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Gaining electricity from *in situ* oxidation of hydrogen produced by fermentative cellulose degradation

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ABSTRACT

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Aim: To exploit the fermentative hydrogen generation and direct hydrogen oxidation for the generation of electric current from the degradation of cellulose.

Methods and Results: Utilizing the metabolic activity of the mesophilic anaerobe *Clostridium cellulolyticum* and the thermophilic *Clostridium thermocellum* we show that electricity generation is possible from cellulose fermentation. The current generation is based on an *in situ* oxidation of microbially synthesized hydrogen at platinum-poly(tetrafluoroaniline) (Pt-PTFA) composite electrodes. Current densities of 130 mA l^{-1} (with 3 g cellulose per litre medium) were achieved in poised potential experiments under batch and semi-batch conditions.

Conclusions: The presented results show that electricity generation is possible by the *in situ* oxidation of hydrogen, product of the anaerobic degradation of cellulose by cellulolytic bacteria.

Significance and Impact of the Study: For the first time, it is shown that an insoluble complex carbohydrate like cellulose can be used for electricity generation in a microbial fuel cell. The concept represents a first step to the utilization of macromolecular biomass components for microbial electricity generation.

Keywords: biohydrogen, cellulose, *Clostridium*, hydrogen oxidation, microbial fuel cells.

INTRODUCTION

With a production rate of 10^{15} kg year^{-1} (Berg *et al.* 2002), cellulose not only is the predominant carbohydrate and most common organic compound on earth, it also forms about one half of the terrestrial biomass. Thus, the formation and the microbial degradation of cellulose represent major energy fluxes in our biosphere. Cellulose also represents one of the main renewable energy sources. Because of its neutral CO_2 balance, the utilization of cellulose and of related biomass constituents represents a major concept for a sustainable energy production.

Currently, microbial fuel cells (MFCs) face strongly growing research efforts as they offer the potential to convert the chemical energy, contained in biomass materials (even in waste streams), into electricity (review by Katz *et al.* 2003). Some recent developments allow high conversion rates and high conversion efficiencies of simple carbohydrates like glucose, in a mediator-less fuel cell operation (e.g. Rabaey *et al.* 2003). Recently we presented a novel anode concept that facilitates the generation of electricity by *in situ* oxidation of microbially synthesized hydrogen. This allowed boosting the power output of microbial electricity generation by more than one order of magnitude (Schröder *et al.* 2003). We could also demonstrate electricity generation based on a complex carbohydrate like starch (Niessen *et al.* 2004a,b).

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The direct oxidation of microbial hydrogen shows a number of potential advantages. Thus, it does not require the separation and purification of the gas for its subsequent conversion in conventional fuel cells. The separation of the hydrogen gas from microbial cultures is considered to be an important issue as a large fraction of the gas remains dissolved in the microbial medium – promoting the growth of hydrogen-consuming micro-organisms and thus further lowering the hydrogen yield. Additionally, high hydrogen partial pressures are known to limit the microbial hydrogen

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synthesis, which is subject to negative feedback inhibition at high partial pressure levels.

The utilization of cellulose for microbial electricity generation is much more difficult than that of low-molecular carbohydrates, or of the storage carbohydrate starch, as the β-glycosidic bonds of the structural carbohydrate cellulose are highly resistant against hydrolysis. On top of this chemical stability, its insolubility as well as the association with lignin make cellulose a recalcitrant substrate for enzymatic or microbial hydrolysis. So, even though MFCs are generally referred to as devices in which biomass is converted to electricity, so far their operation has been demonstrated only with low molecular, soluble substrates such as simple carbohydrates and low-molecular organic acids.

Aerobic and anaerobic micro-organisms use different strategies to feed on cellulose. Whereas aerobes generally make use of single-enzyme components, excreted in high concentrations and in concert in order to hydrolyse the β-glycosidic bonds linking the glucose units, some anaerobes have evolved a more elaborate way – by using an extracellular multi-enzyme complex – the so called cellulosome (Schwarz 2001). Cellulosomes, first discovered in the cellulolytic bacterium *Clostridium thermocellum* (Bayer *et al.* 1983), are cell protuberances that tightly bind to cellulose and thus mediate a close neighbourhood between the cells and their substrate, avoiding unnecessary enzyme losses (Lamed *et al.* 1987). This strategy of the cellulolytic bacteria to tackle their substrate has an inevitable consequence for the development of cellulose-based MFCs. It requires the biocatalyst to be dispersed in the substrate medium, where it can bind to the cellulose, and thus it bans the use of fuel cell concepts, where the biocatalyst is bound to the fuel cell anode in the form of a biofilm.

