



Heat treated soil as convenient and versatile source of bacterial communities for microbial electricity generation

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Abstract

Bacterial communities from heat treated soils are shown to be convenient and versatile biocatalysts for hydrogen mediated microbial electricity generation. The isolation of spore forming species by heat pre-treatment allows the growth of robust hydrogen producing bacterial consortia with which electricity can be generated from a large variety of substrates, including complex carbohydrates. The fermentative hydrogen is efficiently oxidized in the microbial medium at electrocatalytic electrodes coated with platinum – poly(tetrafluoroaniline) bilayer (Pt-PTFA) composites. Coulombic yields up to 30% with respect to the maximum biological hydrogen yield of 4 moles H₂/glucose unit, and maximum current densities of 170–200 mA L⁻¹ were achieved by using chronoamperometric batch and semi-batch experiments.

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1. Introduction

In microbial fuel cells living microorganisms are used as biocatalyst to produce electricity. The electron transfer from the microbial cell to the fuel cell anode can be achieved either directly or indirectly. The direct transfer generally requires the physical contact of the organisms (in most cases attached to the electrode in the form of a biofilm) to the fuel cell anode together with the capability of the organism to directly conduct the electron transfer. An increasing number of microorganisms are so far known to accomplish this transfer [1,2]. Although such a transfer mechanism seems elegant and appealing, the requirement of the physical contact of the involved cells to the electrode limits the achievable density of active cells and thus the achievable power density. As an example, current densities as low as 3 $\mu\text{A cm}^{-2}$ have been reported for a microbial

fuel cell based on biofilm forming iron(II) reducing microorganism *Rhodospirillum rubrum* [3].

Bacteria that are either not capable of using such direct electron transfer route, or that are simply not in physical contact to the anode, e.g., due to an increasing thickness of a biofilm, however, require an indirect electron transfer mechanism that involves electron carriers – so called mediators. The rather impractical concept of using artificial mediators [4] has been abandoned, but many naturally occurring or microbially synthesized compounds can serve as electron carrier. Thus, in benthic fuel cells, the sediment contained iron or sulphide ions are known to facilitate the electricity generation [5]. As recently found, microorganisms even produce their own electron mediators that can be exploited to enhance the electron transfer in microbial fuel cells [6,7].

Reduced metabolic products like ethanol and formate and particularly hydrogen, are excellent electron carriers, provided electrocatalytically active anodes are used that facilitate the metabolite oxidation. As we have shown, microbial fuel cells that are based on the direct oxidation of microbial hydrogen at such highly electrocatalytically

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active and yet robust Pt-polyaniline (or Pt-poly(tetrafluoroaniline) sandwich electrodes yield high current densities [8,9] and allow great versatility with respect to the biological systems and the exploited substrates. Thus, we demonstrated the utilization of complex carbohydrates like starch and cellulose as substrates in microbial fuel cells by exploiting the dark fermentative hydrogen production of different saccharolytic strains of the *Clostridia* genus [10,11]. We further demonstrated the utilization of purple bacteria [12] (photo-fermentation) and green algae [13] (photosynthesis) for microbial electricity generation. The advantage over biofilm based MFCs certainly is the rapid operational readiness of the fuel cell, since no film formation is necessary. The limited coulombic efficiency of dark fermentative hydrogen production can be overcome either by combining dark fermentation with other biological systems, e.g. based on photo-fermentation [12], or by improved fuel cell anodes that allow the additional electrochemical oxidation of reduced organic fermentation products like formate and ethanol [14].

So far, our studies were based on the use of pure bacterial cultures, which allowed high reproducibility and a comparably low degree of complexity – important prerequisites for basic research. Such pure cultures, however, are unsuitable for technical application. Necessity for highly sterile conditions, culture degradation and the costs for purchase are only some points to list. Mixed cultures, or microbial consortia, have been shown to be much more robust and more productive than single strains [15], and their extraction can be easily achieved from natural sources. This applies not only to microbial fuel cells but also to biohydrogen generation [16–18]. Thus, for example, soils and sewage sludge accommodate a great variety of hydrogen producing anaerobic microorganisms, which, however, live in community with hydrogen consumers like methanogens that dissipate the excreted hydrogen [19,20]. In order to extract hydrogen from such communities the hydrogen consumers have to be either eliminated or inhibited [17,21–24]. Heat treatment is a simple and yet effective way that is based on the elimination of all vegetative cells, which leaves the spores of, e.g. hydrogen producing microorganisms like *Clostridia* as the source for germination [25].

