Long-term study of a new bioelectrochemical technology – The BioGenerator

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ABSTRACT

The BioGenerator is a unique microbial fuel cell for the conversion of hydrogen to electricity. It can be used in the hydrogen-based energy storage as a re-electrification device. This paper shows the results of the long-term stability testing of the BioGenerator. Using a bench scale bioreactor and electrochemical cell, it has been shown that the BioGenerator can achieve at least 3.8 years of continuous electricity generation without significant deterioration either in the biological or in the electrochemical components. The only part which was replaced twice a year was the anode in the electrochemical cell. The results of this work are a significant step towards the commercialization of the BioGenerator, especially in the energy storage sector.

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Introduction

Hydrogen and energy storage

The conversion of hydrogen to electricity (ch2e) is a very important process which can have various applications [1]. There are different ways of converting hydrogen to electricity, however, at present only several of them are considered to be viable: burning hydrogen in combustion turbines; burning hydrogen in an internal combustion engine; combining hydrogen and oxygen electrochemically in fuel cells [2]. Unfortunately, in addition to their advantages, each of these three technologies has its own shortcomings. Firstly, due to very different physicochemical and thermodynamic properties of hydrogen in comparison with natural gas, there are significant difficulties in the design of such turbines [3]. Besides, their maximum efficiencies are expected to be within the range of 30% (for single cycle turbines) and 50% (for combined cycle turbines) determined based on the higher heating value (HHV) of hydrogen. Secondly, while there are existing internal combustion engines working with hydrogen as a fuel [4], their HHV efficiency is quite low – approximately 20–25%. Thirdly, despite the fact that the first hydrogen fuel cell (FC) automobile has recently been commercialized in Japan, hydrogen fuel cells for stationary large-scale applications have still been in the research and development phase [5], and their electrical efficiencies are expected to be up to 40% (HHV). Unfortunately, there is no profitable fuel cell company in the world yet [6]. Therefore, the main hurdles...
preventing the wide-spread use of ch2e converters include their technological development challenges and the relatively low efficiencies.

One of the most important applications of ch2e technologies is in the energy storage domain. It is well-understood that the transition from fossil-fuel to renewable primary energy sources is one of the paramount goals in the modern society mainly due to both environmental concerns, and logistical and geopolitical issues. Regrettably, the intermittency and unpredictability of the most common renewable energy sources, the wind and solar, drastically limit their ability for a direct integration into the existing electrical infrastructure [7].

While the first two steps (water electrolysis and hydrogen storage) are relatively well-developed, the re-electrification (ch2e) is not quite yet, as discussed above.

The main objective of this work is to study the long-term stability of a unique bioelectrochemical convertor of hydrogen to electricity. It was named BioGenerator and is described below.

BioGenerator

As aforementioned, one of the most promising technologies for the ch2e is the fuel cell. As in other electrochemical electricity generation/storage devices (i.e. batteries), the fuel cells consist of two separated in space electrochemical half-reactions: one of them liberates electrons, for instance:

\[
\text{H}_2 \rightarrow 2\text{H}^+ + 2e^- \quad (1)
\]

and the other one consumes electrons:

\[
\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O} \quad (2)
\]

When the electrodes of the above two reactions are connected by an electrical conductor, electrons start flowing from the point of production (reaction 1) to the point of consumption (reaction 2), thus generating electrical current. The above two reactions represent the electrochemistry in the most common acidic hydrogen/oxygen fuel cell such as the proton exchange membrane fuel cell. Due to the inherently sluggish kinetics of the considered reactions, both of them require electrocatalysts (usually platinum or platinum-based) to speed up the redox processes at the electrodes. Pt catalyst is especially important for the oxygen reduction (ORR) represented by Eq. (2) because it is slower than the hydrogen oxidation reaction (Eq. (1)) by approximately 4 orders of magnitude [13]. However, the slowness of the reaction (2), even using a Pt electrocatalyst, is the main cause of the serious problems in PEM fuel cells, which include the high cost due to the large amounts of Pt; the relatively low stability due to the poisoning of Pt by impurities present in the atmospheric oxygen; as well as the low electrical efficiency due to the high cathodic overpotential.

Therefore, the performance of the H2/O2 fuel cells can be greatly improved if one can find a way to increase the rate of ORR without using precious-metal-based electrocatalysts [14].

It is a very well known fact that the oxygen-respiring living organisms (mammals and some microorganisms) assimilate oxygen according to the following biochemical reaction:

\[
\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- = \text{H}_2\text{O} \quad (3)
\]

It is interesting to note that the overall respiration reaction (3) is very similar to the cathodic fuel cell reaction (2). However, there is one very distinct and important difference — the rate of the biological respiration (3) is by at least three orders of magnitude faster that the rate of the cathodic oxygen reduction reaction (2), even on Pt electrocatalyst, due to the extremely efficient biocatalysis in living organisms.

