# Supplementary Information

## Revealing of the electrochemically driven selection in natural community derived microbial biofilms using flow–cytometry

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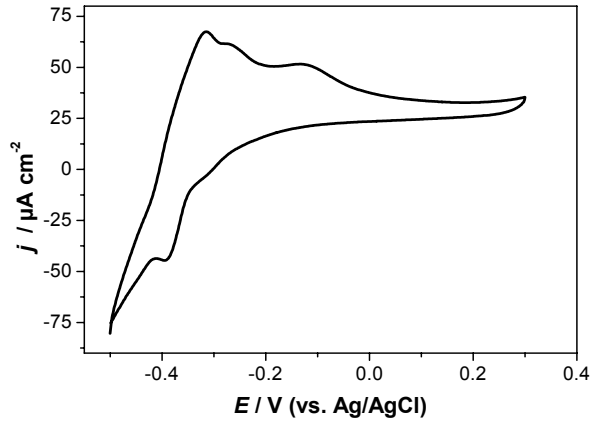
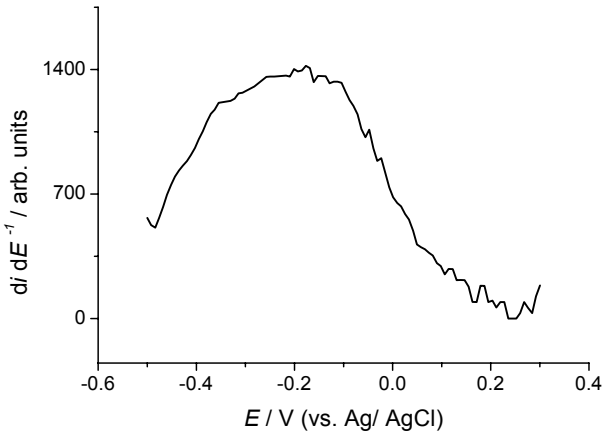
## 1. Results

### 1.1. Electrochemical biofilm analysis




**A**




**B**

Figure SI-1: A) cyclic voltammetric (CV) curve of the non turn-over biofilm (substrate depleted) conditions using a scan rate of 1 mV $s^{-1}$, B) First derivative of the CV- curve for turnover conditions (a shown in Figure 1 C in the manuscript) indicating the formal potential of the active site

### 1.2. T-RFLP-analyses & Sequences:

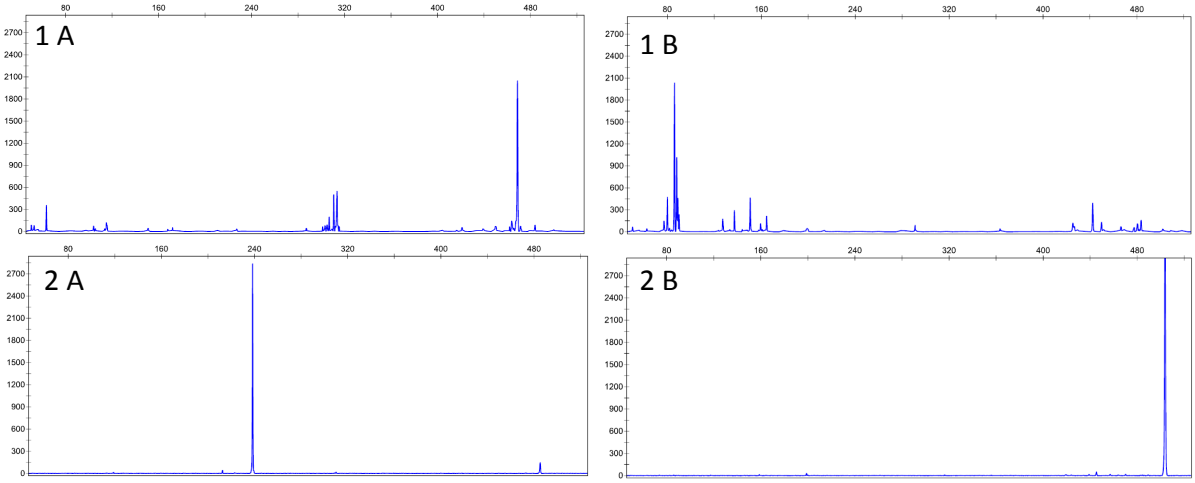



Figure SI-2: T-RFLP chromatograms of amplified 16S rRNA gene fragments from wastewater (1) and anode biofilm (2) samples. The results are shown for the digestion with Rsa I (A) and Msp I (B) (experimental conditions-see below).

## 2. Materials and methods

All chemicals used were of analytical grade and all potentials are reported versus Ag/AgCl (0.195 V vs. SHE) unless stated otherwise.

### 2.1. Growth medium and microbial inoculum

The bacterial growth medium was prepared as reported by Kim et al. (2005). It contained $NH_4Cl$ (0.31 g $L^{-1}$), KCl (0.13 g $L^{-1}$), $NaH_2PO_4.H_2O$ (2.69 g $L^{-1}$), $Na_2HPO_4$ (4.33 g $L^{-1}$), trace metal (12.5 mL) and vitamin (12.5 mL) solutions (Balch et al. 1979; Lovley et al. 1984). Acetate (10mM) served as substrate throughout this study. The pH of the medium was adjusted to 6.8. The growth medium was purged with nitrogen for ≥ 20 min before using it to ensure anoxic conditions. The inoculum used was primary wastewater obtained from wastewater treatment plant Steinhof (Braunschweig).

