What is the Role of Individual Species within Bidirectional Electroactive Microbial Biofilms: A Case Study on *Desulfarculus baarsii* and *Desulfurivibrio alkaliphilus*

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Desulfarculus baarsii and *Desulfurivibrio alkaliphilus* are strictly anaerobic bacteria existing in marine sediments. *D. baarsii* gains energy through reducing sulphate and *D. alkaliphilus* is able to reduce elemental sulphur, thiosulphate and polysulphide in seawater. Both organisms were previously identified as key organisms in sediment derived, bidirectional electroactive biofilms. Here, we investigated the electrochemical performance of these two bacteria in bio-electrochemical systems and their possible involvement in anodic and cathodic reactions. The results show that *D. baarsii* was unable to donate or accept electrons to/from an electrode, while *D. alkaliphilus* was able to catalyse both anodic and cathodic reactions and interact with the electrode through direct or potentially indirect electron transfer. Raman spectra of *D. alkaliphilus* electrode biofilms showed a high similarity to *Geobacter sulfurreducens* biofilms, including the specific bands of cytochromes b and c, explaining the electroactivity of *D. alkaliphilus* in bioelectrochemical reactions.

Introduction

Electrochemically active bacterial biofilms are the key elements in microbial fuel cells and related microbial electrochemical technologies. Besides their ability to exchange electrons with an electrode, the selection of suitable bacterial biocatalysts for a bioelectrochemical application depends on the nature of the target substrate and its desired reductive or oxidative conversion. Especially for environmental applications such as wastewater treatment and benthic fuel cells,[1] also the complex chemical and biological environment, an often ill-defined nature of the target substrates, and predetermined environmental parameters, such as temperature, pH and salinity have to be taken into account. For such applications, the use of bacterial consortia, enriched from the actual target environment – i.e., from the respective wastewater,[2] or from the sediment – is without alternative. Thereby, during the enrichment process, microbial consortia form that are able to exchange electrons with a respective electrode in order to oxidize or reduce the provided substrate. The complexity of enriched electrochemically active consortia can differ significantly – often in response to the complexity of the provided substrates.[3] At microbial fuel cell anodes – including anodes in benthic fuel cells,[1,4] *Geobacter sulfurreducens* or *anodireducens* are often the dominating species, however, in many cases this may be connected with conditions in which acetate is being used as the substrate or is being formed as a key intermediate.[5–6]

Especially in bacterial consortia that are enriched at benthic fuel cells anodes, the bacteria that are involved in the biogeochemical sulphur cycle are often dominant.[7–10] Such bacteria abundantly exist in sediments at the high salinity and alkaline pH of seawater.[11] For some of the key organisms, the involved bioelectrochemical mechanisms have been identified. Thus, e.g., it has been reported that *Desulfobulbus propionicus* was not only able to transfer electrons to Fe (III) but also to graphite anodes.[12] For many other organisms, however, their exact role in the electricity generation has not been identified yet. The same applies to biofilms that have been enriched from marine sediments for the purpose of a reversible energy storage. Thus, in bidirectional biofilms developed by Yates et al,[13] *Desulfarculus baarsii* was identified as the most dominant and *Desulfurivibrio alkaliphilus* as the most active biofilm organism.[14–15] *D. baarsii* is a sulphate reducing bacterium known for its ability to completely oxidize volatile fatty acids (VFAs) to CO_2,[16] whereas *D. alkaliphilus* is a sulphur reducing haloalkaliphile[17–18] for which conductive pili and their possible involvement in extracellular electron transfer has been studied previously.[19]

In order to understand the role of these two organisms in the bidirectional biofilm, we studied their ability and potential mechanisms to exchange electron with electrodes – in both anodic and cathodic direction.

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Experimental Section

Bacterial pre-cultivation

Active cultures of *Desulfarculus baarsii* (DSM 2075) and *Desulfurivibrio alkaliphilus* (DSM 19089) were purchased from DSMZ, Braunschweig, Germany, and were grown strictly anaerobically at 35 °C and under stirring (180 rpm) in the sterile DSMZ medium 193b (*D. baarsii*) and DSMZ medium 1104 (*D. alkaliphilus*) (http://www.dsmz.de). In some serum bottles, iron citrate (20 mM) was used as an electron acceptor during pre-cultivation. Bacterial growth was monitored following the optical density (reaching approximately 0.23 for *D. baarsii* and 0.22 for *D. alkaliphilus*), substrate consumption and, in the case of *D. baarsii*, the precipitation of black FeS. If not stated otherwise, the solution at the end of the third transfer was used for BES inoculation.