Therefore, since the low rate of the electrochemical ORR (Eq. (2)) is the main bottleneck in PEM fuel cells, and since the biological respiration reaction (3) occurs much faster in living organisms, the main idea behind the BioGenerator is to use the respiration of living organisms (microorganisms) in the cathodic oxygen reduction process.

In the BioGenerator [15], the iron-oxidizing microorganisms Acidithiobacillus ferrooxidans or Leptospirillum ferriphilum use the energy of oxygen reduction (respiration) to oxidize ferric to ferrous ions. The latter are then pumped into the cathodic compartment of an electrochemical cell where they serve as oxidant:

\[
\text{Fe}^{3+} + e^- = \text{Fe}^{2+} \quad (4)
\]

Replacing the oxygen as an electron acceptor, as in conventional PEM fuel cell The basics of the BioGenerator is shown in Fig. 1. It can be seen that iron ions are continuously shuttled between the electrochemical cell cathode (where they are reduced) and the bioreactor (where they are re-oxidized/regenerated by microorganisms). Therefore, the only input into the BioGenerator system is hydrogen fuel, oxygen as an oxidant and CO2 as a carbon source for the microorganisms. The outputs include water, electricity, heat, and eventually excess microbial mass that is formed in the bioreactor in the course of operation. The microorganisms, growth medium nutrients and iron salts remain in the system.

The net reaction used by iron-oxidizing autotrophic microorganisms to obtain biological energy is:
\[ 2\text{Fe}^{2+} + \frac{1}{2}\text{O}_2 + 2\text{H}^+ = 2\text{Fe}^{3+} + \text{H}_2\text{O} \quad (5) \]

It can be seen that the energy-supplying reaction does not involve any organic compounds, which would normally be used by heterotrophic organisms as a carbon source for their growth. Therefore, in order to proliferate, the iron-oxidizing microorganisms fix atmospheric carbon dioxide, which is a sole carbon source \[16\]. As a result, the BioGenerator is the first power generation technology of any kind that has the capability of consuming CO\(_2\) from the atmosphere.

The main goal of this work is to study the long-term operation of a bench-scale BioGenerator with an emphasis on the microbial dynamics during operation over the span of several years.

**Materials and methods**

The overall scheme of the BioGenerator used in this study is shown in Fig. 2. It contains two major components: biological (the bioreactor) and electrochemical (the electrochemical cell).

**Bioreactor**

The bioreactor was based on the airlift principle and had a semipermeable separating wall (the immobilized solids bioreactor \[17\]). It was made of 70 cm high glass tube (8 cm internal diameter) tapered at the bottom (Fig. 3) with a liquid volume of 1.4 L. A semipermeable (permeable to liquid, but non-permeable to gas bubbles) vertical wall made of 2.5 cm thick polyurethane foam (Fluval\textsuperscript{®} C3, Baie d’Urfé, Canada) divided the cylinder into two semicylindrical sections. In addition to vertically dividing the bioreactor into two zones – aerated and non-aerated ones, the polyurethane foam served also the role of a support for the immobilization of microorganisms. A sparger made of 7.5 cm long and 0.5 cm in diameter perforated rubber tubing (Great Choice bubble wand, Petsmart, London, Canada) was installed at the bottom of the aerated section. The reactor was capped with a glass funnel having a 2.5 cm opening, which served as a mist collector. The entire bioreactor was immersed in a constant-temperature water bath, keeping the temperature at 40 °C, which was found to be optimal for the used microorganism (\emph{L. ferriphilum}) \[18\]. The air was sparged at a flow rate of 90 L/h thus creating a density difference between the aerated and the non-aerated vertical sections. That density difference resulted in a hydrostatic pressure difference across the polyurethane wall, creating a complex liquid flow (upwards in the aerated section, downwards in the non-aerated one and horizontally though the porous polyurethane wall) \[17\]. The air also supplied microorganisms with oxygen and carbon dioxide.

**Bacterial culture and growth medium**

The microbial culture used was obtained by mixing samples of acid mine drainage from four different sites: Iron Mountain (Richmond Mine), California, USA; Rio Tinto, Spain; copper mines near Kalugerovo, Bulgaria; and Pyhäsalmi Mine, Finland.

A modified 9K growth medium was used for cultivation of the bacterial culture, which contained one tenth of the amounts of nutrients as compared to the composition of the original 9K medium \[19\]. The medium was produced by
dissolving 0.25 g of (NH₄)₂SO₄, 0.05 g of K₂HPO₄, 0.05 g of MgSO₄, 0.001 g of KCl and varying amounts of FeSO₄∙7H₂O in 1 L of deionized water to achieve ferrous iron concentrations between 20 and 50 g/L. The pH of the obtained solution was brought to 0.45 by the addition of concentrated H₂SO₄ depending on the bioreactor design and operation mode.

