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Effect of surface nano/micro-structuring on the early formation of microbial anodes with *Geobacter sulfurreducens*: Experimental and theoretical approaches

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A B S T R A C T

Smooth and nano rough flat gold electrodes were manufactured with controlled Ra of 0.8 and 4.5 nm, respectively. Further nano rough surfaces (Ra 4.5 nm) were patterned with arrays of micro pillars 500 μm high. All these electrodes were implemented in pure cultures of *Geobacter sulfurreducens*, under a constant potential of 0.1 V/SCE and with a single addition of acetate 10 mM to check the early formation of microbial anodes. The flat smooth electrodes produced an average current density of 0.9 A·m⁻². The flat nano rough electrodes reached 2.5 A·m⁻² on average, but with a large experimental deviation of ±2.0 A·m⁻². This large deviation was due to the erratic colonization of the surface but, when settled on the surface, the cells displayed current density that was directly correlated to the biofilm coverage ratio. The micro pillars considerably improved the experimental reproducibility by offering the cells a quieter environment, facilitating biofilm development. Current densities of up to 8.5 A·m⁻² (per projected surface area) were thus reached, in spite of rate limitation due to the mass transport of the buffering species, as demonstrated by numerical modelling. Nano roughness combined with micro structuring increased current density by a factor close to 10 with respect to the smooth flat surface.

Keywords: Bioanode, Surface roughness, Microbial adhesion, Microbial fuel cell, Modelling

1. Introduction

For around two decades, microbial anodes have been opening up fascinating avenues for a huge number of electrochemical processes [1–3]. Microbial fuel cells (MFCs) were the pioneering systems in which microbial anodes were implemented [4,5] and they have since been the source of numerous innovative technological concepts, such as microbial electrolysis cells for hydrogen production [6,7] metal recovery [8,9], microbial autonomous biosensors [10,11] and a microbial snorkel for environmental bioremediation [12,13].

Biofilm development on an electrode surface can be described in three steps: initial reversible contact between cells and the surface, followed by irreversible cell attachment and, finally, biofilm growth due to bacterial division and extracellular matrix production [14,15]. In each of these phases, biofilm formation is strongly dependent on the surface/bacteria interaction. Many studies have pointed out the electrode surface topography as a key parameter of the efficiency of microbial electrodes [16–20]. Roughness has often been used to investigate the effects of surface topography but it remains difficult to extract clear trends, most likely because of the different techniques that have been used to roughen the electrode surfaces, which did not allow direct comparison among the studies [21–23].

Some research teams have structured surfaces by using the accurate tools provided by nano and micro technologies in order to control the surface topography perfectly. Amazing advances have been made in this way. Titanium nanowires and nanotubes have been shown to improve the performance of electroactive biofilms by acting as substitutes for bacterial pili [24–26]. Recessed micro structures, such as holes, revealed a positive effect due to the increased contact area between the cell and the electrode surface, when they were the same size as the cell. Nevertheless, as a side effect, holes that are too deep can induce detrimental segmentation of the biofilm [27–29]. Protuberant micro structures, such as pillars, do not present this drawback. Gold cross shaped micro pillars 40 μm wide and 8 μm high inoculated with *S. cerevisiae* have shown a 4.9 fold increase in current density, mainly explained by an increase of the electrode surface area to volume ratio by a factor of

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Microbial anodes manufactured with 20 μm high and 20 μm diameter cylindrical micro pillars and inoculated with *S. marcescens* have displayed a 1.5 fold increase of the current density [31].

Most of these studies have delivered worthwhile conclusions on mature biofilms. In contrast, almost nothing is known about the mechanism of the early phases of the formation of electroactive biofilms, in which the surface topography should have a crucial impact on the initial steps of biofilm formation. Moreover, it can also be presumed that the final electrocatalytic properties of a biofilm depend strongly on its early formation phases, which establish the interfacial layer between the electrode surface and the biofilm [32].

The purpose of the present study was to gain insight into the impact of surface topography on the early phases of electroactive biofilm formation. Microtechnology techniques were implemented to control the surface roughness of gold electrodes perfectly at the nanometre level and then to pattern the surface with arrays of micro pillars. Gold was chosen as the electrode material because of its suitability for implementing micro technology techniques and because it has been demonstrated to be fully able to support the formation of efficient microbial anodes [16,30,33–36]. Micro structuring of the surfaces was performed with micro pillars having a square cross section. Their height of 500 μm was significantly greater than those generally reported in the literature so far, in order to maximize the chances of detecting their impact.

Many bacteria have been found to possess electrocatalytic properties [37]. Among the models of electroactive bacteria, *Geobacter sulfurreducens* [38] has been widely used and has shown one of the highest levels of performance in terms of current production in pure culture [39,40]. For this reason, this species was chosen for the present study.

### 2. Materials and methods

#### 2.1. Electrode manufacturing

Smooth gold (SG) and Nano rough gold (NG) electrodes were formed on Si (100), P type silica wafers. The SG surfaces were deposited by evaporation of a 250 nm thick gold layer. The NG electrodes were produced by roughening the surface of SG electrodes by electrolytic deposition of gold to achieve a gold layer 1.75 μm thick. The average roughness, measured by AFM was 0.8 nm for the SG surfaces and 4.5 nm for the NG.

Surface micro structuring consisted of arrays of square micro pillars 100 μm wide and 500 μm high. They were created on the same Si (100), P type silica wafers with an epoxy resin (SU 8 3050, MicroChem Corp) patterned by photolithography to form the pillar array. Electrode metalization was performed by electrolytic deposition of gold as for the NG electrodes. A 1 μm thick gold layer was thus created, with a roughness of 4.5 nm identical to that of the NG electrodes. The different pillar array sizes were obtained with spacings of 100 μm (μP100NG), 125 μm (μP125NG) and 200 μm (μP200NG). The three micro structured electrodes had 2500, 2025 and 1156 pillars and an overall geometric surface area of 5.73, 5.03 and 3.26 cm², respectively. Each pillar array was tested in duplicate.