BES operation and electrochemical analyses

All bioelectrochemical experiments were performed in sterilized, double chamber bio-electrochemical reactors, as already used in our previous study.[8] Each compartment was a 130 mL Duran bottle with a net liquid volume of 100 ml, separated with a cation exchange membrane (Fumapem-FKE-PP-75, Fumatech, Germany). A similar medium (volume 85 mL) as used during the pre-cultivation period was used in the compartment of the working electrode and was inoculated with 15 mL of the bacterial medium grown in the serum bottle, reaching the initial optical density of *ca.* 0.02. During anodic conditions, 4 mM butyrate or 30 mM formate was added to the medium of *D. baarsii*, and 5 mM acetate or 22 mM formate was used for *D. alkaliphilus* as sole carbon sources. During cathodic conditions, all organic compounds were removed from the medium, and sulphate (10 mM) and sulphur (10 mM)/thiosulphate (2 mM) were provided as electron acceptors for *D. baarsii* and of *D. alkaliphilus*, respectively. The counter electrode compartment contained the same medium as in the working electrode compartment, yet, without any of the above carbon sources or electron acceptors. In the case of abiotic control experiments, the reactors were operated under completely sterile conditions.

Graphite plates (surface area of 3.14 cm^2) and graphite rods (surface area of 35 cm^2) were used as working and counter electrodes, respectively. If required, graphite felt with the projected surface area of 3.14 cm^2 was used as an alternative working electrode material. Ag/AgCl (saturated KCl, Sensortechnik Meinsberg, Germany, 0.197 V vs. SHE) was used as the reference electrode, placed next to the working electrodes. All electrode potentials provided in this manuscript refer to this reference electrode. All reactor compartments were purged by N_2/CO_2 (95/5) for 40 minutes before starting the experiment to remove the oxygen dissolved in the solution. All experiments and cultivations were performed in triplicates – and in a thermostated anaerobic chamber to assure strictly anaerobic conditions during the experiments. The desired working electrode potentials (0.2 V for anodic conditions and −0.8 V for cathodic conditions) were applied using a multi-channel potentiostat (VMP-3, BioLogic Instruments, France) and a three-electrode arrangement. The potential values were selected according to previous experiment:[8] 0.2 V provided a suitable anodic condition for enriching electroactive microbes and −0.8 V was a threshold of the abiotic H_2 production, confirmed by the abiotic control experiment indicating the lack of H_2 production at electrodes poised at this potential.

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed regularly to investigate potential cell-electrode interactions. CVs were performed at the scan rate of 1 $mV\,s^{-1}$ for two scans, with the second scans being shown here. EIS measurements were performed by setting the amplitude of the sinusoidal excitation signal at 30 mV. The frequencies were between 1000 kHz and 10 mHz. 10 frequency points were recorded per decade by measuring each frequency for 3 times. The AC signal was superimposed with a constant DC potential of 0.2 V or −0.8 V.

Chemical analyses

High performance liquid chromatography (1260 Infinity II chromatograph, Agilent Technologies, USA was used to measure the concentrations of volatile fatty acids such as formate, acetate, propionate, butyrate, valerate and hexanoate, as well as methanol and ethanol in the samples. The machine was equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, USA), a reference index detector, and a diode array detector. A gas chromatography (Thermo scientific, FOCUS GC) equipped with a thermal conductivity detector and a ShinCarbon ST Micropacked column (Restek, USA) was used to detect gas composition (H_2, CO_2 and CH_4) in the reactor headspaces using helium as a carrier gas. Sulphate concentrations in the media were measured using sulphate detection kits (LCK 153, Hach Lange GmbH, Germany).

Microscopic analyses

Speciation analyses of the biofilms were performed using Raman spectroscopy (InVia REFLEX, Renishaw, UK) equipped with a laser wavelength of 532 nm (Nd:Yag laser) and a Leica-Microscope DM 2700 at 20 fold magnification. In order to capture the image of the electrode surface, scanning electron microscopy (SEM, Zeiss EVO LS 10) was used. The electrodes were prepared by fixing in 2% paraformaldehyde (PFA) in aqueous phosphate buffer for 30 minutes.[20] The fixed biofilms were then stored in a 70% ethanol solution at −20 °C. Electrodes were dried and gold-coated before SEM analysis.

Results and Discussion

Anodic behaviour of Desulfarculus baarsii

The anodic growth behaviour of *D. baarsii* was studied using butyrate (4 mM) as the sole electron donor, and a positively polarized (0.2 V) working electrode as the sole electron acceptor. Sulphate was removed from the medium to exclude indirect electron transfer. Over almost 6 days operation, no butyrate consumption was observed, similar to the sterile control reactor. Concurrently, no significant electricity generation was monitored, albeit a slight difference between the biotic reactor and abiotic control (Figure 1). A previous study on SRB in MFC showed the long lag phase, with current generation starting only after almost 2 weeks.[21] In our experiments, however, even after 40 days no butyrate consumption and no anodic current generation was observed. We repeated the experiment using graphite felt as electrode material instead of graphite plate, in order to exploit the fibrous composition as a filter to collect the bacterial cells for an improved biofilm formation. Yet, no electricity generation and organic consumption was observed.

