A Microbial Fuel Cell

A Generation of Microbial Fuel Cells with Current Outputs Boosted by More Than One Order of Magnitude**

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The concept of employing microbial catabolic activity to directly generate electricity from the degradation of organic matter provides access to cheap and environmentally friendly energy sources. The energy conversion can be achieved with the help of microbial fuel cells, in which mostly fermentative microorganisms serve as biocatalysts and from which electrons are diverted and transferred to an electrode to generate electricity. Finding an efficient way to "wire" the microbial activity to an electrode is the key to the success of microbial fuel cells.[1] Hitherto, electricity generation was limited to current densities of about 20 $\mu A\,cm^{-2}$ achieved by the use of dissolved artificial redox mediators[2] and 120 $\mu A\,cm^{-2}$ by using the oxidation of metabolic products at platinum black anodes.[3] Herein we report a microbial fuel cell that continuously generates a current output more than one order of magnitude larger than the known microbial fuel cells (up to 1.5 $mA\,cm^{-2}$). The novel fuel cell concept uses polymer-modified catalytically active anodes which shuttle electrons from the bacterial suspension to the anode.

Until now three concepts have been proposed to wire microbial catabolic activity to electrodes for in situ electricity generation. The differences in these concepts is found at the stage at which electrons are diverted from the microbial catabolic path, and consequently in the way that the electrons are captured: 1) Dissolved artificial redox mediators serve as electron shuttles that penetrate the bacterial cells, divert electrons from the respiration chain and from internal metabolites, and transfer electrons to the fuel cell anode.[4] Current densities of between 5–20 $\mu A\,cm^{-2}$ were reported.[5] However, the low current output, environmental and cost concerns disfavor this concept. 2) Metal-reducing bacteria, such as *Shewanella putrefaciens* that have special cytochromes bound to their outer membrane, can pass electrons directly to an electrode, which mimics the presence of metal ions as the terminal electron acceptors making the presence of artificial electron mediators obsolete.[6] However, the current and power output are rather low. Recently, Park and Zeikus could increase the current output to 16 $\mu A\,cm^{-2}$ by using resting cells of *Shewanella putrefaciens* and enhancing the electron transfer to the anode by using Mn^{4+} ions or neutral red modified anode materials.[7] 3) Fermentation products like hydrogen, methanol, or ethanol have been used for in situ electricity generation. In this case, platinum black electrodes were used as electrocatalytically active anode materials; one such example, a microbial fuel cell based on the hydrogen evolution by immobilized cells of *Clostridium butyricum* was reported.[3] Short circuit currents of 120 $\mu A\,cm^{-2}$ were reached by using lactate as the substrate. The fuel cell concept reported herein overcomes the problems of the previous microbial fuel cells. Central parts of the concept are a novel layered multifunctional anode consisting of a metallic electrocatalyst (present in the form of platinum or platinized carbon cloth electrodes) covered by an electrocatalytic conductive polymer (e.g., polyaniline, poly(neutral red), poly(methylene blue)) and a regenerative-potential program applied to maintain the long-term electrode activity.

The cyclic voltammograms of a polyaniline modified platinum electrode immersed in a stirred anaerobic culture of *Escherichia coli* K12 (Figure 1) demonstrate the electrocatalytic behavior of the electrode material at different stages of the bacterial growth and fermentation. Under fermentative conditions the electrode has a high electrocatalytic activity that is practically constant in the potential range between −0.1 and +0.6 V. The decrease of the activity at more positive potentials is well known for Pt. It is caused by the formation of a PtO layer at the Pt surface. Figure 2 A shows the current measured at the electrode immersed in a freshly inoculated glucose medium when a constant potential of 0.2 V was applied to mimic the presence of a fuel cell cathode. As expected, the current increases as the bacteria pass from the lag phase into the phase of exponential growth. At unmodified carbon electrodes and even at blank platinum electrodes no significant currents were measured. However, the current decreases before the bacterial growth reaches the stationary phase. It does not reach the values obtained in the cyclic

