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Dynamic changes in the microbial community composition in microbial fuel cells fed with sucrose

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Supplemental Material

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Use of a second DGGE for re-amplified 16S rRNA genes of bands from the first DGGE to yield sequences of better quality. Co-migration of DNA molecules of different sequence to the same position in a denaturing gradient is a limiting factor in the analysis of complex microbial communities by DGGE (Gafan and Spratt 2005; Kowalchuk et al. 1997; Satokari et al. 2001; Green et al. 2009), and was also observed in the study described here. The 16S rRNA genes of bands marked with -' - in the DGGE of bacterial community profiles (Fig. S3) were re-amplified by PCR and run on a second DGGE gel with a narrower denaturing gradient because of insufficient quality of sequences (partly overlapping sequences in a chromatogram) obtained from the first DGGEs possibly due to co-migration of several sequences in one band. The second DGGE round (Fig. S3) showed one main band in each sample yielding sequencing results of good quality.

Dynamics of bacterial classes. MFCs B and C showed increasing abundances of *Clostridia* and decreasing abundances of *Bacteroidetes* (Fig. S6 b and c). An increase in the relative proportion of δ -*Proteobacteria* and β -*Proteobacteria* could be seen in MFC-A and MFC-B, respectively (Fig. S6 a and b). At the end of MFC operation, *Clostridia* was the most abundant class in the anodic biofilm of all MFCs ($(41 \pm 5.6) \%$, $n = 3$), followed by δ -*Proteobacteria* (20%) and *Bacteroidetes* (15%) in MFC-A, *Bacteroidetes* (33%) and β -*Proteobacteria* (16%) in MFC-B and *Bacteroidetes* (18%) and γ -*Proteobacteria* (16%) in MFC-C (Fig. S6 a-c).

Decreasing abundances of *Bacteroidetes* were observed both in the anodic suspensions of MFC-B and MFC-C over time (Fig. S6 e and f). At the end of MFC operation, *Clostridia* was the most abundant class also in the anodic suspension of all MFCs ($(46 \pm 3.9) \%$, $n = 3$), followed by

67 δ - (11%) and ϵ -*Proteobacteria* (10%) in MFC-A, β -*Proteobacteria* (20%) and *Bacilli* (20%) in
 68 MFC-B and ϵ - (33%) and β -*Proteobacteria* (8%) in MFC-C (Fig. S6 d-f).

69
 70 **Control MFC.** A control MFC was operated in open circuit, simultaneously with an MFC
 71 connected to an external resistor. Other operational conditions used for these two MFCs were as
 72 described for MFCs A-C, with the exception that the loading rate was 0.05 ml min^{-1} and the
 73 sucrose concentration of the feed was 0.17 g L^{-1} . The anodic bacterial community was sampled
 74 for PCR-DGGE analysis on day 66.

75 Most species detected according to their 16S rRNA sequence in the current-producing MFC
 76 were also found in MFCs A-C (Fig. S7). Some species (bands 3-5) were members of the anodic
 77 biofilm community both in the current-producing and the control MFC (Table S1). Notably, the
 78 species related to *Rhodocyclus* sp. HOD 5 (100% similarity), *Robinsoniella peoriensis* strain
 79 PPC31 (99%) and *Anaerosporeobacter mobilis* strain IMSNU 40011 (100%) were detected only
 80 in the current-producing MFC. However, since the presence or the relative proportions of these
 81 bacteria were not temporarily consistent in the replicate MFCs, it is not possible to deduce that
 82 any specific species would be the main current-producers in the anodic communities.

84 Archaeal community analysis

85 **Results.** Archaea can contribute to the organic removal in MFCs fed with fermentable
 86 substrates, but also cause a loss of electrons through the production of CH_4 . The dynamics of the
 87 archaeal communities in the MFCs was subjected to study by PCR-DGGE, followed by species
 88 identification by sequencing. The DGGE patterns of the archaeal communities of anodic biofilm
 89 and suspension were very similar and most species were already detected in the inoculum (day 0)

(Fig. S8). A small number of different species (1 – 3) were dominant at any time point and no significant change in communities over time was observed, with the exception of MFC-C (Fig. S8 and S9). An archaeon with sequence similarity to *Methanosaeta concilii* (98-99%) was the main species found in all MFCs (bands 1, 2 and 4) (Fig. S8 and S9). In MFC-C, sequences closely related to *Methanosarcina barkeri* (99%) were detected at the end of operation (days 70 and 91) in both the biofilm (band 3) and suspension (band 5). In addition, a species with 98% sequence similarity to the uncultured archaeon clone W30D and distantly related to *Thermoplasma volcanium* (81%) was a member of the suspended microbial community in MFC-C (days 56-91).