The aim of the present study was to investigate the possibility of exploiting cellulose fermentation for microbial electricity generation. We demonstrate that cellulose can in fact serve as a substrate in MFCs. To the best of our knowledge it is the first time that cellulose is reported to serve as fuel in an MFC.

Two strains of the *Clostridium* genus served as biocatalysts for a microbial electricity generation – *Clostridium cellulolyticum*, a mesophilic bacterium that is decisively involved in the decomposition of cellulose-containing biomass (Desvaux *et al.* 2000), and the thermophilic *C. thermocellum*, a model organism for the study of microbial cellulose decomposition.

MATERIALS AND METHODS

Bacterial growths

Clostridium cellulolyticum and *C. thermocellum* were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). *Clostridium cellulolyticum* was grown anaerobically at 36°C for at least 48 h in a CM3 medium (Gehin *et al.* 1995) containing: 1·5 g KH_2PO_4, 2·9 g $K_2HPO_4 \times 3H_2O$, 1·3 g $(NH_4)_2SO_4$, 0·1 g $MgCl_2 \times 6H_2O$, 0·02 g $CaCl_2$, 5 g yeast extract, 25 μl 5% $FeSO_4$ solution, 1 ml 0·2% resazurine, 0·5 g cysteine hydrochloride, 3 g cellulose per litre. The medium was adjusted to a pH of 7·3 (with 6 mol l^{-1} NH_3 solution). *Clostridium thermocellum* was grown anaerobically at 60°C for 3–4 days in a DSMZ medium 122 containing (per litre): 1·3 g $(NH_4)_2SO_4$, 2·6 g $MgCl_2 \times 6H_2O$, 1·43 g KH_2PO_4, 7·2 g $K_2HPO_4 \times 3H_2O$, 0·13 g $CaCl_2 \times 2H_2O$, 6 g Na-β-glycerophosphate, 1·1 g $FeSO_4 \times 7H_2O$, 0·25 g gluthathione (reduced form), 4·5 g yeast extract, 1 mg resazurine, and 3 g cellulose.

The same media were used in the potentiometric and poised potential experiments. For these, the grown cultures were mixed with fresh medium at a ratio of 1 : 1 (v/v). Before inoculation the fresh medium was purged with nitrogen for 10 min in order to remove oxygen. Control experiments were performed using substrate-free medium as well as purely mineral-based medium.

The following compounds are the products of cellulose fermentation by *C. cellulolyticum*: ethanol, lactate, acetate, CO_2 and H_2. Additionally, the cellulose hydrolysis usually leads to the formation of soluble, partially monomeric sugars (Giallo *et al.* 1985). Lactate, acetate and ethanol where detected via HPLC.

Chemicals

All chemicals used in this study were of analytical or biochemical grade. Cellulose powder (D-0, Fluka BioChemika, Fluka Chemie, Buchs, Switzerland) was used as the substrate throughout the experiments.

Electrochemical instrumentation and setup

Experiments under potentiostatic control were performed utilizing a three-electrode arrangement consisting of the working electrode, a Ag/AgCl, saturated KCl (197 mV *vs* standard hydrogen electrode), reference electrode and a counter electrode (platinum wire or a carbon rod electrode). All potentials given in this communication refer to the silver/silver chloride reference electrode. The counter electrode was separated from the bacterial solution by a Nafion®117 perfluorinated membrane (E. L. du Pont de Nemours, Fayetteville, NC, USA). The experiments were conducted with μ-AutolabII, PGSTAT20 and PGSTAT30 Autolab systems (Ecochemie, Utrecht, the Netherlands). Sealed and thermostated fermentation vessels (100 ml) served as electrochemical cells which hosted the fermentation medium and the electrodes. The experiments were carried out at 36°C (*C. cellulolyticum*) or 60°C (*C. thermocellum*).

