Electrochemically Assisted Microbial Production of Hydrogen from Acetate

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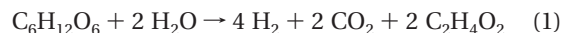and Ion Power, Inc., New Castle, Delaware 19701*

Hydrogen production via bacterial fermentation is currently limited to a maximum of 4 moles of hydrogen per mole of glucose, and under these conditions results in a fermentation end product (acetate; 2 mol/mol glucose) that bacteria are unable to further convert to hydrogen. It is shown here that this biochemical barrier can be circumvented by generating hydrogen gas from acetate using a completely anaerobic microbial fuel cell (MFC). By augmenting the electrochemical potential achieved by bacteria in this MFC with an additional voltage of 250 mV or more, it was possible to produce hydrogen at the cathode directly from the oxidized organic matter. More than 90% of the protons and electrons produced by the bacteria from the oxidation of acetate were recovered as hydrogen gas, with an overall Coulombic efficiency (total recovery of electrons from acetate) of 60−78%. This is equivalent to an overall yield of 2.9 mol H_2/mol acetate (assuming 78% Coulombic efficiency and 92% recovery of electrons as hydrogen). This bio-electrochemically assisted microbial system, if combined with hydrogen fermentation that produces 2−3 mol H_2/mol glucose, has the potential to produce ca. 8−9 mol H_2/mol glucose at an energy cost equivalent to 1.2 mol H_2/mol glucose. Production of hydrogen by this anaerobic MFC process is not limited to carbohydrates, as in a fermentation process, as any biodegradable dissolved organic matter can theoretically be used in this process to generate hydrogen from the complete oxidation of organic matter.

Introduction

The global interest in a hydrogen economy has been stimulated by the promise of clean energy production using hydrogen in fuel cells. A reduction in CO_2 emissions, however, will require sustainable hydrogen production based on renewable energy using solar, wind, and biomass sources. Currently about half of all the hydrogen produced is derived from natural gas, with the balance produced primarily using other fossil fuels, including heavy oils, naphtha, and coal. Only 4% is generated from water using electricity derived from a variety of sources (*1−3*).

Hydrogen can be produced from certain forms of biomass by biological fermentation (*4*), but yields are low. The maximum hydrogen production from fermentation, assuming only acetate or butyrate is produced from glucose, is
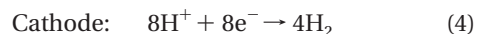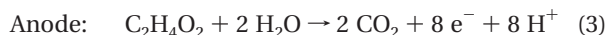
$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 4\ H_2 + 2\ CO_2 + 2\ C_2H_4O_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2\ H_2 + 2\ CO_2 + C_4H_8O_2 \quad (2)$$

We could obtain 4 mol H_2/mol glucose if only acetate was produced, but only 2 mol/mol if butyrate is the sole end product. Current fermentation techniques produce a maximum of 2−3 mol H_2/mol glucose. Thus, most of the remaining organic matter is essentially wasted as a mixture of primarily acetic and butyric acids, despite a stoichiometric potential of 12 mol H_2/mol glucose (*1*). The greatest hydrogen yield theoretically possible using microorganisms (without an external source of energy) is therefore 4 mol H_2/mol glucose based on production of acetic acid. Higher yields can be achieved using photobiological process and supplemental light, or using pure enzymes, but neither of these methods so far show promise for economical production of hydrogen (*5−7*). Moreover, of all the different types of biomass available for making hydrogen, only materials rich in carbohydrates such as glucose are suitable fermentation substrates.

Microbial fuel cells (MFCs) have been discovered as a completely new path to renewable electricity production. In a MFC, microorganisms oxidize organic matter and transfer electrons directly to the anode electrode (*8−9*). Bacteria capable of electron transfer to an electrode, either directly or by endogenously produced mediators, include a wealth of genera including *Geobacter*, *Shewanella*, *Pseudomonas*, and others (*8−10*, *11−13*). Electrons travel to the cathode electrode, where normally they combine with oxygen and protons to form water. The need for oxygen at the cathode is a disadvantage to operation of the system because oxygen can leak into the anode chamber and either lower energy recovery or inhibit the growth of obligate anaerobes. While it is possible to use the electricity produced by a MFC to make hydrogen via electrolysis, such a multi-stage approach would be an inefficient process.