For this communication we have studied heat treated soils as inoculum for hydrogen mediated microbial fuel cells. We show that the performance is comparable to the use of highly productive isolated bacterial strains. The greatest advantages are the flexible substrate utilization and the simplicity of handling, including the ease of storage, no problems with strain degradation, no pre-culturing and, last but not least – soils are available everywhere, free of charge.

2. Experimental

2.1. Electrochemical instrumentation and setup

All electrochemical experiments were carried out under potentiostatic control, utilizing a three electrode arrange-

ment consisting of the working electrode, a Ag/AgCl reference electrode (sat. KCl, Sensortechnik Meinsberg, Germany, 0.195 V vs. SHE) 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 -Autolab II, PGSTAT 20 and PGSTAT 30 Autolab systems (Ecochemie, Netherlands). The PGSTAT 30 potentiostat was equipped with five additional array modules allowing the simultaneous investigation of up to six working electrodes in connection with one reference electrode and one counter electrode. Sealed and thermostated fermentation vessels (100 mL) served as electrochemical cells which hosted the fermentation medium and the electrodes.

2.2. Electrode preparation

As the working electrode we used platinum-poly(tetrafluoroaniline) electrodes (in the following abbreviated as Pt-PTFA) that had proved excellent electrocatalytic activity in our previous study [9]. For the electrode preparation the substrate (platinum net electrodes, 5×10 cm sized, with 1 mm mesh size and platinum sheets, 7.5×20 mm, 0.5 mm thickness contacted with a platinum wire) were first electrochemically platinized at a potential of -0.6 V for 500 s in a stirred acidic solution containing 20 mM H_2PtCl_6 (Fluka). The subsequent deposition of the poly(2,3,5,6-tetrafluoroaniline), PTFA, was performed by electrochemical polymerization at 1.6 V, for 1000 s, from an aqueous solution containing 50 mM tetrafluoroaniline, 0.1 M NaClO_4 and 2 M HClO_4 as described in [9].

2.3. Metabolic analysis

Metabolic substrate consumption and non-gaseous fermentation product formation were followed applying HPLC analysis. The HPLC (Knauer, Berlin, Germany) was equipped with a Rezex[™] ROA-Organic Acid column in combination with the SecurityGuard[™] cartridge AJO-4490 (Phenomenex[®], Aschaffenburg, Germany). The chromatograms were recorded at room temperature with 0.005 N sulphuric acid as the eluent; the detector was a differential refractometer.

The monitoring of the hydrogen content in the headspace was achieved with hydrogen fuel cells (Fuel Cell Junior H_2/Air , h-tec, Germany), connected to the gas outlet of the cell. The cells were operated in short circuit, and the current was measured with a Keithley Integra 2700 digital multimeter (Keithley Instruments Inc., Cleveland, USA) interfaced to a personal computer. To ensure that all hydrogen that may have escaped into the headspace is recorded, the headspace was flushed with nitrogen at the end of the electrochemical measurements. The cathode compartment was continuously supplied with moistened air by a fish pump.

2.4. Chemicals

2,3,5,6-Tetrafluoroaniline was purchased from Across Organics and was used without further purification. All other used chemicals were of analytical or biochemical grade. The beet molasses was kindly provided by Hansa Melasse Handelsgesellschaft mbH, Hamburg, Germany. Its saccharose content was 47%.

2.5. Soil pre-treatment

Soils used for this study were garden compost and foliage-dung soil (Botanical garden, University of Greifswald). All soils were sampled from a depth of 50 cm. The samples underwent a shock heat treatment as described by Logan et al. [26]. For that, the soil was dried in a petri dish at 110 °C for 2 h in order to eliminate all vegetative cells. Then the soil was sieved to remove coarse particles and was stored in sealed plastic bags at 4 °C.

2.6. Substrate solutions and inoculation

A standard growth medium, which contained 2 g NH_4HCO_3 , 3.6 g KH_2PO_4 , 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g NaCl, 0.01 g $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.01 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.015 g $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ and 2.8 mg FeCl_2 per litre was used for all experiment. In some cases peptone (3 g L^{-1}) and yeast extract (1 g L^{-1}) was added to the mineral medium [19]. All substrates (cellulose, starch, molasses, saccharose, lactate, glucose) were added to the solution to reach a concentration of 5 g L^{-1} .