The consumption of propionate and formate were also investigated separately in an anodic compartment. Similar to butyrate, propionate was not consumed over 10 days experimental duration. Different results, however, were observed

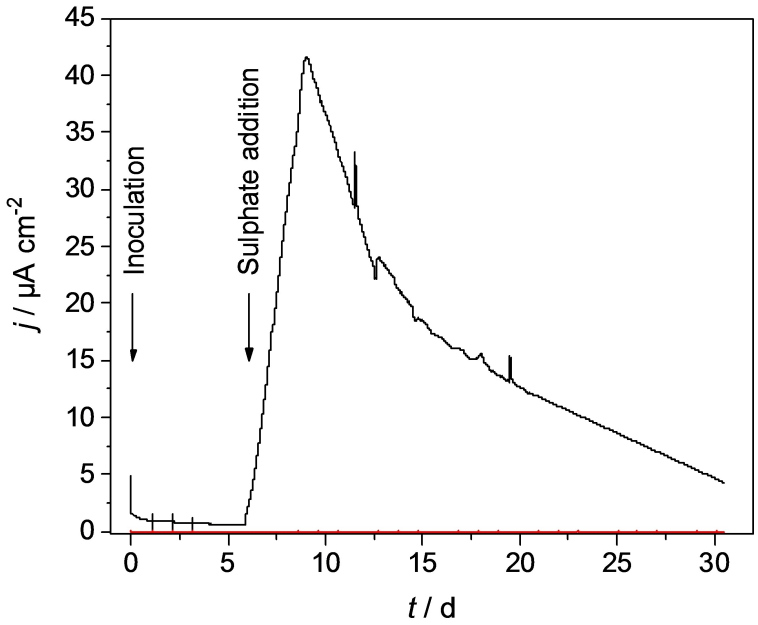

Figure 1. Anodic current generation of a bioelectrochemical reactor inoculated with *D. baarsii.* The applied electrode potential was 0.2 V. The red curve represents an abiotic (sterile) control experiment.

when 30 mM of formate was used as the electron donor (and graphite plate as anode material). Although current generation remained negligible, formate was depleted after 7±1 days – accompanied by the production of acetate (up to 9 mM acetate at the end of the experiment). Very similar trends were observed in control reactors operated under open circuit condition (confirming the none-electrochemical nature of the process) and upon removal of any potential electron acceptors such as minerals from the culture medium. Most likely, the observed formate consumption and acetate production can be attributed to an autotrophic process: Thus, autotrophic growth of *D. baarsii* using formate and CO_2 was reported previously, in which 60% of the bacterial biomass were made of carbon from CO_2 and the rest from formate.[22] According to that study, formate and CO_2 assimilation in *D. baarsii* is similar to carbon fixation in acetogenic bacteria through Wood-Ljungdahl pathway, where formate or H_2 are supplying energy for reduction of CO_2 and production of acetate,[22] which was also observed in our study.

D. baarsii was evidently not capable of performing direct electron transfer (DET) to the polarized anode. This was also reaffirmed by a lack of bacterial cell growth in serum bottles supplied by iron citrate as a sole electron acceptor, as several microorganisms able to donate electrons directly to an anodic electrode were reported to be capable of accepting Fe (III) as an electron acceptor.[12] Therefore, the ability of *D baarsii* towards mediated electron transfer (MET) was investigated by providing 10 mM sulphate to an inoculated BES reactor, containing 4 mM of butyrate as the carbon source and a graphite plate anode, poised at 0.2 V. For comparison, a biotic control reactor was operated at open circuit.

The anodic current generation, depicted in Figure 1, began immediately after sulphate addition, and was accompanied by the consumption of butyrate. A similar trend was also observed in other replicates, as shown in Figure S1. A similar butyrate consumption was also observed under open circuit conditions. The major difference between the anodic and the open circuit reactor lied in the remaining sulphate concentration levels. Under anodic operation, 5.6±1.4 mM of sulphate remained upon butyrate depletion, while no sulphate was remaining in the open circuit reactor. *D. baarsii* is known to consume VFAs such as butyrate to CO_2 when sulphate is provided as a terminal electron acceptor. According to reactions (1) and (2), the oxidation of 2 moles of butyrate requires the reduction of 5 moles of sulphate to sulphide. Therefore, for a complete bacterial mineralization of 4 mM butyrate, *ca.* 10 mM sulphate is required.

$$CH_3CH_2CH_2COO^- + 6H_2O \rightarrow 4\ CO_2 + 19H^+ + 20e^- \quad (1)$$

$$SO_4^{2-} + 8H^+ + 8e^- \rightarrow S^{2-} + 4H_2O \quad (2)$$

This is in agreement with the open circuit experiment, in which the entire amount of sulphate was consumed.

