Studies into design and operation of microbial fuel cells using oxygen gas diffusion electrodes

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Abstract
The use of microbial fuel cells (MFCs) represents a new concept to generate energy by anaerobic biological waste water treatment. Using oxygen gas diffusion electrodes (GDEs) can facilitate the cell design since proton exchange membranes and a cathode chamber are not required. In this laboratory scale study, different GDEs were investigated. GDE type 1 with silver as electro-catalyst and type 2 with carbon nanotubes as electro-catalyst showed the best performance under the chosen conditions. Power density was affected by the electrode material and the availability of organic compounds (acetate, raw waste water). MFC operation resulted in *Geobacteraceae* spp. enrichment at the anode. In a long-term operation with GDE type 1 over ten weeks, biofilm formation also was observed at the GDE cathode, without negative impact on MFC performance. Our results emphasise the consideration of GDEs in up-scaling approaches.

Keywords
Microbial fuel cells; electricity generation; oxygen gas diffusion electrode; cathode

INTRODUCTION

Conventional waste water treatment plants have a high energy demand (Oh et al., 2010). Especially small plants often do not operate a denitrification or an anaerobic digester. In particular for this scenario, the use of microbial fuel cells (MFCs) might represent a promising approach to generate electricity directly from waste water (Aeltermann et al., 2006). Electrochemical processes in combination with biodegradation have already been reported for different applications, e.g. a combined bio-electro-process in which water electrolysis stimulates microbial chloroethene degradation (Lohner et al., 2009; Lohner et al., 2011). In MFCs, bacteria produce electricity from the oxidation of organic matter. One of the advantages of MFCs is the direct conversion of substrate energy into electricity; further, compared with sludge digestion, gas treatment is not necessary because the exhaust gasses are enriched with carbon dioxide. Furtermore MFCs would generate lower amounts of sludge, thus reducing sludge dehydration costs (Hernández-Fernández et al, 2015; Rabaey et al., 2005).

MFCs are typically designed as two-chamber systems with a proton exchange membrane to separate the bacteria-containing anode chamber from the cathode chamber (Figure 1a). In the anode compartment, an active biocatalyst oxidises the organic substrates and produces electrons and protons (Antonopoulou et al., 2010). Through the proton exchange membrane, the protons are conducted to the cathode chamber and the electrons are conveyed through the external circuit (Rahimnejad et al., 2011). In the cathode chamber, protons, electrons and oxygen react to water (Sharma et al., 2010). Two-chamber systems have the disadvantage that they use membranes. However, membranes can be a limiting factor, especially if fouling occurs due to suspended solid and soluble contaminants. Therefore, membranes are an essential cost factor for the construction of an MFC (Hu, 2008).

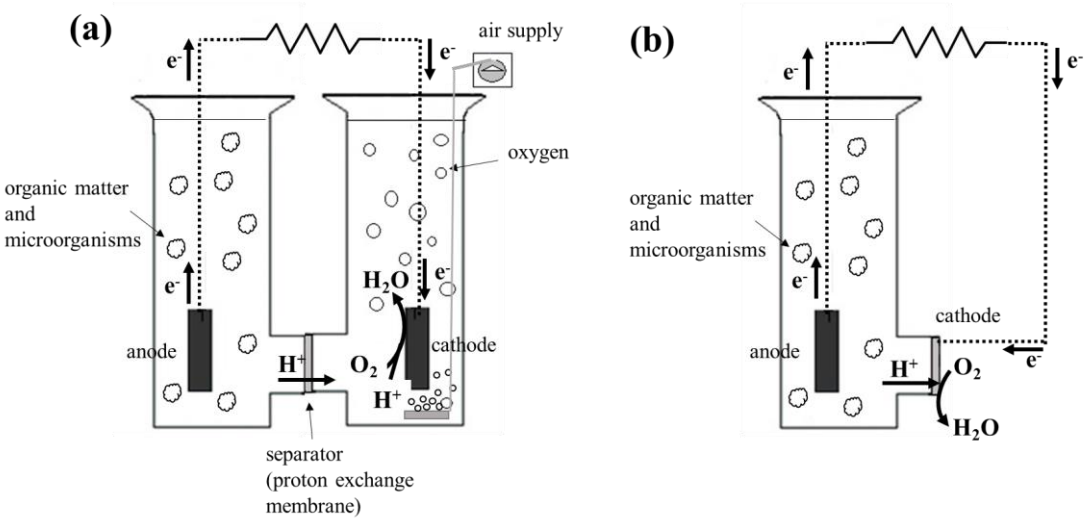


Figure 1. Scheme of a two-chamber MFC system separated by a membrane (a) compared to a one-chamber MFC system equipped with a gas diffusion electrode (GDE) (b).

