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# Spatio-temporal development of the bacterial community in a tubular longitudinal microbial fuel cell

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## Supplementary Information

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## Experimental

### Set up of longitudinal tubular MFC reactors

Each module (A1, A2, B1 and B2) was independently connected to an external load circuit (1 k?) and the voltages across the MFC modules were recorded during operation at 10 minute intervals using LabVIEW™ software and a NI PCI-6224 I/O card (National Instrument Corporation Ltd. Berkshire UK).

Sucrose solution and nutrient buffer medium containing 50mM phosphate buffer (pH 7.0) (Kim et al., 2009) were separately prepared, stored at 4°C and mixed through a Y-connector before delivery through the influent ports of reactors A and B. The two solutions were delivered to each reactor using four channels of a single external peristaltic pump (Watson and Marlow, Falmouth, UK) each set at 0.0175 l/h, maintaining an HRT of 7.1h for a single module (14.2h for each reactor A and B). Antimicrobial tubing (Tygon silver coated tubing 1/8", Cole-Parmer) was used to introduce the influent and remove effluent from the system. The reactors were operated at room temperature (26±2°C).

### PCR gene amplification for bacteria

The partial bacterial 16S rRNA genes were amplified using the bacteria-specific forward primer 341F (*E. coli* 16S rRNA positions 341-357) (Muyzer et al., 1993) and the universal reverse primer 907R (*E. coli* 16S rRNA position 907-926) (Muyzer et al., 1996). A GC-clamp was added to the forward primer at the 5'-end to stabilize the melting behaviour of the DNA fragments in the DGGE (Muyzer et al., 1993). PCR reaction mixtures contained 1× Taq PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, pH 8.3 at 20°C), 200 µM dNTP, 0.2 µM each primer, 0.025 U µL<sup>-1</sup> of Taq DNA polymerase (Roche, UK), 400 ng µL<sup>-1</sup> of bovine serum albumin (BSA, Fermentas, Canada) and nuclease-free water (Promega, UK) to a final volume of 50 µL, to which 1 µL of template was added. PCR was carried out using a GeneAmp PCR System 9700 (PE Applied Biosystems, USA) with the following program: 95°C for 5 min; 25 cycles of 94°C for 0.5 min, 50°C for 1 min and 72°C for 2 min; followed by final extension at 72°C for 7 min.

### PCR gene amplification for archaea

A nested-PCR approach was used for amplifying partial archaeal 16S rRNA genes. First PCR amplification was performed using the archaea-specific primers ArUn4f (*E. coli* 16S rRNA positions 8-25) and Ar958r (*E. coli* 16S rRNA positions 958-967) (Jurgens et al., 2000). The second amplification using the product of the first round of PCR was done with the archaea-specific primers GC-ArchV3f (*E. coli* 16S rRNA positions 340-357) (Ovreas et al., 1997), including a GC-clamp, and Ar958r. PCR reaction mixtures used for archaeal PCR were similar to those described above for bacteria, with the exception that BSA was excluded in the first round of PCR and 0.5 µL of the product of the first PCR was used as template in the second round of PCR. The program for the first PCR was as follows: 95°C for 4 min; 41 cycles of 92°C for 1 min, 57°C for 1 min and 72°C for 2 min; followed by final extension at 72°C for 7 min. The second PCR was performed using the following program: 94°C for 1 min; 25 cycles of 92°C for 1 min, 61°C for 1 min and 72°C for 2 min; followed by 72°C for 7 min.

## Results

### Bacterial community composition dynamics

*Lactococcus* sp., present in the anodic biofilm at high relative proportion by day 97, is capable of coupling carbohydrate oxidation to reduction of extracellular compounds such as Fe<sup>3+</sup> and Cu<sup>2+</sup>, mediated by membrane-bound quinones (Rezaïki et al., 2008). Pure culture of *L. lactis* has also been shown to perform extracellular electron transfer to MFC anode via excretion of soluble quinone and with

simultaneously slightly shifting its metabolism to the production of acetate and pyruvate (Freguia et al., 2009). *L. lactis* was also found to dominate in a MFC fed mainly with acetate (effluent from a glucose-fed MFC) (Chung & Okabe, 2009). In the light of these findings, it can be speculated that *Lactococcus* sp. plays an important part in the electricity generation by the mixed community in the tubular MFCs.

