

# Biofilm Based Microbial Fuel Cell

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## ABSTRACT

Biofilms of natural anaerobic microbial consortia externally grown on Gas Diffusion Electrodes (GDL) in a bioreactor, were investigated within a Membrane-Electrode-Assembly with respect to lifetime and electricity production upon variation of biodegradable materials with an oxygen demand of 1000mg/l as simulated waste water. A remarkable differentiation and plasticity of the films is observed, to resist the toxicity of 0.5 mg/cm<sup>2</sup> Pt used on the inner side of the anode for smooth hydrogen combustion and to recover bioactivity upon alteration of the biodegradable material. Such robust biofilms are intended to provide a new technology for water purification and electricity production from industrial and community wastewater.

**Keywords:** microbial fuel cells, water purification, electricity

## 1 INTRODUCTION

Opposite to Polymer Electrolyte Membrane fuel cells, PEM-FC, Microbial fuel cells, MFC's are not yet commercialized, although such fuel cells could generate electricity and pure water using ubiquitous bacteria and biodegradable waste, instead of expensive catalysts and hydrogen or synthetic fuel generated by electrolysis or petrochemical processes [1].

Future utilisation of bacteria for energy production is not limited to MFC's; devices like e.g., a bioelectrochemically assisted microreactor, BEAMR, or Bacterial Electrolysis BE, offer further possibilities for Hydrogen or electricity production utilising the electron transfer processes in bacteria and organic materials as "fuel" [2].

The working principle of a MFC is to offer the bacteria externally a better acceptor for the electrons transferred during metabolic processes as compared to the internal electron acceptor. In such case, the electrons can be used for electricity generation. The device to be build must ensure a close contact of the enzymatic active centre of the bacteria with some means for electron transfer and an electro acceptor in close contact. As shown in Fig. 1, such an environment is provided by a Membrane Electrode Assembly used in PEM-FC, because the electrode materials are made of carbon, with a high degree of biocompatibility. The biofilm is at the anode, air oxygen is the electron

acceptor, water is the reaction product at the cathode, CO<sub>2</sub> at the anode.

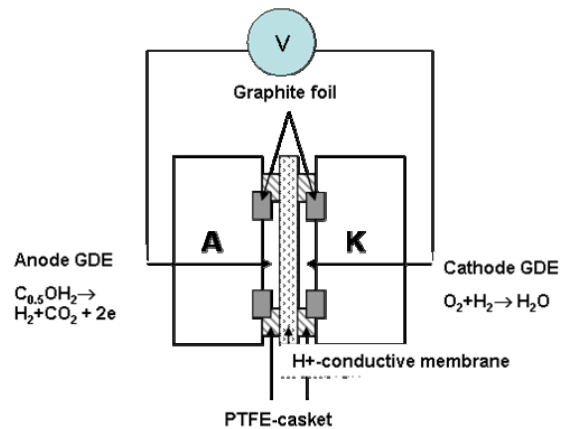


Figure 1: MFC-set up

The difficulty of MFC's, BEMR or BE technology as compared to direct methane production from biomass [3], is based on the lack of deep knowledge about the physiology of bacterial consortia directly interfaced with an artificial, technical system. The variety of communication among such consortia is remarkable: some evidence is given for "wireless" and "wired" communication as well as for processes called "quorum sensing", with partial destruction of the biofilm under stress [1,4].

Therefore, the study presented in this paper is focussed on the investigation of the electricity production as an indicator of plasticity and differentiation within a biofilm-based natural bacterial consortia upon fuel cell operation with varying biodegradable materials as fuel.

