

Exploiting complex carbohydrates for microbial electricity generation – a bacterial fuel cell operating on starch

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Abstract

In this contribution we demonstrate that by combining specially designed anodes, consisting of platinum covered by poly(tetrafluoroaniline) [1,2] and living cells of the biocatalyst *Clostridium butyricum* or *Clostridium beijerinckii* electricity can be generated from a variety of substrates, including starch, one of the major biomass constituents. Current densities between 1 and 1.3 mA cm⁻² are achieved by using glucose, molasses, or starch as fuel.

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1. Introduction

Large amounts of energy contained in different forms of biomass that we refer to as waste usually remain unexploited and subject of microbial degradation. Finding a way to exploit this biological substrate degradation for the generation of electricity is the driving force for the development of microbial fuel cells (MFCs). MFCs make use of the catabolic activity of living cells (“biocatalysts”) for converting chemical energy into electricity. It has already been demonstrated that, at a low level, electricity can be produced from even such unlikely energy sources like sediments [3,4], sewage sludge [5] or waste water streams [6]. Recently a number of advanced concepts have been proposed utilizing glucose as fuel [1,7,8]. The development of microbial fuel cells is, however, still in its infancy with the need of considerable improvements with respect to power output and accessible substrates. Thus, most MFCs still require their fuel to be of low-molecular nature. The majority of herbal bio-

mass, however, consists of macromolecular compounds including complex carbohydrates like starch and cellulose. In this communication, we present a fuel cell that operates on starch producing electricity at high current and power output, which represents a great step towards the exploitation of natural carbohydrate sources. The core of the fuel cell concept is the combination of the highly productive and versatile biocatalysts *Clostridium butyricum* and *Clostridium beijerinckii*, and a novel anode design consisting of a layered conductive polymer/platinum composite material. As we have shown before [1,2], such composite materials allow current densities that are more than one order of magnitude larger than of conventional MFCs. The choice of using *C. butyricum* and *C. beijerinckii* strains as biocatalysts is based on their high production rate and efficiency of hydrogen, the major electron donor in the electricity generation process, and their capability to digest a large number of substrates, reaching from low molecular compounds like lactate, monosaccharides like glucose, to disaccharides like sucrose, and even starch [9], for which the bacteria make use of the extracellular enzymes α -amylase and glucoamylase. This versatility makes them extremely valuable biocatalysts for microbial fuel cells.

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It is important to mention that the human-pathogenic genera of the Clostridia family, e.g., *C. botulinum* or *Clostridium tetanie*, which gain their energy mainly from the fermentation of amino acids, are not capable of digesting complex carbohydrates and are thus, on top of their hazardous nature, not suitable as biocatalysts in microbial fuel cells.

2. Experimental

2.1. Chemicals

All chemicals used in this study were of analytical or biochemical grade. The beet molasses was kindly provided by Hansa Melasse Handelsgesellschaft mbH., Hamburg, Germany. Its sugar content was 47%.

2.2. Electrochemical instrumentation and setup

Experiments under potentiostatic control were performed utilizing a three-electrode arrangement consisting of the working electrode, a Ag/AgCl, sat. KCl, (197 mV vs. SHE) reference electrode and a counter electrode (platinum wire or a carbon rod electrode). The counter electrode was separated from the bacterial solution by a Nafion[®] 117 perfluorinated membrane. The experiments were conducted with μ -AutolabII, PGSTAT20 and PGSTAT30 Autolab systems (Ecochemie, Netherlands). Sealed and thermostated fermentation vessels (100 ml) served as electrochemical cells which hosted the fermentation medium and the electrodes. Current and potential measurements at the model fuel cell system were carried out using a data acquisition system consisting of two Keithley Integra 2700 digital multimeters equipped with 7700 multiplexer (Keithley Instruments, Inc., Cleveland, USA) connected to a personal computer via Keithley KPCI-488 IEEE interface card. For the determination of the power output a variable resistance (0.1 Ω to 1 k Ω) was used as external load. All experiments were carried out at a temperature of 36 °C.

2.3. Electrode materials

Platinum sheets (paddle-shaped, 7.5 \times 20 mm, 0.5 mm thickness), contacted with a platinum wire were used as working electrodes for the potentiostatically controlled current measurements. Woven graphite (purchased from NCBE, University of Reading, UK) was used as the base material for the fuel cell cathode and anode.

2.4. Electrode preparation

For the anode preparation the electrode base material (platinum sheet or graphite felt, respectively) was first

platinized by electrochemical reductive deposition from a 50 mM H₂PtCl₆ solution. Then, an overlay of tetrafluoropolyaniline was deposited. The deposition was achieved by electrochemical polymerization from a 50 mM solution of 2,3,5,6-tetrafluoroaniline in 2 M HClO₄, at constant potential (1.6 V vs. Ag/AgCl) for 1000 s.

