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Inocula selection in microbial fuel cells based on anodic biofilm abundance of *Geobacter sulfurreducens*

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ABSTRACT

Microbial fuel cells (MFCs) rely on microbial conversion of organic substrates to electricity. The optimal performance depends on the establishment of a microbial community rich in electrogenic bacteria. Usually this microbial community is established from inoculation of the MFC anode chamber with naturally occurring mixed inocula. In this study, the electrochemical performance of MFCs and microbial community evolution were evaluated for three inocula including domestic wastewater (DW), lake sediment (LS) and biogas sludge (BS) with varying substrate loading (L_{sub}) and external resistance (R_{ext}) on the MFC. The electrogenic bacterium *Geobacter sulfurreducens* was identified in all inocula and its abundance during MFC operation was positively linked to the MFC performance. The LS inoculated MFCs showed highest abundance (18% ± 1%) of *G. sulfurreducens*, maximum current density [I_{max} = (690 ± 30) mA·m^{-2}] and coulombic efficiency (CE = 29% ± 1%) with acetate as the substrate. I_{max} and CE increased to (1780 ± 30) mA·m^{-2} and 58% ± 1%, respectively, after decreasing the R_{ext} from 1000 Ω to 200 Ω, which also correlated to a higher abundance of *G. sulfurreducens* (21% ± 0.7%) on the MFC anodic biofilm. The data obtained contribute to understanding the microbial community response to L_{sub} and R_{ext} for optimizing electricity generation in MFCs.

1. Introduction

A microbial fuel cell (MFC) encompasses anode and cathode reactions to drive redox processes that result in production of electricity. The core principles of the electricity generation are similar to those in chemical fuel cells, but in MFCs, the reactions rely on bacterial metabolism based on a microbial biofilm on the anode electrode [1]. Fermentative bacteria are needed to convert complex substrates (*e.g.* glucose) into carboxylic acids including acetate, which can then be digested by electrogenic bacteria [2,3]. *Geobacter sulfurreducens*, is an electrogenic bacterium widely found in nature, which means that it can directly transfer electrons to the electrode [4,5]. The performance of MFCs depends therefore on the type and abundance of the microbial consortium in the anode chamber and notably in the anode biofilm. The inoculum source of electrogenic and fermentative bacteria is therefore important in the establishment of the anodic biofilm.

Inocula sources that have been studied in MFCs include pure bacteria [5], domestic wastewater (DW) [6–8] and biogas sludge (BS) [9]. Nevin *et al.* reported that pure cultures of electrogenic bacteria can produce higher maximum power density (MPD = 1900 mW·m^{-2}) than mixed communities (1600 mW·m^{-2}) with acetate as feed [5]. Holmes *et al.* [10] operated MFCs inoculated with marine sediment, salt-marsh sediment and freshwater sediment and showed that the power output was linked to electrogenic bacteria regardless of the salinity. Yates *et al.* [7] examined the microbial community in two-chamber H-shape MFCs inoculated with DW (two sources tested) and lake sediment (LS). They found that the cell voltage reached similar values [(470 ± 20) mV] after 20 operational cycles and that the anodic biofilm community were dominated by *Geobacter* sp.

Previous studies have shown that external resistance (R_{ext}) and substrate concentration affect the power generation and microbial community composition [11–13]. It is known that in a mixed culture, the electrogenic bacteria compete for substrate with fermentative non-electrogenic bacteria [13]. From the available literature, it is clear that a decaying microbiota is required for the MFC to convert organic substrates to electric current *via* electrogenic bacteria, but it is unclear whether the frequently tested DW may be surpassed by denser inocula such as BS and LS. A better understanding of the evolution of electrogenic *versus* fermentative bacteria will aid in improving MFC performance.