## 2 EXPERIMENTAL

### 2.1 Biofilm growth kinetics

The biofilms were grown in a media consisting of 5 g yeast extract, 10 g Pepton from Casein, 5 g NaCl per 1 l de-ionized water. All parts of the reactor were steril (20 min at 121 °C in an autoclave). Sterile filters (0,2 µm pore size, Sartorius) were used in all silicone tubing. The apparatus is shown in Fig.1. Two different substrate materials based on carbon, 2x2 cm<sup>2</sup> or 0,5x2 cm<sup>2</sup> in size were used: Gas Diffusion Electrodes, GDE commonly applied for Polymer

Electrolyte fuel cells (E TEK, 0,5 mg Pt/cm<sup>2</sup>, type A-6-ELAT-SS) and Gas Diffusion Layer, GDL (GDL 10 BB, SGL, Germany). The growth of bacteria in the media was followed by pH-measurements, Optical Density (OD) measurements, (1 OD = 8\*10<sup>8</sup> cells/ml), and by optical microscopy after staining (BacLight Bacterial Viability Kit, Molecular Probe). The biofilms were characterized by fluorescence confocal laser microscopy, living cells were stained with CFDA (5-Carboxyfluorescein-diacetate), defect cells by Propidium iodide (PI). In addition, cryo-SEM was used to see the roughness of the biofilm on the electrodes.

## 2.2 Membrane Electrode Assembly Measurements

Voltage-current measurements with the MEA shown in Fig. 1 were performed under Nitrogen atmosphere with IM6e, Zahner, under constant feed flow. As biodegradable substance acetate, glucose and starch solution were employed, with 1000mg/l CSB (chemical oxygen demand). Gas analysis of the liquid feed at the anode GDL was performed by GC (Agilent 6890).

## 3 RESULTS

The biofilm growth kinetic is characterized by thickness measurements of the film using fluorescence laser scanning microscopy. As shown in Fig 2, different bacteria are present, mainly belonging to Clostridia. The red stain indicates living bacteria.

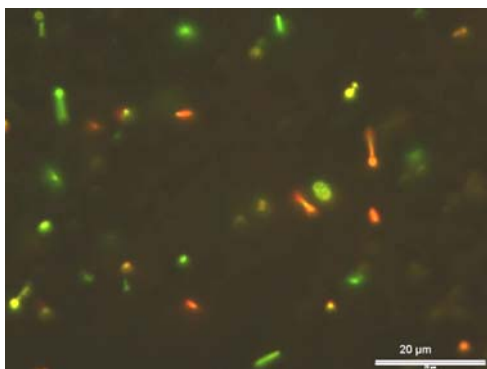


Figure 2. Laser fluorescence image of a typical biofilm: red stain indicates living bacteria

The thickness measurements are related to the electrode material used and to the duration of biofilm growth, as shown in Fig. 3. and Fig.4.

It is clearly seen, that even a very small amount of Pt inhibits significantly the biofilm growth. The biofilm bacteria grow predominantly at the opposite side on the carbon fibre composite, in large colony, as also seen from the cryo-SEM image in Figure 4.

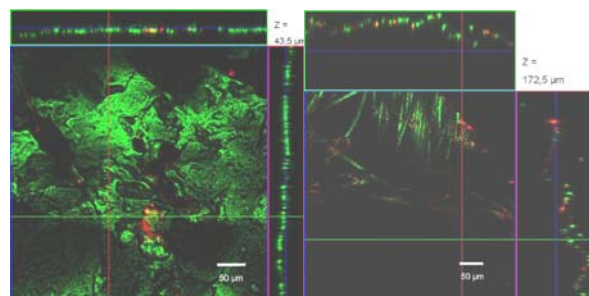
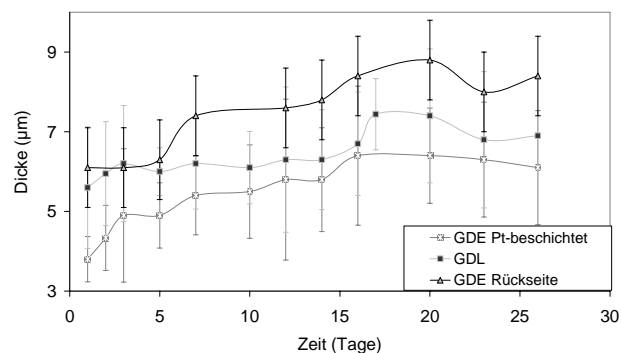