### 2.2. Electrochemical experimental setup & biofilm growth

All experiments in this study were conducted under strictly anoxic conditions at 35°C as half-cell experiments under potentiostatic control (VMP 3, Biologic) (Patil et al. 2010). The cytometric experiments were performed for three independent experiments whereas the T-RFLP analyses were done for one selected setup using different restriction enzymes (more information below). The half-cell experiments were carried out using a conventional three electrode arrangement. Unless stated otherwise, the working electrode was polycrystalline graphite rod (CP Graphite GmbH, Germany), with a projected surface area of 8 $cm^2$. Similar graphite rods were used as counter electrodes, whereas the reference electrode was an Ag/AgCl electrode (sat. KCl, Sensortechnik Meinsberg, Germany, 0.195 V vs. SHE).

The biofilm formation procedure was followed as described by Liu et al (Liu et al. 2008). Primary waste water (4 mL) was inoculated into the sealed electrochemical cell with substrate solution (120 mL) and a constant potential of 0.2 V was applied to the working electrode to facilitate the biofilm formation. The biofilm growth was monitored by measuring the bioelectrocatalytic oxidation current. The substrate (acetate) level was monitored by HPLC analysis. The exhausted substrate solutions were replenished regularly.

### 2.3. Flow-cytometry

**Sample fixation and DNA staining**

Cells were harvested from waste water samples and the anode biofilm by gentle shaking (175 rpm, 10 min). The biofilm dissolved easily into a single cell solution. The samples were washed twice to remove any disturbing substances (cell debris, organic material) with phosphate buffered saline (PBS: 0.4 M $Na_2HPO_4/NaH_2PO_4$, 150 mM NaCl, pH 7.2) by centrifugation at 3,200 x g for 10 min and conserved in fixation buffer (pH 7.0) containing: 5 mM $BaCl_2$ ($BaCl_2$ * $2H_2O$; Laborchemie Apolda, Germany), 5 mM $NiCl_2$ ($NiCl_2$ * $6H_2O$; Merck, Germany) and 10% sodium azide (Merck, Germany) dissolved in PBS (1 ml fixation buffer for app. 3 x $10^8$ cells $ml^{-1}$) for a maximum of 9 days. Aliquots of the fixed samples were washed twice in 2 ml PBS by centrifugation at 3,200 x g for 5 min and treated with 1 ml Tween 20 solution (0.5 g Tween 20 in 100 ml bidistilled water) for 20 min to facilitate dye penetration. Subsequently, the cells were washed, carefully resuspended in 2 ml 4',6-diamino-

2'phenylindole (DAPI) solution (692 µl of 143 µM DAPI stock in 100 ml of 400 mM $Na_2HPO_4$, pH 7.0) and stained for at least 60 min in the dark at 20°C.

**Multiparametric flow cytometry**

Flow cytometric measurements were carried out using a MoFlo cell sorter (DakoCytomation, Fort Collins, CO, USA) equipped with two water-cooled argon-ion lasers (Innova 90C and Innova 70C from Coherent, Santa Clara, CA, USA). Excitation by 580 mW at 488 nm was used to analyze the forward scatter (FSC) and side scatter (SSC) as trigger signal at the first observation point, using a neutral density filter with an optical density of 2.3. DAPI dye was excited by 180 mW of ML-UV (333-365 nm) at the second observation point. The orthogonal signal was first reflected by a beam-splitter and then recorded after reflection by a 555 nm long-pass dichroic mirror, passage by a 505 nm short-pass dichroic mirror and a BP 488/10. DAPI fluorescence was passed through a 450/65 band pass filter. Photomultiplier tubes were obtained from Hamamatsu Photonics (models R928 and R 3896; Hamamatsu City, Japan). Amplification was carried out at linear or logarithmic scales, depending on the application. Fluorescent beads (Polybead Microspheres: diameter, 0.483 µm; flow check BB/Green compensation Kit, Blue Alignment Grade, ref. 23520, Polyscience, USA) were used to align the MoFlo (coefficient of variation – CV value - about 2%). Furthermore, an internal DAPI-stained bacterial cell standard was introduced for tuning the device up to a CV value not higher than 6%. Cell aggregation was not observed, thus clearly separated sub-populations were analyzed.

### 2.4. T-RFLP and Sequencing

For DNA extraction 200 µl of the fixed samples were processed with the Fast DNA Spin Kit for Soil (MP Biomedicals, Illkirch, France). PCR amplification of the 16S rRNA gene fragment with the universal primers 27F-FAM and 1492R (Lane, 1991) and the T-RFLP analyses were performed as described elsewhere (Kleinsteuber et al. 2008). PCR product purification was done with QIAquick PCR Purification Kit (Qiagen, Venlo, The Netherlands). The restriction enzymes BstU I, Hae III, Msp I and Rsp I (New England Biolabs, Schwalbach, Germany) were used with the corresponding buffer. The genemapper V3.7 software (Applied Biosystems, Weiterstadt, Germany) was used to determine the length of the fluorescent terminal

restriction fragments (T-RFs). Only peaks with a relative fluorescence intensity of 50 Units in the range of 50-500 bp were included in the analysis.

The PCR amplification product of the biofilm sample was partial sequenced with the primers 27F and 1492R and the BigDye RR Terminator AmpliTaq Kit 1.1 (Applied Biosystems). An ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) was used for capillary electrophoresis and data collection. The data was analyzed with abi prism dna sequencing analysis software. The sequence endings were trimmed and the two partial sequences of the 16S rRNA gene were compared to published sequences with the blastn tool (www.ncbi.nlm.nih.gov/BLAST) (Altschul et al. 1990).

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