To overcome high internal resistance from membranes, new designs of one-chamber systems with gas diffusion electrodes (GDEs) have been developed and are described in several studies (Santoro et al., 2011; You et al., 2011) (Figure 1b). GDEs are mounted to face one side of the electrode towards the electrolyte and the other side towards the gas phase. Usually, GDEs are used in non-biological alkaline and proton exchange membrane fuel cells (Horst et al., 2016). Up to now, GDEs have predominantly been applied in processes such as fuel cell and chloralkali electrolysis (Bulan et al., 2011). GDEs have advantages especially if a component from the gas phase is involved in the electrochemical reaction or when a desired gaseous component is developed at the electrode. GDEs are porous electrodes; the electrolyte can float from one side and the desired gas can diffuse into the electrode from the other side (Horst et al., 2016). The structure of the GDE represents a large three-phase boundary surface between solid catalysts, electrolyte and gas phase (Horst et al., 2016). A solid catalyst at this interface supports the electrochemical reaction between gaseous and liquid phase (Horst et al., 2016). Important parameters for GDE performance are porosity, hydrophobicity of the electrode material and used catalyst.

As molecular oxygen shows very low solubility in electrolytes, the resulting low oxygen availability can be a limiting factor for the cathodic reaction in MFCs. By using a GDE in MFCs, the mass transfer of oxygen is optimised, because GDEs can use oxygen directly from the air, thereby reducing energy consumption for aeration of the electrolyte. Another advantage of these electrodes is that an extra cathode chamber is not required.

In this study, we investigated electricity generation using different gas diffusion electrodes (GDEs) in a single-chamber MFC without a membrane. Apart from GDEs with carbon as electro-catalyst, we also tested a GDE with precious metal as electro-catalyst. Operation of GDEs was studied with synthetic media and waste water samples from a municipal treatment plant. Additionally, we conducted a long-term operation with the best-performing GDE over several weeks.

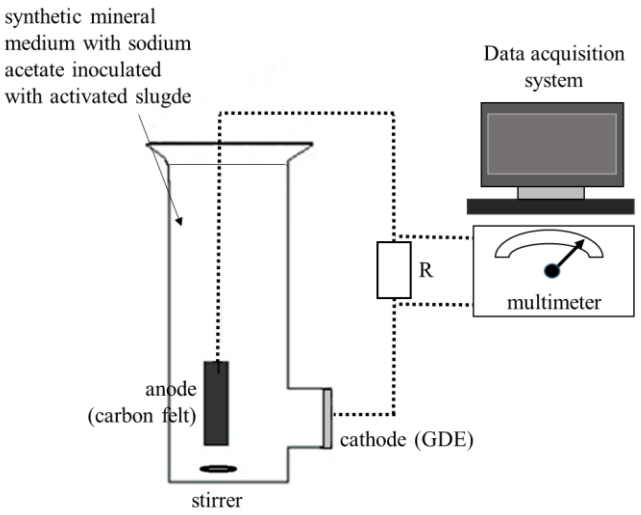
MATERIALS AND METHODS

Chemicals and field samples

Sodium acetate was purchased from Sigma Aldrich Co (analytical grade ≥ 99%). The mineral medium contained the following mineral salts (analytical grade, Merck, Darmstadt, Germany) per litre of demineralised water: 3.17 g KH_2PO_4, 5.68 g Na_2HPO_4, 0.12 g $MgHPO_4$ x 3 H_2O, 0.45 g $(NH_4)_2HPO_4$, 5 mL of trace element solution 1 (400 mg $FeSO_4$ x 7 H_2O, 40 mg $MnSO_4$ x 5 H_2O, 8

mg $CoCl_2$, 40 mg $ZnSO_4$ x 7 H_20, 40 mg $CuSO_4$ x 5 H_2O, 1.06 mg $CaCl_2$, 6 mg H_3BO_3, 8 mg Na_2MoO_4 x 2 H_2O and 2 mL of concentrated H_3PO_4 per litre) and 0.1 mL of trace element solution 2 (50 mg Na_2WO_4 x 2 H_2O, 50 mg Na_2SeO_3 x 5 H_2O and 250 mg of $NiCl_2$ per litre). The medium was adjusted to pH 7.2 ± 0.2, autoclaved at 121°C for 20 min and inoculated with activated sludge from a municipal waste water treatment plant (WWTP).
For waste water experiments, we used sludge liquor from the primary sludge of a WWTP.