#### 2.2. Inocula and media

*Geobacter sulfurreducens* (ATCC 51573) was purchased from DSMZ. The specific growth medium contained, per litre: 0.1 g KCl, 1.5 g NH₄Cl, 2.5 g NaHCO₃, 0.6 g NaH₂PO₄, and 0.82 g CH₃COONa. This medium was sterilized by autoclaving in bottles at 121 °C for 20 min. After sterilization, the medium was completed with 8 g/L sodium fumarate filtered at 0.22 μm, 10 mL/L Wolfe’s vitamin solution (ATCC MD VS) and 10 mL/L modified Wolfe’s minerals (ATCC MD TMS). A pre culture of *G. sulfurreducens* was prepared in a fresh deoxygenated culture medium (2% v/v, 30 °C) for 3 days to reach a final absorbance of around 0.4 at 620 nm. Reactors were then inoculated with this pre culture. The inoculation volume was adjusted to obtain the cell density in each reactor that corresponded to an inoculation ratio of 10% v/v with an inoculum at 0.4 absorbance. Inoculation was performed after 20 min of deoxygenation by bubbling N₂:CO₂ (80:20) through the reactors filled with the specific growth medium, vitamin solution and modified Wolfe’s minerals. In the reactor medium, acetate 10 mM was the sole electron donor and no fumarate was present.

#### 2.3. Experimental set up and electrochemical characterization

All experiments were performed with 3 electrode set ups in single compartment cells. The reactors, each of 500 mL total volume, contained 300 mL solution and were tightly sealed to ensure anaerobic conditions.

A gaseous mix of N₂ and CO₂ (80:20) was continuously and gently sparged into each reactor. The gas sparger was located behind the auxiliary electrode and directed towards the reactor wall to avoid disturbance of the working electrode by gas bubbling. The temperature was kept at 30 °C with a water bath.

The gold electrodes, used as working electrodes, were enclosed in a Teflon based support that exposed a 1 cm² surface area to the medium. A platinum grid (Heraeus SAS, Germany) was used as the auxiliary electrode and a saturated calomel reference electrode as the reference (SCE, potential +0.24 V/SHE). Bioanodes were formed under constant polarizaton at 0.1 V/SCE (VSP potentiostat, Bio Logic SA, France). Current densities were determined based on the projected surface area of the anode, which was always 1 cm². The chronoamperograms were analysed by determining the maximum current density that was reached (jmax) and the time required for the electrodes to start to produce current. This starting time was defined as the time at which current density reached 0.1 A·m⁻².

#### 2.4. Microscopy imaging

At the end of the experiments, the bioanodes were stained with acridine orange 0.01% (A6014 Sigma) for 10 min, then carefully washed with medium and dried at ambient temperature. The flat smooth (SG) and nano rough (NG) electrodes were imaged with a Carl Zeiss Axiovalmager M2 microscope equipped for epifluorescence with an HBO 50 W ac mercury light source and the Zeiss 09 filter (excitor HP450 490, reflector FT 10, Barrier filter LP520). Images were acquired with a monochrome digital camera (Evolution VF). Many different spots were imaged in order to see the general pattern of the colonization. The micro structured electrodes (μP1NG) were imaged with a LEICA TCS SP8 confocal microscope equipped with a 20 W Argon laser and a CS2 UV Optics 1 filter. The active detector was a PMT (500 nm 600 nm). Images were acquired every 2.4 μm along the pillars and the 3D reconstruction was performed using LAS X software.

Epifluorescence images were analysed by greyscale interpretation to calculate the proportion of the electrode surface covered by the bacteria. The grey intensity threshold between the areas covered by the bacteria and the non covered areas was set manually. Grey levels greater than the threshold value were considered to correspond to colonized areas, while grey levels lower than the threshold were considered to show clean areas. Six images, of dimensions 866 by 645 μm, were treated for each electrode to determine the average covering ratio, the edges of the electrodes being excluded to avoid the impact of side effects.

### 3. Results and Discussion

#### 3.1. Nano structuring

Two smooth (SG) and seven nano rough (NG) gold electrodes were implemented separately in pure cultures of *Geobacter sulfurreducens* under applied potential of 0.1 V/SCE. The medium was fed initially with acetate at 10 mM and sparged continuously with N₂:CO₂ (80:20).
The smooth electrodes started to produce current at days 3.2 and 5.5 and reached only modest maximum current densities ($J_{\text{max}}$) of 1.1 and 0.75 A·m$^{-2}$ (Fig. 1.A). The nano rough electrodes displayed high variability. Two NG electrodes showed performance close to the SG electrodes: long starting time of 4.2 and 5.1 days, slow current evolution, which led to low $J_{\text{max}}$ of 0.12 and 0.86 A·m$^{-2}$ (Fig. 1.B). The other five NG electrodes showed very similar, starting times between 1 and 1.3 days and reached high $J_{\text{max}}$ values, but with great variability, in a range of 1.6 to 6.4 A·m$^{-2}$.

These results reveal a significant variability, particularly relating to $J_{\text{max}}$, although the initial surfaces and experimental conditions were strictly identical. Nevertheless, they show that the nano roughness of 4.5 nm favoured the electroactive biofilm formation and performance, leading to average $J_{\text{max}}$ of 2.5 ± 2.0 A·m$^{-2}$, in comparison to smooth surface ($Ra = 0.8$ nm), which produced average $J_{\text{max}}$ of 0.92 ± 0.25 A·m$^{-2}$.

Once the current had fallen to zero, the electrode colonization was observed by epifluorescence microscopy. Coverage ratios are reported in Table 1. On the two SG electrodes, biofilm clusters had formed and covered 65.8 ± 9.6 and 58.7 ± 8.7% of the electrode surface as illustrated in Fig. 2.8. Although the global microbial coverage was significant, the biofilm structures were isolated from one another and no large biofilm patches could be observed. On the NG electrodes, as noted for the current production, a wide range of bacterial colonization patterns was observed (Fig. 2.1 to .7). For the two electrodes producing the lowest $J_{\text{max}}$, NG 1 and NG 2, the observation highlighted the presence of microbial colonies and isolated cells on the surface but no significant biofilm patches were observed (Fig. 2.1 and .2). This resulted in coverage ratios of 18.2 ± 5.2% and 34.2 ± 10.8%. On the NG 3, NG 4 and NG 5 electrodes, producing 1.6, 2.4 and 2.8 A·m$^{-2}$ $J_{\text{max}}$, the coverage ratios rose to respectively 59.6 ± 8.4, 77.2 ± 8.7, and 82.0 ± 7.0% and biofilm structures were present (Fig. 2.3 to .5). The biofilm did not cover the whole surface continuously but was segmented into patches. On the two highest producing electrodes, NG 6 and NG 7, with $J_{\text{max}}$ of 3.2 and 6 A·m$^{-2}$, biofilm covered the whole electrode surface almost uniformly and coverage ratios were 95.2 ± 3.4 and 97.1 ± 3.9% (Fig. 2.6 and .7). The biofilm observed on the highest producing electrode was thicker and its thickness was uniform, showing a higher maturation of the biofilm structures.