**Electrochemical cell**

The bioreactor described above was connected to a single-cell electrochemical cell. The plates of the cell had serpentine flow distribution channels (Fig. 4). The cell active area was 4 × 4.4 cm. The anode was a gas diffusion electrode ELAT LT 140E-W (BASF Fuel Cell Inc., Somerset, USA). The cathode was made of activated graphite felt [20] and had 4.0 × 4.4 cm geometrical area. The catholyte was a bioreactor liquid containing Fe³⁺ as electrochemically active species. It was pumped into the cathodic compartment of the electrochemical cell through a 10 µm pore size cartridge filter (Fig. 2) using peristaltic pump with a flow rate of 100 mL/min. Hydrogen gas (UHP 5.0 grade) was supplied from a high-pressure cylinder. It was recirculated between the anodic compartment of the cell and a flat-plate cooler/condenser (Fig. 2) maintaining a constant temperature of 4 °C in order to remove water vapor from the anodic space. The temperature of the condenser was maintained by a thermoelectric Peltier cooler AHP-300CP (ThermoElectric Cooling America Corp., Chicago, USA). The current drawn from the fuel cell was adjusted manually using a variable resistor (R, Fig. 2) and measured by an ampermeter. The cell voltage was measured by a millivoltmeter (V, Fig. 2).

**Microbial cell counting**

A transmitted light optical microscope Axioskop 40 (Carl Zeiss, Germany) with 1000 × magnification equipped with a camera and QCapture image acquisition software was used to determine the cell concentration through direct manual counting. For the concentrations below 2 × 10¹⁰ cell/L, cell numbers were determined directly in a sample taken from the bioreactor. Otherwise the sample was first diluted 2 to 6 times using deionized water before the microscopic counting. At least ten counts from pictures taken in different spots on the microscope slide (18 mm in diameter) were made to determine the average microbial cells concentration in the bioreactor liquid.

**Determination of ferrous and ferric iron concentrations**

The ferrous iron concentration was determined by titration of a bioreactor liquid sample (2 mL) with 0.1 N K₂Cr₂O₇ (analytical grade) in 10% sulfuric acid and using N-phenylanthranilic acid as a potentiometric indicator. The concentration of ferric iron was titrated with EDTA in an acetic acid-sodium acetate buffer (pH ~ 4) in the presence of 5-sulphosalicylic acid (reagent grade) as a complexometric indicator. The ferric and total iron within the low concentration range (less than 0.15 g/L) were measured spectrophotometrically using a UV–Vis spectrometer Cary 50 (Varian Inc., Sydney, Australia) following the procedure described earlier [21]. The ferrous iron concentration was calculated as the difference between total and ferric iron concentrations.

**Results and discussion**

From the microbial macrokinetics standpoint, the BioGenerator is a unique system in which the substrate is continuously internally regenerated, and no liquid effluent is removed. Since microorganisms, microbial products (except for water due to evaporation) or anything else, other than exhaust air, leaves the system (Fig. 1), the BioGenerator can be considered as a closed system in terms of the liquid phase. There is also no accumulation or withdrawal of the microbial product (Fe³⁺), and no external input of a substrate (Fe²⁺) takes place. Hydrogen and oxygen, entering the system, are
converted to water, which is withdrawn as water vapor with the exhaust air from the bioreactor, as well as a condensate at the anodic side of the electrochemical cell (Fig. 2). The carbon dioxide, entering the bioreactor with air, is converted to microbial mass. Hence, the only component in the BioGenerator, which is expected to be changing in time (accumulating), is the microbial mass.

The dynamics of microbial growth will be analyzed below, since microorganisms are the only component which is accumulating in the BioGenerator. Most microorganisms, including *L. ferriphilum*, increase their mass by the process of binary fission, dynamics of which can be described as:

\[
dX/dt = \mu X
\]

where \(X\) is the concentration of microorganisms at time \(t\) and \(\mu\) is the growth constant (the specific growth rate). The specific growth rate is constant if the following conditions are satisfied: (1) the limiting substrate concentration (in our case, Fe\(^{2+}\)) is much higher than the half saturation constant. While the Fe\(^{2+}\) concentration in the bioreactor fluctuated around 0.5 g/L, the half-saturation constant is 0.0067 g/L [18]; (2) No product concentration limitation. That was the case, based on the kinetics of our process [18]; (3) Both pH and temperature are constant. They were kept close to 0.8 and 40 °C, respectively for the entire period of the study; (4) No oxygen limitation. The O\(_2\) concentration was maintained around 4 mg/L which is much above the limiting one [22]; (5) No limitation by nutrient salts concentration. We regularly added 9K medium to the bioreactor; (6) No removal of microorganisms from the system, which was the case; (7) No diffusion limitations in the biofilm. According to visual observations, the biofilm thickness in our bioreactor was below approx. 0.5 mm, which is in general, below the oxygen penetration limit for different biofilms [23]. Therefore, since \(\mu\) was kept close to constant, the amount of microbial mass should increase in time exponentially:

