Research Paper

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Comparative Study of Biological Hydrogen Production Using Various Microbial Strains

Life Science

KEYWORDS : Alternative energy; Biohydrogen production; Microbial fermentation; Hydrogen

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ABSTRACT

Hydrogen is a promising alternative fuel for the future because its non-polluting and inexhaustible nature, it has high heating power and the potential to eradicate all the environmental problems. As an importantly and specially industrial gases, hydrogen has a wide application and potentiality in petrochemical, metallurgic industry, electronics, food processing, refined organic synthesis and aviation. Among the various hydrogen production pathways, use of biological methods of hydrogen production has attracted increasing global attention, due to its potential for inexhaustible, low-cost and renewable source of clean energy. The aim of this work was to observe the hydrogen production yield of 16 different pure strains and mixed culture, which contains hydrogen-producing strains and non-hydrogen-producing strains. The experimental results compared the hydrogen production yield of these pure strains. In addition, The biohydrogen production in mixed culture of Enterobacter cloacae ATCC 13047, Kluyveromyces marxianus 15D and Clostridium acetobutylicum ATCC 824 was greatly enhanced up to 33.7 mL/h-1.L-1, indicating that biohydrogen production in mixed culture is preferable for the fermentative hydrogen-producing system. These results confirmed Enterobacter cloacae ATCC 13047, Kluyveromyces marxianus 15D and Clostridium acetobutylicum ATCC 824 of hydrogen-producing bacteria has potential and efficient applications in biohydrogen production.

1. Introduction

Biological hydrogen-production technology is one of the most important means of solving the energy and environmental crises and has drawn increasing attention, becoming the research focus of scientists around the world [1-3]. Hydrogen-producing microbes are the core of biological hydrogen-production technology and play important roles in the process of biological hydrogen production. To date, hydrogen-producing fermentative microbes have been extensively studied and widely reported by domestic and foreign researchers. However, the ideal fermentative bacteria for hydrogen production have not been identified. The shortage of ideal hydrogen-producing fermentative microbes has become one of the biggest bottlenecks hindering the development of biological hydrogen-production technology. Therefore, screening, isolating, and identifying highly efficient hydrogen-producing bacteria and enhancing the efficiency of hydrogen production not only provide important microbial resources for the genetic improvement of hydrogen-producing bacteria and for physiological and biochemical studies but also facilitate the industrialisation of biological hydrogen production.

To identify bacteria for efficient fermentative hydrogen production, domestic and foreign researchers have isolated a large number of hydrogen-producing bacteria, which belong mainly to the genera Clostridium and Enterobacter [4-6]. Zhao et al. have found that the addition of silver nanoparticles to anaerobic reactors enhances the ability of Clostridium butyricum to produce hydrogen fermentatively. A maximum hydrogen yield of 2.48 mol/mol glucose was achieved when the concentration of silver nanoparticles reached 20 nmol L^{-1} [7]. Other studies have shown that hydrogen-production technology using mixed microfloras gives superior results compared to technology using a single microbial species [8-10]. Zhang et al. have studied the effects of disulphonate on fermentative hydrogen production by mixed microfloras; in an experimental system consisting of a mixed culture of Clostridium beijerinckii and Enterobacteriaceae with xylose as the substrate, the hydrogen yield reached a maximum of 52.3% [11]. Therefore, screening pure microbial strains of the genera Clostridium and Enterobacter and establishing hydrogen-production systems with mixed microfloras will lay the foundation for subsequent applied research.

In the present study, 16 microbial strains of different origins, including hydrogen-producing strains (belonging to the genera Clostridium and Enterobacter) and non-hydrogen-producing strains (belonging to the genus Bacillus and to the Saccharomyces and edible fungi), were selected, and their 16S/18S ribosomal RNA (rRNA) sequences were identified. In addition, the microscopic morphology of the microbial colonies and cells was examined, and the hydrogen-production efficiencies of hydrogen-production systems composed of pure microbial strains were determined. Of the 16 microbial strains, 8 pure strains were selected for further study. The dynamics and electrochemistry of hydrogen-production systems composed of each pure strain were analysed, and the pure strains that were most suitable for building mixed microfloras were identified. This systematic study of the electrochemical parameters of fermentative hydrogen-production systems, mathematical description of the experimental data, and establishment of an experimental model will provide the basis for establishing quality-control parameters in subsequent applied research.

2. Materials and methods

2.1 Microorganisms

A total of 16 microbial strains of different origins were selected

to build a screening library. The microbial strains are summarised in Table 1. Of the 16 microbial strains, 11 were represented by 16S/18S-rRNA nucleic-acid sequences in GenBank. Electrochemical parameters were determined for the following 8 strains: Clostridium acetobutylicum ATCC 824, Enterobacter cloacae ATCC 13047, Bacillus stearothermophilus U2, Bacillus subtilis B7, Kluyveromyces lactis B9, Rhodotorula lactosa C8, Kluyveromyces marxianus D10, and Kluyveromyces marxianus 15D.

2.2 Media

2.2.1 The composition of the modified Gifu anaerobic medium (GAM) was as follows: proteose peptone 15.0 g, pancreatic casein peptone 10.0 g, yeast extract 5.0 g, beef powder 2.0 g, digestive serum powder 13.5 g, bovine liver extract powder 1.2 g, glucose 3.0 g, potassium dihydrogen phosphate 2.5 g, sodium chloride 3.0 g, soluble starch 0.3 g, L-cysteine 0.3 g, and sodium thioglycollate 0.15 g. The medium was prepared by adding 1.0 L distilled water to 74.0 g modified GAM and autoclaving at 1.05 Kg/cm2 for 20 min.

2.2.2 The composition of the liquid cornmeal-infusion media was as follows: corn flour 5.0 g, peptone 0.1 g, glucose 1.0 g, and distilled water 1.0 L (pH 7.0). The medium was autoclaved at 1.05 Kg/cm2 for 20 min.

2.2.3 The composition of the nutrient broth was as follows: peptone 10.0 g, beef extract 3.0 g, NaCl 5.0 g, agar 20.0 g, and distilled water 1.0 L. The medium was adjusted to pH 7.0 with 5 mol/L sodium hydroxide (approximately 0.2 ml) and autoclaved at 1.05 Kg/cm2 for 20 min.

2.2.4 The composition of the potato-dextrose agar was as follows: potato 200.0 g, dextrose 20.0 g, agar 20.0 g, and distilled water 1.0 L (pH 7.0). The potatoes were washed, peeled, and sliced. The sliced potatoes (200.0 g) were boiled in water for 30 min, and the potato broth was filtered with gauze. Distilled water was added to bring the volume of the broth to 1.0 L. Dextrose was then added, and the broth was heated to aid dissolution. The medium was divided into aliquots and autoclaved at 1.05 Kg/cm2 for 20 min.

2.3 Reagents

The reagents used included peptone (OXIDE), beef extract (BBI), agar (BBI), GAM medium (Qingdao Hi-tech Industrial Park, Haibo Biotechnology Co., Ltd.), and potato and corn flour (commercially available). Other reagents were analytical grade.

2.4 Analytical procedures

2.4.1 Identification of microbial 16S/18S rRNA

Microbial genomic DNAs were extracted from 8 mL of liquid culture containing microbes in the logarithmic-growth phase (OD600nm = 0.6) using genomic-DNA extraction and purification kits. The microbial 16S/18S rRNA was then sequenced by the Beijing Genomics Institute (BGI) and Jiangnan University. The sequence data were submitted to the GenBank sequence database using the program Sequin 11.90 and the SequinMacroSend Direct Submission Tool.

2.4.2 Morphological characterisation of microbial colonies and cells

Each microbial strain was spread on the surface of the solid media using the streak-plate technique. After appropriate incubation, the resulting single colonies were picked and stained with methylene blue [12]. The microscopic morphology of the microbial colonies and cells was photographed using a Canon Powershot A3300/S Digital Camera (Japan). The microscopes included an OPTON universal microscope (West Germany) and an XSM-20 biological microscope (Ningbo Sunny Instruments Co., Ltd.).

2.4.3 Fermentative hydrogen-production experiments

The 16 microbial strains were inoculated onto solid slant media and cultured. Two loopfuls of microbes were scraped off each slant using a sterile inoculating loop, inoculated into test tubes containing 10 mL of liquid medium, and cultured in a constant-temperature oscillator. The resulting liquid cultures were inoculated into flasks containing 100 mL of liquid medium utilising a 10% inoculum volume, incubated in a constant-temperature oscillator, and allowed to undergo substrate conversion. The hydrogen-production experiments were carried out in a slightly modified glass syringe according to a previously described protocol [13]. The 20-mL glass syringes were sealed at the needle port using rubber caps, and 10 mL of medium was added to each syringe barrel. Liquid culture containing microbes in the logarithmic-growth phase was added at 10% v/v to each syringe and cultured at appropriate temperatures. Hydrogen yield was represented as the volume of hydrogen gas at 1.013×105 Pa (1 atm). The average hydrogen-production rate was calculated according to the following formula

$$Average\ hydrogen-production\ rate(mL/h^{-1}\square L^{-1}) = \frac{Total\ volume\ of\ hydrogen\ produced/mL}{Culture\ time \times 10mL} \times 1000 \quad (1)$$

The hydrogen content was determined using a GC-9A gas chromatograph with a thermal conductivity detector (Shimadzu, Japan). The carrier gas was N^2, and the injection volume was 1 mL. The hydrogen content was calculated using the external-standard method.

