Supporting Information

A dual-chamber reactor to assess the saccharification capability of the cellulytic microflora from straw waste

Cheng-Jiao Xu [a, b], Guang-Li Cao [a, c*], Lei Zhao [a], Ai-Jie Wang[a], Lin-Na Chen[a], Nan-Qi Ren[a,*]

[a]*State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China*
[b]*College of Resources and Environment, Northeast Agricultural University, Harbin 150030, China*
[c]*Department of Life Science and Technology, Harbin Institute of Technology, Harbin 150008, China*

Experimental Section

Seed microflora source

Environmental samples collected from forest soil (FS), decay wood (XF), decay bark (FC), sub lacustrine decay branch (YH) in Yichun city, anaerobic composting in the suburb of Harbin city (MX), and hot spring sludge in Benxi city (BW), were used as sources of inoculum. Samples were transported to the laboratory in sterile polypropylene bags and stored at -20^{o}C until use.

Enrichment of cellulolytic anaerobic microflora

For enrichment, samples were first crushed and passed through a 100 mesh screen. 10 g mashed samples were subsequently added into serum bottles which contained 100ml sterilized anearobic water and shaked at 250 rpm/min for 30 minutes. Then 10 ml dispersed solutions were transferred into 90 ml enrichment medium as described by Wolin et al 1963, [1] 0.2 g/L sodium dodecyl benzene sulfonate was added into the medium to inhibit the growth of methane-producing bacteria, 5g/L rice straw of 5 mm segment was served as carbon source. The enriched mixed cultures were transferred continuously every 7days into the fresh straw medium and shaken at 60^{o}C to enhance the cellulose biodegradability and maintain stabilization of the microflora. Variation of pH, straw weight loss, volatile fatty acids (VFAs), and biogas production were used as an indication to determine the stability of the mixed fermentation community. The microflora with strong ability of straw degradation but weak in biogas production was selected as target microflora.

Screening of functional saccharification consortium

An exponential serially dilution method was applied to screen the consortium towards reducing sugar accumulation as described by Wang et al., 2010.[2] The selected microflora MX after enrichment was transferred into the Avicel medium with the same components as described before except the carbon source was substituted by Avicel PH101. The consortia from Avicel medium were serially diluted from the ratio of 10^{-1} to 10^{-9} with anaerobic sterilized water. 1ml solution from each dilution was

*Corresponding author: Tel/ Fax: +86-0451-86418180; E-mail: rnq@hit.edu.cn

then inoculated into 9 ml Avicel medium and cultured at 60^{o}C for 72 h on a shaking incubator at 150 rpm. Reducing sugar production from each dilution rate was detected at every 24h.

Analytical methods

The concentrations of reducing sugars from rice straw saccharification as well as the sugar consumption during hydrogen production fermentation were determined using high performance liquid chromatography (HPLC) system (Zhao et al. 2012, 2013, 2012). [3, 4, 5] The hydrogen content were measured as the method depicted by Zhao et al 2012. [6] The concentrations of volatile fatty acids and alcohols were detected by gas chromatography (4890D, Agilent Cooperation, USA) equipped with steel column packed with GDX103 (60/80 mesh) (Zhao et al. 2013).[7]

References

1 E. A. Wolin, M. J. Wolin and R. S.Wolfe, *Journal of Biological Chemistry*, 1963, 238, 2882–2886.

2 A.J. Wang, L. F. Gao, N. Q. Ren, J. F. Xu, C. Liu and D. J. Lee, *Int. J. Hydrogen Energy*, 2010, 33, 1-6.

3 L. Zhao, G. L. Cao, A. J. Wang, H. Y. Ren, D. Dong, Z. N. Liu, X. Y. Guan, C. J. Xu and N. Q. Ren, *Bioresour. Technol.*, 2012, **114**, 365-369.

4 L. Zhao, G. L. Cao, A. J. Wang, H. Y. Ren, C. J. Xu and N. Q. Ren, *GCB Bioenergy*, 2013, 5(5), 591-598.

5 L. Zhao, G. L. Cao, A. J. Wang, W. Q. Guo, B. F Liu, H. Y, Ren, N. Q. Ren and F. Ma, *Int. J. Hydrogen Energy*, 2012, **37**, 162-166.

6 L. Zhao, G. L. Cao, J. Yao, H.Y. Ren, F. Ma, N. Q. Ren and A. J. Wang, *RSC Advanc.*, 2012, 2, 7391-7395.

7 L. Zhao, G. L. Cao, A. J. Wang, W. Q. Guo, H. Y, Ren and N. Q. Ren, *Bioresour. Technol.*, 2013, 145, 103-107.