The detection of remaining sulphate in the solution of the current producing BES reactor (in combination with the inability of *D. baarsii* to produce currents in the absence of sulphate) strongly indicates the role of the sulphate/sulphide couple as an electron shuttling system, with the (abiotic) electrochemical re-oxidation of microbially formed sulphide being the actual current producing step. In contrast to an electron transfer based on secondary metabolites – such as phenazines or flavins – this is an example of a primary metabolite based mediated electron transfer.[23–24]

An ideal shuttling process would require the complete re-oxidation of sulphide to sulphate – with the maximum number

of liberated electrons and the recovery of the microbial terminal electron acceptor (reaction 3).

$$S^{2-} + 4H_2O \rightarrow SO_4^{2-} + 8H^+ + 8e^- \quad (3)$$

Yet, depending on the electrochemical conditions (electrode potential, electrode material, etc.), the electrochemical sulphide oxidation can also lead to an incomplete sulphide oxidation (see reactions 4 and 5, for example).

$$2S^{2-} + 3H_2O \rightarrow S_2O_3^{2-} + 6H^+ + 6e^- \quad (4)$$

$$S^{2-} \rightarrow S^0 \left(\text{or } S_n^{2-}\right) + 2e^- \quad (5)$$

Thus, in our experiments, the slight yellow colour of the medium at the end of the batch cycle indicates polysulphide formation; whereas the presence of elemental sulphur at the electrode surface was confirmed using Raman spectroscopy (Figure S2).

Our hypothesis was further investigated by performing abiotic control experiments with only sulphide or only sulphate under sterile conditions and applied electrode potentials of 0.2 V. Sulphate concentration was completely stable during 14 days of abiotic control experiment, while almost 50% of sulphide was changed to sulphate.

At the end of the bioelectrochemical experiments, scanning electron microscopy was used to analyse a potential biofilm formation at the working electrodes. Predictably, no biofilm was observed at the electrode when no sulphate was provided in the medium. However, bacterial biofilms were observed when sulphate was provided during the experiments, supporting the ability of *D. baarsii* to grow under the conditions of electrochemical sulphate regeneration (Figure S3).

Cathodic behaviour of Desulfarculus baarsii

The ability of *D. baarsii* in catalysing cathodic reactions was investigated by starting the bioelectrochemical reactors without any organics in the medium. 10 mM sulphate was supplied as a microbial electron acceptor. However, no sulphate consumption (samples collected on days 1, 2, 3 and 6) was obverse and, apart from an initial reductive current during the first day of operation, no significant cathodic current was generated over 6 days of the experiment (Figure 2). Similar trend was also observed in other replicates (Figure S4). It is likely that the reductive current during day 1 is caused by the reduction of components in the microbial growth medium. On day 6, formate was added to the medium to provide an additional carbon source for the bacterial cells than only bicarbonate, however, no change in cathodic current was observed, indicating the probable lack of bacterial abilities in electron uptake from the electrode.

Anodic behaviour of Desulfurivibrio alkaliphilus

The anodic growth behaviour of *D. alkaliphilus* was studied at a constant electrode potential of 0.2 V and using 5 mM acetate as a sole electron donor. All potential electron acceptors were removed from the culture medium. As shown in Figure 3A, current generation in the reactors began within the first day after the inoculation and reached the peak current after almost

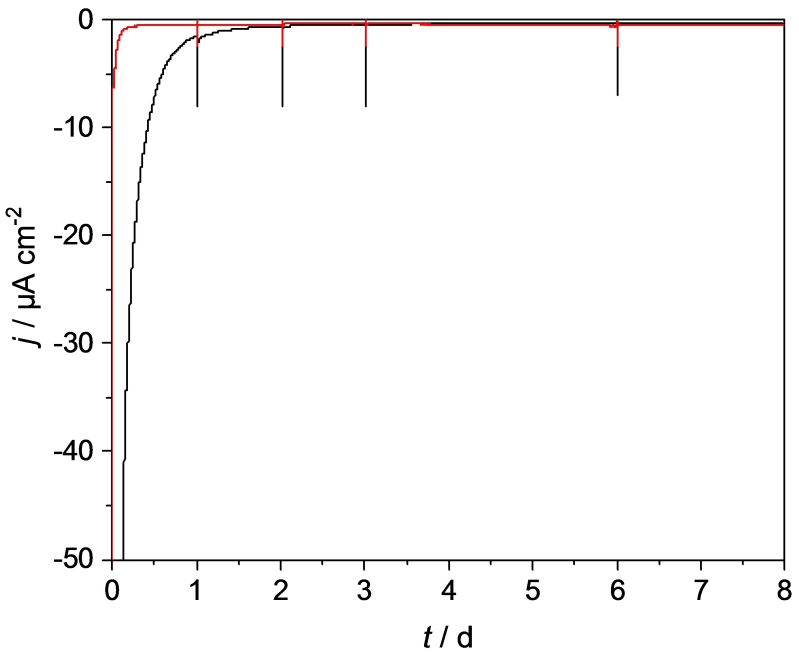

Figure 2. Cathodic current generation at an applied electrode potential of −0.8 V, recorded from a bioelectrochemical reactor inoculated with *D. baarsii* (black curve). The blue arrow indicates the time of formate addition to the medium. The red curve represents an abiotic (sterile) control experiment.