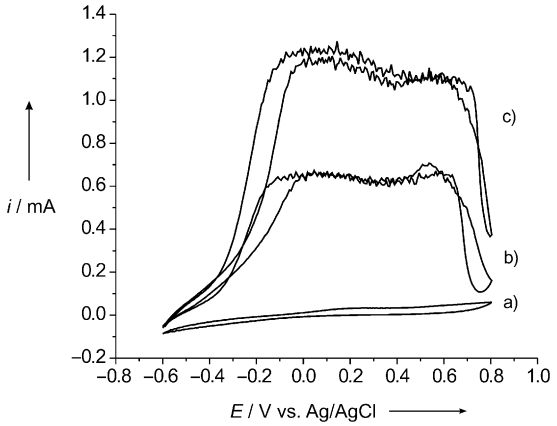

Figure 1. Cyclic voltammograms of a polyaniline-modified platinum electrode (1 cm^2 surface area) placed in a stirred anaerobic culture of *Escherichia coli* K12 in a standard glucose medium ($c_{glucose} = 0.55\ mmol\,L^{-1}$). The experiment was carried out at 37 °C; scan rate 5 $mV\,s^{-1}$. The voltammograms were recorded at different stages of the bacterial growth: a) sterile medium; b) during the exponential growth; c) during the stationary phase of the bacterial growth.

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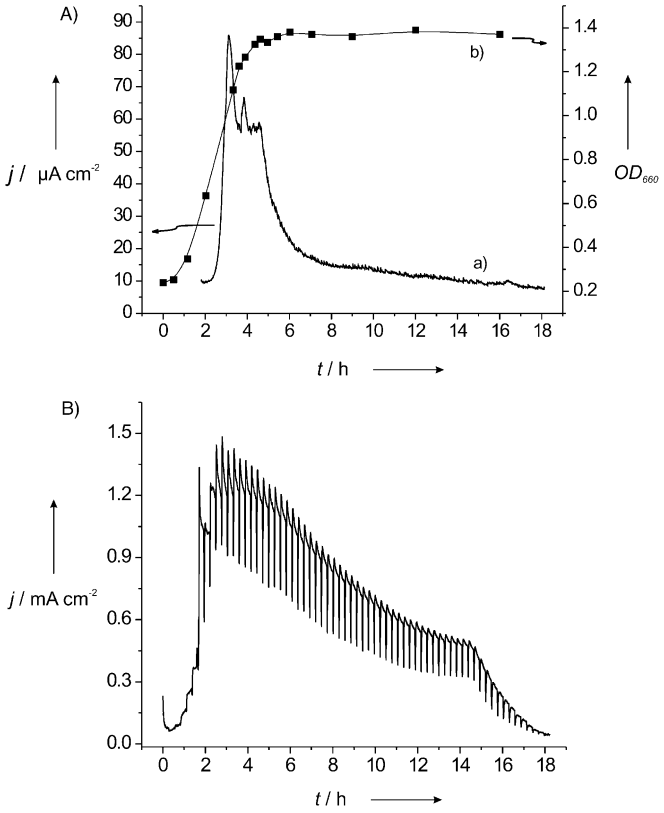

Figure 2. A) Curve a): Chronoamperometric plot of a polyaniline modified platinum electrode placed in a stirred anaerobic culture of *Escherichia coli* K12 in a standard glucose medium ($c_{glucose} = 0.55$ mmol L^{-1}). The potential applied to the electrode was 0.2 V. Curve b): Optical density of the bacterial suspension determined at the wavelength of 660 nm throughout the chronoamperometric experiment. The experiment was carried out at 37 °C. B) Chronoamperometric plot of the modified platinum electrode under the same conditions as those of 2A). Additionally, in an interval of 1000 s potential pulses of 1 V, 5 s were applied to prevent the diminution of the electrode activity. During the potential pulsing the current recording was interrupted.

voltammetric experiment (Figure 1). This phenomenon is the consequence of a diminution of the electrocatalytic activity of the working electrode caused by microbial catabolic by-products and products of the electrocatalytic oxidation and is presumably similar to the deactivation known from direct methanol fuel cells, which is caused by the incomplete oxidation of methanol to form carbon monoxide as an adsorbed layer at the surface of the platinum electrode. In comparison to the unmodified platinum electrode, which was found to be fully blocked, the polymer layer slows down the deactivation but cannot fully prevent it. However, the anode deactivation can be reversed, the performance can be greatly enhanced, and the long-time stability of the electrodes can be maintained with the help of a regenerative-potential program, which can be conveniently performed in situ, that is, within the bacterial medium. A procedure that proved to be most effective is to regularly apply short oxidative-potential pulses to the anode. During the potential pulses, chemisorbed species are presumably oxidized and stripped off from the electrode surface, which reactivates the surface of the metallic electrocatalyst.[8] This procedure prevents the rapid diminution of the anodic currents and increases the current densities to the expected values of 1–1.5 mA cm^{-2} (Figure 2B), which represents an immense progress and offers new prospects for microbial electricity generation.