Experimental setup
The experiments were conducted in batch cells made of glass under anaerobic conditions (Figure 2). As anode material, we used carbon felt (SIGRACELL® KFD2.5EA, SGL Group, Germany). The geometric area of the anode was 19 cm². Different gas diffusion electrodes with different catalyst materials based on precious metal or carbon were obtained from Covestro AG (Leverkusen, Germany) and tested as cathode material. The geometric area of GDE cathodes was 7 cm² and the distance between the anode and cathode was 6 cm. The electrodes were connected with platinum wire through a resistance decade (10 Ω -1 kΩ) and two multimeters. For tests with synthetic media, the electrolyte consisted of 1 L mineral medium containing 2 g/L sodium acetate inoculated with activated sludge from a waste water treatment plant (300 mL). Six different GDEs were used as cathodes and placed with direct contact to the anode chamber. GDE type 1 contained silver, GDE type 2 carbon nanotubes, GDE type 3 graphite, GDE type 4 carbon black, GDE type 5 carbon fibre and GDE type 6 glassy carbon as electro-catalyst. All experiments were conducted at room temperature (20°C) and atmospheric pressure.



tested GDE materials

	electrocatalyst
type 1	silver
type 2	carbon nanotubes
type 3	graphite
type 4	carbon black
type 5	carbon fibre
type 6	glassy carbon

Figure 2. Experimental set up of the single-chamber MFC system used in this study and list of the tested GDE materials.

Calculations
Voltage (V) across the external resistor (R) and current (I) in the circuit of the microbial fuel cell were recorded at 1 hour intervals using a multimeter (34411A, KEYSIGHT, Agilent Technologies) connected to a personal computer. Power density (P) was calculated according to the formula
$P = I * V$ and normalised by cathode projected area ($A = 7$ cm²).

DNA extraction and PCR analysis
For PCR analysis, 30 mL of process liquid were filtered through a 0.2 µm Supor-200 membrane filter (47 mm diameter) (Pall Life Science) at the beginning and the end of the experiment. Additionally, the anode material of the carbon felt was cut into small pieces (approx. 0.3 g) and the wet weight was determined (dry mass approx. 12 %) at the end of the experiment. The membranes

and pieces of carbon felt were stored at -20°C until DNA extraction and analysis. Total DNA was extracted directly from the membranes and the carbon felt by using the FastDNA® Spin Kit for soil (MP Biomedicals) according to the manufacturer´s instructions.
Geobacteraceae spp. and *Shewanella* specific DNA sequences as well as bacterial 16S rDNA were enumerated using quantitative real-time PCR (qPCR). We performed qPCR using RotorGene (Corbett) and 9 µL reaction mixture [1× SYBR SensiMixTM (Bioline), 0.5 µM of each primer and 1 µL of template] with a temperature program of 10 min at 95°C (initial denaturing and Hot Start Taq activation), followed by 40 cycles of 25 s, 95°C / 30 s at the annealing temperature / 20-50 s, 72°C (optical window on), followed by a final dissociation stage. Primers and annealing temperatures are listed in Table 1. All samples and standards were analysed in duplicate. Calibration was performed with serial dilutions of a known quantity of linearized plasmids containing according gene fragments. Amplification products were verified via QIAxcel Advanced system.