2.4.4 Electrochemical kinetics

Electrochemical parameters were measured using an FJA-3 electrochemical ion analyser (Nanjing Chuan-Di Instrument & Equipment Co., Ltd.). The fermentation broth was collected periodically, and the pH/Eh and electrical conductance/conductivity were determined. The measurements were repeated 5 times, and the average values were used for data analysis.

2.4.5 Biomass determinationThe biomass was determined by measuring the absorbance of each microbial culture at a 600-nm wavelength using 1-cm cuvettes and a UV-1800PC UV spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). Un-inoculated liquid medium was used as a reference. The measurements were repeated 5 times, and the average values were used for data analysis.

2.5 Data analysis and calculation methods

2.5.1 Analysis of pH/Eh by the kinetic-potentiometric method

To study the electrochemical characteristics of the hydrogen-production systems composed of pure microbial strains, the Nernst equation was employed to calculate the equilibrium potential (E) of specified redox couples relative to the standard potential (E^{φ}).

$$E = E^{\circ} - \frac{RT}{nF}\ln\frac{{}^{a}red}{{}^{a}ox} \Leftrightarrow E = E^{\circ} - \left(\frac{RT}{nF}\right)\ln\frac{{}^{a}ox}{{}^{a}red} \quad (2)$$

At room temperature (25°C = 298.15 K), the following formula holds:

$$\frac{RT}{F}\ln 10 \approx 0.05916 \quad (3)$$

Therefore, at 25°C, the Nernst equationca be simplified as follows:

$$E = E^{0} - \frac{0.05916}{n}\lg\frac{[red]}{[ox]} \Leftrightarrow E = E^{0} + \frac{0.05916}{n}\lg\frac{[ox]}{[red]} \quad (4)$$

In this formula, R is the gas constant, which is equal to 8.31441 J.K-1.mol-1; T is the temperature (in kelvins); a is the activity of the oxidising and reducing agents (activity = concentration × activity coefficient); [ox] represents the concentration of oxidising agents; [red] represents the concentration of reducing agents; F is the Faraday constant (1 F = 96487 C.mol-1); and n is the number of transferred electrons in a half-reaction (in moles).

Using pH/Eh as parameters, the hydrogen-producing fermentative systems were analysed by the kinetic-potentiometric method. The entire fermentative system was considered as a meas-

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urement cell. The electromotive force of the fermentative system was calculated using the following formula:

$$E_x = E^{o\prime} + \left(\frac{2.3026RT}{F}\right) pH_x \quad (5)$$

2.5.2 Analysis of electrical conductance/conductivity by the kinetic-potentiometric method

In hydrogen-production systems composed of pure microbial strains, when all other conditions are constant, the electrical conductance of the biohydrogen-producing solution is determined by the number of ions, their electric charges, and their mobility. The electrical conductance of the biohydrogen-producing solution is the sum of the electrical conductances of the various ions in the solution. Therefore, in the biological hydrogen-production process, the electrical conductance and conductivity can be utilised to determine the concentrations of the components in a hydrogen-production system and to optimise hydrogen-production conditions.

The conductance of a solution can be calculated using the folow ing formula:

$$G = \sigma \frac{A}{l} = \frac{1}{K}\sigma \quad (6)$$

In this formula, l is the length of the conductor (cm); A is the cross-sectional area of the conductor (cm2); σ is the electrical conductivity (or specific conductance), which represents the conductance of a conductor with a volume of 1 cm^3 (in $(\Omega \bullet cm)^{-1}$ or S/cm); G is conductance in siemens (S) ($1S = 1A \bullet V^{-1} = \Omega^{-1}$); and K represents the conductivity cell constant (in cm-1), which is the ratio of the distance between the 2 pole pieces of the conductivity electrodes (l) to the area of the pole pieces (A).

The dynamics of the electrical conductance/conductivity of the hydrogen-production systems composed of pure microbial strains showed that the curve representing the relationship between the electrical conductance of the biohydrogen-producing solution and the concentration (electrical conductivity) of the solution was approximately linear. This curve could be expressed by the following linear-regression equation:

$$y = bx + a \quad (7)$$

In this equation, y is the electrical conductance; x is the concentration of the biohydrogen-producing solution (or the electrical conductivity of the solution); b is the regression coefficient (slope); and a is the regression constant (intercept). Thus, the electrical conductivity of the solution can be calculated using the following formula

$$x = (y - a)/b \quad (8)$$

2.5.3 Analysis of cell-growth kinetics

The hydrogen-production efficiency is correlated with the growth of the microbial strains. Studying the growth kinetics of various microbial strains using mathematical models helps to further optimise hydrogen-production systems. In the present study, the following exponential-decay model was utilised to evaluate the growth of each microbial strain:

$$y = y_0 + A_1 e^{-x/t_1} + A_2 e^{-x/t_2} \quad (9)$$

3. Results and Discussion

3.1 Analysis and identification of the 16S/18S rRNA of the microbial strains

Except for the commercially available microbial strains, all microbial strains obtained via mutation or isolation were identified according to Bergey's Manual of Determinative Bacteriology (Eighth Edition) and A Guide for the Identification of Fungi. The microbial strains submitted for 16S/18S rRNA sequencing included Clostridium acetobutylicum ATCC 824, Enterobacter cloacae ATCC 13047, Bacillus stearothermophilus U2, Bacillus subtilis B7 and B7-S, Kluyveromyces lactis B9, Rhodotorula lactosa C8, Kluyveromyces marxianus D10 and 15D, Cantharellus, Boletus edulis, and Clavicorona pyxidata.

3.2 Comparison of the fermentative hydrogen-production rates of the 16 initial microbial strains

The hydrogen-gas yields produced by the 16 microbial strains via fermentation were analysed and compared. As seen in Figure 1, the anaerobic microbial strains displayed the most efficient hydrogen production. Among the anaerobic strains, the hydrogen-production efficiency of Clostridium acetobutylicum ATCC 824 (13.6 $mL/h\text{-}^1.L\text{-}^1$) was significantly higher than that of Clostridium butyricum T11 (8.1 $mL/h\text{-}^1.L\text{-}^1$). Among the bacterial strains, Enterobacter cloacae ATCC 13047 showed the most efficient hydrogen production, reaching 12.5 $mL/h\text{-}^1.L\text{-}^1$. Among the yeast strains, Kluyveromyces marxianus 15D showed the most efficient hydrogen production, at 8.9 $mL/h\text{-}^1.L\text{-}^1$. The 3 strains of filamentous fungi showed the lowest hydrogen-production efficiency; all 3 had a yield of less than 1.5 mL/h-1.L-1. The 8 microbial strains that showed the best hydrogen-production efficiency were selected, and their microbiological characteristics were further studied.

3.3 Colonial and cellular morphology of the selected microbial strains

Based on detailed comparisons of the hydrogen-production efficiencies of the 16 microbial strains, the colonial and cellular morphologies of 8 selected microbial strains were further examined. The results are shown in Figures 2 and 3.

3.4 Electrochemical analysis of pH/Eh in hydrogen-production systems composed of the selected microbial strains

Electrochemical analysis was employed to determine the pH of the hydrogen-production systems composed of the 8 selected microbial strains (Figure 4). In the hydrogen-production system composed of the anaerobic bacterial strain Clostridium acetobutylicum ATCC 824, the pH value was basically stable at 5.3. In the hydrogen-production systems composed of the other 3 bacterial strains, the overall trend for pH over time was upward (i.e., more basic). The pH values gradually increased from 7.0 to approximately 8.0. These results were related to the decomposition of polypeptides, such as peptone, in the culture media. The final products of in-vitro polypeptide decomposition are amino acids, which are further broken down by the microbes to provide energy. The breakdown products are ultimately released in the forms of organic amines, amine salts, and ammonia [14], which affect the pH of hydrogen-production systems. In the hydrogen-production systems composed of the 4 yeast strains, an overall declining trend for pH was observed, with the pH changing from 5.5 to approximately 2.5. These results were related to the catabolism of starch and glucose in the media. The starch-decomposition process is summarised in formula (3-9) [15]:

$$C_6H_{10}O_5(l) + 7H_2O(l) \longrightarrow 12H_2(g) + 6CO_2(g) \quad (3\text{-}9)$$

Compared with the control systems (containing culture medium only), the regulatory effects of the 4 yeast strains on the pH of the hydrogen-production systems were clear. In contrast, the changes in pH over time in the hydrogen-production systems composed of the 4 bacterial strains were mostly consistent with those in the control systems. Therefore, a 2-step hydrogen-production process consisting of the establishment of mixed microfloras by co-culturing bacteria and yeast and the fermentative conversion of organic substrate to hydrogen by anaerobic microbial strains is feasible. The redox potential can be affected by various environmental factors. First, the redox potential is influenced by the partial pressure of oxygen. High oxygen partial pressure normally results in high redox potential, whereas low oxygen partial pressure corresponds to low redox potential. Second, reducing substances such as hydrogen and hydrogen sulphide, which are generated by microbes during the oxidation and metabolism of organic compounds, may decrease the environmental Eh value. Finally, the environmental pH value may also affect the redox potential. The redox potential increases when the pH value decreases and decreases when the pH value increases [16].

