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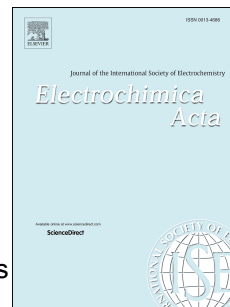
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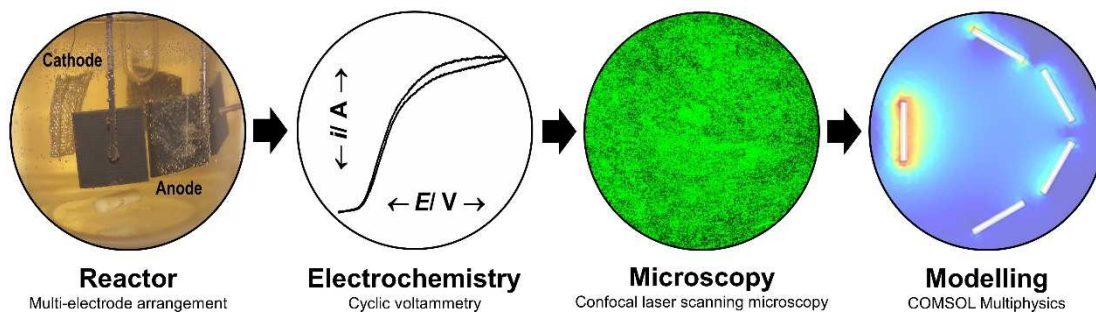
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Graphical abstract

On the consensus of the anode area that contributes to microbial current via:



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1 On the actual anode area that contributes to the current density 2 produced by electroactive biofilms

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10 Abstract

11 Even with an increasing interest in scaling-up Microbial Electrochemical Technologies
12 (MET), it is still common to focus on their “fundamentals”. An important example is the
13 production of current density (j_{max}) by microbial anodes in a three-electrode arrangement
14 (3EA) configuration, e.g.: a graphite plate of well-defined projected (or geometric) surface
15 area (PSA) and a cathode, both parallel to each other.

16 With such type of anode within a 3EA configuration, j_{max} 's calculation is expected to be
17 straightforward. Nonetheless, certain issues prevail. Occasionally, j_{max} is wrongly
18 overestimated neglecting the surface of the anode that does not directly face the cathode.

19 Here, grown biofilms of the novel electroactive bacterium *Geoalkalibacter subterraneus*
20 showed that the actual area of anode that contributes to j_{max} is the total PSA (or apparent
21 geometric area) immersed in the electrolyte available to form a biofilm regardless the side of
22 the anode that faced or opposed the cathode even in a medium with low conductivity such as
23 urban wastewater, a niche of application for METs.

24 For the sake of normalization, researchers (and especially a “freshman” microbial
25 electrochemist) are encouraged to: A) use the total PSA (or apparent geometric area)
26 immersed in the electrolyte to calculate j_{max} or B) to cover edges and faces hidden of the
27 anode with an electrical insulator to allow the flow of current on the side of the anode that
28 directly faces the cathode prior calculation of j_{max} . This normalization can be conducted when
29 the main goal is to quantify (and thus properly report) j_{max} produced when using (e.g.): a novel
30 i) electroactive bacterium, ii) electrode material or iii) reactor design.

31 **Keywords:** *Electroactive biofilm* • *Geoalkalibacter subterraneus* • *COMSOL Multiphysics*[®] •
32 *Electrode surface area* • *Microbial electrochemical technologies* • *Current density*

33 1. Introduction

34 Microbial Electrochemical Technologies (METs) take advantage of the electron transfer (ET)
35 interactions occurring between electroactive bacteria (EAB) and electrode materials [1]. The
36 use of these interactions impacts several rapid evolving technological fields ranging from the
37 treatment of wastewater by the integration of METs into conventional treatment technologies
38 [2], via the desalination of brackish water in microbial desalination cells [3], to the production
39 of value-added molecules such as acetate from CO₂ via microbial electrosynthesis [4].

40 Although several parameters are used to evaluate the performance of METs, such as the
41 product yield or the coulombic efficiency, the calculation of the maximum current density
42 (j_{max}) per projected (or apparent geometric) surface area (PSA) of anode material is among
43 one of the most widely accepted parameters [5]. Despite the efforts to standardize the
44 calculation of j_{max} to use the reported value for comparison among different studies, certain
45 issues prevail regarding its proper calculation.