The importance of the polymer layer for an efficient and sustained electrode activity, as well as for the stability of the electrode function, can be derived from Figure 3. As expected, at the unmodified carbon cloth no microbiologically induced current flow is observed (curve a). A comparison of the platinized only carbon electrode (curve b) and the additionally polyaniline-modified electrode (curve c) shows that whereas the polyaniline-modified electrode gives a stable current flow with a high current density the values for the unprotected electrode lie about 50% below those of the polymer modified electrode. The segments between the individual regeneration pulses show a strong current decrease leading to the pronounced sawtooth structure of the plot. It is caused by a fast deactivation of the unprotected platinum layer.

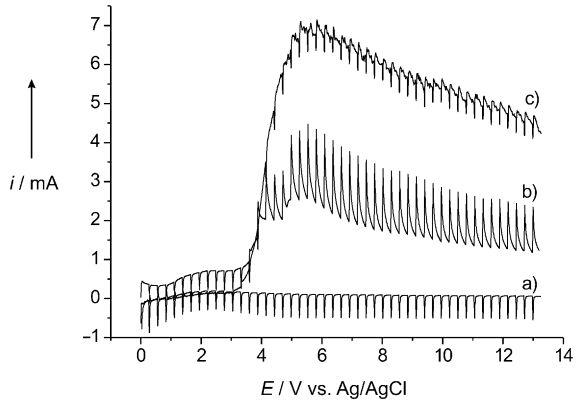

Figure 3. Chronoamperometric plots of differently modified carbon cloth electrodes (25 × 25 mm sized, 0.045 g weight) placed in a stirred anaerobic culture of *Escherichia coli* K12 in a standard glucose medium ($c_{glucose} = 0.55$ mmol L^{-1}). The potential applied to the electrode was 0.2 V. In an interval of 1000 s potential pulses of 1 V, 5 s were applied. Curve a): unmodified carbon cloth; b) platinized carbon cloth, and c) platinized carbon cloth with an overlay of polyaniline. During the potential pulsing the current recording was interrupted. The regular negative current peaks, seen in curve a) and b), are caused by the relaxation of the system after the application of the positive potential pulse.

The conductive polymer has not only a protective function but also directly contributes to the current flow. As presented in Table 1 at purely polyaniline-modified carbon electrodes

Table 1: Mean current densities at differently modified pyrolytic carbon. The electrode was placed in a stirred anaerobic culture of *Escherichia coli* K12 in a standard glucose medium ($c_{glucose} = 0.55$ mmol L^{-1}). The potential applied to the electrode was 0.2 V.

Electrode modification	Current density [$mA^{-1}\,cm^{-2}$]
Polyaniline	0.29
platinum black	0.84
platinum black + polyaniline overlay	1.45

current densities of 0.29 mA cm^{-2} can be achieved. This result means that whereas the platinum black accesses the oxidation of metabolites, the conductive polymer layer fulfils a multitude of tasks. Consisting of molecular units similar to the conventionally used redox mediators, the polymers form a redox active biocompatible layer that takes the function of the dissolved mediators, thus banishing these artificial compounds from the bacterial medium. Because of their reversible redox behavior and electronic conductivity, the polymers are also involved in the oxidation of excreted metabolites. Additionally to these functions, the polymer layers serve as a barrier allowing metabolic products like H_2, one of the major electroactive products, to diffuse to the electrocatalytically active electrode surface but blocking large molecules from accessing the electrode surface and therewith preserving the underlying electrocatalyst from becoming poisoned. Similar protective effects have been observed for platinum–polymer composites, used for the oxidation of methanol.[9]

The performance of the novel fuel-cell anodes was tested in a model fuel-cell system (Figure 4A) that consisted of a reactor containing an anaerobically growing suspension of