In modules A2 and B2 the two dominant species *Lactococcus* sp. and *P. propionicigenes* were probably migrated from modules A1 and B1 by continuous flow condition. In addition to sucrose, *K. oxytoca* can also use acetate (Holt, 1994) and almost all strains of *Klebsiella* sp. are able to use lactate which can be converted from sugar such as sucrose by lactic acid fermentation (Grimont et al., 1992), which explains the abundance of *K. oxytoca* in successive MFC modules receiving different substrate levels. In contrast, some species showed spatial divergence, probably due to different substrate availability in successive modules, but although they were present at relatively low proportion, they could have an important functional role in electricity production. These could include species with 97% sequence similarity to *Geobacter* sp. Ply1 which became detectable in modules A2 and B2 by day 97. *Geobacter* sp. oxidize organic acids using Fe(III) as electron acceptor and are known exoelectrogens reported to be present in many MFC anodes (Bond et al., 2002; Jung & Regan, 2007; White et al., 2009; Xing et al., 2009). Therefore, the anode-reducing activity by these species in modules A2 and B2, fed mainly by acetate, seems likely. The disappearance of aerobes (as indicated by sequences related to *Acidovorax* sp. and *Pseudomonas* sp.) and the detection of the strictly anaerobic *Geobacter* (dissimilatory metal-reducer) at the late phase of MFC operation in the anodic biofilm of modules A2 and B2 could be related to the variation of the redox conditions in these modules over time, which may have affected the community structure. In addition, a low relative proportion of genus related to *Desulfovibrio desulfuricans* was detected in modules A1 and B1 by day 41 when MFC voltage had reached stability. Electricity generation from lactate by *D. desulfuricans* has been previously demonstrated (Zhao et al., 2009).

### Analyses of archaeal community composition dynamics

The DGGE analysis of the archaeal communities of A1, A2, B1 and B2 showed similar patterns (Figure S3). Until day 41, the archaeal community composition did not differ from that of the inoculum (day 0), and only one archaeal species could be detected at any time point. A species with sequence similarity to *Methanosaeta concilii* (98-100%) was the predominant archaea until day 70, when a species closely related to *Methanocorpusculum parvum* (98-100%) started to become predominant (Figure S3 and Figure S4).

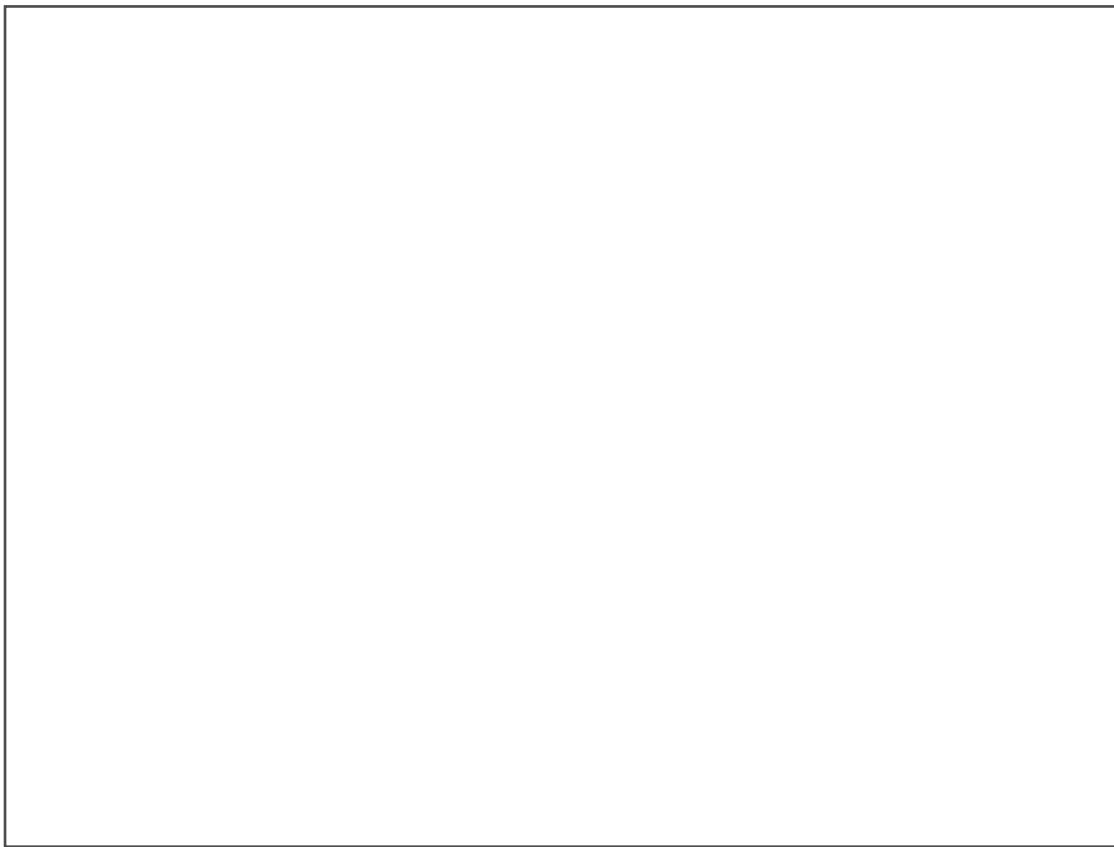
Archaeal community showed no spatial difference in tubular MFCs. Only one dominant archaeal species was detected at any time with clear succession from the dominance of the *Methanosaeta concilii* related acetate-fermenting archaeon to the *Methanocorpusculum parvum* related hydrogen-oxidizing archaeon from day 70 onwards (Garcia et al., 2006). The reason for a shift from acetate-utilizing methanogens to H<sub>2</sub>-utilizing methanogens remains unclear. Previous studies suggest the existence of competition between methanogens and electricity producing bacteria for H<sub>2</sub> (Freguia et al., 2008) and acetate (Virdis et al., 2009). Methane production has been observed in MFCs fed with fermentable substrates (glucose and ethanol) (Freguia et al., 2007; Kim et al., 2007; Lee et al., 2008; Parameswaran et al., 2009), and in acetate fed MFCs at the early stages of enrichment when using digester sludge inoculum (Kim et al., 2005). However the serial transfer of enriched electrodes under exo-electrogenic conditions diminishes methane production in acetate fed MFCs, suggesting that acetate-oxidizing exoelectrogens are capable of out-competing acetoclastic methanogens as it would be thermodynamically favourable (Lovley & Phillips, 1988). Using short-term kinetic tests with H<sub>2</sub>, Freguia et al. (2008) showed that part of the anodic H<sub>2</sub> produced by glucose fermentation was used by hydrogen-oxidizing methanogens. By electron

