Demonstration of hydrogenase electrode operation in a bioreactor

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\textbf{A R T I C L E   I N F O}

Article history:
Received 21 July 2011
Received in revised form 31 August 2011
Accepted 31 August 2011

Keywords:
Dark fermentative hydrogen production
Hydrogenase electrode
Electricity generation from biohydrogen

\textbf{A B S T R A C T}

This work describes the first step towards combination of the bioreactor with a starch-degrading microbial consortium and hydrogenase electrode (HE) in one unit for electricity generation. For this purpose, the bioreactor for microbial fermentation was designed with a set of electrodes (pH-sensor, Ag/AgCl reference electrode, Pt-electrode, and HE) inside the bioreactor. Potentials of all electrodes and H$_2$ accumulation were monitored in the system under the precise pH control. Results obtained with the hydrogen-producing microbial consortium indicated that HE generates the potential equal to the H$_2$/2H$^+$ equilibrium potential. Furthermore, HE was able to catalyze the current generation (200 $\mu$A) by consuming H$_2$ gas produced in the microbial consortium from starch. After 220 h of operation, HE retained at least 81% of the initial activity. Calculations of carbon balance indicated that fermentation products were similar in microbial cells without HE and with HE generating the current due to H$_2$ consumption.

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1. Introduction

Accumulation of organic wastes in nature requests a development of appropriate methods of their decomposition. On the other hand, organic wastes contain energy. As an example, domestic wastewater may contain 7.6 kJ L$^{-1}$ \cite{1}. Simultaneous decomposition of wastes and generation of energy is a challenge for scientists. The application of methanogenic microbial consortium for waste degradation and generation of methane is already a proven, practical process. Other biological methods for renewable energy generation from organic wastes are also under investigation. One of them is a two-stage process that includes decomposition of organics to organic acids through fermentation in the first stage and consumption of organic acids by purple photosynthetic bacteria in the second stage with generation of H$_2$ gas during both stages \cite{2}. A treatment of wastes by microbial fuel cell for electricity generation is also a very promising approach \cite{3}. Different combinations of fuel cells and bioreactors for electricity generation from biologically produced H$_2$ have been used. In the first, the Pt-based fuel cell was connected to a bioreactor incorporating chemotrophic bacteria \cite{4,5}, purple bacteria \cite{6} or green alga \cite{7,8}. Hydrogen produced in the bioreactor was then directed to a separate fuel cell. In all these examples, the system worked successfully for several days. In the second, a proton-exchange-membrane fuel cell with Pt electrodes was incorporated into the photobioreactor with purple bacteria and proved to be efficient for a short time \cite{9}. However, Pt-based electrodes are susceptible to deactivation by various poisons produced in microbial reactors, like H$_2$S, CO, some sulfur containing amino acids, etc. Hence, the platinum-independent hydrogen electrodes are required for successful coupling of fuel cells with microbial hydrogen-producing reactors.

Bioelectrocatalysis by enzyme electrodes is a valuable alternative to platinum electrocatalysis. Hydrogen enzyme electrodes (HEs) with hydrogenases achieve hydrogen equilibrium potential \cite{10}. Current–voltage characteristics of hydrogen electrooxidation from aqueous solutions by enzyme electrodes are similar to Pt \cite{11}. In addition, HEs are insensitive to H$_2$S and even able to sense hydrogen directly in microbial media \cite{11}. Thus, available data suggest that HEs are promising candidates for conversion of renewable energy into electricity via hydrogen production in the system integrating enzyme and microbial technology. However, no data exist on operation of HEs in microbial environment for electricity generation.

This work describes the first step towards combination of the bioreactor with a microbial consortium and HE in one unit for electricity generation: (1) design and test of the system combining a H$_2$–producing microbial consortium with a hydrogen enzyme electrode, (2) studies of simultaneous operation of hydrogen producing microbial consortium and hydrogen enzyme electrode, and (3) analysis of carbon recovery in the integrated system during fermentative degradation of starch.

2. Materials and methods

2.1. Strain and media for cultivation

Experiments were performed with a microbial consortium previously obtained from a silo pit liquid and characterized for starch fermentation at 37 $^\circ$C \cite{12}.

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0141-0229/– see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2011.08.007
Microbial consortium was obtained by several re-inoculations in the starch-containing media. The cultivation of the inoculum for experiments was performed in 50 ml flasks filled with the medium described previously [12] with additions of KCl at 7.45 g.L⁻¹ and starch at 20 g.L⁻¹. The addition of KCl was important for decreasing the electrical resistance of the medium. Inocula were pre-treated at 100 °C for 10 min to suppress H₂-consuming and methanogenic bacterial activities.