The objective of this work is to assess the electrochemical performance, stability and microbial consortium development using three inocula including DW, BS and LS, respectively. It was expected that a denser inoculum would allow an increase in power generation and make the process more robust to substrate changes. Based on the optimal inocula,

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the effect on the microbial evolution of a variation of R_{ext} and substrate loading (L_{sub}) was examined to improve MFCs performance. The process analysis was performed with thorough microbial analysis, chemical analysis and electrochemical impedance spectroscopy (EIS).

2. Materials and Methods

2.1. MFCs configuration

The H-shaped reactors used in this study were constructed by two cylindrical acrylic glass bottles with a volume of 300 cm^3 for each of the compartments (220 cm^3 liquid), which were connected with a tube with an inner diameter of 30 mm [6]. A proton exchange membrane (Nafion™ N117, Dupont Co., USA) with an area of 7.1 cm^2 was placed between the chambers. The two chambers were tightened with rubber rings. Both anode and cathode electrode were made of paralleled carbon paper sheets (TGPH-020, Fuel Cells Etc, USA) of 3 cm × 8 cm ($A = 24\ cm^2$) and a thickness of 0.35 mm.

2.2. Inoculation and operational conditions

The basic anolyte consisted of M9 medium containing per litre: 6 g Na_2HPO_4, 3 g KH_2PO_4, 1 g $NaHCO_3$, 1 g NH_4Cl, 0.5 g NaCl, 0.247 g $MgSO_4 \cdot 7H_2O$, 0.0147 g $CaCl_2$ and 1 cm^3 trace element solution [6]. pH could be maintained at 7.0 due to the high buffer capacity of the M9 medium (64 $mmol \cdot dm^{-3}$ of phosphate buffer + 12 $mmol \cdot dm^{-3}$ of carbonate buffer). The carbon source (sodium acetate or xylose) was added to the medium. The cathode solution was 100 $mmol \cdot dm^{-3}$ of $K_3Fe(CN)_6$ and 100 $mmol \cdot dm^{-3}$ of phosphate buffer (pH 6.7) and was replaced at the beginning of each cycle. All the MFCs were operated at 30 °C in an incubator with magnetic stirring [6].

Reactors (triplicates) were inoculated with three types of inocula: DW obtained after the fine separation process on a domestic wastewater treatment plant (Lyngby Taarbæk Community, Denmark); LS collected from Sorø lake (55°25′20.8″N, 11°32′22.7″E); and BS from Hashøj Biogas (Dalmose, Denmark). The LS sample was on March 11th, 2014, collected at 40 cm water depth and was a suspension of anaerobic surface sediment and water. pH, electric conductivity (EC), dry matter (DM) and chemical oxygen demand (COD) of these inocula are shown in Table 1. The reactors were inoculated in a 1:1 ratio of medium to inocula and fed with sodium acetate (1 $g \cdot dm^{-3}$ of COD) using R_{ext} of 1000 Ω. Feeding was done every 5 days (equal to one cycle) with fresh medium and corresponding substrates. Due to start up time, the first cycle lasted for 7 days. After 2 to 3 batch cycles, stable power generation was obtained in all the reactors. The acetate substrate was at beginning of Cycle 4 changed to xylose to study the adaptability of the microbial community to a fermentative substrate still using 1 $g \cdot dm^{-3}$ of COD content.

Table 1
Chemical parameters of the inocula including pH, electric conductivity (EC), dry matter (DM) and chemical oxygen demand (COD)

Inocula	pH	EC/$mS \cdot cm^{-1}$	DM/$mg \cdot g^{-1}$	COD/$g \cdot dm^{-3}$
DW	7.2	2.1	1.2	0.4
LS	7.4	0.9	3.8	2.1
BS	8.2	37.0	20.5	16.5

Based on the inocula test, four reactors (duplicate) inoculated with the optimal inoculum (LS) were operated in batch mode testing R_{ext} of 200, 500, 800 and 1000 Ω. Anode solution was replaced every 5 days, which equals to one cycle. From second cycle, all the reactors were fed with fresh medium and sodium acetate. After 3 batch cycles, stable power generation was obtained and different L_{sub} (0.5, 1, 1.5 and 2 $g \cdot dm^{-3}$ of COD) were tested in the MFCs. Operational cycles and corresponding R_{ext} and L_{sub} are outlined in Table 2.