Table 1. Target genes, primer sequences and amplicon sizes for quantitative real-time PCR analysis

Target	Primer sequence (5'-3')	Amplicon size in bp	Reference	Annealing temperature in °C	Elongation time in s
16S rRNA gene of bacteria	F-CCT ACG GGA GGC AGC AG R-ATT ACC GCG GCT GCT GGC	160	Smits et al., 2004	58	20
16S rRNA gene of *Geo-bacteraceae* spp.	F- AGG AAG CAC CGG CTA ACT CC R- GGC ACT GCA GGG GTC AAT A	330	Holmes et al. 2002 (forward) and Cummings et al., 2003	50	20
16S rRNA gene of *Shewanella*	F- CGC GAT TGG ATG AAC CTA G R- GGC TTT GCA ACC CTC TGT A	1084	Todorova et al., 2006	55	50

RESULTS AND DISCUSSION

We tested GDEs with different catalysts as cathode material in synthetic media and without separate proton exchange membranes. After an adaption time of five days, the measured cell voltage increased and settled to a constant value for all tested GDEs (Figure 3). All investigated electrodes were able to generate electricity. Cell voltage was depending on the cathode material; GDE type 1 with silver as electro-catalyst and type 2 with carbon nanotubes as electro-catalyst showed the best performance under the chosen conditions.
In previous studies, using an air-cathode single chamber MFC with acetate or glucose, output voltages in the same order of magnitude were observed (Wang et al., 2013; You et al., 2011; Borole et al., 2009; Logan et al., 2007; Liu et al., 2005a; Liu et al., 2004). In most studies, platinum (Pt) was the most used and efficient cathodic catalyst for oxygen reduction (Santoro et al., 2013). The results of our study show that power generation in air-cathode MFC systems is also possible with different cathodic catalysts based on carbon or silver.

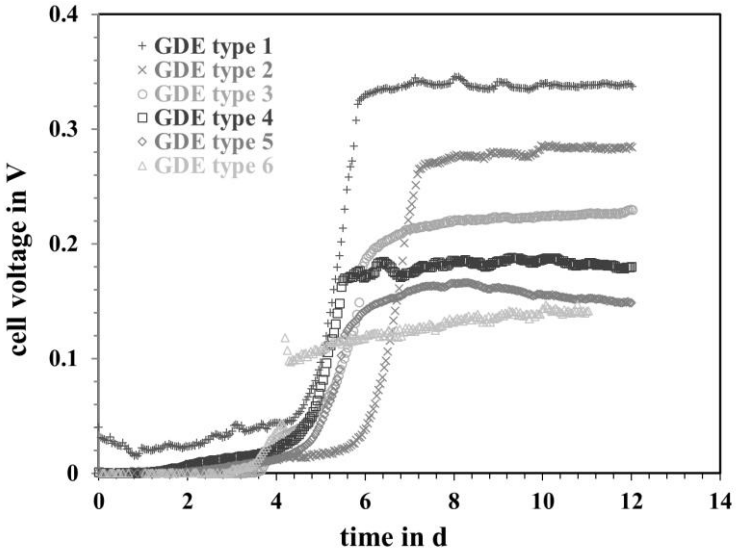


Figure 3: Cell voltage of six different gas diffusion electrodes (GDEs) with acetate (1000 Ω resistor) inoculated with activated sludge (30%).

Additionally, PCR analyses were conducted for all experiments with different GDEs. DNA sequences for bacteria species which are able to transfer electrons to electrodes (Reguera et al., 2005) were enumerated as well as the bacterial 16S rDNA. To compare the microbial communities, we determined the ratios of specific DNA sequences of *Geobacteracea* spp. and *Shewanella* to the total bacterial 16S rDNA (Figure 4). *Geobacteracea* spp. accumulated at the anode surface over the test duration, regardless of the GDE material (Figure 4a). The increasing proportion of *Geobacteracea* spp. correlated with greater cell voltage, indicating that *Geobacteracea* spp. influence electricity generation. However, DNA sequences of *Shewanella* were ≤ 1% compared to the bacterial 16S rDNA at the anode surface over the test duration (Figure 4b), indicating that this bacteria group grows poorly under the chosen conditions. Similarly, Chae et al. (2009) and Jung and Regan (2007) reported the dominance of various *Geobacter*-like species, but no detection of *Shewanella* sp. in their MFCs fed with acetate, glucose and lactate as substrates.