2.2. Hydrogen enzyme electrode (HE) preparation

Carbon filament material (CFM) 1.5 cm = 0.5 cm (TVS-300M, Alten’ Company, Moscow Region, Russia) was used as a working electrode. The CFM-specific electrical resistance was 50–70 mΩ cm⁻¹. HEs were prepared by enzyme absorption from an aqueous solution of HydSL hydrogenase from Thiothrix roseopersicina BBS (2–4 mg mL⁻¹) for 12 h at 4 °C in 0.005 M K-phosphate buffer, pH = 7.0 onto the CFM electrodes modified with poly(MPDPB) as described previously [13]. This enzyme was selected for hydrogenase electrode preparation due to its high stability against oxygen [14]. Five electrodes with currents of 400–750 μA under 100 mV over-voltage (against Pt electrode) were used in this study.

2.3. The system for integration of starch degrading microbial consortium with HE

The system (Fig. 1) consists of a bioelectrochemical cell (bioreactor), devices for measurements of pH, a potential of the Pt electrode, potentiostat (IPC-Compact, VOLTA, Russia), accessories for pH control including peristaltic pump (Masterflex) with alkaline solution (0.5 M NaOH) vessel, a graduated cylinder for H₂ accumulation, and gas supply equipment including an H₂ generator and argon lines. All devices are connected to a PC for data accumulation.

The bioreactor (Fig. 2A) has two chambers separated by a Nafion membrane (250 μm thick). One chamber contains the auxiliary electrode (CFM) connected to a potentiostat. The Nafion membrane is connected to chambers via silicon sealants (not shown). Another chamber (65 cm × 40 cm × 32 cm) contains ports for electrodes, for sampling and adding gases and for H₂ output. The cell was installed inside a box with a constant temperature (37 °C). Before each installation of a Pt electrode (EPV-01, Gomel, USSR) in the system, the electrode was activated using abrasive paper with 2000 mesh. An overall view of the bioreactor is shown in Fig. 2B.

All electrodes have one reference electrode (Ag/AgCl) built in the combined pH electrode (InPro3030/120, Mettler Toledo). The single reference electrode was important for direct and accurate comparison of potentials on Pt and HE. To ensure that measuring devices would not have mutual impact, we checked the system using CFM without hydrogenase (Fig. 3). The cell was filled by 50 mM K-P buffer with KCl at 7.45 g.L⁻¹ (pH 7.00) and flushed by argon. At the time indicated in Fig. 3 by the first arrow, H₂ was introduced into the cell (3 Lh⁻¹). The potential on the Pt electrode decreased to —602 mV. In different experiments the potential of the Pt electrode varied from —602 to —615 mV (against Ag/AgCl reference electrode) but the form of the curve was identical. The second arrow indicates the time when H₂ was replaced by argon. During this time pH was monitored and the pH-meter did not change readings (data not shown). The potential on the CFM electrode measured by the potentiostat did not depend on the H₂ addition and after 0.8 h of incubation the potential was stabilized (Fig. 3). The connection and disconnection of devices did not change each other’s readings (not shown). Thus, the multiple electrode system with a single reference electrode showed sufficient stability and correct measurements.

2.4. Experiments with microbial consortium

The cell was assembled by connecting all electrodes and a magnetic bar, filled by the medium described above (60 ml), connected to the pH control line, gas line, upside down cylinder for H₂ accumulation, and installed on a magnetic stirrer. Then, the cell was flushed by H₂ up to the activation of the enzyme electrode. Before the inoculation, the current–voltage dependence for HE was recorded. After that, the cell was flushed by argon to remove H₂ until the potential on Pt and HE became higher than —400 mV (against reference electrode), and the gas line was disconnected. The
cell was inoculated by microbial consortium (1.7%). All data were recorded using a PC.
The concentration of H₂ in the cylinder was measured by a gas chromatograph as described earlier [12]. All data present the volume of produced H₂ at 25°C and ambient pressure. All potentials presented on figures and in the text measured against reference electrode. Experiments were done 5–7 times with different enzyme electrodes but with similar results. The figures represent data from one experiment.

2.5. Other analytical methods

Gas production by bioreactors was measured by the water displacement method and H₂ percentage was analyzed by GC. The concentrations of acetate, propionate, butyrate, isobutyrate, and alcohols were determined by gas chromatography as described earlier [15]. Lactate concentrations were determined by an enzymatic method and monitored as NAD⁺ reduction at 340 nm [16]. The concentration of hexose equivalents after hydrolysis by sulfuric acid was assayed using anthrone reagent [17].