It may be suspected that bacteria that came in contact with the smooth surface ($Ra = 0.8$ nm) had great difficulty in linking with it. On such a smooth surface, during the first phase of adhesion, cells were probably entrained away from the surface before achieving an irreversible attachment and starting to create an extracellular matrix. Actually, the shift of the cells from the planktonic to the biofilm (sessile) phenotype is triggered by quorum sensing exchanges [41]. In the case of a smooth surface, the shift to the biofilm phenotype was probably delayed by the slowness of the primary colonization. Smooth surfaces may consequently increase the time needed for an electroactive biofilm to develop. As it was difficult for the cells to adhere to the smooth gold surface, early biofilm development may have mainly taken place by the adhesion of cells to the few existing microcolonies rather than to the clean surface. In addition, the absence of asperities can also reduce biofilm swarming [42,43]. In consequence, SG electrodes showed isolated biofilm clusters and their shape showed no significant swarming pattern. The smooth surface was a hindrance to biofilm formation during the expansion and connection phase.

In contrast, on the nano rough electrodes, e.g. NG 3 electrode (Fig. 2.3), many small bacterial colonies could be seen, which were all interconnected, thus initiating the formation of good biofilm coverage. The bacterial colonization varied significantly on the different NG surfaces but a clear correlation could be established between the coverage ratio and the maximum current density (Fig. 2). The only electrode that did not show such correlation was the one presenting a mature, uniform biofilm (NG 7). In such cases, the coverage ratio, close to 100%, is no longer the relevant parameter and the biofilm volume should be considered [44]. This relation showed that, in the early stage of electroactive biofilm formation, G. sulfurreducens cells were equally efficient for current production whatever their colonization pattern. Small or dense microbial colonies, biofilm patches or uniform biofilm demonstrated the same correlation between coverage ratio and current density produced.

It should be noted that a different type of behaviour has been reported with G. sulfurreducens cells colonizing stainless steel cathodes. Increasing the average roughness, Ra, from 2 to 4 μm increased current density but, in this case, isolated cells and small colonies were observed to provide higher current density than dense colonies [19]. However, these observations were made in very different conditions from those of the present study: the electrode was operated as a cathode and the electrode material was different. For instance, the semi-conductive properties of stainless steel may have impacted the electroactive behaviour of the cells [45]. Moreover, the roughness range was not at the same level: these previous studies dealt with micrometre scale roughness, i.e. roughness that was of similar magnitude to the cell size, while the results presented in this section addressed nanometre roughness. Such different behaviours observed with the same microbial species exposed to different experimental conditions point out the difficulty of extracting universal rules about the early-stage formation of electroactive biofilms.

As noted above, the current densities produced by the six NG electrodes exhibited great variability with a standard deviation of ±2.0 A·m$^{-2}$ around an average value of 2.5 A·m$^{-2}$. Actually, epifluorescence imaging of the electrodes clearly showed that this variability was related to the very different levels of surface colonization. The phase of early microbial settlement on these nano rough, flat surfaces was consequently far from controlled. In contrast, the proportional relationship established between biofilm coverage ratio and current density demonstrated a nice reproducibility of the electron transfer rate that was ensured by cells settled on the electrode surface. The variability observed on the chronoamperograms was due to the early colonization process but, when settled, the microbial cells displayed reproducible electrochemical characteristics.

The correlation between coverage ratio and current production, established here with NG electrodes, was no longer valid for SG electrodes. The SG electrodes showed microbial coverage ratios of 65.8

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Fig. 1. Chronoamperograms recorded with (A) smooth (SG, Ra = 0.8 nm) and (B) nano-rough (NG, Ra = 4.5 nm) gold electrodes under polarization at 0.1 V/SCE with G. sulfurreducens and a single batch of acetate 10 mM.
and 58.7% and they produced current densities of 0.92 A·m⁻² on average while, in the same range of biofilm coverage ratios, the NG electrodes produced around twice as much current (Fig. 2). This result suggests that nano roughness may influence the electron transfer rate. The asperities of 4.5 μm on average implemented here may facilitate cell anchoring and, when the cells have settled on the electrode surface, may also improve electron transfer. Nevertheless, further experimental investigations are still required to confirm this hypothesis.

### 3.2. Surface patterning with micro pillars

Gold electrodes were designed with micro pillars of square cross section 100 × 100 μm and a height of 500 μm. Three patterns were manufactured with pillar spacings of 100, 125, and 200 μm, noted μP100NG, μP125NG and μP200NG, respectively. The surface roughness was always 4.5 nm, like that of the nano rough (NG) electrodes used in the previous section. Six μPNG electrodes, two with each pillar spacing, were implemented to form G. sulfurreducens anodes. Chronoamperograms displayed a quick start of current production on all electrodes, after between 1.1 and 1.7 days (Fig. 3). The μP100NG, μP125NG and μP200NG electrodes reached maximum current densities of 8.4, 7.5 and 6.3 A·m⁻², respectively (duplicates gave 8.5, 8.2 and 5.9 A·m⁻², respectively). These values represent improvements of up to 3.4 fold compared to the average current density provided by the NG electrodes (2.5 A·m⁻²).