Discussion. Archaeal communities showed much less diversity than the bacterial communities. *Methanosaeta concilii* was the dominant archaeal species in the anodic biofilm and suspension of all replicate MFCs, with the exception of *Methanosarcina barkeri* and an uncultured archaeon clone detected in MFC-C at the end of operation (Fig. S8 and Fig. S9). *M. concilii* is an acetate-fermenter whereas *M. barkeri* grows either on acetate or hydrogen (Garcia et al. 2006). Thus, in these sucrose-fed MFCs, methanogenic archaea would compete with exoelectrogenic bacteria mainly for acetate, as shown in acetate-fed MFCs (Virdis et al. 2009). In studies with glucose, ethanol or cellulose as substrate, H₂-oxidizing methanogens were found to compete or even out-compete the H₂-utilizing exoelectrogens, whereas acetoclastic methanogenesis was not detected (Freguia et al. 2008; Ishii et al. 2008; Parameswaran et al. 2009). Acetoclastic and hydrogenotrophic methanogens accounted for around 19% and 5%, respectively, of the total microbial community in a glucose-fed MFC (Chung and Okabe 2009). Both acetoclastic (*Methanosaeta* sp.) and hydrogenotrophic (*Methanobacterium* sp. and

Methanospirillum sp.) methanogens were also members of anodic biofilm community in a glucose- and lactate-fed MFC (Borole et al. 2009). The presence of acetoclastic methanogens in the sucrose-fed MFCs of this study represents a loss of electrons to methane, giving rise to a need of suppressing these methanogens e.g. by periodic aeration in order to promote the growth of acetate-oxidizing exoelectrogens (e.g. facultative denitrifiers would not be suppressed by aeration) and to improve the current output of MFCs.

References

- Borole AP, Hamilton CY, Vishnivetskaya TA, Leak D, Andras C, Morrell-Falvey J, Keller M, Davison B (2009) Integrating engineering design improvements with exoelectrogen enrichment process to increase power output from microbial fuel cells. *J Power Sour* 191:520–527
- Chung K, Okabe S (2009) Continuous power generation and microbial community structure of the anode biofilms in a three-stage microbial fuel cell system. *Appl Microbiol Biotechnol* 83:965–977
- Freguia S, Rabaey K, Yuan Z, Keller J (2008) Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. *Environ Sci Technol* 42:7937–7943
- Gafan GP, Spratt DA (2005) Denaturing gradient gel electrophoresis gel expansion (DGGE) - an attempt to resolve the limitations of co-migration in the DGGE of complex polymicrobial communities. *FEMS Microbiol Lett* 253:303–307
- Garcia J-L, Ollivier B, Whitman WB (2006) The Order Methanomicrobiales. *The Prokaryotes*. Springer Science, New York, USA, pp 208–230
- Green SJ, Leigh MB, Neufeld JD (2009) Denaturing gradient gel electrophoresis (DGGE) for microbial community analysis. In: Timmis KN (ed) *Microbiology of hydrocarbons, oils, lipids and derived compounds*. Heidelberg, Germany, pp 4137–4158
- Ishii S, Shimoyama T, Hotta Y, Watanabe K (2008) Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell. *BMC Microbiol* 8:6
- Kowalchuk GA, Stephen JR, de Boer W, Prosser JI, Embley TM, Woldendorp JW (1997) Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and

sequencing of PCR-amplified 16S ribosomal DNA fragments. *Appl Environ Microbiol* 63:1489–1497

Parameswaran P, Torres CI, Lee H-S, Krajmalnik-Brown R, Rittmann BE (2009) Syntrophic interactions among anode respiring bacteria (ARB) and non-ARB in a biofilm anode: electron balances. *Biotechnol Bioeng* 103:513–523

Satokari RM, Vaughan EE, Akkermans ADL, Saarela M, de Vos WM (2001) Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 67:504–513

Virdis B, Rabaey K, Yuan Z, Rozendal RA, Keller J (2009) Electron fluxes in a microbial fuel cell performing carbon and nitrogen removal. *Environ Sci Technol* 43:5144–5149

Figure S1. An exemplar polarization curve (●) and power density curve (■) of MFC-D on day 91.