2.5. Bacterial growths

Clostridium butyricum and *C. beijerinckii* were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). Both strains were grown anaerobically at 36 °C for at least 24 h in a medium containing 10 g substrate (starch, glucose, lactate or molasses), 5 g yeast extract, 10 g peptone, 0.5 g L-cysteine-HCl, 8 mg CaCl₂, 8 mg MgSO₄, 40 mg KHSO₄, 80 mg NaCl, 0.4 g NaHCO₃, and 1 mg resazurine per litre. The same medium served as the anolyte solution in the poised potential experiments and fuel cell experiments. For these experiments 25–50 ml of an overnight culture were inoculated into 100 ml fresh medium. Before inoculation the solutions were purged with nitrogen for 10 min in order to remove oxygen.

Beside hydrogen and carbon dioxide, *C. butyricum* and *C. beijerinckii* produce the following fermentation products: (i) fermentation of glucose: ethanol, butyrate, acetate and propionate; (ii) fermentation of starch: mainly butyrate.

3. Results and discussion

Fig. 1 shows the electricity generation in freshly inoculated anaerobic cultures of *C. butyricum* containing starch (curve A) and molasses (curve B) as substrates. The anode was potentiostatically poised at a potential of 0.2 V (vs. Ag/AgCl) in order to mimic the presence of a fuel cell cathode. Both current curves, shown in Fig. 1, are the results of batch experiments in which the starch and the molasses containing solutions were inoculated with the biocatalyst. As it can be seen, both cultures quickly start fermentation and reach a maximum current output of about 1.1 mA cm⁻² (molasses) and 1.3 mA cm⁻² (starch), values, which lie more than one order of magnitude above the current output of conventional microbial fuel cells. The current decreases after approximately 5 h due to substrate exhaustion in the medium. In a semi-batch experiment, the replacement of 80% of the exhausted solution by fresh medium led to an almost instantaneous recovery of the current generation (see inset in Fig. 1).

Similar results are also obtained with *C. beijerinckii*. It too is capable of accessing a large number of substrates, including starch. A comparison of the electricity production of both strains for different substrates is given in Table 1. It shows that both strains are excellent

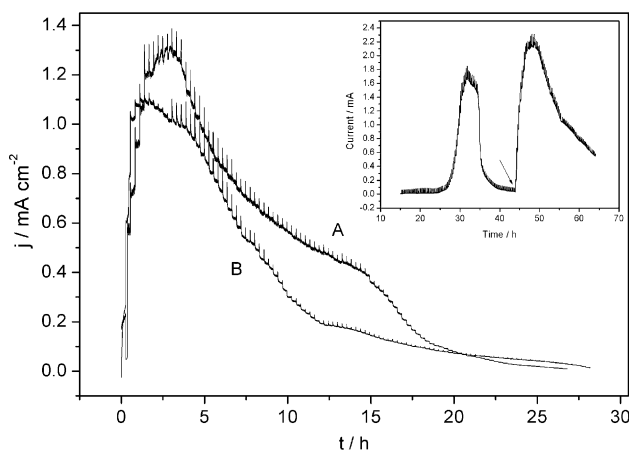


Fig. 1. Current generation of a freshly inoculated anaerobic culture of *Clostridium butyricum* measured at a polytetrafluoroaniline modified platinum electrode (batch experiment). (A) The substrate was starch and (B) the substrate was molasses. The substrate concentration is 10 g l^{-1} . The electrodes were potentiostatically poised at a potential of 0.2 V vs. Ag/AgCl (sat. KCl). The experiment was carried out at $36 \text{ }^\circ\text{C}$. Inset: Fed batch experiments in which the starch containing solution was inoculated with the biocatalyst, and in which (indicated by arrow) 80% of the bacterial medium was replaced by fresh substrate solution.

Table 1

Mean current generation of *Clostridium beijerinckii* and *Clostridium butyricum* at polytetrafluoroaniline modified platinum electrodes

Substrate	Current density (mA cm^{-2})
<i>Clostridium beijerinckii</i>	
Glucose	1.33
Lactate	0.7
Starch	0.8–1
<i>Clostridium butyricum</i>	
Molasses	1.1
Starch	1.3

The electrodes were placed in the freshly inoculated stirred anaerobic culture containing the substrate in the concentration of $c_{\text{substrate}} = 10 \text{ g l}^{-1}$. A potential of 0.2 V (vs. Ag/AgCl sat. KCl) was applied.

biocatalysts capable of generating electricity at high rate. However, in our experiments *C. butyricum* appeared superior with respect to the utilization of starch.