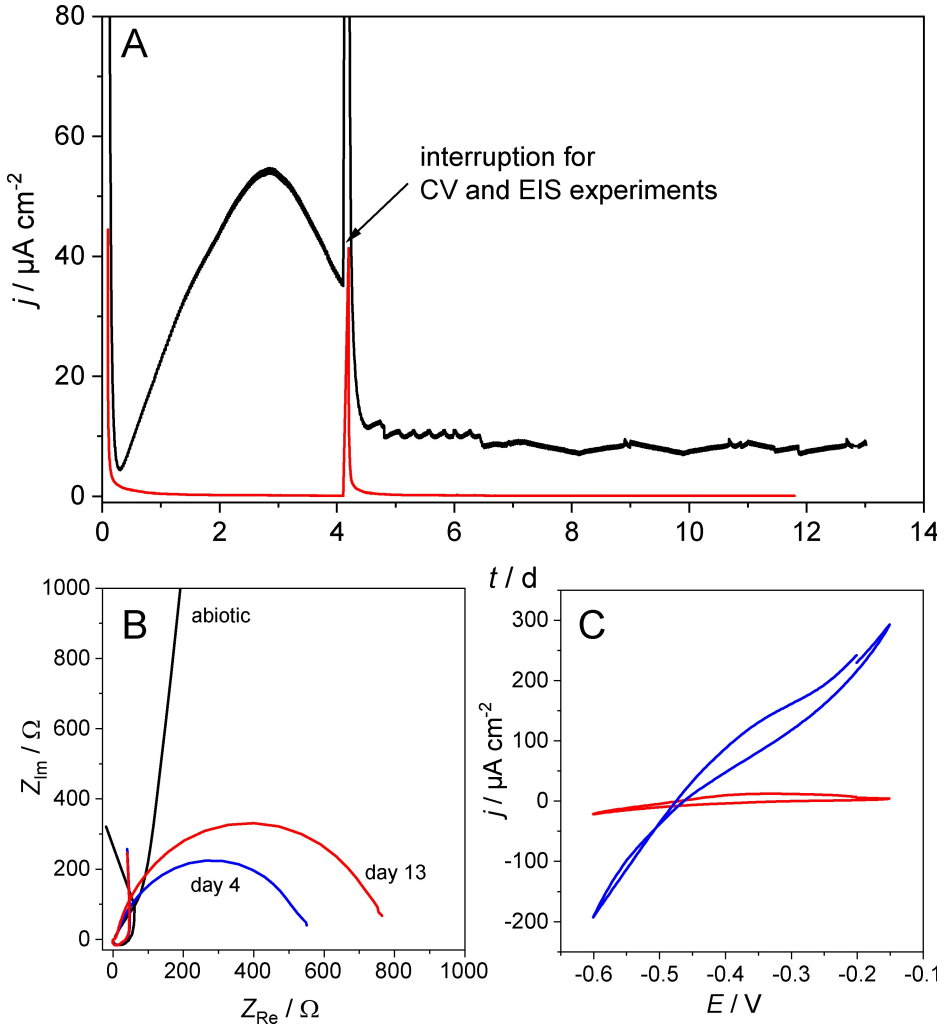

Figure 3. (A) Anodic current generation of a bioelectrochemical cell inoculated with *D. alkaliphilus*, using acetate as sole electron donor. The applied electrode potential was 0.2 V. The red curve represents an abiotic (sterile) control experiment. **(B)** Nyquist plots of electrochemical impedance spectroscopy data recorded during the experiment; **(C)** Cyclic voltammograms recorded during turnover (blue curve) and at the end of the batch experiment (red curve), at a scan rate of 1 mV s^{-1}.

3 days (the results of other replicates are presented in Figure S5).

Hereby, with a Coulomb efficiency of 93 ± 3%, the cumulated charge corresponded well with the amount of consumed acetate. After about three days of operation, the current declined to a stationary value of about 10 µA cm^{-2}. Since at that point only 10% of the provided acetate was consumed, the decay cannot be attributed to substrate depletion.

EIS data depict the bioelectrochemical experiment from the viewpoint of the internal resistances of the system, reflecting its ability to perform a bioelectrochemical reaction. Thus, Figure 3B shows that the abiotic control (black curve) is dominated by a straight line with a phase shift angle of approaching 90° – representative for a purely capacitive behaviour without any charge transfer processes. In the presence of *D. alkaliphilus*, a semi-circle occurred that is typical for a charge-transfer process and combines faradaic and capacitive behaviour. It reached a low value during the phase of maximum bioelectrochemical activity (here represented for EIS data recorded at day 4 – blue curve) and increased again in the course of the experiment (red curve in Figure 3B). The reasons for the decline in current density (Figure 3A) and the increase of charge-transfer resistance (Figure 3B) are so far unclear – could, however, potentially be found in the properties of the biofilms formed by *D. alkaliphilus*: In their mature state, the thick reddish biofilms (Figure S6) are soft, and with only low adherence to the electrode surface. A resulting low cell-to-cell and cell-to-electrode interaction may thus lead to the decrease in bioelectrochemical performance.[25–26] A more profound picture of the underlying limitations, however, requires additional analysis, e.g., by studying the viscoelastic properties of the growing biofilms.[27]

The weak attachment to the electrode surface (in contrast to strongly attached biofilms of other electroactive bacteria, such as *Geobacter sulfurreducens*[28]) and the fragility of the biofilms led to their detachment from the electrode after transferring the electrodes to the fixing solution prepared for SEM analysis, preventing conventional biofilm fixing. Therefore,

the biofilms were dried for 1 hour after termination of the reactors, and SEM images were recorded from the gold-coated dried biofilms without fixation. Although bacterial cells are not clearly visible due to the omission of fixation steps, the SEM images show a thick biofilm layer at the electrode surface (Figure S7).