Electrochemical analysis was also employed to determine the Eh of the hydrogen-production systems composed of the 8 selected microbial strains (Figure 5). In the hydrogen-production system composed of Clostridium acetobutylicum ATCC 824, the redox potentials ranged from 60 to 82 mV. The survival of anaerobic microbes requires an environment with a relatively low redox potential (Eh value) because certain dehydrogenases in the microbes, including coenzyme I, ferredoxin, and flavoprotein, require a low-Eh environment to maintain their activities. In the hydrogen-production systems composed of the other 3 bacterial strains, the Eh values ranged from -80 to 15 mV. In the hydrogen-production systems composed of the 4 yeast strains, the Eh values ranged from 70 to 250 mV. These results indicate that the hydrogen-production systems composed of the yeast strains had the highest redox potentials, followed by the hydrogen-production system composed of the anaerobic bacterial strain. The hydrogen-production systems composed of the other 3 bacterial strains displayed the lowest redox potentials.

3.5 Dynamics of pH/Eh in hydrogen-production systems composed of the selected microbial strains

The electromotive force (Ef) of each hydrogen-production system was calculated using the Nernst equation. Figure 6 shows that the Ef of the hydrogen-production system composed of the anaerobic bacterial strain was 39 mV. In the hydrogen-production systems composed of the 4 yeast strains, Ef ranged from 30 and 33 mV; in those composed of the remaining 3 bacterial strains, Ef was 52-53 mV. In addition, the biological hydrogen-production systems were most likely to remain stable when the Ef value was approximately 30 mV. Therefore, the hydrogen-production systems composed of the yeast strains showed the highest stability, followed by the system composed of the anaerobic bacterial strain. The systems composed of the remaining 3 bacterial strains were less stable. Based on these Ef values, the hydrogen-production system composed of Enterobacter cloacae ATCC 13047 was superior to those composed of the other 2 aerobic bacterial strains, and the hydrogen-production systems composed of the yeast strains Kluyveromyces lactis B9 and Kluyveromyces marxianus 15D were superior to those composed of the other 2 yeast strains.

3.6 Electrochemical analysis of electrical conductance/conductivity in hydrogen-production systems composed of the selected microbial strains

The changes in electrical conductance were analysed in the hydrogen-production systems composed of the 8 selected microbial strains. Figure 7 shows that the changes in electrical conductance in the hydrogen-production system composed of the anaerobic bacterial strain Clostridium acetobutylicum ATCC 824 were basically consistent with those in the control system. In the hydrogen-production system composed of Clostridium acetobutylicum, the electrical conductance changed with the temperature. This pattern clearly differed from those of the other hydrogen-production systems and may be related to the anaerobic environment of the system. The conductance trends in the hydrogen-production systems composed of the other 3 bacterial strains were similar, while those in the hydrogen-production systems composed of the 4 yeast strains were basically the same. After 50 h of fermentation, the electrical conductivity of the hydrogen-production systems composed of yeast strains had increased significantly. This phenomenon is related to the fact that these yeast strains are facultatively anaerobic. After 50 h of fermentation, glycolysis was enhanced, resulting in an increased partial pressure of CO^2 and enhancing the systems' hydrogen-production capacity. Figure 8 shows that the electrical conductivity of the hydrogen-production system composed of the anaerobic bacterial strain Clostridium acetobutylicum ATCC 824 changed smoothly over time. The results suggest that the hydrogen-production system composed of Enterobacter cloacae ATCC 13047 was superior to those composed of the other 2 aerobic bacterial strains and that the hydrogen-production systems composed of the yeast strains Rhodotorula lactosa C8 and Kluyveromyces marxianus 15D were superior to those composed of the other 2 yeast strains.

3.7 Dynamics of electrical conductance/conductivity in hydrogen-production systems composed of the selected microbial strains

¡n the biological hydrogen-production process, electrical conductance and conductivity can be used to determine the concentration of each component in the hydrogen-production system and to regulate the hydrogen-production conditions. Figure 9 shows the dynamics of electrical conductance/conductivity in the hydrogen-production systems composed of the selected microbial strains. The experimental data display a linear relationship ($R^2 > 0.90$), indicating a positive correlation between electrical conductance and electrical conductivity in the hydrogen-production systems composed of these microbial strains.

3.8 Growth kinetics of the selected microbial strains in the hydrogen-production systems

The growth kinetics of the 8 selected microbial strains in the hydrogen-production systems are shown in Figure 10. For Clostridium acetobutylicum ATCC824, Enterobacter cloacae ATCC 13047, and Kluyveromyces marxianus 15D, the best-fit nonlinear curves are consistent with the experimental data. However, the best-fit curves for the other microbial strains do not fully match the experimental data. The discrete data points deviate from the fitted curves with large residuals. Among the 8 microbial strains, Clostridium acetobutylicum ATCC 824, Enterobacter cloacae ATCC 13047, and Kluyveromyces marxianus 15D were similar in the parameters of the fitted curves and in their growth and habitat parameters. Therefore, these 3 strains are suitable for the construction of mixed microfloras.

3.9 Comparison of the fermentative hydrogen-production rates of the selected microbial strains

The hydrogen-production efficiencies of the 8 selected microbial strains were analysed and compared, and the results are shown in Figure 1. The 3 microbial strains with the highest hydrogen yields were Clostridium acetobutylicum ATCC 824 (13.6 mL/h^{-1}.L^{-1}), Enterobacter cloacae ATCC 13047 (12.5 mL/h^{-1}.L^{-1}), and the yeast strain Kluyveromyces marxianus 15D (8.9 mL/h^{-1}.L^{-1}). Therefore, these 3 strains were selected for the construction of mixed microfloras.

4. Conclusion

The present study established a screening library using 16 microbial strains of different origins. After sequencing the microbial 16S/18S rRNA, examining the microscopic morphology of the microbial colonies and cells, and determining the hydrogen-production efficiencies of hydrogen-production systems composed of the pure microbial strains, 8 microbial strains were selected for further study. The colonial and cellular morphologies of these strains were examined to provide a foundation for the preliminary screening of suitable microbial strains for the construction of mixed microfloras. The electrochemical parameters of the 8 selected strains were analysed and compared. The pH, Eh, electrical conductance and conductivity, electrochemical dynamics, and growth kinetics of the selected microbial strains were analysed using mathematical tools. Based on these results and on the comparative hydrogen-production efficiencies of the microbial strains, 3 strains were identified as suitable for use in hydrogen-producing mixed microfloras. These strains were Clostridium acetobutylicum ATCC 824, Enterobacter cloacae ATCC 13047 and Kluyveromyces marxianus 15D.

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Tables and Figures

Table 1. The 16 microbial strains initially screened in the present study

Strains	Source	GenBank accession number	Culture medium	Temperature
Clostridium acetobutylicum S512	ATCC 824	JQ086380	[1]	37°C
Clostridium butyricum T11	CGMCC 1.335	ND	[2]	35°C
Enterobacter cloacae Y219	ATCC 13047	JQ086381	[3]	37°C
Enterobacter aerogenes E9	CGMCC 45103	ND	[3]	30°C
Bacillus stearothermophilus U2	CGMCC 1.1865	ND	[3]	45°C
Bacillus subtilis B7	CGMCC 1.210	JQ086378	[3]	30°C
Bacillus subtilis B7-S	CCTCC M 2011162	JQ086379	[3]	30°C
Pseudomonas putida P85	ATCC 17485	ND	[3]	30°C
Kluyveromyces lactis B9	CGMCC 2.1494	JQ086382	[4]	25°C
Rhodotorula lactosa C8	CGMCC 2.680	JQ086383	[4]	25°C
Kluyveromyces marxianus D10	CGMCC 2.1440	JQ086384	[4]	25°C
Kluyveromyces marxianus 15D	GIBT-01	JQ086385	[4]	34°C
Rhodotorula rubra R530	CGMCC AS2.530	ND	[4]	28°C
Cantharellus KX560JY	GIBT-02	JQ086387	[4]	28°C
Boletus edulis KX920NG	GIBT-03	JQ086386	[4]	28°C
Clavicorona pyxidata KX320SH	GIBT-04	JQ086388	[4]	28°C