Two grams of the heat treated soil was used to inoculate the substrate solutions. Prior to that the sterilized substrate solution was purged with nitrogen for 20 min to remove oxygen.

3. Results and discussion

Fig. 1 shows the plots of the redox potentials, measured at Pt-PFTA electrodes in different substrate solutions, as a measure of the development of the metabolic activity of the bacterial culture over time. All solutions were freshly inoculated with heat treated soil.

The initial redox potential of the freshly inoculated substrate solutions ranged between 350 and 400 mV (with the exception of the starch solution). Despite of the different substrates (glucose, molasses, starch, cellulose) all solutions show a similar feature – the relatively steep potential drop after approximately 15 h. The only exception is the medium based on glucose – the microbially most easily utilizable substrate. Here, the potential drop is observed after already 8 h. After approximately 19 h, the potentials measured in the cultures growing on glucose, molasses and starch drop to about –480 to –490 mV. As to be expected, the growth medium with least accessible substrate, cellulose, reaches this level only after thirty hours. The potential drop originates from the starting fermentative hydrogen production

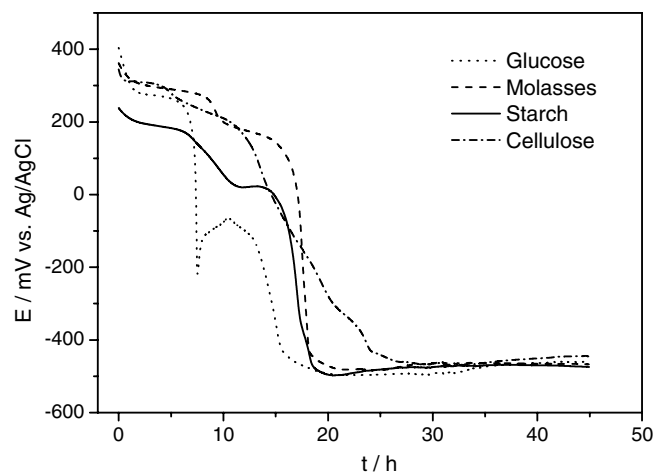


Fig. 1. Redox potentials measured in different substrate solutions, freshly inoculated with heat treated compost soil. The substrate concentration was 5 g L^{-1} . The sensing electrodes were Pt-PFTA electrodes. The experiment was carried out at 37 °C.

of the growing microbial culture that leads to an increasing hydrogen partial pressure and a thus shifting ratio of the redox couple $\text{H}_2/2\text{H}^+$. A constant potential is expected to be reached when the hydrogen concentration approaches the solubility of the gas in the electrolyte solution and the further produced gas is left into the solution headspace. In water the solubility of hydrogen is 0.73 mM (for $\vartheta = 37 \text{ }^\circ\text{C}$, $1 \text{ atm}_{\text{H}_2}$ [27]); using the Nernst equation the redox potential for a hydrogen saturated solution, 37 °C, pH 7, can be calculated to be –462 mV (vs. Ag/AgCl). This value lies 30 mV above the experimental potentials, a difference that can be explained with an over-saturation of the produced hydrogen in the solution. The oversaturation may arise from the high biological hydrogen production rate in combination with an impeded bubble nucleation or mass transport to the bubbles that do not allow the concentrations between the solutions and the above gas phase to equilibrate. When the biological activity has faded after 30–35 h due to substrate exhaustion (see Figs. 1 and 3) the equilibration is allowed to take place and the potentials in all solutions reach values between –460 and –465 mV, which is in good agreement with theory.

We have already demonstrated the excellent electrocatalytic properties of Pt-PFFA electrodes towards hydrogen oxidation in pure cultures of *Escherichia coli* [9,11], *Clostridium butyricum* and *Clostridium beijerinckii* [11]. As the chronoamperometric plots in Fig. 2 illustrate this also applies to soil based bacterial mixtures. This figure shows a comparison between a platinum electrode and a Pt-PFTA electrode. As expected from our previous studies the Pt-PFTA sandwich material shows superior performance. Regeneration procedures are not required to maintain the electrode activity.