By electrochemically augmenting the cathode potential in a MFC circuit (*14*) it is possible to directly produce hydrogen from protons and electrons produced by the bacteria. This approach greatly reduces the energy needed to make hydrogen directly from organic matter compared to that required for hydrogen production from water via electrolysis. In a typical MFC, the open circuit potential of the anode is ∼ −300 mV (*15, 16*). If hydrogen is produced at the cathode, the half reactions occurring at the anode and cathode, with acetate oxidized at the anode, are as follows:

$$\text{Anode:} \quad C_2H_4O_2 + 2\ H_2O \rightarrow 2\ CO_2 + 8\ e^- + 8\ H^+ \quad (3)$$

$$\text{Cathode:} \quad 8H^+ + 8e^- \rightarrow 4H_2 \quad (4)$$

Producing hydrogen at the cathode requires a potential of at least $E^0 = -410$ mV(NHE) at pH 7.0 (*17*), so hydrogen can theoretically be produced at the cathode by applying a circuit voltage greater than 110 mV (i.e., 410−300 mV). This voltage is substantially lower than that needed for hydrogen derived from the electrolysis of water, which is theoretically 1210 mV at neutral pH. In practice, 1800−2000 mV is needed for water hydrolysis (under alkaline solution conditions) due to overpotential at the electrodes (*18*). Thus, by deriving the protons and electrons from organic matter instead of water, we can directly generate hydrogen at a low voltage using a type of MFC device that does not

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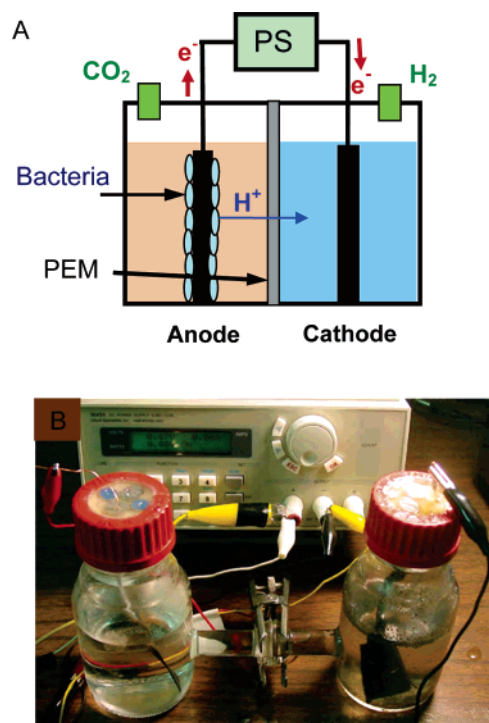† Penn State University.
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FIGURE 1. (A) Generalized schematic of the electrochemically assisted MFC, showing two chambers separated by a proton exchange membrane (PEM) with the voltage set using a power supply (PS). Gas was sampled via sampling ports at the top of the reactors. (B) Laboratory-scale prototype of the reactor based on using two bottles with the PEM held in a tube between the two chambers.

require oxygen. The use of a completely anaerobic process should increase the Coulombic efficiency of the MFC process using mixed cultures as oxygen diffusion through the cathode is avoided (*15*).

We report here our preliminary findings using this system that demonstrate for the first time hydrogen produced directly from protons and electrons produced by the bacteria from the complete oxidation of organic matter with greatly reduced energy needs compared to the water electrolysis process.