ND means no detection

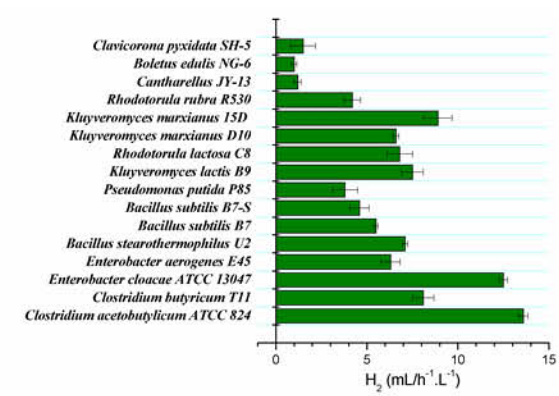
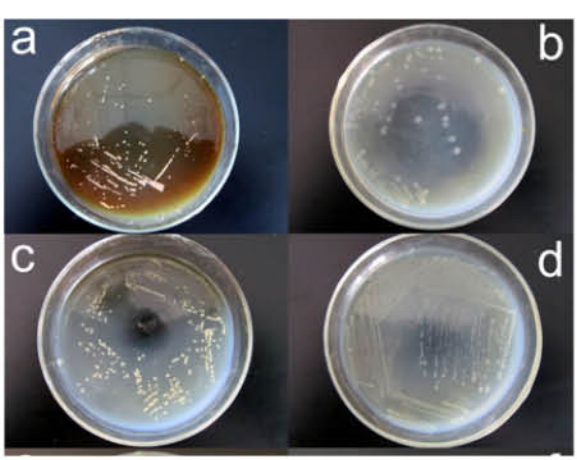


Figure 1 Analysis and comparison of the hydrogen-production efficiencies of the 16 microbial strains



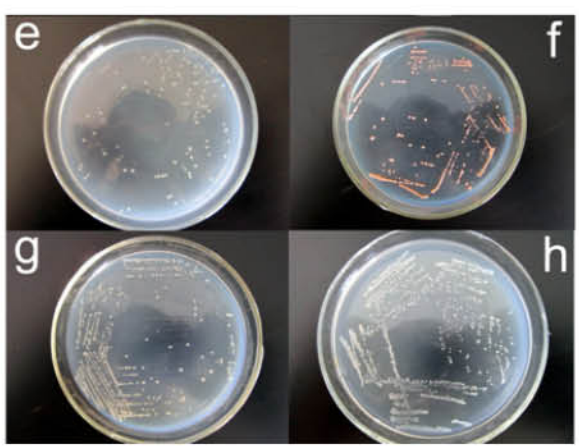
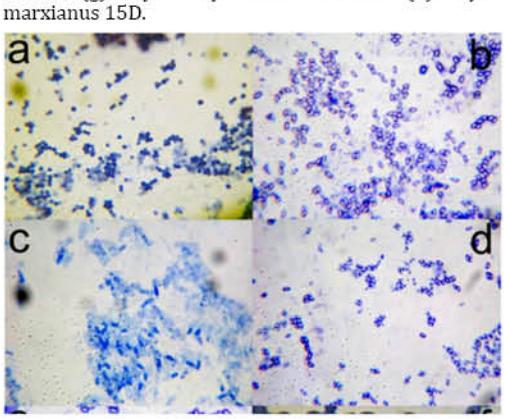


Figure 2 Colony morphologies of the 8 selected microbial strains

(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



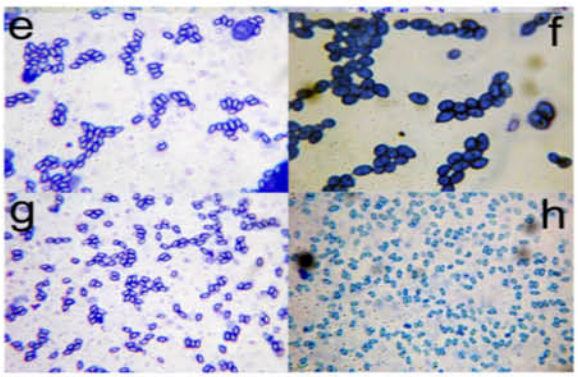
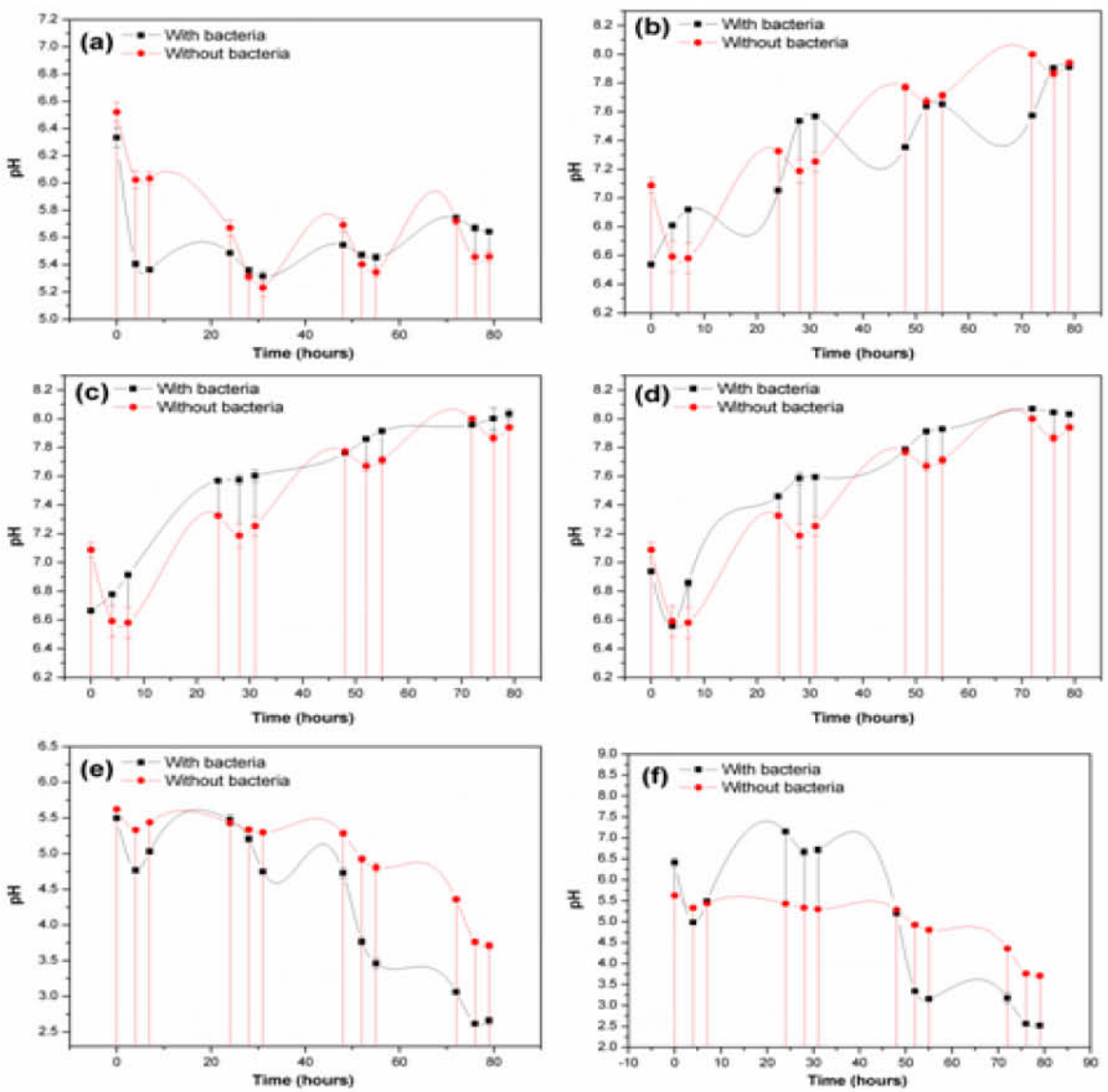


Figure 3 Microscopic morphology of the 8 selected microbial strains
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.