The increase in current production obtained with the micro pillar electrodes with respect to the flat NG electrodes was related with the larger surface area created by the addition of the pillars. All the electrodes, both NG and μPNG, had a projected surface area of 1 cm², but the geometric surface area that was really offered to the microbial cell anchoring and, when the cells have settled on the electrode surface, was always 4.5 nm, like that of the nano rough (NG) electrodes used in the previous section. The presence of pillars ensured a effective and reproducible bacterial colonization even though the pillars were covered with the same nano rough gold surface (Ra = 4.5 nm) as the NG electrodes, the high variability observed with the NG electrodes was no longer observed with the μPNG electrodes. The μ pillar surface patternning considerably decreased the experimental deviation due to microbial adhesion.

Microbial colonization was imaged on the full height of the pillars, from the bottom to the top, by confocal microscopy. It was thus possible to determine that all μPNG electrodes presented coverage ratios above 90% (Fig. 4). The biofilm was well established at the bottom of the electrode and all along the pillars. The biofilm coverage seemed to be close to 100% at the bottom of the pillars but it was not possible to make a numerical measurement to confirm this qualitative observation.

Considering the great height of the micro pillars designed here, with respect to the cell size of a few micrometres or with respect to the height of 8 to 20 μm for similar micro structures reported in the literature so far [30,31], it is noteworthy that the biofilm fully covered the bottom interface. The significant height of these pillars did not hamper the full colonization of the electrode surface, right down to their bases. It can now be kept in mind that protruding micro structures intended to increase the surface area available for biofilm development can be of significant height. The ratio of height to separation distance is likely to be a key parameter that should be optimized in order to maximize the interfacial area. Here 500 μm high pillars set 100 μm apart allowed the biofilm to colonize the pillar sides and the base surface almost uniformly.

The presence of pillars ensured an efficient and reproducible bacterial colonization even though the pillars were covered with the same nano rough gold surface (Ra = 4.5 nm) as NG electrodes. The great experimental deviation observed on the chronoamperograms with NG electrodes was considerably mitigated by the presence of the micro pillar arrays. A micro pillar array offered a quiet environment for microbial cell adhesion by reducing the local shear stress. In the absence of significant solution stirring, as was the case here, the microbial cells engaged in the interfacial adhesion. Here 500 μm high pillars set 100 μm apart allowed the biofilm to colonize the pillar sides and the base surface almost uniformly.

<table>
<thead>
<tr>
<th>Electrode surface</th>
<th>Roughness Ra (nm)</th>
<th>Geometric area (cm²)</th>
<th>( J_{\text{max}} ) (A·m⁻²) projected</th>
<th>( J_{\text{max}} ) (A·m⁻²) geometric</th>
<th>Starting time (days)</th>
<th>Coverage ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth</td>
<td>0.8</td>
<td>1</td>
<td>1.1</td>
<td>1.1</td>
<td>3.2</td>
<td>65.8 ± 9.8</td>
</tr>
<tr>
<td>Nano-rough</td>
<td>4.5</td>
<td>1</td>
<td>6.4</td>
<td>6.4</td>
<td>1.1</td>
<td>97.1 ± 3.9</td>
</tr>
<tr>
<td>100 μm spaced</td>
<td>4.5</td>
<td>5.73</td>
<td>8.5</td>
<td>8.4</td>
<td>1.48</td>
<td>97.1 ± 2.8</td>
</tr>
<tr>
<td>micropillar</td>
<td>4.5</td>
<td>5.03</td>
<td>8.4</td>
<td>8.4</td>
<td>1.48</td>
<td>97.1 ± 2.8</td>
</tr>
<tr>
<td>125 μm spaced</td>
<td>4.5</td>
<td>3.26</td>
<td>6.3</td>
<td>6.3</td>
<td>1.81</td>
<td>98.3 ± 3.0</td>
</tr>
<tr>
<td>micropillar</td>
<td>4.5</td>
<td>3.26</td>
<td>6.3</td>
<td>6.3</td>
<td>1.81</td>
<td>98.3 ± 3.0</td>
</tr>
<tr>
<td>200 μm spaced</td>
<td>4.5</td>
<td>3.26</td>
<td>6.3</td>
<td>6.3</td>
<td>1.81</td>
<td>98.3 ± 3.0</td>
</tr>
<tr>
<td>micropillar</td>
<td>4.5</td>
<td>3.26</td>
<td>6.3</td>
<td>6.3</td>
<td>1.81</td>
<td>98.3 ± 3.0</td>
</tr>
</tbody>
</table>
excellent way to increase the surface area available for biofilm development, on the one hand, and to improve the biofilm formation process, on the other. The μPNG electrodes thus improved the reproducibility of biofilm formation and subsequently of current production with respect to the NG electrodes, when current densities were calculated with respect to the 1 cm² projected surface area.

Considering the overall geometric surface areas of 5.73, 5.06 and 3.26 cm² the current densities gave $J_{\text{max}}$ of 1.48, 1.56 ± 0.10 and 1.87 ± 0.10 A·m⁻² for 100, 125 and 200 μm pillar spacing, respectively (Table 1). As mentioned above, these values were lower than the average current density produced by the NG electrodes. Moreover, considering that the biofilm coverage of the whole geometric surface area of the μPNG electrodes ranged from 90 to 100%, the correlation in Fig. 2 shows that NG electrodes would produce around 3 A·m⁻² with similar biofilm coverage. This confirms that the μPNG electrodes produced less current per unit area if the whole geometric surface was considered. Moreover, the 100 μm spaced pillar array produced the lowest current density and the highest was achieved by the electrodes with pillars 200 μm apart. These two observations strongly suggest the occurrence of mass transport limitation, which was more marked with the densest micro pillar array. The diffusion of either acetate or micronutrients, which were in lower concentrations, may have been hampered nearer to the bottom of the micro pillar array. On the other hand, mass transport limitation can also affect the local pH. Acetate oxidation induces local acidification of the electrode:

$$\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}$$  \hspace{1cm} (1)
the chronoamperograms of the μP200NG electrodes only. With the less dense micro pillar array, the gradient profile resulting from the diffusion/reaction balance was established faster than in the more compact arrays.

3.3. Mass transport beyond the diffusion layer

A simple approach of mass transport modelling is proposed here to assess whether mass transport inside the micro pillar array is a major cause of rate limitation, as supported by the experimental data described above.