In Fig. 3a chronoamperometric batch and semi-batch experiment using soil inoculated saccharose substrate solutions are presented. The main figure shows the typical current curve of freshly inoculated batch systems. The lag time

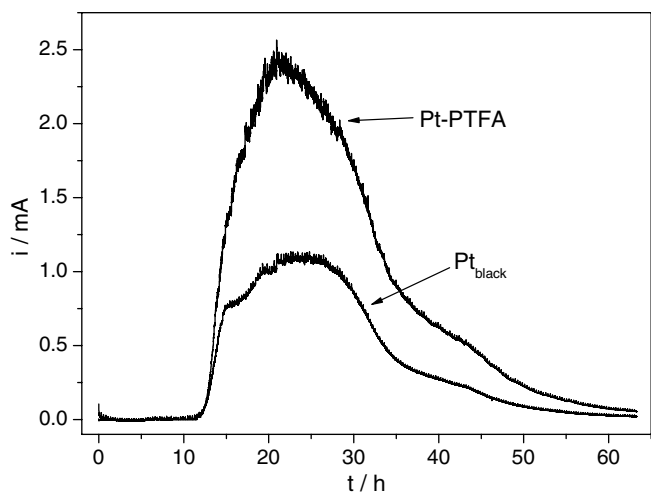


Fig. 2. Chronoamperometric batch experiment performed in a glucose medium (5 g L^{-1}), freshly inoculated with heat treated compost soil. The working electrodes were a Pt black electrode and a Pt-PTFA sheet electrode. The electrode potential was 0.2 V . The experiment was carried under stirring, at 37°C .

was shorter than 10 h , followed by a sharp increase of the oxidative current to a peak current of 18.5 mA and the subsequent decrease due to substrate depletion. As shown in the inset figure for the example of the semi-batch experiments, the replenishment of exhausted substrate leads to an almost instantaneous recovery of the current without significant lag times. The integration of the current over time of the chronoamperometric batch experiment (Fig. 3, main graph) yields 630 C . Based on the assumption that the current exclusively originates from the oxidation of hydrogen this electric charge would correspond to a yield of 2.4 moles of hydrogen per mole saccharose (i.e. 1.2 moles of hydrogen per mole glucose). The monitoring of

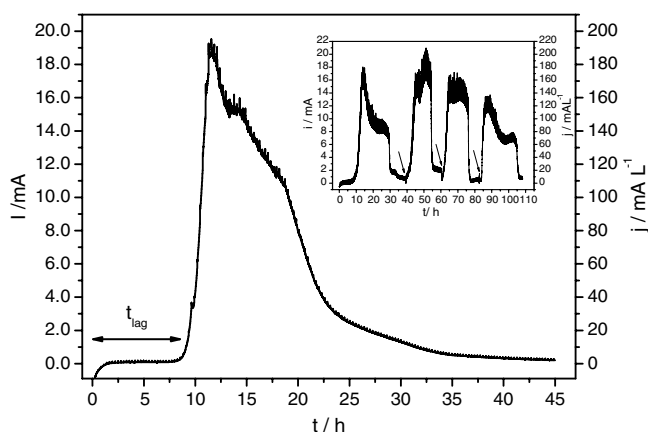


Fig. 3. Chronoamperometric batch experiment (main figure) performed in a molasses medium (10 g L^{-1}), freshly inoculated with heat treated compost soil. The working electrode was a Pt-PTFA mesh electrode, $5 \times 10 \text{ cm}$ sized, mesh size 1 mm . The electrode potential was 0.2 V . The experiment was carried under stirring, at 37°C . Inset figure: Fed batch experiments under the same conditions as in the main figure. Indicated by arrow, 80% of the bacterial medium was replaced by fresh substrate solution.

the gaseous fermentation products exhausting from the gas outlet of the electrochemical cell did not show measurable amounts of hydrogen, which indicates a complete oxidation of the microbial hydrogen in the bacterial solutions.

The right y -axes in Fig. 3 represent the current densities related to the volume of the substrate solutions. Such normalisation is generally used as a measure to describe the performance of bioreactor systems like microbial fuel cells. Table 1 lists the respective current densities for various substrates. Molasses, glucose and sucrose show the best performance with current densities of up to 200 , 180 and 170 mA L^{-1} , respectively. With the here used soils and the growth medium composition the current densities for cellulose are considerable lower than reported in our previous study [10,11].

