Biohydrogen production by the co-culture of *Clostridium beijerinckii* and *Rhodobacter capsulatus* with acid resistance

Yang Zhang[1*] , Jifeng Yuan[1], Liejin Guo[2]

1 School of Life and Science, Xiamen University, Xiamen, 361102, P.R. China

2 State Key Laboratory of Multiphase Flow in Power Engineering, Xi'an Jiaotong University, Xi'an, 710049, P.R. China

ABSTRACT

The co-culture of dark- and photo-fermentative bacteria has been regarded as a promising way for bio-hydrogen production. However, the low hydrogen production performance of photo-fermentative bacteria at acidic condition badly limits the co-culture hydrogen production. In this work, we obtained five mutants of *Rhodobacter capsulatus* with acid resistance by Tn7 transposon, which is an efficient genetic tool for random mutation. The mutant of *R. capsulatus* ZYac2 with optimum hydrogen production performance at low pH was selected for co-culture fermentation with *Clostridium beijerinckii* YA001. A four-factor and four-level orthogonal experimental array was designed and conducted to study the effects of the ratio of dark- to photo-fermentative bacteria, initial pH, phosphate concentration, and light intensity on hydrogen yield. The highest hydrogen yield of 3948.3 mL/L was obtained at the ratio of dark- to photo-fermentative bacteria of 1:1, light intensity of 9000 lux, initial pH of 7.5, and phosphate buffer concentration of 40 mM. And the significance of the four parameters on hydrogen yield was listed in high-to-low order as: initial pH, ratio of dark- to photo-fermentative bacteria, phosphate concentration, and light intensity. At last, a high hydrogen yield of 326 mL/g-cornstalk was obtained by this co-culture system using cornstalk pretreated by diluted acid as a substrate.

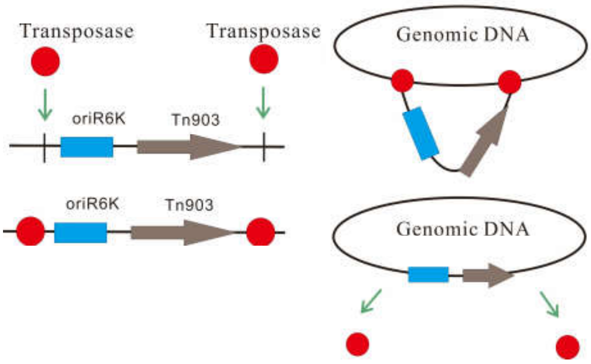

Fig.1- Schematic diagram of Tn7 transposon mutation

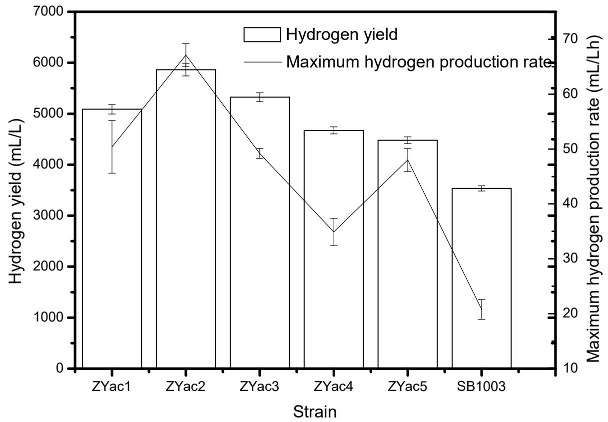

Fig.2- Hydrogen production by transposon mutants

Table 1- A L_{16} (4^4) orthogonal array and experimental results

Ratio of dark- and photo-fermentative bacteria (A)	Light intensity (B) /lux	Initial pH (C)	Phosphate buffer concentration (D)/mM	Hydrogen yield / mL/L	Maximum hydrogen production rate (V_m) /mL/Lh
1:0.2	3000	7	20	2170.6±51.2	80.2±2.1
1:1	7000	8	20	3049.5±62.8	93.6±3.6
1:3	9000	8.5	20	2267.4±43.8	73.5±5.1
1:0.5	5000	7.5	20	2063.3±59.8	71.2±9.2
1:0.5	3000	8.5	40	2628.6±68.3	79.4±7.3
1:3	3000	8	30	2641.3±46.1	78.6±5.8
1:1	3000	7.5	50	2579.2±50.5	73.1±6.4
1:0.2	7000	8.5	50	2397.1±39.6	72.4±2.9
1:3	5000	7	50	2534.8±31.5	80.4±3.7
1:0.2	5000	8	40	3059.7±67.1	80.3±2.4
1:0.5	9000	8	50	3559.7±74.9	86.4±6.9
1:0.5	7000	7	30	2430.9±64.4	73.6±12.1
1:1	9000	7	40	2761.3±84.1	82.2±6.9
1:1	5000	8.5	30	3269.4±81.9	96.1±4.6
1:3	7000	7.5	40	2724.5±91.2	80.4±5.8
1:0.2	9000	7.5	30	3126.8±83.6	93.8±7.1

Table 2- Variance analysis of different factors on hydrogen yield

Factors	Sum of square	Freedom	Mean of square	*F*	*P*	Significance
A	0.688E6	3	0.196E6	1.015	0.034	Yes
B	0.375E6	3	0.125E6	0.671	0.034	Yes
C	0.811E6	3	0.270E6	1.451	0.028	Yes
D	0.555E6	3	0.185E6	0.992	0.021	Yes
Error	0.559E6	3	0.186E6		α = 0.05	

The significance of the four parameters on hydrogen yield was listed in high-to-low order as: initial pH, ratio of dark- to photo-fermentative bacteria, phosphate concentration, and light intensity

Table 3- Verification tests for orthogonal experiments under the ratio of dark- to photo-fermentative bacteria of 1:1, initial pH of 7.5, and phosphate buffer concentration of 40 mM

Light intensity /lux	V_m/mL/Lh	Hydrogen yield/mL /L	Final pH
3000	119.5±22.1	3359.4±100.7	6.21
5000	114.2±11.4	3451.8±126.4	6.34
7000	107.9±24.7	3851.7±153.7	6.61
9000	104.8±10.7	3948.3±116.5	6.70

Table 4- Hydrogen production by different fermentation systems using cornstalk pretreated by diluted acid

Fermentation type	Hydrogen yield /mL/g-cornstalk	V_m/mL/L/h
Photo-fermentation	242.6±6.8	65.1±5.9
Dark-fermentation	105.8±5.7	131.7±10.7
Two-step fermentation	291.3±10.1	
Co-culture fermentation	326.5±12.3	102.7±5.9