Major anode reaction is the catalytic oxidation of hydrogen, product of the fermentative substrate decomposition of the *Clostridia* biocatalyst. *C. beijerinckii* and *C. butyricum*, which synthesize hydrogen via a ferredoxin-linked pathway of the butyric acid fermentation, are known as hydrogen producers, forming up to two moles of hydrogen per glucose unit in the fermentation of starch [9,10]. The efficient hydrogen synthesis, along with the ability to feed on a desired carbon source, in fact represent main criteria for the selection of microorganisms as biocatalysts for the here presented fuel cell concept. Certainly, this microbially formed hydrogen could be processed in an external, conventional hydrogen fuel cell [11,12]. However, there are good reasons

for an in situ electricity generation [13,14], i.e., an electrochemical depletion of the microbially synthesised hydrogen in the bacterial solution. From the technological viewpoint, this would make the hydrogen collection and the usually costly cleaning procedures unnecessary. More important, however, is a possible assistance of the biological hydrogen synthesis: thus it is known that hydrogen synthesis pathways are sensitive to H_2 concentration and are subject of end-product inhibition. As H_2 concentrations increase, H_2 synthesis decreases resulting in larger concentrations of reduced organic compounds [11]. Vice versa, when hydrogen is removed from the microbial medium (usually by bubbling with nitrogen) higher yields of oxidized compounds and lower yields of reduced organic products are formed indicating an increased hydrogen formation [15]. So, the in situ electrochemical depletion of the microbially formed hydrogen could potentially even enhance the hydrogen and thus electricity yield.

But what electrode material an in situ oxidation could be achieved with? Platinum is a very efficient electrocatalyst for hydrogen oxidation. Its extreme liability to poisoning, however, would forbid its use as an anode material in a microbial fuel cell. In order to use platinum for in situ electricity generation it would be essential to find means to protect the metal from becoming deactivated by the partially high levels of electrode poisoning fermentation by-products. Recently we have shown that by providing platinum with an overlay of the conductive polymer polyaniline its tolerance against poisoning can be considerably increased and the catalytic activity of the precious metal can be kept at high level even under the “dirty” conditions of a bacterial growth medium [1]. For the present study, however, a further improved material was used, a composite of platinum modified with a layer of the perfluorinated form of polyaniline, polytetrafluoroaniline [2]. This conductive polymer not only improves the catalytic activity as well as the biological and chemical stability of the anode material, its effective interaction with platinum also increases the tolerance of the electrocatalyst against poisoning in such a way that the formerly necessary potential pulsing for electrode regeneration becomes obsolete. For the removal of chemisorbed species from the electrode surface it now suffices to shortly interrupt the current flow, which due to the reducing conditions in the bacterial solution leads to a reductive stripping of chemisorbed species from the catalyst surface.

The performance of the electricity generation was tested in a fuel cell system comprised of a reactor containing 200 ml of an anaerobically growing suspension of *C. beijerinckii* in a starch medium ($10 \text{ g starch per l}$), and a small fuel cell unit of two fuel cells electrically connected in series. The fuel cell units consisted of an anode compartment through which the bacterial medium was pumped and a cathode compartment through

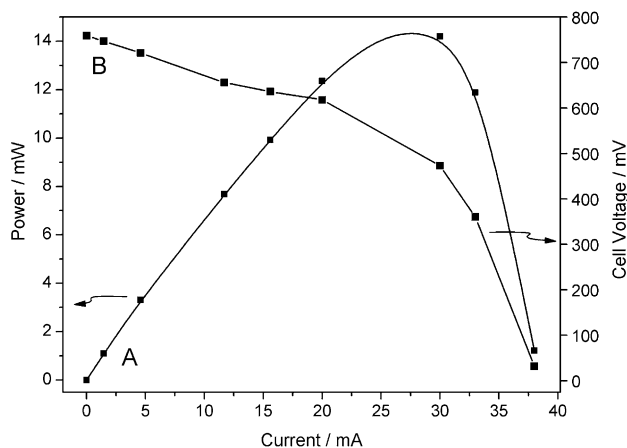


Fig. 2. Polarization curves of the model microbial fuel cell. (A): Power output P (mW), and (B): cell voltage, E_{cell} , as a function of the current output i of the fuel cell. Anolyte solution was an anaerobic culture of *Clostridium butyricum* containing 10 g l^{-1} starch.

which the catholyte was pumped. Anode and cathode compartment were separated by a Nafion™ proton conducting membrane. The anode was a woven graphite cloth, 30×25 -mm sized, with a weight of 0.05 g, which was platinized and subsequently tetrafluoropolyaniline modified. The cathode was unmodified woven graphite and the catholyte was a 50 mM ferricyanide solution in a pH 7 phosphate buffer. The ferricyanide catholyte was chosen as it allows the reversible electron transfer at unmodified carbon electrodes. Potentially, it will be replaced by an oxygen electrode. The open circuit potential of the fuel cell was measured with 759 mV. Under short circuit conditions the steady state current was 38 mA, and maximum currents of 120 mA were measured. Fig. 2 shows the polarization curve of the fuel cell system as a function of the measured steady state currents. The maximum power output was 14 mW, corresponding to a current of 30 mA at a potential of 473 mV.

4. Conclusions

High current output and flexible fuel utilization, including substrates of macromolecular nature, are prerequisites for a successful implementation of microbial

fuel cells in biomass and waste utilization. With the here presented concept we hope to be able to get one step closer to this aim. The concept also shows that the interfacing of biology and electrochemistry may become of utmost importance for developing sustainable energy concepts as well as to cope with environmental problems of waste use [16].

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