Figure 3: Average biofilm thickness as function of growth time and carbon substrate material (top); fluorescence image of morphology and density of living cells (red stained)

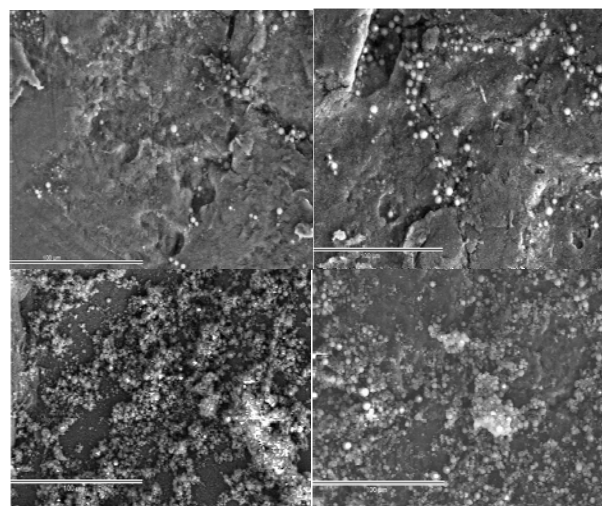


Figure 4: Cryo-SEM image of the Pt-side of a biofilm-GDE-anode after 2 days (right) and 1 week (top) as compared to a GDL as substrate: the amount of bacteria colonies is significantly depleted at the Pt-side of the GDE and increased at GDL as substrate, 100 μm scale bar

Roughness measurements of the substrate indicate, that the depletion at the Pt-containing side of the anode GDE could also be caused by the smoother surface and reduced number of large pores at this side of the electrode. Biofilm

However, the bioactivity of such Pt-containing GDE-anode in the MEA-measurement is still significantly higher as compared to a biofilm grown on a Pt-free GDL, as shown in Figure 5.

One can assume, that the contact between the bacterial enzyme system and the electrode is comparably good in both cases, but in the presence of Pt the hydrogen produced by the bacteria undergoes faster “cold combustion” as compared to the Pt-free system, therefore the overall reaction yield is higher.

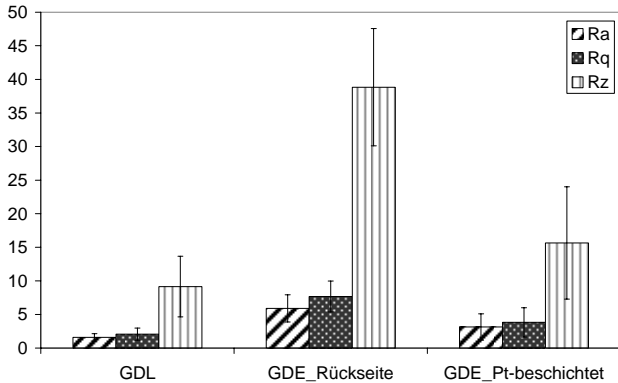


Figure 5: Roughness of the substrates for biofilm growth

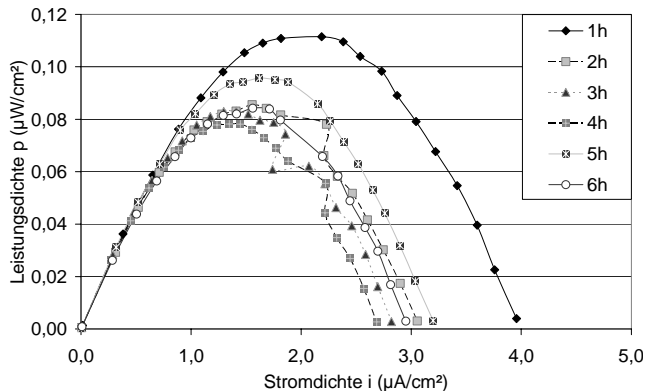


Figure 6: Power production of a biofilm as function of time elapsed from media change, feed contains 1000 mg/l acetate

The MEA measurement reveal, that it needs several hours for the biofilm-bacteria to accommodate their bioactivity to a changing biodegradable organic substrate. The biofilm is grown on the anode in a different media as compared to the one used in the MEA-measurement. At the beginning of the measurement, some residue of this media is still present in the porous substrate material, therefore bioactivity is high, as reflected by the voltage-current curve shown in Figure 6. As soon as the bacteria “recognize” the change in media, their activity drops significantly. After a certain period of time, it “recovers”, therefore we assume, that some reorganization of the microbial colonies has taken place.

