

## Supporting Information

### Optimization of Immobilized Parameters of *Thermoanaerobacterium thermosaccharolyticum* W16 on a New Carrier for Enhanced Hydrogen Production

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#### Experimental Section

##### Microorganisms

The strain for hydrogen production was *T. thermosaccharolyticum* W16.<sup>1</sup> It was routinely grown at 60 °C in a medium containing (L<sup>-1</sup>): 1.0 g NH<sub>4</sub>Cl, 3.0 g K<sub>2</sub>HPO<sub>4</sub>, 1.5 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g MgCl<sub>2</sub> 6H<sub>2</sub>O, 2.0 g NaCl, 0.2 g KCl, 0.5 g cysteine-HCl, 2.0 g yeast extract, 2.0 g tryptone, 10 g glucose, 1 ml trace element solution, 1 ml vitamin solution.<sup>2</sup> The strain at its exponential-growth phase was used as the inoculum.

The strain which is used to form mycelia pellets was *Aspergillus niger* Y3 from Environmental Biotechnology Laboratory of HIT. Mycelia pellets was obtained by cultivate the spore suspension in a liquid medium at 30 °C in a gyratory incubator-shaker at a rotational speed of 140 rpm/min as described by Zhao et al (2012).<sup>3</sup>

##### Hydrogen production with immobilized cells in batch culture

The mycelia pellets were washed with sterile water to prevent agar debris and conidiophores in the spore suspension after the moderate size were formed. Then the pellets were poured into 250 ml glass serum bottles with 150 ml basal medium mentioned before in nitrogen atmosphere. Each glass serum bottle was inoculated with 5 ml of *T. thermosaccharolyticum* W16 suspension at its exponential-growth phase for hydrogen production. All hydrogen production tests were performed at 60 °C, initial pH 7.0 under anaerobic condition. Cell density, pH, residual substrate concentration, and quantity and compositions of produced biogas were determined. All treatments were carried out in triplicate to check data reproducibility.

##### Optimization of immobilized parameters and the experimental design

Response surface methodology (RSM) of central composite design (CCD), which is effective for sequential experimentation that gives information about interaction between independent variables in relation to the dependant variable, was chosen for the experimental design.

The independent variables of mycelia pellets dosage, mycelia pellets size and culturing time (coded as  $X_1$ ,  $X_2$ , and  $X_3$ ) were optimized for enhanced hydrogen production in this research. Based on the design, a total number of twenty experiments with six replicas at the center point were employed. The hydrogen yield was considered as the dependent variable or response. For predicting the optimal point, a second-order polynomial function was fitted to evaluate the correlativity between independent variables and response as follows:

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j$$

where  $Y$  is the predicted response;  $b_0$  is the interception coefficient;  $b_i$  is the linear coefficients;  $b_{ii}$  is the quadratic coefficients;  $b_{ij}$  is the cross product coefficients; and  $X_i$  represent the independent variables studied.

The Design Expert (Version 7.4.1.0, Stat-Ease Inc., Minneapolis, USA) and STATISTICA v.7.0 (Statsoft, USA) software were carried out by predicting the optimum response of different factors and graphical analyses of the data obtained.

### Scanning electron microscope (SEM) observation

The mycelia pellets samples with immobilized cells at the optimum conditions were examined and photographed by SEM (JEOL JSM 5800) using 5kV accelerating voltage and 10mm distance. Digital images were captured using 1280×960 resolutions and 160s dwell time.<sup>3</sup>

### Analytical methods

The sugar consumption during fermentation was detected by a high performance liquid chromatography (LC-10A, Shimadzu Corporation, Kyoto, Japan) with a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The gas samples were measured by gas chromatography (4890D, Agilent Cooperation, USA) as described by Zhao et al (2012).<sup>4</sup> The soluble metabolites were detected by gas chromatography (4890D, Agilent Cooperation, USA) equipped with a hydrogen flame-ionization detector (FID).

### References

- 1 G. L. Cao, N. Q. Ren, A. J. Wang, D. J. Lee, W. Q. Guo, B. F. Liu, Y. J. Feng and Q. L. Zhao, *Int. J. Hydrogen Energy*, 2009, **34**, 7182-7188.
- 2 S.V. Mohan, G. Mohanakrishna, S. S. Reddy, B. D. Raju, K. S. Rama Rao and P. N. Sarma, *Int. J. Hydrogen Energy*, 2008, **33**, 6133-6142.
- 3 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.
- 4 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.