Fig.4



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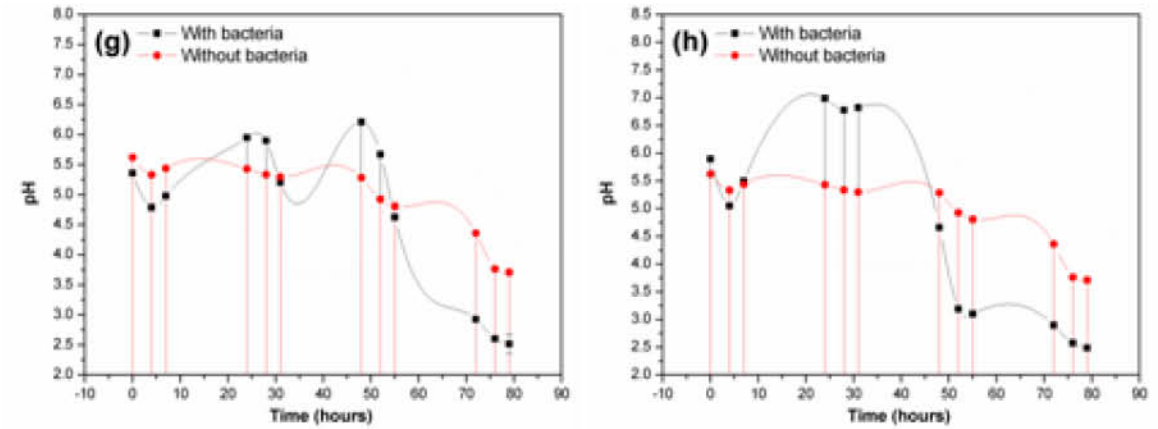
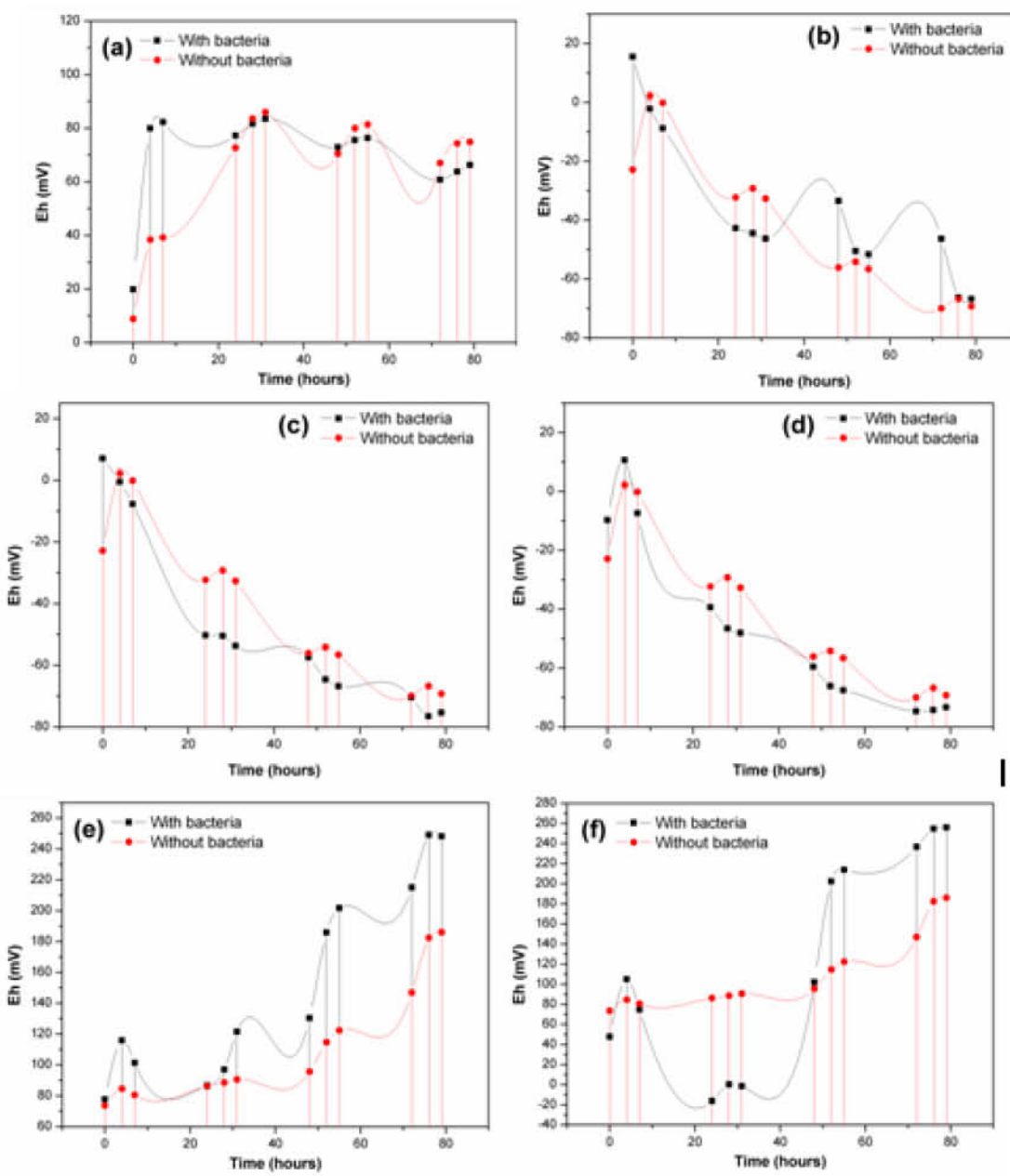


Figure 4 Electrochemical analysis of pH in hydrogen-production systems composed of the 8 selected microbial strains
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



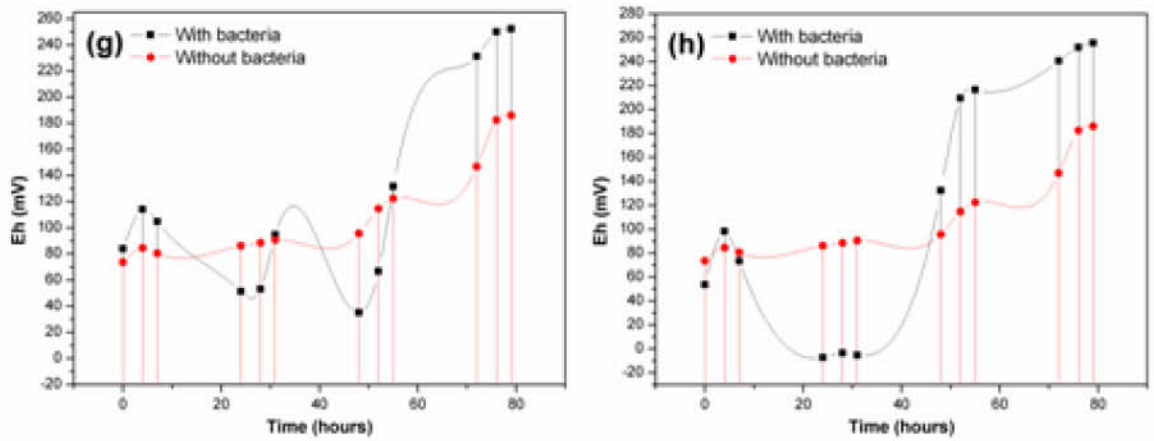
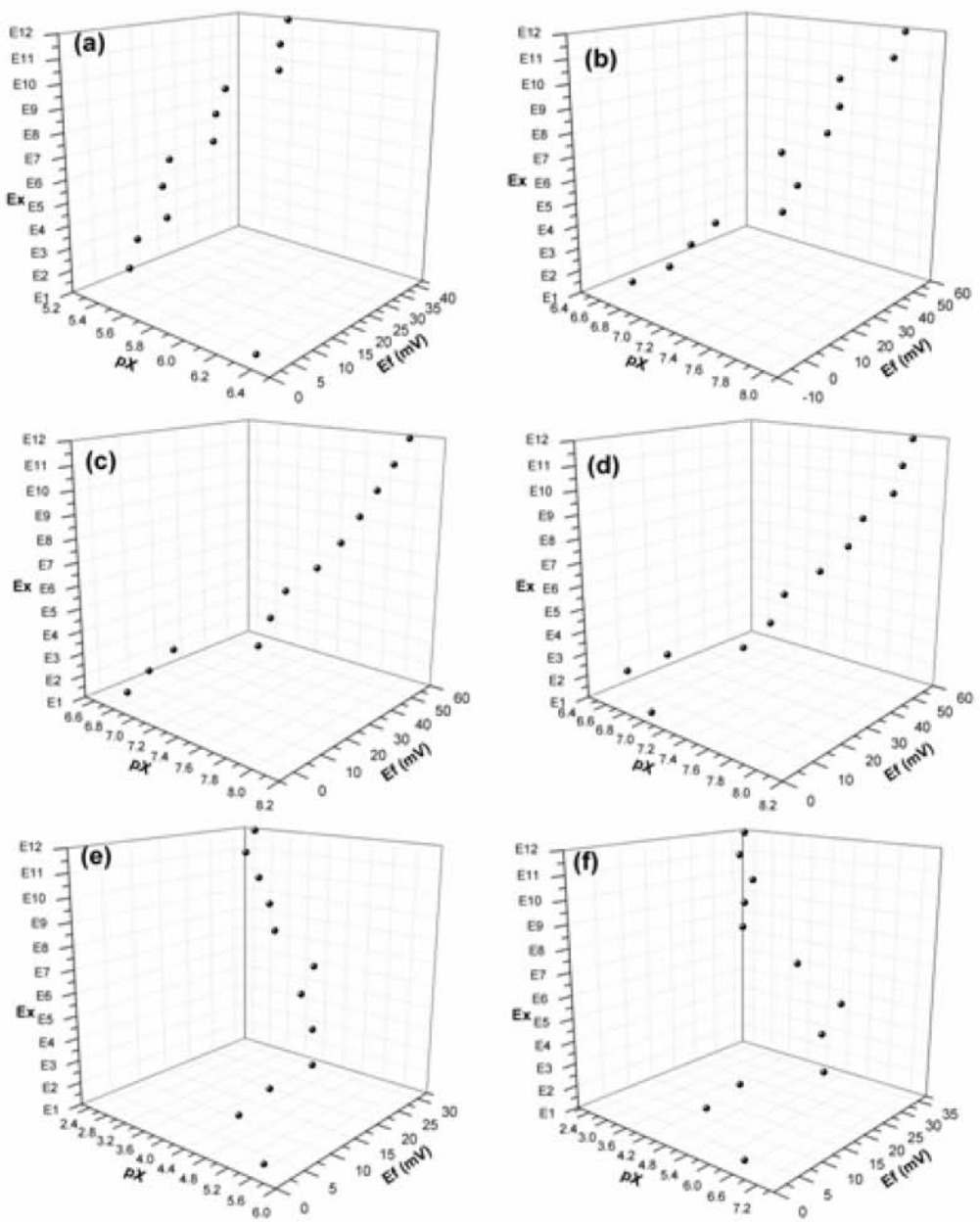