Clearly visible in all figures is the occurrence of a lag time after inoculation – before the currents begin to flow. This lag time is typical for all microbial fuel cells when they are set up and inoculated with a biocatalyst – be it in the form of single bacterial strains or consortia. The microorganisms are then required to reproduce and, in many cases, start forming biofilms at the anode. The biofilm formation has been reported to take between 6 and 15 days until a stable current generation was obtained [3,28,29]. In some cases enrichment procedures with durations of 5 months have been proposed [30]. Such long start-up times may be disadvantageous when a fast operational readiness is required. Even without such film formation, however, every bacterial culture that is transferred into a new environment requires a certain time span for adaptation before the cells start reproducing and generating electricity. With the pre-cultured strains of different fermenting bacteria (e.g. *E. coli* K 12 [8], *Clostridia* species [10,11]) that we used in our previous studies the lag times were usually between four and twenty hours. For the heat treated soil inoculum we expected considerably longer lag times, since the remaining bacterial spores need to germinate and form vegetative cells. As our experiments show, however, this is not the case. Lag times between 8 and 15 h are comparable to our previous studies and are considerably shorter than reported for biofilm based microbial fuel cells.

Fig. 4 shows the results of a chronoamperometric batch experiment performed in a soil inoculated glucose medium. The figure correlates the current generation with substrate

Table 1

Peak current densities determined from chronoamperometric batch experiments as described in Fig. 3

Substrate	Current density (mA L^{-1})
Glucose	180
Saccharose	170
Molasses	200
Starch	130
Cellulose	40

All substrates were used in initial concentrations of 5 g L^{-1} (The initial molasses concentration was 10 g L^{-1}); the inoculum was heat treated compost soil.

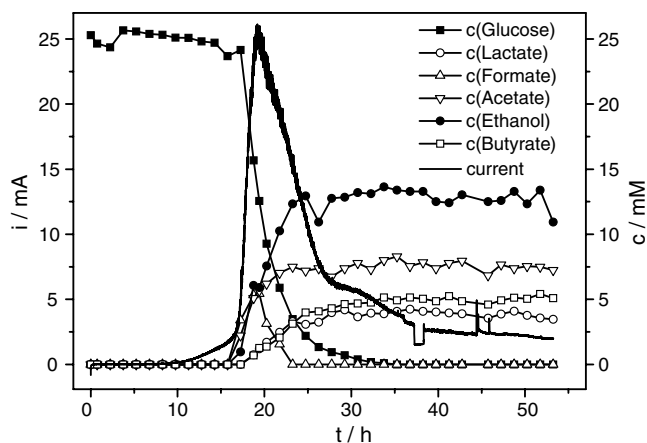


Fig. 4. Chronoamperometric batch experiment (main figure) performed in a glucose medium (5 g L^{-1}), freshly inoculated with heat treated foliage/dung soil. The working electrode was a Pt-PTFA mesh electrode. The electrode potential was 0.2 V . The experiment was carried under stirring, at $37 \text{ }^\circ\text{C}$. Right axis: Concentrations of the substrate (glucose) and of the non-volatile fermentation products as determined with HPLC.

exhaustion and fermentation product formation. Clearly visible is the close connection between the decrease of the glucose concentration and anodic current. The maximum of the current curve coincides with the maximum of the glucose consumption rate (derivative $-\Delta c/\Delta t$, not shown). Also depicted in Fig. 4 are the concentrations of the organic fermentation products, as determined via HPLC. The analysis of the fermentation products can give information on the bacterial genera that grow in the medium and may be involved in the current generation processes. It thus represents an important tool for the investigation of the microbial ecology and how it may be affected by electrochemistry. This analysis, however, is currently in full process.

4. Conclusions

Soil accommodates a great variety of hydrogen producing microorganisms. In this study we have shown that after heat treatment it can be used as a convenient and versatile source of bacterial mixed cultures for electricity generation in microbial fuel cells. Extensive and careful culturing of the biocatalyst becomes obsolete, strain degradation is not observed. Further studies, however, are required and currently undergoing, to investigate the structure of the bacterial communities, to uncover and reduce substrate scavenging microbial side processes and to increase the overall energetic and coulombic efficiency.

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