This behavior is independent of the feed used, however the time and extend of recovery differs, as shown in Figure 6 for acetate and figure 7 for glucose and starch. Because the concentration of the biodegradable material was kept constant in each case with respect to the CSB, a direct comparison is justified.

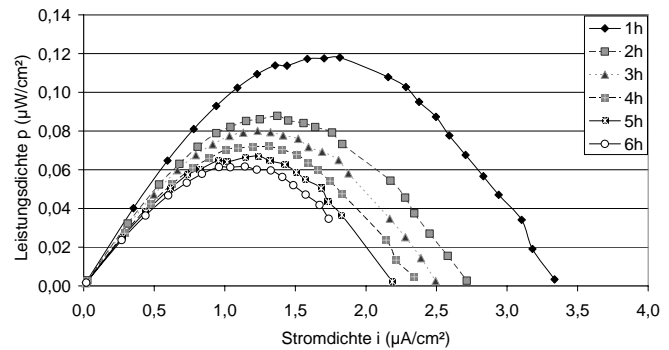
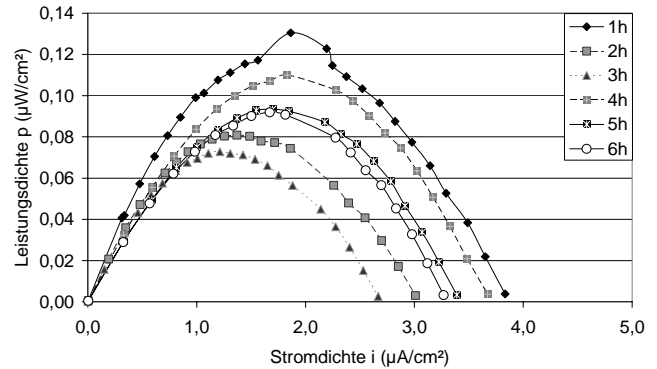


Figure 7: Power production of a biofilm as function of time elapsed from media change, feed contains 1000 mg/l glucose (top) or starch (bottom)

The results of gas analysis measurements in the anode-liquid confirm bacterial metabolism as the source of electricity production, as shown in Table 1.

	CO <sub>2</sub> der Gasphase (ppm)		H <sub>2</sub> der Gasphase (ppm)		CH <sub>4</sub> der Gasphase (ppm)	
Glukose	672,67	x	x	x	38,43	x
Glukose in MBZ	731,80	739,47	x	1,93	40,41	39,56
Glukose GDL MBZ	716,10	666,56	x	1,71	39,95	39,29
Stärke	601,45	x	0,75	x	41,91	x
Stärke MBZ	740,81	906,49	x	2,63	43,24	40,57
Stärke MBZ 4 W	771,99	934,30	x	1,26	40,34	39,22
Acetat	1723,25	x	x	x	38,19	x
Acetat MBZ	862,34	785,86	x	2,18	39,29	38,52
	1h	4h	1h	4h	1h	4h

x = nicht gemessen

Table 1: GC-gas analysis results at the anode liquid.

More quantitative measurements as well as a full analysis of errors upon MEA-measurements have been reported in [6]. In future, membrane materials will be introduced, which have an increased biocompatibility as compared to the ones used in the present study [7,8], in order to increase the bioactivity of the biofilm at the inner side of the anode and study the maximum possible energy output but releasing the limitation of biodegradable material to 1000mg/l, which was intentionally applied in the work reported here.

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