Figure 5 Electrochemical analysis of Eh in hydrogen-production systems composed of the 8 selected microbial strains
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



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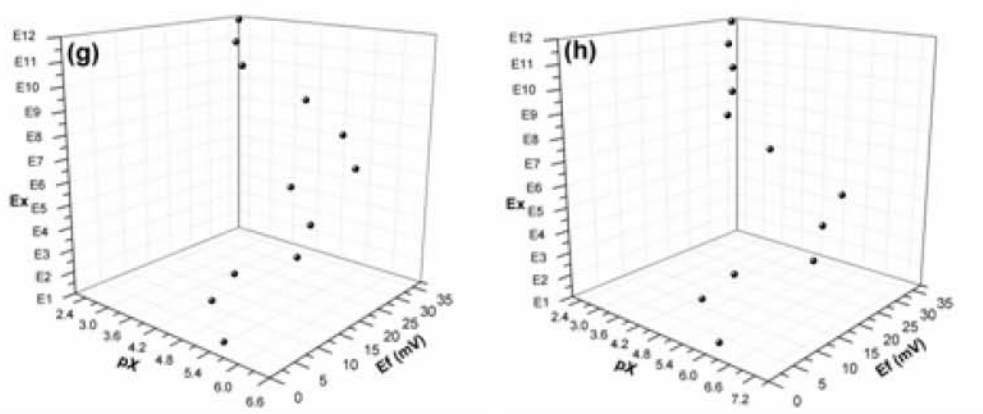
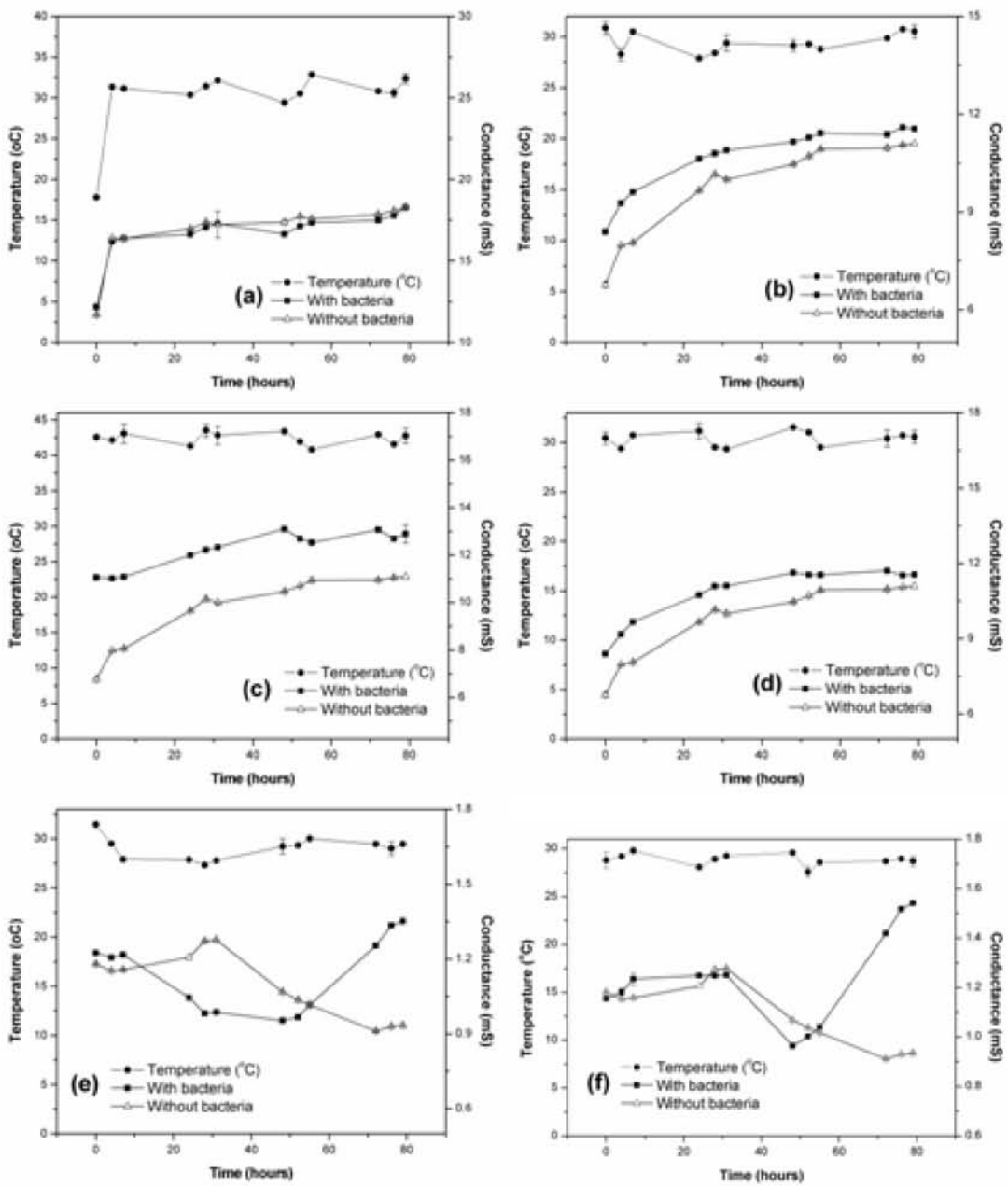


Figure 6 Dynamics of pH/Eh in hydrogen-production systems composed of the 8 selected microbial strains (Ef = electromotive force)
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



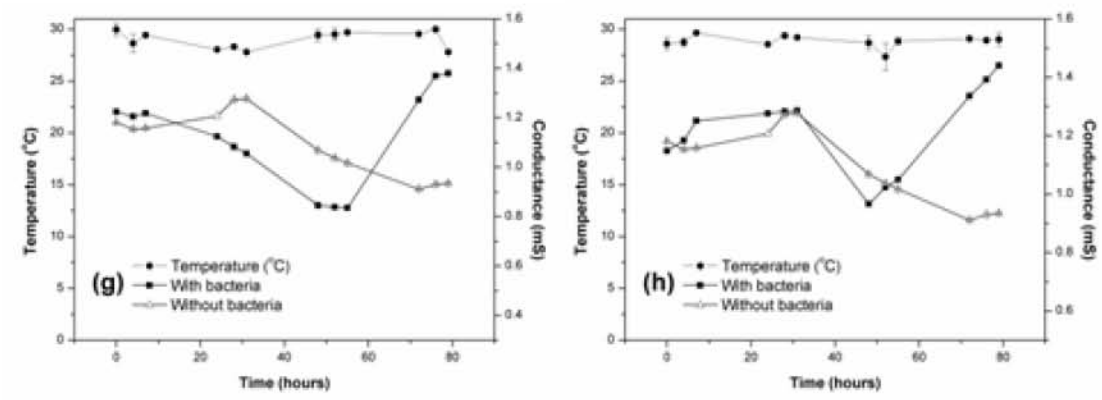
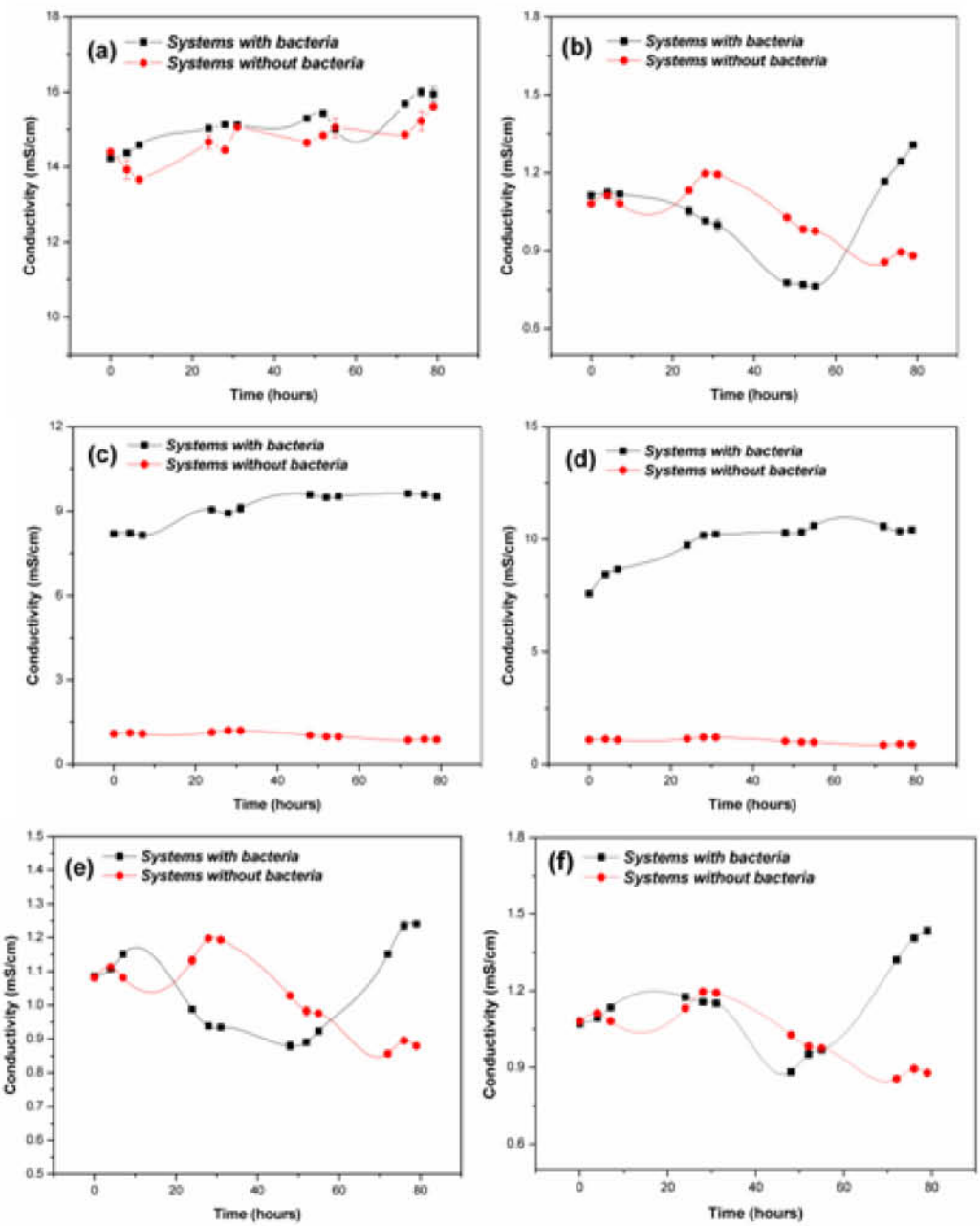