46 In three-electrode arrangement (3EA) experimental set-ups where a two-side planar anode
47 (hereafter WE from “working electrode”) is used in parallel to a cathode (henceforth CE from
48 “counter electrode”), j_{max} is occasionally calculated neglecting the surface of the WE that does
49 not directly face the CE [6]. This causes j_{max} to be wrongly overestimated, which in turn
50 prohibits an objective comparison of results among studies.

51 When considering for example the case of a WE with dimensions of 2.5 x 2.5 x 0.25 cm,
52 some might use only the side of the WE that faces the CE to calculate j_{max} (i.e.: 6.25 cm²).
53 However, in multiple experiments it is usually observed that the biofilm grows on all the
54 immersed anode material which is in contact with the electrolyte (i.e. both electrode sides plus
55 edges: 15.00 cm², see Fig. A7 in Appendix A). Depending on the methodology used to
56 calculate the surface area, j_{max} can therefore vary by a factor of 2.4.

57 This overestimation might be due to the direct extrapolation of the information extracted from
58 strictly electrochemical electrode processes’ books. Such information is usually employed for
59 the interpretation of microbial electron transfer mechanisms between electrodes and EAB that
60 might be governed by completely different phenomena. As an example of this, it is reported in
61 text books of fundamental electrochemistry that j_{max} strongly depends on the WE’s proximity
62 to the CE [7]. Thus, it is usually assumed that the current produced on the side of the WE that
63 directly faces the CE will be significantly higher than the current produced on the back side of
64 the WE that does not directly faces the CE [8] (please see Fig. A1 in Appendix A).

65 This manuscript proposes a consensus on the actual total PSA of anode that contributes to
66 j_{max} . Experiments were carried out with biofilms of the novel EAB *Geoalkalibacter*
67 *subterraneus* and subjected to a multi-approach analysis with techniques that span from
68 electrochemistry via microscopy to modelling. This is not an attempt to evaluate the overall
69 performance of a bioelectrochemical device by its produced anodic current density alone as
70 would be typical for MFC research.

71

72 2. Experimental

73 2.1. Overall experimental strategy

74 A three-electrode arrangement was used (Fig. 1A). Four graphite plate anodes (WE) faced a
75 single platinum-iridium cathode (CE). In the middle of the reactor a SCE reference electrode
76 (RE) was placed. While WE1, WE2 and WE3 were chronoamperometrically (CA) and
77 individually controlled at +200 mV vs. SCE, WE4 was not. A N-Stat (N) configuration was
78 used, as similarly described by [9]. The N-Stat configuration allowed each WE to be
79 connected to a separate potentiostat channel sharing the same RE and CE according to
80 BioLogic's connection mode protocol. In the N-Stat configuration, the multi-channel
81 potentiostat individually controls each anode with respect to a single RE.

82 On all WEs the growth of pure culture anodic biofilms of the novel EAB *Geoalkalibacter*
83 *subterraneus* (henceforth *Glk. subterraneus* [10]) was followed by Confocal laser scanning
84 microscopy (CLSM). Biofilm growth on anodes was allowed (green "tick" symbol in Fig. 1A)
85 or limited by an electrical insulator (red "cross" symbol in Fig. 1A).

86 -Please insert **Figure 1** here-

87 2.2. Maintenance and growth of *Geoalkalibacter subterraneus*

88 The growth and maintenance of *Glk. subterraneus* was carried out exactly as previously
89 detailed elsewhere [10]. *Glk. subterraneus* was chosen here as a model EAB due to its shared
90 similar electrochemical characteristics with the extensively well characterized EAB *Geobacter*
91 *sulfurreducens* in terms of its j_{max} and its electron transfer mechanism [11].

92 All chemicals were of analytical or biochemical grade and were purchased from Sigma-
93 Aldrich and Merck. All media preparations were adjusted to pH 7, vigorously flushed with N₂
94 gas (purity ≥ 99.9999, Linde France S.A.) for at least 30 min using a commercial air stone (or
95 aquarium bubbler) and then autoclaved (121°C for 20 min).

96 Sterile growth medium FRR was used for routinely culture maintenance and contained (per
97 L): 17.0 g of NaCl, 4.50 g of MgCl₂·6H₂O, 0.35 g of CaCl₂·2H₂O, 1.00 g of NH₄Cl, 0.08 g
98 KH₂PO₄, 3.50 g of NaHCO₃, 3.00 g of Yeast extract, 1 mL of trace element solution, 1 mL of
99 selenite-tungstate solution and 1.00 g of CH₃COONa as electron donor.