Raman microscopy (Figure 4) performed at the end of the experiment showed the presence of cytochrome b and c in both oxidised and reduced forms within the *D. alkaliphilus* biofilms[29] – which also explains the reddish colour of the biofilms, similar to the previous observations by Sorokin et al.[17] Thereby, the Raman spectrum of *D. alkaliphilus* resembled the spectrum of *Geobacter sulfurreducens*. Judging from the extraordinary electroactivity of *Geobacter sulfurreducens*, this resemblance could indicate a direct interaction of *D. alkaliphilus* cells with electrodes via cytochromes. In addition to the Raman results, *D. alkaliphilus* was reported to have larger pili than *Geobacter* but with comparable conductance.[19] It was also reported that porin-cytochrome genes of *D. alkaliphilus* were highly related to those in *Geobacter* recognised for their involvement in extracellular electron transfer.[30] Our observations in addition to the previous studies could explain the growth and electro-activity of *D. alkaliphilus* cells under anodic conditions.

Based on the physiological similarity of *D. alkaliphilus* and *G. sulfurreducens*, a related voltammetric behaviour was expected. However, the cyclic voltammograms obtained for *D. alkaliphilus* biofilms (Figure 3C) were comparatively undefined, preventing the determination of characteristic redox potentials. Thereby, the slope of the turnover voltammetric curve indicates a dominating internal biofilm resistance, as already indicated by the EIS data.

In addition to acetate, consumption of formate (22 mM) as a substrate was also investigated. It seemed that anodic formate consumption is more feasible for bacterial cells, as they consumed almost 80% of formate over 14 days of experiment, at a coulombic efficiency of 85.9 ± 0.5% (Figure S8). Although it is also possible that *D. alkaliphilus* is able to oxidise sulphide for chemoauthotroph growth,[18] an (abiotic) electrochemical sulphide oxidation would overlap with such biotic process. We therefore did not study sulphide as an electron donor.

In parallel with the anodic cultivation, control reactors operated at open circuit showed no organic consumption and no visible biofilm formation, supporting the critical role of the anode as an electron acceptor for bacterial growth.

The anodic biofilm cultivation was also investigated at a lower electrode potential of −0.2 V in order to supress the oxidation of possible sulphur compounds transferred with inoculum in the anolyte and their effect on the anodic current generation.[8] However, a similar biofilm growth and bioelectrochemical behaviour was obtained for both conditions (data not shown).