The mass transport situation far from the electrode surface must be grasped in order to define the ionic fluxes that reach the front of the electrode. In a general way, mass transport is the result of three contributions: diffusion, migration and convection. In our electrochemical reactors, solution movement was caused only by gentle gas bubbling through a gas disperser, which was deliberately set behind the auxiliary electrodes in order not to disturb the bioanode zone. In such conditions, the convective contribution can be neglected in the bioanode zone. Consequently, outside the diffusion layer of the bioanode, mass transport was mainly ensured by migration. For each ion, the molecular flux driven by migration can be easily calculated by using the transport numbers. The concept of transport numbers and the way to calculate them has been recalled recently in the context of microbial electro technology [51]. The calculation of the ionic composition of the solution used in the electrochemical reactors is described in the Supplementary Information. It led to the transport numbers reported in Table 2.

The transport numbers give the percentage of current that was transported through the solution by the motion of each ionic species. Here, most of the current was transported through the solution by the motion of Na⁺ and NH₄⁺ to the auxiliary electrode (cathode) and of Cl⁻ to the bioanode. Using the transport numbers, the molecular balance sheet of the ionic species can be calculated directly. For instance, taking the exchange of 100 electrons as the calculation basis, the molecular flux of each ion is equal to its transport number expressed as a percentage. It is just necessary to remember to divide the transport number by the charge for multivalent ions (Fig. 5).

When 100 electrons are extracted from the bioanode, 12.5 molecules of acetate are oxidized according to Reaction (1). Migration brings 3.8 molecules of acetate from the bulk to the bioanode. For the process to be stable, 8.7 molecules of acetate must be brought to the bioanode by diffusion.

Moreover, when 100 electrons are extracted from the bioanode, 25 molecules of HCO₃⁻ and 112.5 protons are produced. To be stable, the process also requires the 112.5 protons be neutralized to avoid local acidification of the bioanode. At pH around neutrality, the concentration of protons is extremely weak. For instance, even accepting significant local acidification to pH 5.0, proton concentration is only 0.01 mM. With such a low concentration, neither migration nor diffusion is efficient to drive the protons away from the interface at a sufficient rate. Protons must consequently be neutralized by the transport of buffering species towards the bioanode. Here, with 112.5 protons produced, 25 at most can be neutralized by the HCO₃⁻ molecules produced by the reaction. In addition, migration drives 13.6 molecules of HCO₃⁻ from the bulk to the bioanode together with 0.8 molecules of HPO₄²⁻, which can also help to neutralize the protons (in the case of divalent ions, the molecular flux is half the charge flux). In total, reaction and migration can neutralize a maximum of 39.4 protons. To be stable, the process requires mass transport by diffusion to bring 73.1 molecules of buffering species to the bioanode.

It can be concluded that migration from the bulk of the solution, far from the electrode surface, did not ensure the mass transport situation that would be required for the bioanode to operate in stable conditions. In such conditions, steep concentration gradients develop close to the electrode surface and diffusion must ensure the majority of mass transport. Here, diffusion must play the main role for the transport of both acetate and the buffering species to the bioanode. Diffusion must bring only 8.7 acetate molecules but 73.1 molecules of buffering species to the bioanode. In consequence, it is much more likely that mass transport limitation appeared because of insufficient diffusion of the buffering species than of substrate.
Table 2
Concentrations and transport numbers of the ionic species contained in the electrochemical reactors after inoculation and pH stabilization by N₂:CO₂ bubbling. Fumarate and succinate came from the pre-culture used as the inoculum (see Supplementary Information).

<table>
<thead>
<tr>
<th>Ion</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>NH₄⁺</th>
<th>HCO₃⁻</th>
<th>H₂PO₄⁻</th>
<th>HPO₄²⁻</th>
<th>Acetate</th>
<th>Cl⁻</th>
<th>Succinate²</th>
<th>Fumarate²</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (mM)</td>
<td>55</td>
<td>1.3</td>
<td>28</td>
<td>29.6</td>
<td>3.6</td>
<td>1.4</td>
<td>9</td>
<td>29.3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>qₜ (%)</td>
<td>28.4</td>
<td>1.0</td>
<td>21.2</td>
<td>13.6</td>
<td>1.3</td>
<td>1.6</td>
<td>3.8</td>
<td>23.0</td>
<td>4.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

3.4. Mass transport through the diffusion layer and inside the micro pillar array

According to the global approach developed in the previous section, diffusion close to the electrode should play the main role in mass transfer of the substrate and buffering species.

The impact of diffusion above the micro pillar array can be assessed by assuming a diffusion layer of constant thickness above the micro pillar structure (Fig. 6). In the stationary state, for each species, the diffusion flux, which is driven through the diffusion layer by the concentration gradient, is equal to the flux consumed by the bioanode:

\[ -\frac{D}{\delta_d} \left( c^{bulk} - c^{bulk} \right) \frac{J}{n \cdot F} \]  \hspace{1cm} (2)

where \( D \) (m²·s⁻¹) is the diffusion coefficient of the species, \( \delta_d \) (10⁻⁴ m) is the thickness of the diffusion layer above the micro pillar structure, \( c^{bulk} \) and \( c^l \) (mol·m⁻³) are the concentrations in the bulk and at the top of the micro pillar array, \( J \) (A·m⁻²) is the current density expressed with respect to the electrode surface area, and \( F \) (96 485 C·mol⁻¹) is the Faraday constant.

Dividing by \( n \cdot F \) transforms the current density into the molar flux of the species consumed by the bioanode. For acetate, \( n \) is obviously equal to 8 according to Reaction (1). The case of HCO₃⁻ is slightly more complex. In the stationary state, pH is stable inside the micro pillar structure, so each proton produced by the electrochemical reaction must be extracted by a molecule of HCO₃⁻ that has penetrated into the array and reemitted in the form of H₂CO₃. For 8 electrons produced, 9 protons have to be neutralized, but Reaction (1) produces 2 HCO₃⁻ molecules, so only 7 molecules of HCO₃⁻ must penetrate into the array from the bulk, and the parameter \( n \) is equal to 8/7.