Methods

To prove the efficiency of this bioelectrochemically assisted process, we constructed two different hydrogen-generating reactors by adapting MFC reactor designs currently used in our laboratory for electricity production (*15, 19*). Both reactors were two-chamber MFCs with the anode and cathode each in a chamber separated by a proton exchange membrane (PEM; NAFION 117) (Figure 1A). The anode electrodes were plain carbon cloth and the cathode electrodes were made of carbon paper containing 0.5 mg Pt/cm^2. The first system was a two-bottle reactor (310-mL capacity each; Wheaton Scientific) with the PEM held by a clamp in the tube separating the chambers, with electrodes spaced 15 cm apart [modified from the design of Oh et al. (*19*)]. Instead of sparging the cathode chamber with air, the chamber was sealed and analyzed periodically for hydrogen gas production. Each electrode was 12 cm^2 and the proton exchange membrane was 3.5 cm^2, and the bottles were filled to 200 mL (Figure 1B).

The second reactor was based on the tubular-reactor design of Liu and Logan (*15*). This reactor was converted into a two-chamber system by inserting a PEM into the middle of a 4-cm-long cylindrical chamber (3-cm diameter, formed of Plexiglass) sealed on both sides to avoid air leaking into the system. The two electrodes (7 cm^2 each) were placed in

the chambers on opposite sides of the PEM, with each electrode set at a distance 0.25 cm from the PEM. This arrangement is essentially that shown in Figure 1A except the chambers did not contain a headspace: gas produced in the cathode chamber was released into a sealed gas bottle (120 mL) that was analyzed periodically for hydrogen as described below.

The bacteria used in the anode chamber were enriched in a conventional microbial fuel cell with domestic wastewater as the inoculum, using techniques as previously described (*15*). After enrichment, the anode was removed and placed into the anode chamber of one of the hydrogen-producing reactors described above. The anode chamber contained a phosphate buffer (50 mM, pH 7.0) and a nutrient medium while the cathode chamber contained only buffer (*15*). Acetate was used as an energy source in all tests. Both chambers were initially purged with N_2 gas to remove oxygen.

The anode potential, which is set by the potential of the respiratory enzymes used to make energy for the cell from the oxidation of organic matter, was measured by multimeter using a reference electrode (Ag/AgCl). A voltage in the range of 250−850 mV (first reactor) or 450−850 mV (second reactor) was applied to the circuit by connecting the positive pole of a programmable power supply (3645A, Array Elec. Co. Ltd) to the anode, and the negative pole to the cathode. A potential of 250 mV (first reactor) or 450 mV (second reactor) was then used until the anode potential increased above zero. In tests where the applied voltage was varied, the anode chamber solution was replaced with new medium and the voltage was increased in 100-mV intervals.

Acetate in the solution was measured at the end of a test by analysis of the sample using a gas chromatograph (Agilent, 6890) equipped with a flame ionization detector and a fused-silica capillary column (DB-FFAP) as described previously (*15*). The volume of gas produced in the cathode chamber (or collected in the bottle attached to the cathode chamber) was measured using a glass syringe (10-mL capacity; Perfektum Syringes; Popper & Sons, Inc.) (*20*). Hydrogen concentration was measured using a gas chromatograph (GC; model 310, SRI Instruments, Torrence, CA) equipped with a thermal conductivity detector and a molecular sieve column (Alltech Molesieve 5A 80/100) with nitrogen as the carrier gas. (*20*).

The Coulombic efficiency was calculated as CE = C_p/C_T × 100%, where C_p is the total Coulombs calculated by integrating the current over time. C_T is the theoretical amount of coulombs that can be produced from acetate, calculated as $C_T = FbSv/M$, where F is Faraday's constant (96 485 C/mol electrons), $b = 8$ is the number of moles of electrons produced per mol of acetate substrate, S is the substrate concentration, v is the liquid volume, and $M = 82$ is the molecular weight of acetate.