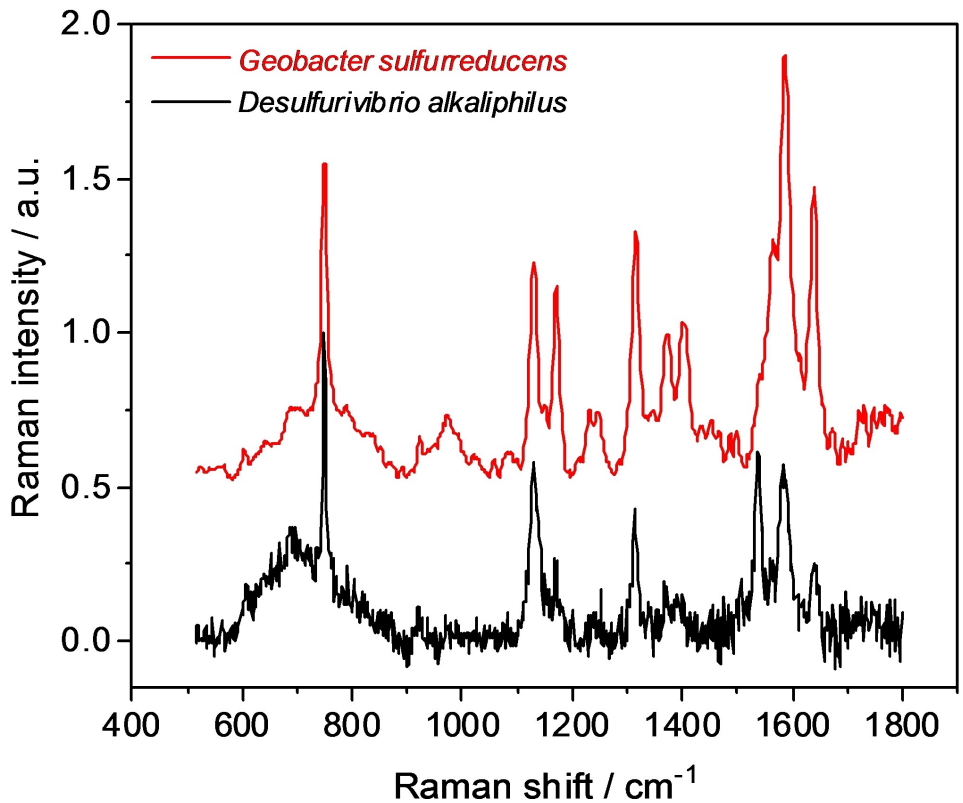

Figure 4. Raman spectrum of a *Desulfurivibrio alkaliphilus* biofilm (black curve) formed after 13 days of anodic cultivation in comparison to the spectrum of a *Geobacter sulfurreducens* biofilm (red curve, data derived from[31]). The height of the spectra was normalized to 1 using the Raman peak at 749 cm^{-1}.

Cathodic behaviour of Desulfurivibrio alkaliphilus

D. alkaliphilus are able to utilize inorganic carbon (CO_2) to grow by exploiting elemental sulphur or thiosulphate (depending on the genes involved) as electron acceptors.[32] They are also able to fix CO_2 following Wood-Ljungdahl pathway, however due to the lack of genes required for the conversion of acetyl-CoA to the end product acetate, CO_2 is eventually converted into biomass.[32] To investigate whether *D. alkaliphilus* can also catalyse cathodic reactions, bioelectrochemical reactors were operated in the absence of organic compounds and at a cathodic electrode potential of −0.8 V (Figure 5A).

Over the first 5 days, elemental sulphur was provided as the sole electron acceptor. The negligible cathodic current during this time (Figure 5A) was likely related to the lack of solubility of elemental sulphur and therefore its limited microbial availability (the results of other replicates are demonstrated in Figure S9). On day 5, thiosulphate (final concentration 2 mM) was added to the cathodic compartment, which resulted in a steady increase of the cathodic current to a maximum current density of *ca.* −47 μA cm^{-2} on day 24. After day 26, slight yellow colouration began to appear in the solutions, likely due to the production of polysulphides. During the experiment no organic compound was detected in the electrolyte. In addition, no H_2 production was observed in the reactors.

Cyclic voltammograms recorded during the cathodic cultivation (Figure 5B) show – with progressing cultivation time – increasing peak currents. However, their shape and characteristics (e.g., considering the unusually large peak-to-peak separation, the missing peak overlap and the missing catalytic behaviour) are untypical for a bioelectrochemical system. Comparative measurements of the abiotic controls (Figure S10) support their abiotic nature. Based on the similarity in the characteristics of the voltammograms with previous publications (see, e.g.[8]), the voltammetric system can most likely be ascribed to the electrochemical oxidation of sulphide ions to e.g., elemental sulphur and its re-reduction. Hereby, sulphide originates from the microbial reduction of thiosulphide by *D. alkaliphilus* (in the biotic system) and by a slow chemical