Figure 7 Electrical conductance of hydrogen-production systems composed of the 8 selected microbial strains
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



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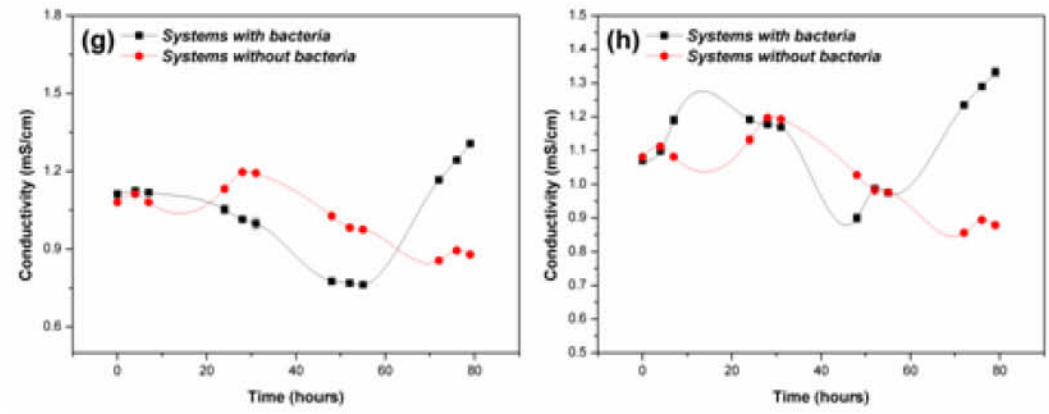
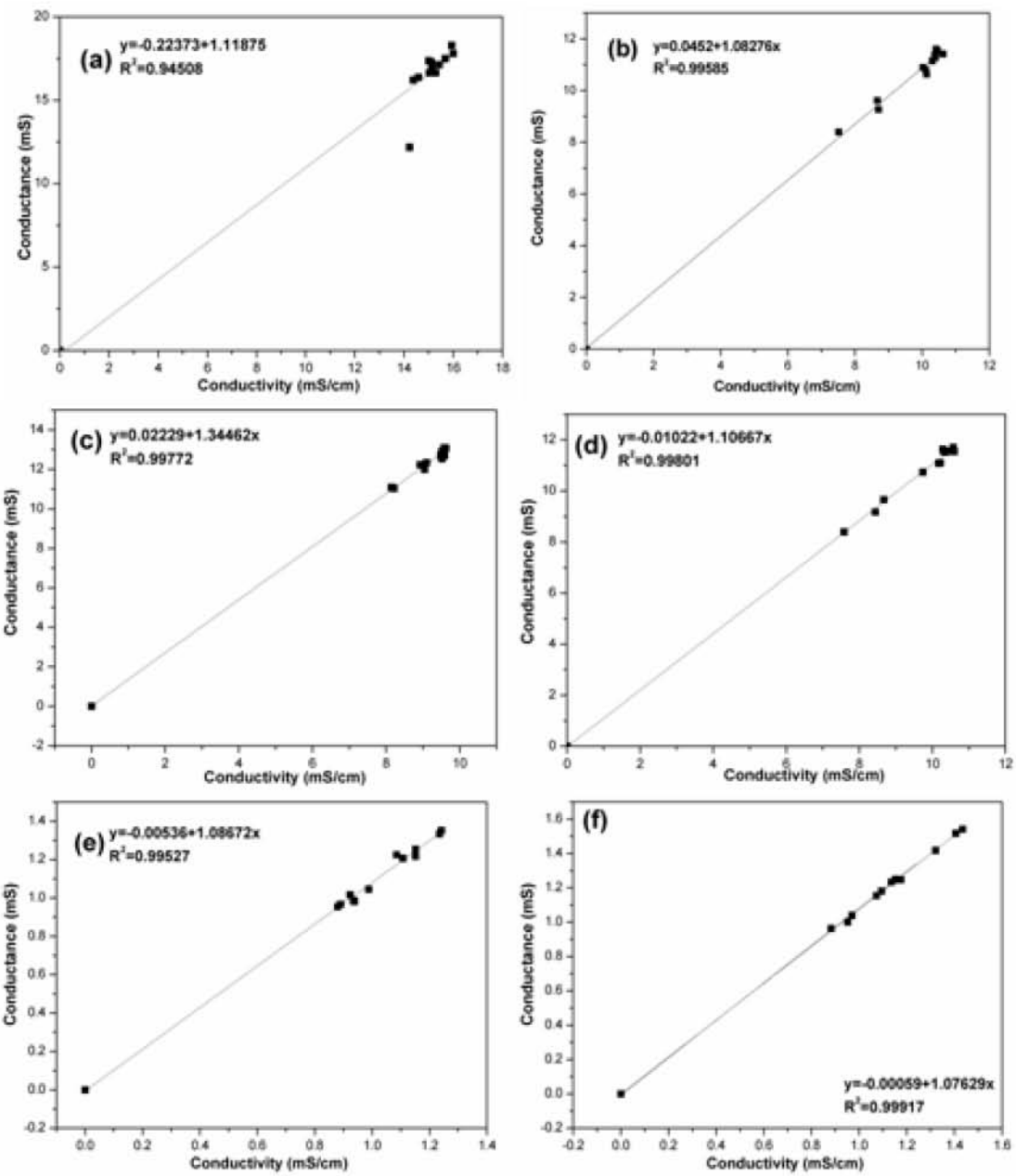


Figure 8 Electrical conductivity of hydrogen-production systems composed of the 8 selected microbial strains
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