Results and Discussion

Hydrogen production was detected when the applied voltage was greater than 250 mV. Although theoretically hydrogen can be produced at the cathode by applying a circuit voltage greater than 110 mV, in practice greater voltages are needed due to overpotential at the electrode. Current density increased with the applied voltage from 0.15 A/m^2 (250 mV) to 0.88 A/m^2 (850 mV) (Figure 2). The anode potential decreased with the voltage added from −291 mV (250 mV) to −275 mV (850 mV) (Figure 2). Over 95% of the acetate was typically degraded by the end of a batch cycle (when the reactor was refilled with fresh medium). This reactor was run for over four months, under various operating conditions, to demonstrate that hydrogen generation was stable in this type of reactor.

The recovery of electrons as hydrogen was over 90% (Figure 3). The Coulombic efficiency, defined as the recovery
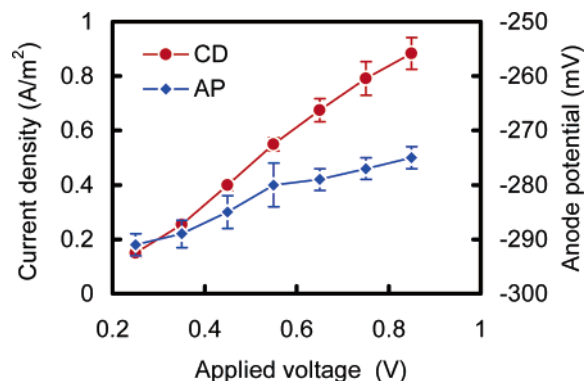
FIGURE 2. Current density (CD) and anode potential (AP) increased with the applied voltage in a two-chambered hydrogen generating system [two-bottle system based on the design of Oh et al. (*21*)]. (Error bars ±SD based on averages measured during stable conditions in three separate batch experiments).
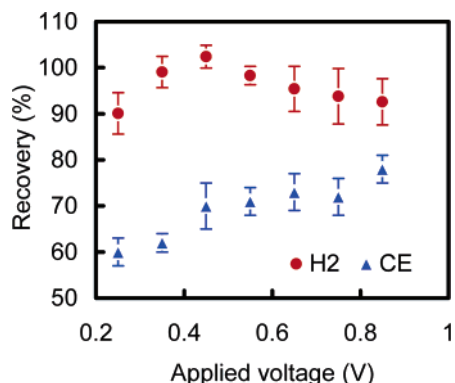

FIGURE 3. Hydrogen recovery and Coulombic efficiency (CE) as a function of the applied voltage in a two-chambered hydrogen generating system [two-bottle system based on the design of Oh et al. (*21*)]. (Error bars ±SD based on averages measured during stable conditions in three separate batch experiments).

of total electrons in acetate as current (eq 1), ranged from 60 to 78% depending on the applied voltage (Figure 3). This range in Coulombic efficiency is similar to that obtained in some aerobic MFCs (*21, 22*). Assuming a maximum possible production of 4 mol H_2/mol acetate, a 78% Coulombic efficiency, and 92% current recovery as hydrogen, the overall hydrogen yield was 2.9 mol H_2/mol acetate. The recovery of electrons is affected by many factors, including biomass production, substrate conversion to polymers and storage, and methanogenesis of hydrogen and acetate to methane. Pure culture tests have reported Coulombic efficiencies as high as 98.6% (*9*). Thus, the use of selected strains might increase overall electron recovery and hydrogen production where such systems can be used under sterile conditions.

The overall efficiency and cost of the system can be improved as current densities are increased and the reactor size is decreased. The system described above achieved a current density <1 A/m² at applied voltages as large as 850 mV. To increase the current density, we modified a MFC (*15*) having a lower internal resistance, due to an increased membrane surface area and decreased spacing between the electrodes, to produce hydrogen. In preliminary tests with this system, hydrogen generation was stable over a two-month period. Using this system, we achieved higher current densities (1.4–7.1 A/m²; Figure 4) than those obtained using the first system and Coulombic efficiencies in a typical range of 65–76%. However, overall hydrogen recovery was reduced to 60–73%, likely due to increased loss of H_2 through its diffusion into the anode chamber.
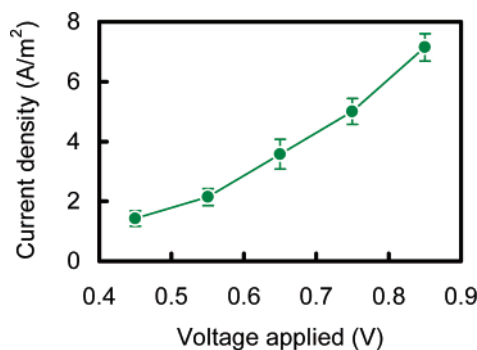