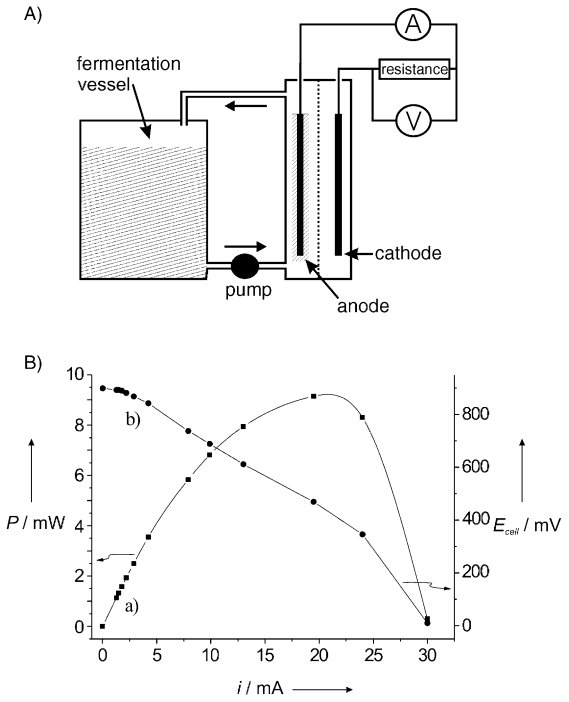

Figure 4. A) Schematic drawing of the novel microbial fuel cell; B) Polarization curves of the model fuel cell. Curve a: Power output *P* [mW], and curve b: cell voltage, E_{cell}, as a function of the current output *i* of the fuel cell.

Escherichia coli K12 in a standard glucose medium (55 mM glucose), and the fuel cell consisting of an anode compartment through which the bacterial medium was pumped and a cathode compartment through which the catholyte was pumped. The anode and cathode compartments 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 polyaniline modified. The cathode was unmodified woven graphite and the catholyte was a 50 mM ferricyanide solution in a phosphate buffer identical to the buffer in the bacterial medium. The ferricyanide catholyte was chosen for these experiments as it is easier to handle than an oxygen electrode. For practical applications the ferricyanide electrode will be replaced by an oxygen electrode. The open circuit potential of the fuel cell was 895 mV. Under short circuit conditions the steady state current was 30 mA, and maximum currents of 150 mA were measured. Figure 4B shows the polarization curve of the fuel-cell system as a function of the measured steady-state currents. The maximum power output was 9 mW, which corresponds to a current of 19.5 mA at a cell potential $E_{cell} = 469$ mV. The power output was sufficient to operate continuously a ventilator driven by a 0.4 V motor.

With this concept for a microbial fuel cell, we have raised the limits of microbial electricity generation by more than one order of magnitude. Thanks to a combination of a novel anode design and the applied in situ electrode-generation procedure, a high current and power output can be achieved. As the use of dissolved artificial redox mediators is unnecessary, the operation costs are low and the fuel cell works at a low emission level.

Experimental Section

Electrochemical instrumentation: All systematic electrochemical investigations of the new anode materials were carried out under potentiostatic control by using a three electrode arrangement consisting of the working electrode, a saturated Ag/AgCl reference electrode, and a platinum wire serving as the counter electrode. The counter electrode was separated from the working electrode by a Nafion 117 perfluorinated membrane. The experiments were conducted with a μ-Autolab II and with a PGSTAT 30 Autolab system (Ecochemie, Netherlands). Current and potential measurements at the model fuel-cell system were carried out by using digital multimeters (Keithley 2700). For the determination of the power output a variable resistance (10–1 kΩ) was used as external load. Electrode preparation: For the anode preparation the electrode base material was first platinized by electrochemical reductive deposition from a 50 mmol L^{-1} H_2PtCl_6 solution, then an overlay of a conduction polymer was deposited. The deposition of the conducting polymers was carried out by electrochemical polymerization from the respective monomer solutions by following standard literature procedures, such as the deposition of polyaniline by potential cycling between −0.1 and 1.2 V from a solution containing 0.1 mol L^{-1} H_2SO_4 and 0.1 mol L^{-1} aniline.[10]

Bacterial growths: *Escherichia coli* K12 was grown aerobically at 37 °C for 12–24 h in a standard medium containing 10 g glucose, 5 g yeast extract, 10 g $NaHCO_3$ and 8.5 g NaH_2PO_4 per litre. The same medium served as the anolyte solution in the chronoamperometric and the fuel cell experiments. For experiments 1 mL of an overnight culture were inoculated into fresh medium. By blocking the access of oxygen the bacteria were now cultivated under anaerobic, fermentative conditions.

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