balance and finding that the hydrogenotrophic methanogenic genus Methanobacteriales was the only methanogen present in the anodic biofilm (~4% of the total microbial community), Parameswaran et al. (2009) demonstrated that the electrons available in H<sub>2</sub> from ethanol fermentation were routed to CH<sub>4</sub>. A hydrogenotrophic methanogen, *Methanobacterium bryantii*, dominated the archaeal community in a cellulose-fed MFC (Ishii et al., 2008), whereas acetoclastic methanogens accounted for ~19% and hydrogenotrophic methanogens accounted only for ~5% of the total microbial community in a glucose-fed MFC (Chung & Okabe, 2009). The results of the present study suggested that over time the acetate-oxidizing bacteria could out-compete the acetoclastic methanogens, thus preventing electrons from acetate sinking to CH<sub>4</sub>. The appearance of hydrogenotrophic methanogens coincided with a significant decrease in the relative proportion or disappearance of *Eubacterium* sp., capable of homo-acetogenic production of acetate from H<sub>2</sub>+CO<sub>2</sub>, suggesting that H<sub>2</sub>-oxidizing methanogens were competing with (or gradually out-competed) the homo-acetogenic bacteria. However, other bacteria able to oxidize H<sub>2</sub> (*Pantoea*, *Klebsiella*, *Desulfovibrio* and *Geobacter*) were found in increased relative proportions at the end of the process and could also have been competing for H<sub>2</sub> with methanogens. Parameswaran et al. (2009; 2010) showed the abundance of homo-acetogens and electron flow from H<sub>2</sub> to current via acetate in ethanol-fed MFC only when methanogenesis was inhibited. Therefore they suggested promoting of growth of homo-acetogens in anodic biofilm based on their higher tolerance to oxygen and ability to out-compete hydrogenotrophic methanogens at low temperature and pH in order to prevent electron flow to CH<sub>4</sub> (Parameswaran et al., 2010). These means could also possibly provide a practical way for improving fuel efficiency and the power density of the tubular MFCs.

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**Figure S1** SEM pictures of the anode electrode in the first and second module of longitudinal tubular reactor

[pic]

Figure S2 Bacterial DGGE profile of longitudinal tubular reactors according to time; Std: Standard mix;  
Inoculum: anaerobic sludge; \*: chimeras. Numbered bands were excised for sequence analysis.