Calculations were made with current densities \( J \) of 8.5 and 6.1 A·m⁻² for micro pillar spacing of 100 and 200 μm, respectively. The diffusion layer thickness was assumed to be 100 μm, which is a common value for an electrochemical process in quiescent solution [52, 54]. The diffusion coefficients of acetate and HCO₃⁻ were extracted from the literature. The results reported in Table 3 show a limited impact of the diffusion layer, diminishing the concentration of acetate from 9 mM in the solution bulk to 8.0 mM at the interface with the top of the micro pillar array. Similarly, the concentration decrease was not important for HCO₃⁻, which diminished from 29.6 to 23.1 mM. For both compounds, the maximum gradient was obtained with the 100 μm spacing, which was consistent with the higher current density \( J \) produced by this configuration.

Mass transport inside the micro pillar array can be approached by a diffusion reaction equation. The micro pillar pattern offers a highly symmetric topography composed of adjacent elementary units bounded by the central axes of four neighbouring pillars (Fig. 6). The mass transport situation is exactly the same in all these elementary units (i.e. the first derivative of the concentration of each species is nil at the frontier between two adjacent units), so each unit can be modelled as an individual pore. The diffusion reaction equation was established in the x direction perpendicular to the electrode surface in such a unit, i.e. parallel to the micro pillars.

For acetate, the differential mass balance in a section of the elementary unit at the stationary state is:

\[ -S \cdot D_{ac} \frac{d c^{ac}}{dx} = -J_{lac} \frac{J_{lac}}{n \cdot F} \]  \hspace{1cm} (3)

where \( S \) (m²) is the section of the elementary unit that is free for diffusion, \( D_{ac} \) (m²·s⁻¹) is the diffusion coefficient of acetate, \( c^{ac} \) (mol·m⁻³) is the acetate concentration, \( J_{lac} \) (m) is the electrochemically active length in the section of the elementary unit (Fig. 6), \( J_{lac} \) (A·m⁻²) is the local current density, i.e. expressed with respect to the overall geometric surface area, \( n = 8 \) is the number of electrons produced per molecule of acetate consumed (Reaction (1)), and \( F \) (96 485 C·mol⁻¹) is the Faraday constant. The width of the micro pillars was always 100 μm, so \( J_{lac} \) was equal to 400 μm for all the configurations. In contrast, the free section \( S \) depended on the pillar spacing and was equal to (Fig. 6):

\[ S = (d + 0.10 \times 10^6)^{-3} \]  \hspace{1cm} (4)

where \( d = 100, 125 \) or 200.10⁻⁶ m is the distance between pillars.

At the upper surface of the electrode (\( x = 0 \)), the acetate concentration was the interfacial concentration (\( c_{ac}^{0} \)) calculated above:

\[ x = 0, c_{ac} = c_{ac}^{0} \]  \hspace{1cm} (5)

At the bottom of the elementary unit (\( x = \delta \)), acetate was consumed in the same way as along the electroactive wall:

\[ x = \delta, \frac{d c_{ac}}{dx} = -\frac{J_{bac}}{n \cdot F} \]  \hspace{1cm} (6)

This assumption was justified by the full biofilm coverage of the bottom of the micro structure electrodes.

To reach a standard approach and reduce the number of parameters to be treated, the equations were transformed into non dimensional form, with the dimensionless variables:

\[ \bar{c}_{ac} = \frac{c_{ac}}{c_{ac}^{0}}, \quad \bar{x} = \frac{x}{\delta} \]  \hspace{1cm} (7)

Fig. 5. Molecular balance sheet of the main ionic species involved in charge transport through the bulk on the basis of 100 electrons exchanged. K⁺, H₂PO₄⁻, succinate and fumarate, which had minor roles, are not represented.
leading to:

$$\frac{d^2 \bar{C}_A}{dx^2} = \frac{l_e \delta \delta J_{loc}}{D_{Ae}C_{Ae} nF} \frac{d \bar{C}_A}{dx}$$ (8)$$

$$X = 0, \bar{C}_A = 1$$ (9)

$$X = 1, \frac{d \bar{C}_A}{dx} = \frac{\delta J_{loc}}{D_{Ae}C_{Ae}} \frac{d \bar{C}_A}{dx}$$ (10)

Two dimensionless numbers appear, one (\(\zeta\)) is a geometric ratio expressing the overall electrochemically active area in the elementary unit with respect to the section available for diffusion:

$$\zeta = \frac{l_e \delta}{S}$$ (11)

the other is the modified Damköhler number [55]:

$$D_{Amod} = \frac{J_{loc}}{nF} \frac{\delta}{\frac{D_{Ae}C_{Ae}}{\delta}}$$ (12)

which expresses the rate of consumption by the electrochemical reaction with respect to the maximum diffusion rate. Solving Eq. (8) with the boundary conditions (9) and (10) gives the concentration profile of acetate in the micro pillar array:

$$\bar{C}_A = 1 + \zeta D_{Amod} \frac{X^2}{2} - \zeta D_{Amod} \left(\frac{1}{\zeta} + 1\right) X$$ (13)

where \(X\) varies from 0 at the top of the micro pillar to 1 at the bottom.

The same equation was derived for HCO₃⁻. As detailed above, the current density was transformed into the molar flux of the HCO₃⁻ that was consumed by the electrochemical reaction by using \(n = 8/7\). It may be recalled that, in this context, HCO₃⁻ was indirectly consumed by the reaction because, to maintain a stationary state, HCO₃⁻ is assumed to neutralize the protons produced by the electrochemical reaction (Reaction (1)). This is the condition sine qua non for maintaining a stable pH inside the micro pillar array at the stationary state.

Assuming that HCO₃⁻ and H₂CO₃ have identical diffusion coefficients, mass conservation indicates that the sum of their concentrations remains equal along the whole diffusion pathway. Calculating the concentration of HCO₃⁻ along the micro pillars thus directly gives the concentration of H₂CO₃ along the micro pillars and the pH profile with the acid dissociation equilibrium:

$$\text{pH} = \text{pKa} + \log \left(\frac{C_{HCO_3^-}}{C_{H_2CO_3}}\right)$$ (14)

For acetate, the values of \(\zeta D_{Amod}\) (Table 3), were always less than 1, which indicates that the consumption rate by the electrochemical reaction was slower than the maximum diffusion rate (Eq. (12)). The profile of the dimensionless concentration of acetate in the micro pillar array (Fig. 7.A) confirmed that acetate did indeed reach the bottom of the structure and was consequently not a significant source of rate limitation.