Electrode materials

Platinum sheet electrodes (Paddle-shaped, 7·5 × 20 mm, 0·5 mm thickness), and platinum net electrodes (5 × 10 cm) contacted with a platinum wire were used as working electrodes for the potentiostatically controlled current measurements.

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 stirred 50 mmol l^{-1} H_2PtCl_6 solution (−0·6 V, 500 s, room temperature). Then, an overlay of tetrafluoropolyaniline was deposited. The deposition was achieved by electrochemical polymerization from a 50 mmol l^{-1} solution of 2,3,5,6-tetrafluoroaniline in 2 mol l^{-1} $HClO_4$, at a constant potential of 1·6 V for 1000 s (Niessen *et al.* 2004a,b).

RESULTS

Figure 1 shows the redox potential measured in two suspensions containing cellulose and inoculated with *C. thermocellum* (solid curve) and *C. cellulolyticum* (dashed curve). Despite the rather different temperature conditions of both strains the course of the potential in both solutions is almost identical. The major feature is a steep drop of the potential approx. 13–14 h after inoculation. The potential drop results from the cellulose fermentation by the *Clostridia* biocatalysts: as for all fermentative-oxidative substrate degradation processes, the lack of inorganic electron sinks (like oxygen) requires parts of the substrate to become partially or fully reduced. Thus, depending on the fermentative path, hydrogen is produced. The hydrogen formation can be monitored by measuring the redox potential in the bacterial solution with the help of a platinum electrode. An increasing hydrogen partial pressure leads to a shift of the redox potential towards negative values by shifting the ratio of the redox couple $H_2/2H^+$ – resulting in a potential drop as shown in Fig. 1. Due to the high temperature necessary for *C. thermocellum* fermentation this strain is unsuitable for MFCs. Thus, the following results will focus on the mesophilic *C. cellulolyticum*.

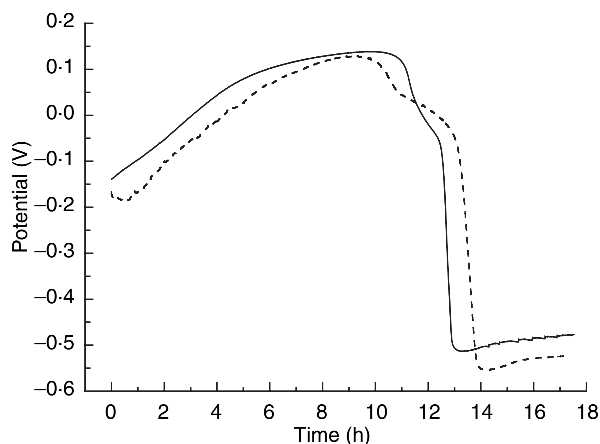

Fig. 1 Redox potential of freshly inoculated anaerobic cultures of *Clostridium thermocellum* (solid curve) and *C. cellulolyticum* (dashed curve) in a cellulose medium. The sensing electrode was a platinized platinum electrode. The experiment was carried out at 60°C (*C. thermocellum*), and 36°C (*C. cellulolyticum*) respectively

As we have recently demonstrated, it is possible to electrochemically oxidize chemotropically and phototropically formed hydrogen directly in the bacterial environment to use it for an *in situ* electricity generation (Schröder *et al.* 2003; Niessen *et al.* 2004a,b; Rosenbaum *et al.* 2005). We could demonstrate the efficient and complete depletion of the microbially formed hydrogen from the microbial medium. Such a procedure not only spares the hydrogen collection and the usually costly cleaning procedures, it may also assist the biological hydrogen synthesis: thus it is known that the microbial hydrogen synthesis is often end-product inhibited, and 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 (Harper and Pohland 1986). In order to effectively oxidize the microbially synthesized hydrogen in the microbial environment, anodes are required that (i) possess a high catalytic activity under the physiological conditions of the bacterial cultures, and (ii) are insensitive against deactivation (poisoning) by fermentation by-products. For that we developed layered composite materials based on an electrocatalyst layer (here platinum) covered by a conductive polymer like polyaniline or its fluorinated forms. Platinum itself is an effective electrocatalyst, however, in a 'dirty' bacterial environment it swiftly becomes deactivated. The polymer layer, deposited on top of the electrocatalyst, serves to protect it from fouling and further improves the catalytic activity of the electrode material.