Figure S2. DNA concentrations in (a) anodic biofilm and (b) suspension of MFC-A (●), MFC-B (□) and MFC-C (▲).

Figure S3. DGGE of re-amplified 16S rRNA genes of bands marked with '-' in the DGGE of bacterial community profiles of MFCs A-C, with denaturing gradients ranging from (a) 40 to 55% and (b) 40 to 60%.

Figure S4. Proportion trends of the most abundant species in the anodic biofilm of MFC-A and MFC-B based on band intensities of DGGE community profiles and identification of excised bands by sequencing and comparison to known 16S rRNA sequences in Genbank.

Figure S5. Proportion trends of the most abundant species in the anodic suspension of MFC-A and MFC-B based on band intensities of DGGE community profiles and identification of excised bands by sequencing.

Figure S6. Relative abundances of bacterial classes in the anodic biofilm of MFCs A (a), B (b) and C (c) and in the suspended culture of MFCs A (d), B (e) and C (f): α - (●), β - (■), γ - (▲), δ - (▼) and ϵ -*Proteobacteria* (◆); *Clostridia* (○), *Bacteroidetes* (□), *Spirochaetes* (Δ), *Actinobacteria* (∇), *Flavobacteria* (◇), *Bacilli* (*), and unknown (☆).

Figure S7. DGGE profile of anodic bacterial communities of an MFC connected to external resistor (left) and a control MFC operated in open circuit (right) analyzed by DGGE of PCR-amplified genes coding for 16S rRNA. Numbers indicate bands identified by sequence analysis.

Figure S8. DGGE profile of archaeal communities of the anodic biofilm (a) and suspended culture (b) analyzed by DGGE of PCR-amplified genes coding for 16S rRNA; Std: Standard mix; Inoc: anaerobic sludge. Arrows indicate bands excised for sequence analysis. Same numbering is used for identical sequences.

Figure S9. Phylogenetic tree based on partial archaeal 16S rRNA gene sequences of anodic biofilm and suspended culture of three replicate sucrose-fed MFCs. Sequences found only in biofilm or suspension are marked with 'b' or 's', respectively. The tree was constructed using distance matrix and neighbour joining algorithm with 1,000 bootstrappings. The bootstrap values $\leq 99\%$ are shown. A bacterium *Clostridium indolis* (Y18184) was used as out-group. The scale bar represents 10% sequence divergence.

Table S1. Bacterial 16S rRNA gene sequences of bands excised from the DGGE gel of anodic biofilm bacteria in a control (open-circuit) and electricity-producing MFCs.

Band	MFC	Accession no.	GenBank match (accession no.)	Simil. (%)	Class
1	Current-producing	JF330839	<i>Anaerospirrobacter mobilis</i> strain IMSNU 40011 (AY534872)	100	Clostridia
2	Current-producing	JF330840	<i>Robinsoniella peoriensis</i> strain PPC31 (AF445285)	99	Clostridia
3	Control/Current-producing	JF330841	<i>Clostridium indolis</i> strain DSM 755 (Y18184)	99	Clostridia
4	Control/Current-producing	JF330842	<i>Ochrobactrum anthropi</i> strain YZ-1 (EU275247)	100	α -Proteobacteria
5	Current-producing	JF330843	<i>Rhodocyclus</i> sp. HOD 5 (AY691423)	100	β -Proteobacteria
6	Control/Current-producing	JF330844	<i>Petrimonas sulfuriphila</i> strain BN3 (AY570690)	97	Bacteroidetes
7	Control	JF330845	<i>Ochrobactrum anthropi</i> strain CCUG 50899 (AM490609)	100	α -Proteobacteria
8	Control	JF330846	<i>Luteibacter</i> sp. CCUG 25036 (FM212561)	99	γ -Proteobacteria
9	Control	JF330847	<i>Gordonia</i> sp. IFM 10067 (AB476396)	99	Actinobacteria

Figure S1.

Figure S2.

Figure S3.

Figure S4.

Figure S5.

284 **Figure S6.**

Figure S7.

Figure S8.

Figure S9.