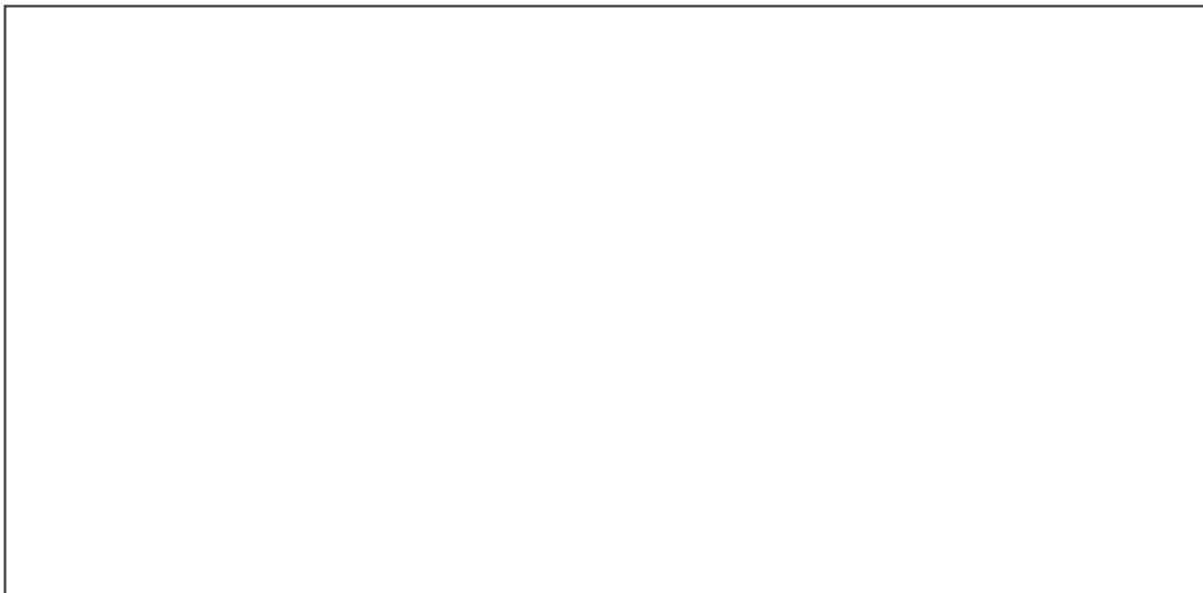


Figure S3 Archaeal DGGE profiles of longitudinal tubular reactors according to time; (A) modules A1 and A2 of reactor A, and (B) modules B1 and B2 of reactor B; Std: Standard mix; Inoc: anaerobic sludge. Numbers indicate the bands excised for sequence analysis.

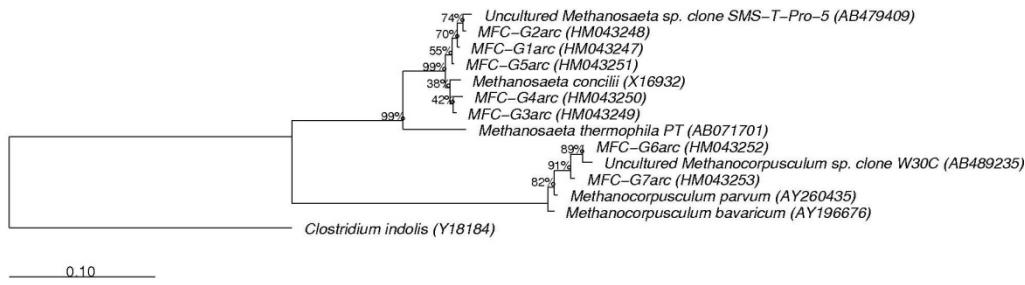


Figure S4 Phylogenetic tree based on partial archaeal 16S rRNA gene sequences of anodic biofilm of tubular MFCs. The tree was constructed using distance matrix and neighbour joining algorithm with 1,000 bootstrappings. Bootstrap values ? 99% are shown. A bacterium *Clostridium indolis* (Y18184) was used as out-group. The scale bar represents 10% sequence divergence.

Sample	std	A1			A2			B1			B2			inoc
Days		21	41	97	21	41	97	21	41	97	21	41	97	
0														

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A

B

Methanomicrobiales

Methanosarcinales