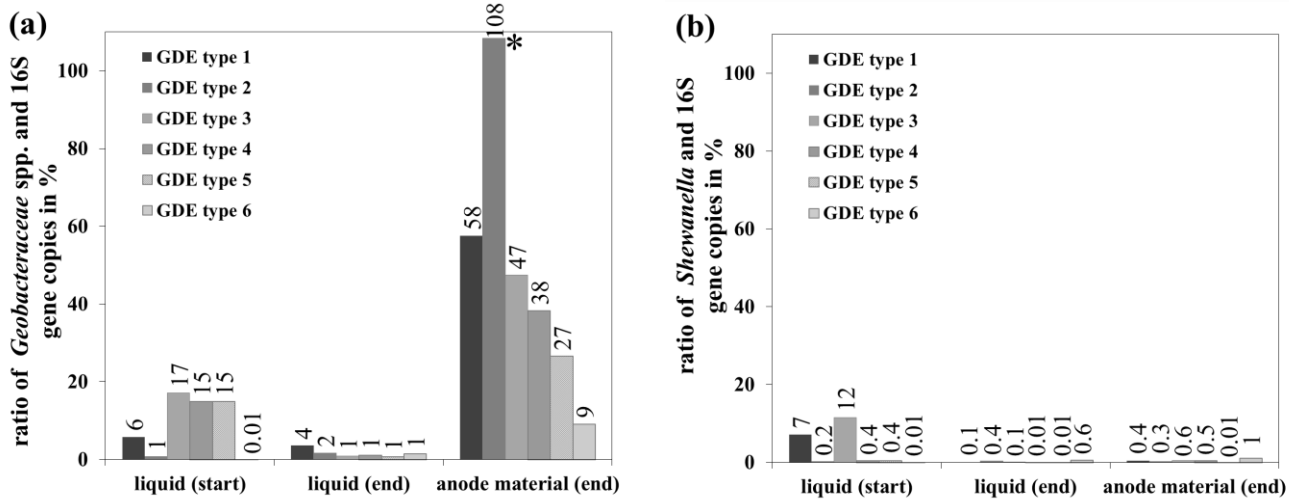


Figure 4. Ratio of *Geobacteraceae* spp. and 16S gene copies (a) and ratio of *Shewanella* and 16S gene copies (b) in process liquid at the beginning (start) and end (end) of the experiment as well as at the anode material at the end of the experiment. The microbial fuel cell (MFC) was operated with six different gas diffusion electrodes (GDEs); asterisk indicates longer test duration (1.5-fold) compared to the other experiments.

Because of the highest voltage output with GDE type 1, corresponding to Figure 3, we performed polarisation and power density curves as well as a long-term test. By changing the circuit resistance from 10 Ω to 10 kΩ, the polarisation curve of the MFC and a maximum power density of 353 mWm^{-2} at 2,245 mAm^{-2} (100 Ω), normalised to the cathode area, were obtained (Figure 5).
In this study, power generation was lower than in previous studies with novel cathode structures (501 mWm^{-2}; Santoro et al., 2011 and 766 mWm^{-2}; Cheng et al., 2006a). It is difficult to compare power output directly with other MFC performances in the literature due to different microorganisms involved. The main goal of this study was to investigate the effects of different cathodic catalysts (six different materials) on power generation and microbial communities in an air-cathode MFC system. For optimisation of MFC configuration, future studies should consider additional factors such as the distance between the electrodes (Cheng et al., 2006b), anode/cathode chamber configurations, MFC volume, pre-colonised anodes (Santoro et al., 2012), substrate types and solution conductivity (Sharma et al., 2010; Liu et al., 2005b).

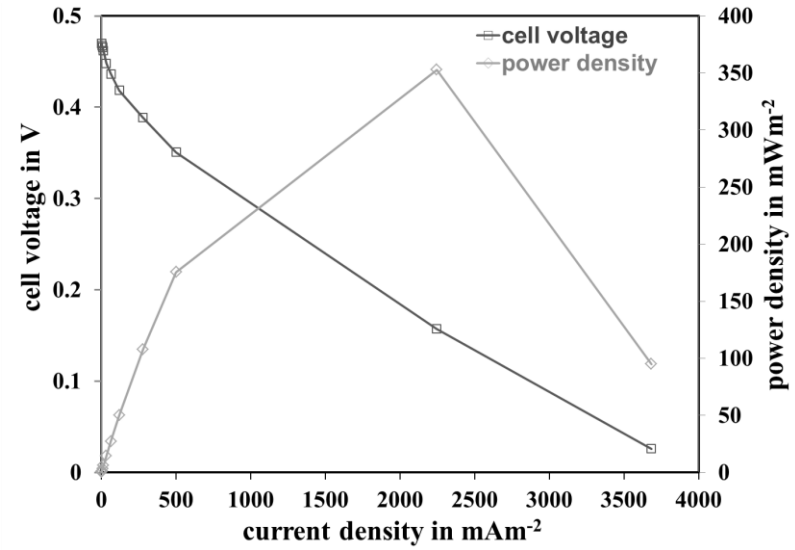


Figure 5. Polarisation and power density curves of MFC with GDE type 1 after 27 days.