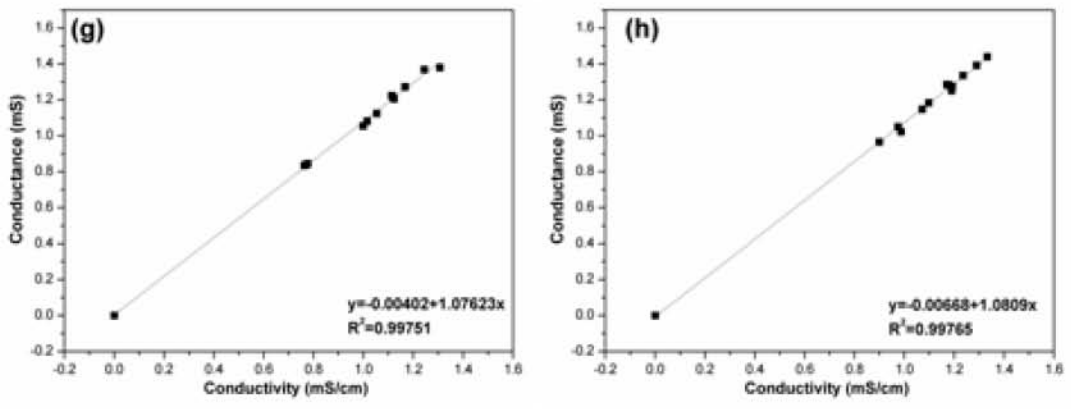
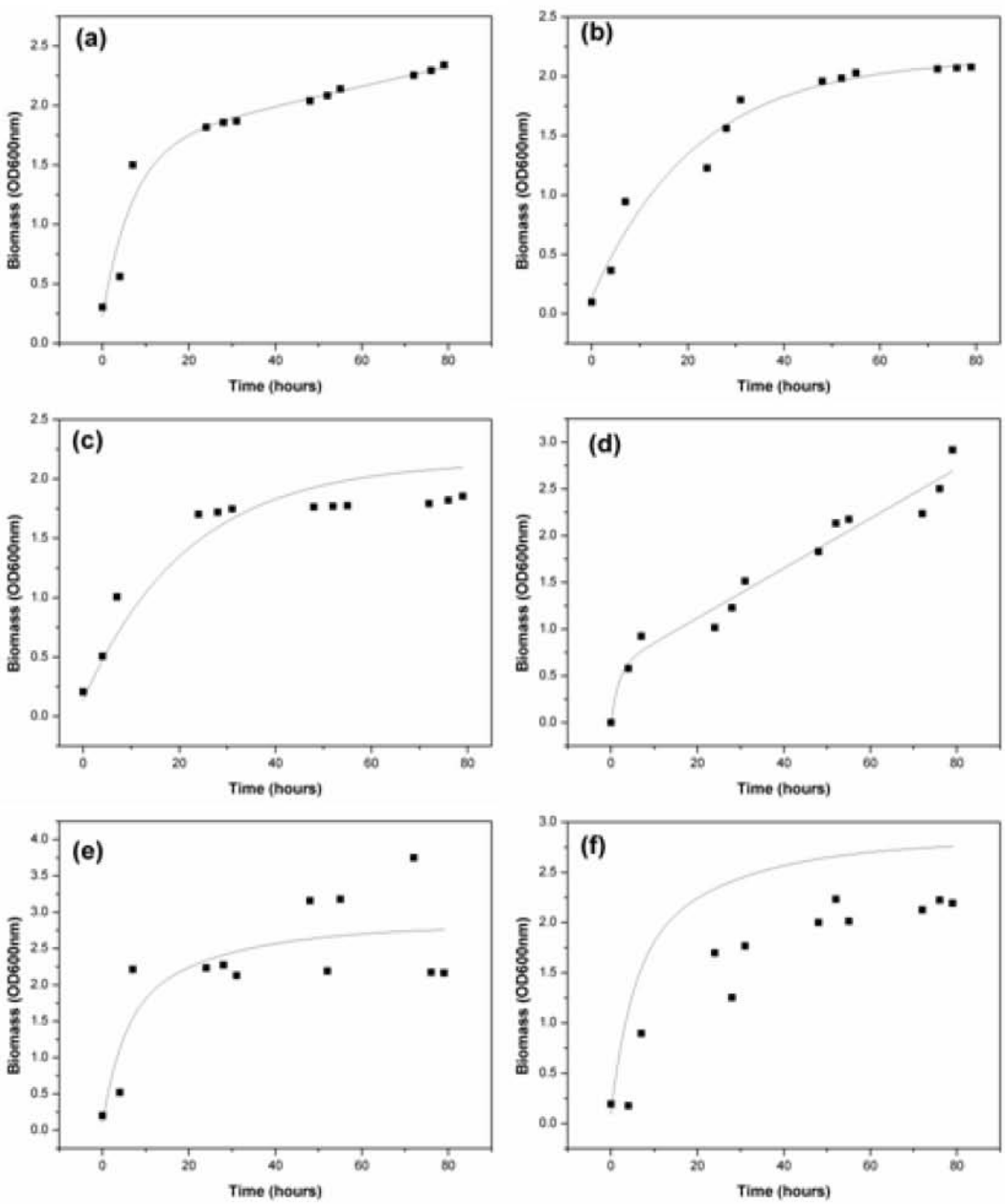


Figure 9 Dynamics of electrical conductance/conductivity in hydrogen-production systems composed of the 8 selected microbial strains

(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.



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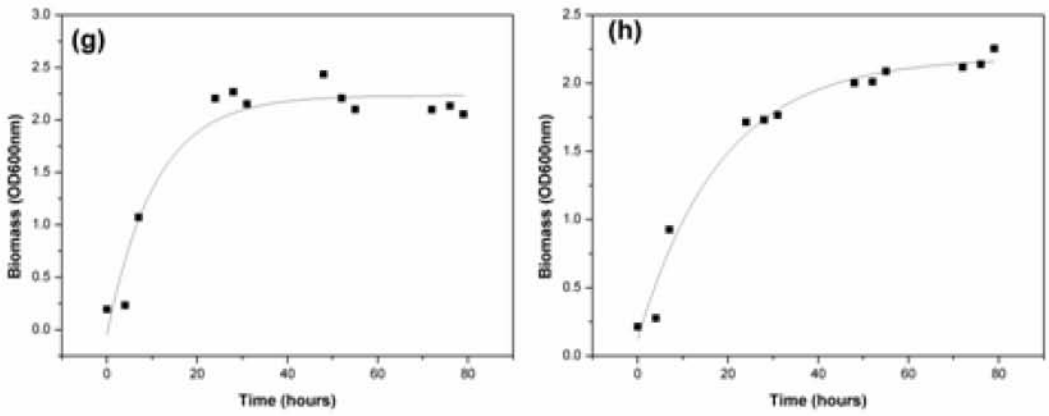

Figure 10 Growth kinetics of the 8 selected microbial strains in the hydrogen-production systems
(a) Clostridium acetobutylicum ATCC 824; (b) Enterobacter cloacae ATCC 13047; (c) Bacillus stearothermophilus U2; (d) Bacillus subtilis B7; (e) Kluyveromyces lactis B9; (f) Rhodotorula lactosa C8; (g) Kluyveromyces marxianus D10; (h) Kluyveromyces marxianus 15D.

REFERENCE

[1] Wang H, Fang M, Fang Z, Bu H. Effects of sludge pretreatments and organic acids on hydrogen production by anaerobic fermentation. Bioresour Technol. 2010;101:8731-5. | [2] Venkata Mohan S, Reddy MV, Subhash GV, Sarma PN. Fermentative effluents from hydrogen producing bioreactor as substrate for poly(beta-OH) butyrate production with simultaneous treatment: an integrated approach. Bioresour Technol. 2010;101:9382-6. | [3] Spagni A, Casu S, Farina R. Effect of the organic loading rate on biogas composition in continuous fermentative hydrogen production. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2010;45:1475-81. | [4] Sarkar S, Kumar A. Large-scale biohydrogen production from bio-oil. Bioresour Technol. 2010;101:7350-61. | [5] Rouvalis A, Iliopoulou-Georgudaki J. Comparative assessment of olive oil mill effluents from three-phase and two-phase systems, treated for hydrogen production. Bull Environ Contam Toxicol. 2010;85:432-6. | [6] Perera KR, Nirmalakhandan N. Enhancing fermentative hydrogen production from sucrose. Bioresour Technol. 2010;101:9137-43. | [7] Zhao W, Zhang Y, Du B, Wei D, Wei Q, Zhao Y. Enhancement effect of silver nanoparticles on fermentative biohydrogen production using mixed bacteria. Bioresource technology. 2013;142:240-5. | [8] Nissila ME, Tahti HP, Rintala JA, Puhakka JA. Effects of heat treatment on hydrogen production potential and microbial community of thermophilic compost enrichment cultures. Bioresour Technol. 2011;102:4501-6. | [9] Hwang JH, Choi JA, Abou-Shanab RA, Min B, Song H, Kim Y, et al. Feasibility of hydrogen production from ripened fruits by a combined two-stage (dark/dark) fermentation system. Bioresour Technol. 2011;102:1051-8. | [10] Prakasham RS, Sathish T, Brahmaiah P. Biohydrogen production process optimization using anaerobic mixed consortia: a prelude study for use of agro-industrial material hydrolysate as substrate. Bioresour Technol. 2010;101:5708-11. | [11] Zhang X, Ye X, Finneran KT, Zilles JL, Morgenroth E. Interactions between Clostridium beijerinckii and Geobacter metallireducens in co-culture fermentation with anthrahydroquinone-2, 6-disulfonate (AH2QDS) for enhanced biohydrogen production from xylose. Biotechnology and bioengineering. 2013;110:164-72. | [12] Chen L, Ge X, Dou Y, Wang X, Patel JR, Xu P. Identification of hydrogen peroxide production-related genes in Streptococcus sanguinis and their functional relationship with pyruvate oxidase. Microbiology. 2011;157:13-20. | [13] Liu B, Wang H, Zhao J, Xu H, Li X. Ability of hydrogen production by three strains and their synergistic effect. Food and Fermentation Industries. 2003;29:23-6. | [14] Wang L, Chen Y, Ye Y, Lu B, Zhu S, Shen S. Evaluation of low-cost cathode catalysts for high yield biohydrogen production in microbial electrolysis cell. Water Sci Technol. 2011;63:440-8. | [15] Zhang YH, Evans BR, Mielenz JR, Hopkins RC, Adams MW. High-yield hydrogen production from starch and water by a synthetic enzymatic pathway. PLoS One. 2007;2:e456. | [16] Li Y, Ren nq, Ding J, Lin H, Zheng G, Cui Y. Biohydrogen□. The engineering and applications. Advance in earth sciences. 2004:542-6. |