3. Results and discussion

3.1. Operation of HE in the bioreactor with zero current generation

After the inoculation of the bioreactor by microbial consortium, the potential measured by Pt and HE electrodes increased for 2 h (Fig. 4). After that, the potential of both electrodes started to decrease. Judging from the potential of Pt and HE electrodes, H₂ appeared in the medium 2 h after inoculation. However, visual accumulation of H₂ was observed after 13 h of operation. After 15 h the pH reached a value of 5.45 (in different repetitions it was observed after 8–20 h of operation). At this point, the system controlled pH by adding an alkaline solution. We selected pH for the control equal to 5.5 since microbial consortium produced H₂ with maximum rate at pH 5.4–5.8 [11], and HE had maximum activity at pH 5.5–6.5 [18]. After 28 h of incubation more than 75% of the total hydrogen was produced (in different repetitions 75% of H₂ was produced after 14–30 h). From 15 to 52 h of operation (when the pH was equal to 5.45) Pt and HE showed stable potentials (−544 mV relative to Ag/AgCl electrode) with differences not more than 3 mV. The average value of accumulated H₂ from 4 independent repetitions was 2.44 ± 0.15 L per L of the medium. It should be noted here that HE potential showed a lower value at the start of the system operation as compared with the Pt electrode.

This experiment showed that HE is able to generate the potential equal to the equilibrium potential for H₂│H⁺ couple and can be used as a H₂ sensor in a microbial environment. A similar conclusion has been reached previously [11]. In some experiments HE showed lower potentials (5–8 mV) than the Pt electrode at the end of the H₂ production phase (not shown). This might be attributed to the gradual inactivation of Pt in the medium with the microbial consortium.

3.2. HE operation with current generation

After the experiment described in Section 3.1 the system was refilled with a fresh medium under argon flow. After the stabilization of Pt and HE electrode potentials the system was flushed by H₂, and the current–voltage dependence of HE was recorded. Then, the system was flushed by argon up to the HE potential higher than −500 mV (relative to Ag/AgCl reference electrode), and all parameters were recorded from this moment. The current of HE was fixed at 0 μA (Fig. 5). As in the previous experiment, the HE potential was lower than the Pt potential. However, the HE potential did not show any increase at the start of operation. This might be attributed to the presence of a microbial consortium inside filaments of HE, which can produce H₂. The pH was decreased down to the stabilization point (pH = 5.45) after 12 h (6.5–15 h in different repetitions). The potentials of Pt and HE electrodes were equalized when pH was stabilized at 5.45.

When the HE and Pt potentials were equalized, the current generation by the HE electrode (the current was fixed at I = 200 μA) was activated using potentiostat (indicated by the arrow in Fig. 5). This resulted in the increase of the HE potential by app. 50 mV (35–55 mV with different HEs). During the entire experiment this difference was stable and slightly increased at the end of H₂ production phase.

After 23 h of operation, the microbial consortium produced 2.23 L H₂ per L of the bioreactor. Hydrogen production stopped after 43 h of operation for data presented in Fig. 5 (34–60 h for different repetitions) with the total H₂ equal to 3.43 L.L⁻¹ (the average value from 6 independent repetitions was 2.79 ± 0.51 L.L⁻¹, Table 1). Using our system we could not estimate the efficiency of catalytic current generation. Instead, if we suggest that this efficiency is close to 100%, it means that 1 mA of catalytic current consumes 1.87 × 10⁻² mmoles H₂ h⁻¹. Based on this suggestion one could estimate the quantity of H₂ that was consumed by HE. During 33 h of current generation (Fig. 5) HE consumed 0.12 mmoles of H₂ from the hydrogen-producing bioreactor (60 mL). Recalculating to 1 L of the bioreactor and taking into account that H₂ collecting system was at room temperature (25°C), 2.06 mmmoles H₂ (50.9 mL) was consumed during the experiment described in Fig. 5. So, the total H₂ production by the system for this particular experiment was 3.49 L.L⁻¹, which is in the same range with H₂ production by the system without HE or without current generation (Table 1).

This experiment showed that HE is able to consume H₂ for catalytic current generation simultaneously with H₂ production by a microbial consortium.

3.3. The stability of HE in the system

To test the operational stability of HE we recorded current–voltage dependencies before the experiment and immediately after it. One electrode was used in two experiments as the sensor and in three experiments with current generation. Current–voltage dependencies at the start of operation and after all experiments (220 h) are presented in Fig. 6. After all experiments HE showed an even higher current compared to the start of operation, when the potential higher than 65 mV (relative to H₂│H⁺ electrode) was applied. In contrast, at the potential lower than −65 mV HE showed a lower current after 220 h of operation than before it. This apparent contradiction can be explained by the
medium properties. At the start of the experiments, the medium contains 2% of hydrophilized starch. As the result, this medium apparently has higher viscosity. After dark fermentation of the starch by microbial consortium, the viscosity decreased. It is possible that at high potentials generated by HE, the current in the medium with starch was limited by the rate of H₂ diffusion to the surface of HE. When the starch was consumed, the viscosity decreased, and HE generated a higher current. At potentials lower than 60 mV the current was defined by the catalytic activity of HE and after 220 h of operation this activity decreased (Fig. 6). However, at any potential (5–50 mV) the residual HE activity was not lower than 81% of the initial activity.