\[
X = X_0 e^{\mu t}
\]

where \(X_0\) is the initial concentration of microorganisms (at \(t = 0\)). Since there was no harvesting of microorganisms from the BioGenerator, it is expected that their number would increase drastically, taking into account that the average doubling time for *L. ferriphilum* is approximately 9 h, corresponding to \(\mu_{\text{max}} = 0.075 \text{ h}^{-1}\) [18]. This is a very important feature that needs to be taken into account during the long-term (months and even years) operation of the BioGenerator.

To start the bioreactor, initially it was filled with a modified 9K growth medium, as described above, containing 20 g/L Fe\(^{2+}\) and having a pH of 1.0. It was inoculated with 150 mL of bacterial culture suspension (2 × 10\(^9\) cells/mL). The bioreactor was operated in a batch regime. After a complete conversion of ferrous to ferric iron was achieved, the bioreactor was switched to continuous mode. The feed solution was introduced with a flow rate of 0.10 L/h. It contained 9K medium containing 50 g/L Fe\(^{2+}\) and had a pH of 0.45. Under steady-state conditions the pH in the bioreactor was kept constant at 0.8. The difference between pH of the feed solution (0.45) and in the bioreactor (0.8) is due to the fact that protons are consumed during the microbial iron oxidation (Eq. (5)), which results in an increase of the pH. Once 99% of Fe\(^{2+}\) oxidation was reached, the bioreactor was connected to the electrochemical cell and the flow of fresh ferrous iron solution was stopped permanently. At that stage, the microbial substrate (Fe\(^{3+}\)) was produced by the reduction of the microbial product (Fe\(^{3+}\)) in the electrochemical cell and was entering the bioreactor with the recirculating liquid at the flow rate of 6 L/h (Fig. 1).

Once the BioGenerator reached steady state, initially the electrical current drawn from the electrochemical cell of the system was kept between 1.0 and 1.4 A, controlled by the electrical resistance R (Fig. 2). However, that current resulted in a significant and sudden increase in the concentration of ferrous iron and, consequently, in lowering the pH down to 0.6 in the bioreactor liquid. The pH decreased because, according to Eq. (5), when the reaction is moving towards the left (Fe\(^{3+}\) is reduced to Fe\(^{2+}\)) protons are formed. However, pH of 0.6 is well below the optimal range for the growth of *L. ferrooxidans*. In order to improve microbial kinetics, the electrical current was decreased to 0.5 A, and then slowly brought back to the original value (1.0 A) in a stepwise manner (0.1 A increments) until the steady state pH stabilized in the region of 0.8. Under the new steady-state conditions, the electrochemical cell voltage remained relatively constant, fluctuating around 3.5 V. To remove any water, crossing the membrane and accumulating in the anodic chamber, the anode of the electrochemical cell was purged with dry hydrogen on a weekly basis. Apart from that, once in two weeks the anode was dried by blowing dry compressed air for about 2 h. The time profile of the electrical current and voltage are shown in Figs. 5 and 6, respectively. It can be noted that both the voltage and current time profiles had the shape of zigzag curves, fluctuating around 0.35 V and 0.58 A, respectively. The reason for that was that after every purging/drying cycle, the performance of the electrochemical cell improved (both the voltage and current increased), which was followed by a gradual decrease until the next drying cycle.

A typical polarization curve of the BioGenerator during the steady state period is shown in Fig. 7. The cell voltage did not
The long-term stability of a novel bioelectrochemical converter of hydrogen to electricity - the BioGenerator - was tested on a laboratory scale. It was found that over the course of 3 years and 10 months, the BioGenerator had a stable performance, producing nearly constant electrical current of $0.59 \pm 0.11$ Amps and voltage of $0.35 \pm 0.06$ V. A highly unusual feature of the system was observed during the entire multiyear period, the amount of microorganisms in the bioreactor remained constant despite the fact that the BioGenerator is a closed system in terms of microbial dynamics, and no microorganisms were withdrawn from the system. It was found that the reason for the stable microbial population was that in addition to the main iron-oxidizing species Leptospirillum ferriphilum, there was another species – the red alga Cyanidium caldarium. While L. ferriphilum is a strict autotroph, C. caldarium is a heterotroph. Thus, when the microbial cells of the autotroph die and degrade, the heterotroph consumes the products of the microbial degradation. This symbiotic relationship allows to avoid overpopulation by maintaining the long-term stability of the system, without removing microorganisms from the BioGenerator.
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