Table 2
Overview of the operational parameters in 4 MFCs (duplicates) testing R_{ext} and L_{sub}

Batch No.	R_{ext}/Ω	COD conc (L_{sub})/$g \cdot dm^{-3}$
Cycle 1	200, 500, 800 and 1000	1
Cycle 2	200, 500, 800 and 1000	1
Cycle 3	200, 500, 800 and 1000	1
Cycle 4	200	1
Cycle 5	200	0.5, 1, 1.5 and 2
Cycle 6	200	0.5, 1, 1.5 and 2

2.3. Microbial community analysis

Biofilm samples from the anode chamber were obtained by cutting 0.5 cm^2 of the anode electrode at the end of each cycle [6]. Genomic DNA extraction followed by polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) were conducted as previously described [6,14]. Similarity between the samples was analysed by using BioNumerics software v.7.1 (Applied Maths, Sint-Martens Latem, Belgium) [6].

A clone library for providing a phylogenetic affiliation of the DGGE bands was constructed and resulting sequences have been submitted to EMBL Nucleotide Sequence Database (Accession No. LN650984–LN651064) by Sun et al. [6]. Subsequently the unique clones were amplified by PCR as described above. The PCR products were then run in a DGGE gel to identify the bands formed by biofilm samples [6].

2.4. Scanning electron microscopy

In order to examine biofilms on the anode surfaces, the anodic electrode (~1 cm^2) was removed without touching its surface. Small samples (1 cm × 1 cm) were fixed in 50 $dm^3 \cdot m^{-3}$ glutaraldehyde + 20 $dm^3 \cdot m^{-3}$ paraformaldehyde in 0.1 $mol \cdot dm^{-3}$ Na-acetate in deionized water (pH 7.2). After fixation, the samples were dehydrated in aqueous ethanol using: 20%, 40%, 60%, 80%, 90% and 100% for 20 min in each solution. Subsequent dehydration was performed in 33%, 66% and 100% acetone in ethanol before samples were critical point dried using Agar E3000 critical point dryer (Agar Scientific, Stansted, UK) with liquid CO_2 as drying agent. Following coating with gold using an Emitech E5000 sputter coater, samples were observed using a Philips XL30 ESEM scanning electron microscope at 50 to 10000 times of magnification [15].

2.5. Chemical, electrochemical and statistical analysis

The COD concentration and dry matter content were measured similar to Sun *et al.* [6]. Concentrations of monomeric sugars and volatile fatty acids (VFA) were measured by HPLC (high-performance liquid chromatography) [6]. pH and electrical conductivity were tested by multimeter (Multi 3430, WTW, Germany).

Electric current was recorded every 15 min by a data logger (Model 2700, Keithley Inc.). In polarisation tests, R_{ext} was varied between 30 Ω and 50 kΩ. The current density (I) and maximum current density (I_{max}) were calculated by dividing the current with the electrode surface area ($A = 48\ cm^2$) including both sides. EIS was carried out with a potentiostat (SP-150, BioLogic, France). The anode polarisation resistance was measured by connecting the MFCs to the potentiostat in the three-electrode mode within the frequency range from 10 kHz to 0.1 Hz with amplitude of 10 μA. Lower frequencies were not tested since it can disturb the microbial process due to a long test period (>1 h). The anode and cathode were used as working electrode and counter electrode, respectively. The third lead was attached to a reference electrode (Ag/AgCl; #MF2079; Bioanalytical Systems Inc.) inserted in the anode chamber. Zview (Scribmer Associates Inc.) was used for EIS data fitting. Coulombic efficiency (CE) was calculated as the ratio of accumulative charges produced from the MFCs to the charges released from substrate degradation. Statistics analysis by ANOVA (one-way;