The current–potential dependencies showed that HE after the operation in the microbial medium for 220 h retained significant activity and was not destroyed by proteases produced by the microbial consortium. This observation supports conclusions made previously that hydrogenase T. roseopersicina BBS is highly stable against different proteases [19]. Furthermore, data suggest that at given pH (5.45) hydrogenase on the electrode have satisfactory stability and adsorption of the enzyme to the electrode was not affected significantly.

3.4. Products of microbial fermentation

To understand a possible influence of HE on the microbial consortium, products of fermentation by the system operated without HE (control) and with HE operated at I=200 μA were analyzed (Table 1).

In both cases the major products were CO₂, H₂, acetic acid, and butyric acid. Carbon recovery (90–99%, Table 1) indicates that all major products of fermentation were taken into account. The system produced same quantity of all products with and without HE. This means that, on the one hand, the presence of HE did not affect microbial consortium, and, on the other hand, excluding app. 1.4%
Table 1
Profile of the final fermentation products.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Content</th>
<th>Reactor without HE</th>
<th>Reactor with HE generating 200μA current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial starch (as glucose)</td>
<td>moles L⁻¹</td>
<td>0.094 ± 0.01</td>
<td>0.089 ± 0.01</td>
</tr>
<tr>
<td>g⁻¹</td>
<td></td>
<td>16.9 ± 1.8</td>
<td>16.0 ± 1.7</td>
</tr>
<tr>
<td>Fermentation products H₂ yield</td>
<td>moles L⁻¹</td>
<td>0.13 ± 0.027</td>
<td>0.115 ± 0.019</td>
</tr>
<tr>
<td>from initial glucose</td>
<td>moles mole⁻¹ initial glucose</td>
<td>1.42 ± 0.27</td>
<td>1.29 ± 0.21</td>
</tr>
<tr>
<td>CO₂ yield</td>
<td>moles L⁻¹</td>
<td>0.1285 ± 0.075</td>
<td>0.117 ± 0.019</td>
</tr>
<tr>
<td>LT⁻¹</td>
<td></td>
<td>3.313 ± 1.88</td>
<td>2.84 ± 0.52</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>moles L⁻¹</td>
<td>0.047 ± 0.007</td>
<td>0.040 ± 0.001</td>
</tr>
<tr>
<td>g⁻¹</td>
<td></td>
<td>2.57 ± 0.4</td>
<td>2.18 ± 0.05</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>moles L⁻¹</td>
<td>0.0003 ± 0.00005</td>
<td>0</td>
</tr>
<tr>
<td>g⁻¹</td>
<td></td>
<td>0.03 ± 0.005</td>
<td>0</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>moles L⁻¹</td>
<td>0.0576 ± 0.0088</td>
<td>0.0595 ± 0.0019</td>
</tr>
<tr>
<td>g⁻¹</td>
<td></td>
<td>4.95 ± 0.76</td>
<td>5.11 ± 0.16</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>moles L⁻¹</td>
<td>0.0009 ± 0.0015</td>
<td>0.0009 ± 0.0012</td>
</tr>
<tr>
<td>g⁻¹</td>
<td></td>
<td>0.08 ± 0.14</td>
<td>0.08 ± 0.11</td>
</tr>
<tr>
<td>iso-Butyric acid</td>
<td>moles L⁻¹</td>
<td>0.0097 ± 0.0076</td>
<td>0.012 ± 0.0076</td>
</tr>
<tr>
<td>g⁻¹</td>
<td></td>
<td>1.78 ± 1.39</td>
<td>2.2 ± 1.39</td>
</tr>
<tr>
<td>Residual starch (as glucose)</td>
<td>moles L⁻¹</td>
<td>0.94 ± 15.0</td>
<td>98.7 ± 10.1</td>
</tr>
<tr>
<td>Carbon recovery, %</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Conclusion

A system was designed with a bioreactor for producing fermentative hydrogen by a microbial consortium with monitoring of potentials on Pt and HE electrodes and with a pH and temperature control. HE showed the ability to work as an H₂ sensor, showing similar potentials to the Pt electrode and did not affect the microbial consortium. HE can generate current due to H₂ consumption directly in the bioreactor. The results of this study open possibilities for further research of prolonged HE operational stability and experiments with higher current densities.

Acknowledgement

This work was supported by the Russian Academy of Sciences Program for Basic Research (Chemical aspects of energetic).

References