The situation was not so obvious for HCO₃⁻, which displaced \(\zeta D_{Amod}\) greater than 1 for the 100 \(\mu\)m spaced micro pillars (Table 3). In this case, diffusion may be rate limiting. This was confirmed by the concentration profile (Fig. 7). For the most compact micro pillar array, HCO₃⁻ did not reach the bottom of the structure. Due to the protons produced by the electrochemical reaction, HCO₃⁻ was fully consumed at around 80% of the depth of the structure. In this case, HCO₃⁻ was not able to maintain a stable pH on the whole micro pillar array. The concentration and pH profiles showed that diffusion limitation was not so severe in the 200 \(\mu\)m spaced micro structure. HCO₃⁻ was able to reach the bottom of the array (Fig. 7). Nevertheless a significant pH gradient occurred in side (Fig. 7), with a value of 5.76 at the bottom.

The pH of the solution bulk was 6.8 and the calculated value was around 6.5 at the top of the micro piller structure for the two configurations. For the 100 \(\mu\)m spaced micro structure, the model revealed great

![Fig. 6. Scheme of the integration elementary unit. The electroactive length (\(l_e\)) is represented by the continuous red line of the frontier; a scheme of concentration profile is represented on the side view.](Image 131x606 to 455x741)

Table 3

<table>
<thead>
<tr>
<th>d ((\mu)m)</th>
<th>S ((m^2))</th>
<th>J (A·m⁻²)</th>
<th>Jloc (A·m⁻²)</th>
<th>n</th>
<th>D (m²·s⁻¹)</th>
<th>Cbulk (mol·m⁻³)</th>
<th>C (mol·m⁻³)</th>
<th>(\zeta)</th>
<th>D_{Amod}</th>
<th>(\zeta D_{Amod})</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3 (10^8)</td>
<td>8.5</td>
<td>1.48</td>
<td>8</td>
<td>1.09 (10^0)</td>
<td>9</td>
<td>8.0</td>
<td>6.67</td>
<td>0.11</td>
<td>0.73</td>
</tr>
<tr>
<td>200</td>
<td>8 (10^8)</td>
<td>1.87</td>
<td>8/7</td>
<td>1.18 (10^0)</td>
<td>29.6</td>
<td>23.1</td>
<td>6.67</td>
<td>0.25</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3 (10^8)</td>
<td>8.5</td>
<td>1.48</td>
<td>8/7</td>
<td>1.18 (10^0)</td>
<td>29.6</td>
<td>24.9</td>
<td>2.5</td>
<td>0.29</td>
<td>0.72</td>
</tr>
</tbody>
</table>
acidiﬁcation along the micro pillars, which was probably the main cause of rate limitation. This severe limiting effect explained the low local current density ($j_{loc}$, expressed with respect to the overall geometric surface area) of 1.48 A·m$^{-2}$, while flat electrodes with the same nano roughness and with similar full bioﬁlm coverage produced current densities ranging from 2.5 to 3 A·m$^{-2}$ (Fig. 2). Mass transport of the buffering species was less limiting in the case of the 200-μm spaced micro pillars. Nevertheless, a signiﬁcant pH gradient was calculated, with pH falling to 5.76 at the bottom of the micro pillars. This signiﬁcant acidification explained why the local current density of 1.87 A·m$^{-2}$ was higher than that of the 100-μm spaced structure but did not reach the value of the ﬂat surface.

4. Conclusion

By mastering the roughness of gold surfaces at the nanometre scale, it has been possible to reveal that nano roughness impacts the electron transfer rate of the cells settled on the surface. A roughness of 4.5 nm may improve the electron transfer rate with respect to smooth surface ($R_a = 0.8$ nm). Great experimental deviation was observed on the production of current. Nevertheless, regardless of the progress of the microbial colonization on the surface, current density was always directly proportional to the bioﬁlm coverage. The experimental deviations were not due to the electron transfer process but to the difﬁculty for the microbial cells to settle on flat surfaces.

The experimental reproducibility was considerably improved by patterning the electrode surface with arrays of micro pillars 500 μm in height. The micro pillar array offered the cells an environment that allowed them to establish irreversible adhesion. Moreover, the current density related to the projected surface area was increased around 3.4 fold with respect to the flat electrodes with identical surface roughness (NG electrodes with $R_a = 4.5$ nm), because of the larger surface area available for bioﬁlm development.

In conclusion, with respect to the ﬂat smooth surface, nano roughness increased the current density by a factor of 2.8, due to improved electron transfer, and micro structuring improved it by a factor 3.4, due to the area increase. The surface design of microbial electrodes should consequently include nano roughness to favour electron transfer and micro roughness of the order of tens or hundreds of micrometres to increase the surface available for bioﬁlm growth and create a quiet environment favouring cell settlement. The optimal micro roughness must be now designed by balancing the largest possible surface area and the lowest possible mass transport limitation, particularly of the buffering species.

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Declaration of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2018.02.005.

References

Effect of surface nano/micro-structuring on the early formation of microbial anodes with *Geobacter sulfurreducens*: experimental and theoretical approaches

Supplementary information

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Calculation of the ionic composition and the transport numbers of the solution implemented in the electrochemical reactors.

The inoculum was prepared by pre-culturing the *G. sulfurreducens* cells with sodium acetate (CH₃COONa) 10 mM, used as the electron donor, and sodium fumarate (Na₂C₄H₂O₄) 50 mM, used as the electron acceptor. Acetate was oxidized into hydrogen carbonate, producing 8 electrons per molecule:

\[
\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^- \quad (1)
\]

and fumarate was reduced to succinate, consuming 2 electrons per molecule:

\[
\text{C}_4\text{H}_2\text{O}_4^{2-} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{C}_4\text{H}_4\text{O}_4^{2-} \quad (2)
\]

Considering the electron balance, the overall transformation was:

\[
\text{CH}_3\text{COONa} + 4\text{C}_4\text{H}_2\text{O}_4\text{Na}_2 + 4\text{H}_2\text{O} \rightarrow \text{NaHCO}_3 + \text{H}_2\text{CO}_3 + 4\text{C}_4\text{H}_4\text{O}_4\text{Na}_2 \quad (3)
\]

Assuming that acetate was completely oxidized at the end of the pre-culture, the samples used for inoculating the electrochemical reactors contained sodium hydrogen carbonate 10 mM, succinate 40
mM, with 10 mM fumarate remaining. Actually, to be fully rigorous, the ratio between $\text{HCO}_3^-$ and $\text{H}_2\text{CO}_3$ should be calculated as a function of the pH, including the equilibrium of the phosphate species contained in the medium and the possible dissolution of CO$_2$ from the gas. Nevertheless, this correction would have had a very minor impact on the assessment of the concentrations of the major ions that composed the solution in the electrochemical reactors.

