Batch mixed fermentations with different inoculation methods were conducted, and the results were listed in Table 2. Ethanol yield from glucose by ethanol fermentation was 0.46 g/g, 90.20 % of the maximum theoretical yield (0.51 g/g). Total solvents yield from mixed sugars by ABE fermentation was 0.38 g/g, which was lower than the yield of ethanol fermentation. For mixed fermentation with different inoculation methods, the total solvents yields ranged from 0.37 to 0.41 g/g. The total solvent yields increased when the *S. cerevisiae* was inoculated earlier. The highest total solvents yield was achieved when *S. cerevisiae* was inoculated 24 h earlier than *C. acetobutylicum* CH02, with the inoculation levels of 5 %. At this condition, the total solvents yield was 0.41 g/g, compared to 0.38 g/g in ABE fermentation, increased of 7.9 %. Without doubt, more glucose was consumed by *S. cerevisiae* when *S. cerevisiae* was inoculated earlier, and consequently, higher solvents yield was achieved. Compared with ethanol fermentation, the residual xylose concentrations in mixed fermentations were much lower, which suggested that the xylose utilization defect was overcome in mixed fermentation. The final solvents titers in mixed fermentations ranged from 17.50 to 19.62 g/L, which were much higher than ethanol titer of 11.45 g/L in ethanol fermentation.

The profiles of sugars utilization and solvents production during the mixed fermentation processes are shown in Fig. 2. In these inoculation methods, *S. cerevisiae* was inoculated 24, 12, and 0 h before *C. acetobutylicum*, respectively, and the seed culture conditions and inoculation levels of *S. cerevisiae* and *C. acetobutylicum* in these inoculation methods were the same as described in "Materials and Methods." The fermentation time for mixed

Table 2 Results of mixed fermentation with different inoculation methods

Inoculation methods	Products (g/L)				Residual sugars (g/L)	(g-ABE/g-sugars)
	Acetone	Butanol	Ethanol	ABE		
SC	0	0	11.46 ± 0.08	11.46 ± 0.08	24.08 ± 0.05	0.46 ± 0.00
CA	5.62 ± 0.12	11.22 ± 0.20	1.61 ± 0.13	18.45 ± 0.42	1.56 ± 0.25	0.38 ± 0.01
SC-CA-24 h	2.50 ± 0.01	5.29 ± 0.09	11.82 ± 0.21	19.62 ± 0.36	1.57 ± 0.15	0.41 ± 0.01
SC-CA-12 h	2.32 ± 0.21	5.30 ± 0.15	11.47 ± 0.16	19.09 ± 0.15	1.77 ± 0.20	0.40 ± 0.00
SC-CA-0 h	2.39 ± 0.09	5.40 ± 0.07	11.40 ± 0.44	19.19 ± 0.39	2.05 ± 0.31	0.40 ± 0.01
CA-SC-12 h	3.61 ± 0.31	7.29 ± 0.37	7.49 ± 0.13	18.39 ± 0.52	3.07 ± 0.41	0.39 ± 0.01
CA-SC-24 h	3.42 ± 0.13	8.33 ± 0.24	5.90 ± 0.07	17.64 ± 0.30	3.22 ± 0.13	0.38 ± 0.01
CA-SC-36 h	4.45 ± 0.19	10.19 ± 0.38	2.82 ± 0.18	17.46 ± 0.48	2.56 ± 0.35	0.37 ± 0.01
CA-SC-48 h	4.73 ± 0.33	10.44 ± 0.43	2.33 ± 0.14	17.50 ± 0.48	3.29 ± 0.11	0.37 ± 0.01

SC, *S. cerevisiae*; *CA*, *C. acetobutylicum*; *SC-CA-24 h*, *S. cerevisiae* was inoculated 24 h earlier than *C. acetobutylicum* CH02

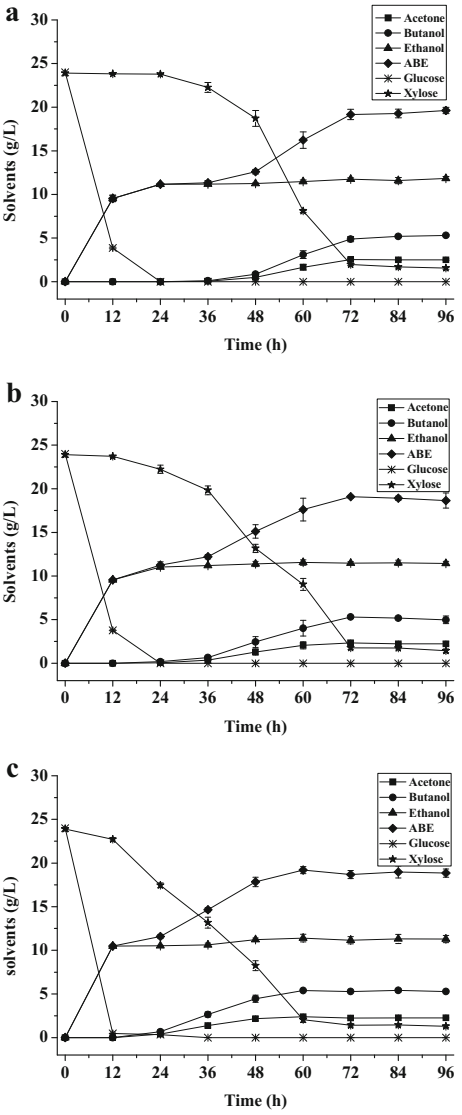

Fig. 2 Sugars utilization and solvents production during mixed fermentation with different inoculation methods (**a**, SC-CA-24 h; **b**, SC-CA-12 h; **c**, SC-CA-0 h)