FIGURE 4. Current from acetate oxidation as a function of applied voltage in a second two-chambered hydrogen-producing system [modified from the design of Liu and Logan (*11*) to contain two chambers separated by a PEM]. (Error bars ±SD based on averages measured during stable conditions in three separate batch experiments).

Implications for Using this Process for Renewable Hydrogen Production. The advantages of these bioelectrochemically assisted systems over production of hydrogen using conventional water electrolysis and nonrenewable sources of electricity are multifold, particularly when the possibilities for hydrogen production from waste biomass sources are considered. First, the microbial oxidation of the organic matter provides a renewable source of energy for hydrogen production, and overall greatly reduces the energy needed to produce hydrogen compared to using electricity produced by other means. The electricity energy need using our approach could be as low as 0.6 kwh/m³ H_2 (assuming an overall added potential of 250 mV), corresponding to an energy use of 0.2 mol hydrogen energy (121 kJ/g) per mole of hydrogen produced. This is much lower than the typical energy requirement of 4.5–5 kwh/m³ (or 1.5–1.7 mol hydrogen energy per mole of hydrogen produced) for water electrolysis. Second, precious metal catalysts needed at the anode for water electrolyzer systems are completely replaced by self-sustaining microbial biocatalysts. Although Pt is still needed for the cathode in this system, other research in our laboratory with aerobic MFCs suggests that the Pt content may be reduced to as little as 0.1 mg/cm² without affecting energy production (*23*). The effect of Pt loading, and the use of alternatives to Pt at the cathode, should be more fully investigated. Third, hydrogen can be recovered from the byproducts of fermentation, thereby increasing the overall yield of hydrogen from sugars in a fermentation system coupled with a bioelectrochemically assisted microbial reactor process. We have previously shown that electricity can be efficiently produced using either acetate or butyrate in a MFC (*15*). Assuming that we could achieve between 2 and 3 mol H_2/mol glucose from fermentation, and that we obtain from the fermentation end products the equivalent of 3 mol H_2/mol acetate (i.e., two moles of acetate produced per mole of glucose during fermentation; see eq 1), we estimate that a combined fermentation and a bioelectrochemically assisted anaerobic MFC has a potential to produce as much as 8–9 mol H_2/mol glucose.

With this bioelectrochemically assisted reactor, hydrogen can be produced from any type of biodegradable organic matter, although the Coulombic efficiencies and power densities will vary depending on the substrate (*16, 22*). Virtually any type of biodegradable organic matter has been shown to produce electricity in a MFC, including carbohydrates (*15–22*), amino acids and proteins (*24–25*), and animal and human wastewaters (*16, 26*). The same potential benefits of using MFCs to generate electricity from the treatment of wastewater (*16*) apply here to hydrogen generation. It should be possible for the first time to produce large yields of

hydrogen from domestic wastewater using this approach. Combining hydrogen production and wastewater treatment should result in a more economical venue for hydrogen generation as the infrastructure needed for wastewater treatment can be used to effectively subsidize the cost of the hydrogen generation. The large scale engineering of such technologies would provide methods of generating electricity or hydrogen not only in industrialized nations, but in any area of the world where wastewater treatment systems are needed, especially in developing nations. While there is likely insufficient waste biomass to sustain a global hydrogen economy, this form of renewable energy production may help offset the substantial costs of wastewater treatment as well as provide a contribution to nations able to harness hydrogen as an energy carrier.

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