Before inoculation, the solution in the electrochemical reactors was the same as the medium used for the cell pre-culture except that fumarate was omitted to force the cells to use the electrode as the sole electron acceptor. This composition is recalled in the first column of Table 1. Inoculating with the pre-culture at a 10% (v/v) ratio modified the initial composition of the solution because of the presence of additional compounds in the inoculum sample (hydrogen carbonate, fumarate and succinate) and the absence of some others (acetate). The concentrations in the electrochemical reactors after inoculation are reported in column 2 of Table 1. The columns to the right give the contribution of each component in terms of ionic species.

**Table 1.** Composition of the solution in the electrochemical reactors before (first column) and after (second column) inoculation with the cell pre-culture (10% v/v). Micro-nutriments and vitamins have been omitted. The 10 columns to the right give the contribution of each compound to the ionic concentration after inoculation.

<table>
<thead>
<tr>
<th></th>
<th>Initial Medium (mM)</th>
<th>After inoc. (mM)</th>
<th>Ionic contribution after inoculation (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO$_3$</td>
<td>30</td>
<td>31</td>
<td>$\text{Na}^+$ $\text{K}^+$ $\text{NH}_4^+$ $\text{HCO}_3^-$ $\text{H}_2\text{PO}_4^-$ $\text{HPO}_4^{2-}$ $\text{Acet}^-$ $\text{Cl}^-$ $\text{Suc}^{2-}$ $\text{Fum}^{2-}$</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>CH$_3$COO</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>NaH$_2$PO$_4$</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>C$_4$H$_2$O$_4$Na</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C$_3$H$_4$O$_2$Na</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>ion concentration after inoculation (mM)</strong></td>
<td>55 1.3 28 31* total : 5</td>
<td>9 29.3 4 1</td>
<td></td>
</tr>
<tr>
<td><strong>ion concentration after pH adjustment with N$_2$:CO$_2$ (mM)</strong></td>
<td>55 1.3 28 29.6 3.6 1.4 9 29.3 4 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>transport number (%)</strong></td>
<td>28.4 1 21.2 13.6 1.3 1.6 3.8 23 4.8 1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A part of the carbonate species should be in non-dissociated form, H$_2$CO$_3$.

The phosphate and carbonate compounds were initially brought into the solution only in the forms of $\text{H}_2\text{PO}_4^-$ and $\text{HCO}_3^-$, respectively. The ratios between the species $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ and $\text{HCO}_3^-$ and $\text{H}_2\text{CO}_3$ were then controlled by the pH of the solution. The pH was maintained at 6.8 by constant bubbling of N$_2$:CO$_2$. The concentrations of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ were calculated by using the acid dissociation constant:
\[
10^{-7.2} = \frac{HPO_4^{2-}}{H_2PO_4^-}
\] (4)

which led, at pH 6.8, to \(HPO_4^{2-}\) 1.4 mM and \(H_2PO_4^-\) 3.6 mM, giving the total phosphate concentration of 5 mM (Table 1). Then, the concentration of \(HCO_3^-\), which had been disturbed by \(CO_2\) gas bubbling, was corrected by using the overall charge balance:

\[
[HCO_3^-] + [H_2PO_4^-] + 2[HPO_4^{2-}] + [\text{Acetate}^-] + [\text{Cl}^-] + 2[\text{Succinate}^2^-] + 2[\text{Fumarate}^2^-]
= [\text{Na}^+] + [\text{K}^+] + [\text{NH}_4^+]
\] (5)

Assuming that acetate, succinate and fumarate were in their fully dissociated forms led to a concentration of \(HCO_3^-\) of 29.6 mM. The \(HCO_3^-/H_2CO_3\) acid dissociation constant (\(pK_a = 6.3\)) indicated that the concentration of \(H_2CO_3\) was 9.4 mM. The total concentration of the carbonate species, when the pH was stabilized at 6.8 by gas bubbling, was consequently 39 mM. Gas bubbling was responsible for an increase of 8 mM of the concentration of the carbonate species.

These concentration values were then used to calculate the transport number \(t_i\) of each ionic species expressed as [1]:

\[
t_i = \frac{\lambda_i C_i}{\sum \lambda_k C_k}
\] (6)

where \(\lambda_i\) is the molar ionic conductivity \((\text{m}^2\cdot\text{S} \cdot \text{mol}^{-1})\) and \(C_i\) the concentration \((\text{mol} \cdot \text{m}^{-3})\) of species \(i\). The transport numbers reported in the last line of Table 1 were calculated with the values of molar ionic conductivities extracted from the literature and reported in Table 2.

**Table 2. Molar ionic conductivities from [2]**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Na$^+$</th>
<th>K$^+$</th>
<th>NH$_4^+$</th>
<th>HCO$_3^-$</th>
<th>H$_2$PO$_4^-$</th>
<th>HPO$_4^{2-}$</th>
<th>Acetate$^-$</th>
<th>Cl$^-$</th>
<th>Succinate$^{2-}$</th>
<th>Fumarate$^{2-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_i) ((10^{-4} \text{ m}^2 \cdot \text{S} \text{ mol}^{-1}))</td>
<td>50.1</td>
<td>73.5</td>
<td>73.5</td>
<td>44.5</td>
<td>36</td>
<td>114</td>
<td>40.9</td>
<td>76.3</td>
<td>117.6</td>
<td>123.6</td>
</tr>
</tbody>
</table>

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References