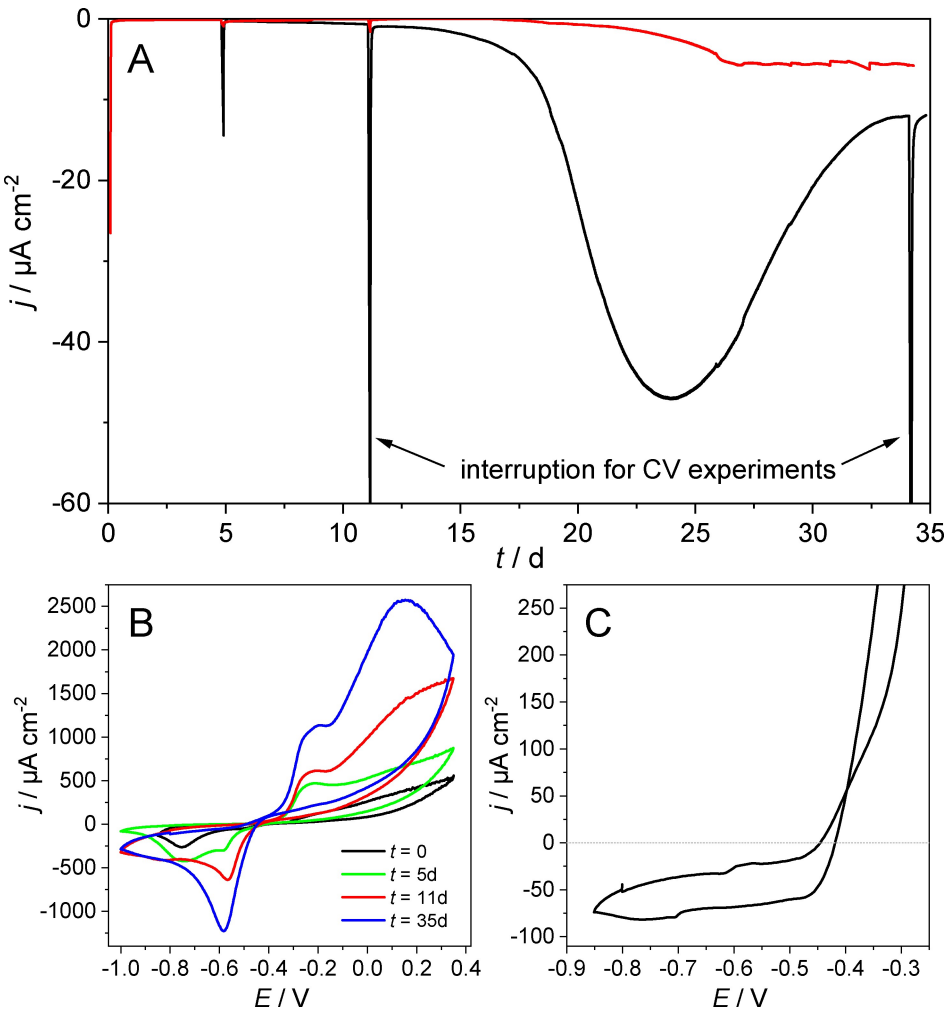

Figure 5. (A) Cathodic current generation of a bioelectrochemical cell inoculated with *D. alkaliphilus*. The applied electrode potential was −0.8 V. The red curve represents an abiotic (sterile) control experiment. **(B)** Cyclic voltammograms recorded during the experiment depicted in (A) – at t = 0 and at days 5, 11 and 35 after inoculation. The scan rate was 1 mV s^{-1}. **(C)** Cyclic voltammograms recorded for the abiotic control reactor.

thiosulphide disproportionation[33] (abiotic system) – explaining the increase of the voltammetric currents over time.

Although no cathodic H_2 was detected in the headspace of the abiotic control reactor, it cannot be excluded that small amounts of H_2 were generated during the cathodic electrode polarization. It can thus not be ruled out that H_2 was produced abiotically and was consumed by microbes as an energy source, leading to H_2 mediated electron transfer. However, Raman spectroscopy showed the presence of cytochromes in the cathodic *D. alkaliphilus* biofilms (Figure S11) – indicating cytochrome-based electron transfer. Therefore, similar to a previous biocathode study,[34] both direct and indirect microbe-cathode interaction may take place. In order to isolate a potential electrochemical contribution of a cytochrome-based electron transfer from the electrochemical sulphide system, we limited the potential window of the voltammetric experiment to a region below −0.2 V (Figure 5C). The resulting voltammogram exhibits a reductive plateau between −0.45–0.8 V, indicating cathodic bioelectrocatalytic activity in this potential range. The transition to oxidative currents (most likely also involving sulphide oxidation) takes place at about −0.43 V. At this point, unfortunately, the determination of a formal potential from the turnover curve (see also[35]) was not hindered by the overlapping abiotic sulphide oxidation.

Conclusion

D. baarsii and *D. alkaliphilus* are marine microorganisms that were previously identified as dominating organisms in bidirectional electrochemically active biofilms developed.[13–14] The target of this study was to investigate their bioelectrochemical properties. Our results illustrate a very different electrochemical behaviour of the two organisms. Thus, *D. alkaliphilus* was highly active for catalysing both anodic and cathodic reactions. Raman spectroscopy revealed the presence of cytochromes b and c and showed similarity with *Geobacter*, indicating a cytochrome based direct electron transfer ability – making *D. alkaliphilus* a typical electrochemically active bacterium.[36] *D. alkaliphilus* can not only oxidise organic acids like acetate and formate, but should, based on literature information,[18] also be able to utilize inorganic electron donors such as hydrogen or sulphide for growth and electricity generation. Cathodic growth and electrochemical activity were possible through autotrophic growth using a polarised cathode as an energy source and sulphur compounds such as thiosulphate as electron acceptors.

D. baarsii was unable to interact directly with electrodes. Its abundance in the sediment based electrochemically active biofilm[14] can be thus ascribed to its ability to reduce sulphate to sulphide, which in turn could be electrochemically re-oxidized – establishing a mediated electron transfer and allowing *D. baarsii* to grow anodically. The exploitation of a sulphate based mediated electron transfer seems especially feasible for environments with high abundance of sulphate, such as marine systems with sulphate concentration levels of about 2500 mg L^{-1}.[11] The combination of biochemical sulphide reduction and electrochemical sulphide re-oxidation also provides the opportunity of decoupling both steps for an intermittent energy storage – e.g., via the accumulation of sulphides[37] or elemental sulphur[8] at the electrode surface.

Although we did not study possible syntrophies between *D. baarsii* and *D. alkaliphilus*, our results indicate that both species within mixed culture biofilms could potentially interact via two pathways: (i) Thus, the ability of *D. alkaliphilus* for using sulphide as an electron donor may potentially allow an interspecies electron transfer from *D. baarsii* to *D. alkaliphilus* via the sulphide/sulphate couple. (ii) In addition, when formate is available, *D. baarsii* could consume it through the Wood-Ljungdahl pathway and produce acetate, which could serve as an energy and carbon source for the anodic metabolism of *D. alkaliphilus*.

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Conflict of Interest

The authors declare no conflict of interest.

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