fermentation with the inoculation methods of SC-CA-24 h, SC-CA-12 h, and SC-CA-0 h was 96, 72, and 60 h, respectively. The fermentation time was prolonged when the *S. cerevisiae* was inoculated later than *C. acetobutylicum* CH02 (data not shown). The total solvents productivities obtained from the mixed fermentation with the inoculation methods of SC-CA-24 h, SC-CA-12 h, and SC-CA-0 h were 0.20, 0.27, and 0.32 g/L/h, compared with the productivity of 0.15 g/L/h obtained from ABE fermentation, increased of 33 %, 80 %, and 113 %, respectively. The CCR in mixed fermentation was alleviated when the glucose was metabolized quickly by *S. cerevisiae*, and consequently, the productivities were improved dramatically.

Conclusions

In this work, the feasibility of mixed fermentation of *S. cerevisiae* and *C. acetobutylicum* CH02 from a mixture of glucose and xylose for solvents production was investigated, and the results showed that the method was successful. The xylose utilization defect in ethanol fermentation was overcome by mixed fermentation. The fast utilization of glucose by *S. cerevisiae* alleviated the CCR in mixed fermentation, and therefore, the fermentation time was reduced and the solvents productivity was improved. The solvents yield in mixed fermentation was improved as a result of the high ethanol yield from glucose by *S. cerevisiae*.

Acknowledgments This study was supported by Plan Project of National Science and Technology (2012BAD32B07), the Science and Technology Project of Guangdong Province (2013B010404036), project of Guangzhou Science and Technology (2013 J4300031), National Natural Science Foundation of China (51378486), Natural Science Foundation of Guangdong Province (S2012040007546), the Project of Jiangsu Province Science and Technology (BE2013083), the Foundation of Xuyi Center of Attapulgite Applied Technology Research Development & Industrialization, Chinese Academy of Sciences (20121004), and Foundation of Director of Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences (y407r41001).

References

1. Dellomonaco, C., Fava, F., & Gonzalez, R. (2010). The path to next generation biofuels: successes and challenges in the era of synthetic biology. *Microbial Cell Factories*, *9*, 1–15.
2. Aristidou, A., & Penttilä, M. (2000). Metabolic engineering applications to renewable resource utilization. *Current Opinion in Biotechnology*, *11*, 187–198.
3. Saha, B. C. (2003). Hemicellulose bioconversion. *Journal of Industrial Microbiology & Biotechnology*, *30*, 279–291.
4. Ezeji, T., Qureshi, N., & Blaschek, H. P. (2007). Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. *Biotechnology and Bioengineering*, *97*, 1460–1469.
5. Karhumaa, K., Fromanger, R., Hahn-Hägerdal, B., & Gorwa-Grauslund, M. F. (2007). High activity of xylose reductase and xylitol dehydrogenase improves xylose fermentation by recombinant *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, *73*, 1039–1046.
6. Dürre, P. (2007). Biobutanol: an attractive biofuel. *Biotechnology Journal*, *2*, 1525–1534.
7. Lin, X. Q., Wu, J. L., Jin, X. H., Fan, J. S., Li, R. J., Wen, Q. S., Qian, W. B., Liu, D., Chen, X. C., Chen, Y., Xie, J. J., Bai, J. X., & Ying, H. J. (2012). Selective separation of biobutanol from acetone–butanol–ethanol fermentation broth by means of sorption methodology based on a novel macroporous resin. *Biotechnology Progress*, *28*, 962–972.
8. Dong, H. J., Tao, W. W., Dai, Z. J., Yang, L. J., Gong, F. Y., Zhang, Y. P., & Li, Y. (2012). Biobutanol. *Advances in Biochemical Engineering/Biotechnology*, *128*, 85–100.
9. Shao, L.J., Hu, S.Y., Yang, Y., Gu, Y., Chen, J., Yang, Y.L., Jiang, W.H. &Yang, S. (2007). Targeted gene disruption by use of a group II intron (targetron) vector in *Clostridium acetobutylicum*. *Cell Research*, *17*, 963–965.
10. Jang, Y. S., Malaviya, A., Cho, C., Lee, J., & Lee, S. Y. (2012). Butanol production from renewable biomass by *clostridia*. *Bioresource Technology*, *123*, 653–663.
11. Lin, X. Q., Xiong, L., Qi, G. X., Shi, S. L., Huang, C., Chen, X. F., & Chen, X. D. (2015). Using butanol fermentation wastewater for bio-butanol production after removal of inhibitory compounds by micro-mesoporous hyper cross linked polymeric adsorbent. *Acs Sustainable Chemistry and Engineering*, *3*, 702–709.
12. Maes, C., & Delcour, J. A. (2001). Alkaline hydrogen peroxide extraction of wheat bran non-starch polysaccharides. *Journal of Cereal Science*, *34*, 29–35.
13. Liu, Z. Y., Ying, Y., Li, F. L., Ma, C. P., & Xu, P. (2010). Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran. *Journal of Industrial Microbiology & Biotechnology*, *37*, 409–501.
14. Xiao, H., Gu, Y., Ning, Y. Y., Yang, Y. L., Mitchell, W. J., Jiang, W. H., & Yang, S. (2011). Confirmation and elimination of xylose metabolism bottlenecks in glucose phosphoenolpyruvate-dependent phosphotransferase system-deficient *Clostridium acetobutylicum* for simultaneous utilization of glucose, xylose, and arabinose. *Applied and Environmental Microbiology*, *77*, 7886–7895.