In the long-term test, we observed constant power density over ten weeks (Figure 6). To avoid substrate limitations, a regular dose of sodium acetate (1 g/L) was added three times (arrows). Power density rapidly decreased after ceasing the feeding with sodium acetate. This experiment demonstrated that feeding has an important influence on the MFC output. The results are in accordance with previous studies (Chae et al., 2009; Wang et al., 2013). Most of the studies with air-cathode MFC systems were conducted in short operational periods (e.g. several hours or days). In this study, we monitored the performance of an air-cathode MFC system with GDE type 1 over several weeks. The increased biofilm at the inner cathode surface had no influence on MFC performance. The tested GDE material seemed very stable during MFC operation without a separation membrane. For practical application, e. g. in waste water treatment plants, using low-maintenance material represents an economic advantage.

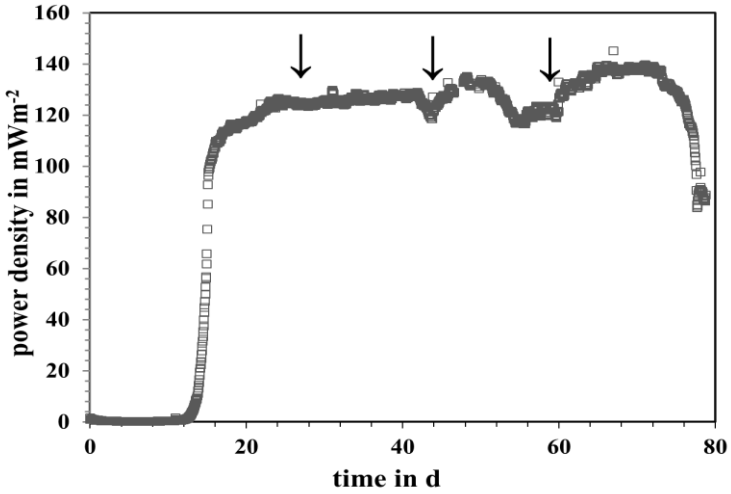


Figure 6. Power density in mWm^{-2} refers to cathode area for GDE type 1 (1000 Ω resistor); arrows indicate feeding with sodium acetate.

Additionally, an experiment with sludge liquor from primary sludge was conducted. Figure 7 demonstrates the power densities referred to the projected cathode area for the synthetic medium with sodium acetate and with sludge liquor. Compared to the use of synthetic medium, the elapsed time before power density increases was longer with sludge liquor. The generated power was approx. 50% lower than that with sodium acetate. This difference was probably caused by lower amounts of easily biodegradable organic substrates in the sludge liquor. The results revealed a direct correlation between power and substrate concentration and are in agreement with data from previous studies using raw waste water and waste water with added sodium acetate for MFC operations (Santoro et al., 2013). However, operation of an MFC with a complex substrate medium (sludge liquor) and GDE type 1 was possible.

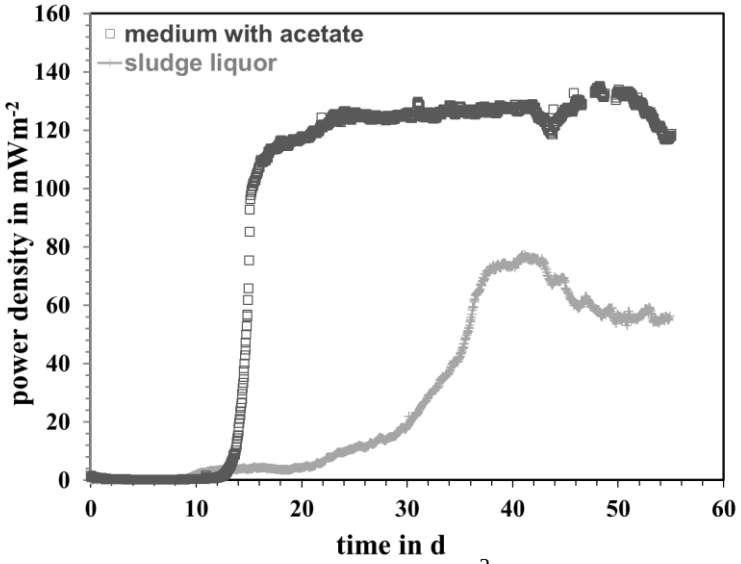


Figure 7. Power densities in mWm^{-2} referred to the cathode area for MFC operation with the synthetic acetate medium in comparison to the sludge liquor as real water matrices.