Figure 2 shows the electricity generation in a freshly inoculated anaerobic culture of *C. cellulolyticum* containing cellulose as the substrate. The anode was potentiostatically poised at a potential of 0·2 V in order to mimic the presence of a fuel cell cathode. The curve is the result of a semi-batch experiment in which a cellulose suspension was inoculated with the biocatalyst. The culture starts fermentation and current generation after 14 h. It reaches the maximum current output of about 10 mA after 16 h. As the biocatalyst is dispersed in the medium and is not bound to the electrode surface, and the electrode surface is large compared with the

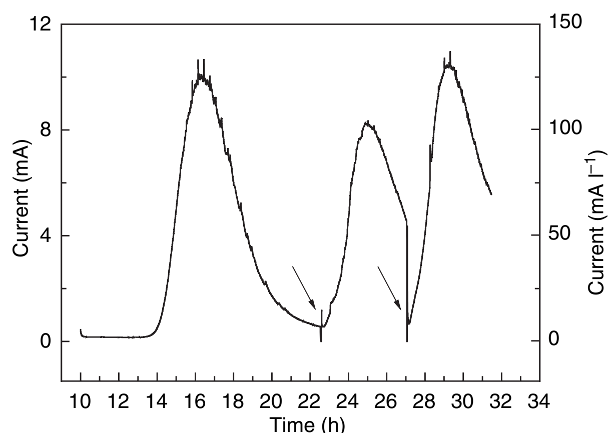

Fig. 2 Current generation of an anaerobic culture of *Clostridium cellulolyticum* in a cellulose medium (80 ml, containing 3 g l^{-1} cellulose) measured at a Pt-PTFA electrode (5 × 10 cm mesh). The electrode was potentiostatically held at a potential of 0·2 V. The experiment was carried out at 36°C. The current registration was started 10 h after inoculation. The arrows indicate the replacement of 80% of the bacterial medium by fresh, cellulose-free medium

volume of the suspension, this value corresponds rather to the volume of the bacterial suspension (130 mA l^{-1}, see right *y*-axis, Fig. 2) than to the surface area of the electrode. The current decreases after approx. 6 h, when cellulose is still present in the system. When parts of the bacterial medium are replaced (indicated by the arrows, Fig. 2) by fresh, cellulose-free medium (the cellulose being mostly precipitated at the bottom of the cell by slowing down stirring) the current generation recovers quickly. Electrode fouling was not observed, and the anode could be used in further experiments without noticeable activity loss.

DISCUSSION

Cellulose is not an ideal substrate for electricity generation in MFCs. Its insolubility and chemical stability limit the rate of microbial substrate decomposition, and thus the current output that can be achieved. Insolubility may also prevent using cellulose as substrate in fuel cells in which the biocatalyst is bound to the anode surface (biofilm-anodes). However, we demonstrate that electric current can be generated from microbial cellulose degradation. The concept is a first step to the utilization of insoluble macromolecular biomass components for electricity generation in MFCs.

Major issue to be solved for practical application is to overcome the activity loss and incomplete cellulose fermentation – illustrated by the fast current decreases shown in Fig. 2. Such activity losses are typical for bacteria of the *Clostridium* family and are most likely caused by the increasing concentrations of acidic fermentation products, leading to changes in the fermentation path away from hydrogen synthesis towards alcohol generation and finally to the inhibition of further substrate decomposition (Desvaux *et al.* 2000; Hawkes *et al.* 2002; Valdez-Vazquez *et al.* 2004). Such change of the metabolic path was observed by HPLC fermentation product analysis, revealing an increased ethanol production.

In order to increase the current and power output and to achieve reasonable conversion efficiencies, further work will have to be emphasized on achieving a more complete cellulose consumption. This may for instance be achieved by utilizing chemostatically controlled semi-batch or flow conditions.

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