Additionally, PCR analyses of the waste water matrices were conducted to compare microbial communities. Figure 8 demonstrates the ratio of specific DNA sequences of *Geobacteracea* spp. and *Shewanella* to the bacterial 16S rDNA as described above. *Geobacteracea* spp. accumulated at the anode surface over the duration of the test with sludge liquor, similar to experiments with

synthetic media with acetate. *Shewanella* constituted less than 1% compared to 16S rDNA.

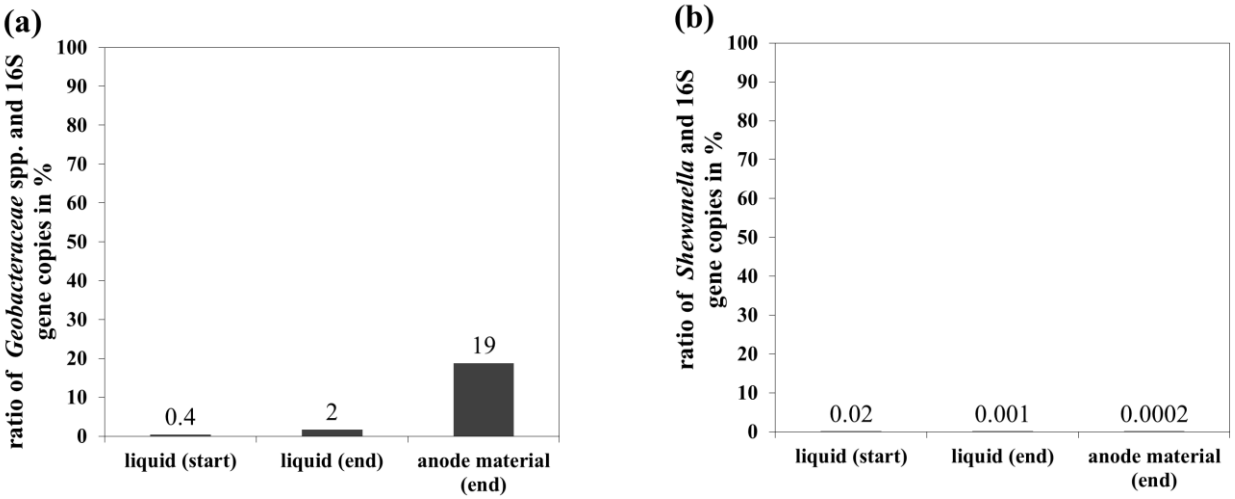


Figure 8. Ratio of *Geobacteraceae* spp. and 16S gene copies (a) and ratio of *Shewanella* and 16S gene copies (b) in process liquid at the beginning (start) and end (end) of the experiment as well as at the anode material at the end of the experiment. MFC was operated with sludge liquor and GDE type 1.

CONCLUSION

In this study, we compared six GDEs with different cathodic catalysts in an air-cathode MFC system. Our results demonstrate that electricity generation with all examined GDEs is possible. GDE type 1, with silver as electro-catalyst, showed the best performance in comparison of GDEs with carbon-based catalysts. During the test duration, a typical bacteria group for electron transfer, *Geobacteraceae* spp., accumulated at the anode, and gene copy numbers correlated to power generation of the different GDE materials. Bacteria belonging to the *Shewanella* group were also detected, but constituted less than 1% compared to the bacterial 16S rDNA. The availability of organic compounds affected electricity generation. In a long-term test over several weeks, biofilm formation was observed at the GDE with a cathodic catalyst of silver, without negative impact on MFC performance.

Based on these promising results, further studies are encouraged. In particular, tests in continuously operated flow cells and with optimised parameters, e.g. electrode distance, should be considered in future studies.

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