100 2.3. Electrode preparation and Bioelectrochemical set-up

101 Preparation of electrodes and bioelectrochemical reactors was exactly conducted in agreement
102 to a procedure reported elsewhere [10]. In brief: working electrodes were 2.5 cm x 2.5 cm x
103 0.25 cm planar graphite plates (C000440/15, Goodfellow SARL, 229 Rue Solférino, F-59000
104 Lille, France) screwed onto 2 mm diameter, 15 cm long titanium rods (TI007910/13,
105 Goodfellow) that ensured electrical connection. Planar graphite electrodes were used as
106 delivered by the provider. Counter electrodes were 90% Platinum-10% Iridium grids joint by
107 heating in a blue flame with a 0.5 mm diameter, 15 cm long 90% Platinum-10% Iridium rod
108 (Heraeus PSP S.A.S., Contact Materials Division, 526, Route des Gorges du Sierroz, 73100
109 Grésy-sur-Aix France). If not stated otherwise, all potentials provided in this manuscript refer

110 to the Saturated Calomel reference Electrode (SCE) (KCl 3.0 M, +240 mV vs. SHE, Materials
111 Mates, La Guilletière 38700 Sarcenas, France).

112 To determine the contribution on j_{max} by both sides of a planar WE as part of the
113 electrochemical set-up, four WEs were placed in the same electrochemical reactor facing a
114 CE (Fig. 1A). However, not all the surface of the WE was available for the EAB to form a
115 biofilm. To accomplish this, one side of the WE material was electrically insulated with a
116 commercial seal (Siljoint, Bardahl®) to make the covered WE's surface inaccessible for the
117 EAB. Furthermore, to assure comparability and reproducibility all four WEs were
118 simultaneously measured in one electrochemical reactor.

119 2.4. Current density (j) calculation

120 Current is calculated throughout this manuscript as i) per projected surface area (j_{PSA}), which
121 takes into account the absolute current ($i_{absolute}$) divided by the projected surface area (PSA),
122 an adequate calculation for compact and smooth electrodes with low degree of surface
123 imperfections, such as the planar graphite plate electrodes used here, since they have a very
124 well defined geometric surface area [12]. The mathematical modelling was conducted taking
125 into account j_{PSA} . Maximum current density (j_{max}) refers to the maximum value extracted from
126 the Chronoamperometric curve only.

127 Additionally, for sake of completeness current is also reported in Fig. 2 as ii) absolute current
128 ($i_{absolute}$), which is the current without taking into account the available surface area of anodic
129 electrode material; and iii) current density as per electrochemically active surface area (j_{EASA}),
130 that is based on the obtained charge by acetate oxidation during Chronoamperometry (i.e.,
131 integrated current over time). In an attempt to normalize the current to the real surface area of
132 electrode available for the EAB to form a biofilm the electrochemically active surface area
133 (EASA) was calculated as previously reported from the Anson equation [12]:

$$134 \quad Q_d = 2 n F EASA C D^{1/2} \pi^{1/2} t^{1/2} \quad (\text{Equation 1})$$

135 Where Q_d is the charge (C), i.e., current integrated over time from the Chronoamperometric
136 curve; n the number of electrons transferred (8 mol e^- /mol acetate); F is the Faraday constant
137 (96485 C/ mol e^-); $EASA$ is the electrochemically active surface area (cm^2); C is the
138 concentration of the substrate (1×10^{-5} mol acetate/ml); D the diffusion coefficient of the
139 substrate (1.21×10^{-5} cm^2/s); and t is time (s). $EASA$ was later used to calculate the current
140 density with respect to the electrochemically active surface area and plotted in Figure 2C. The
141 Excel tool used for the calculation of $EASA$ is provided as supplementary information in
142 Appendix B.

143 2.5. Cyclic voltammetry data and its processing

144 Cyclic voltammetry (CV) of *Glk. subterraneus* biofilms was carried out under
145 bioelectrocatalytic substrate consumption (the so-called "turnover conditions").
146 Chronoamperometric (CA) maximum current densities (j_{max}) of established microbial biofilms
147 were calculated considering the total PSA immersed in the electrolyte and due to the presence
148 of visible apparent reddish biofilms that covered all the electrode. Here all data are based on

149 experiments of at least two independent biofilm replicates [5] and standard deviations are
150 presented throughout the manuscript.

151 **2.6. Confocal laser scanning microscopy (CLSM) to measure biofilm electrode coverage** 152 **and thickness**

153 *Glk. subterraneus* biofilms grown on WEs were examined by CLSM after staining with
154 nucleic acid-specific fluorochromes exactly as reported elsewhere [10]. Whole electrodes
155 were stained using the LIVE/ DEADs BacLight Bacterial Viability Kit (Invitrogen) and
156 confocal images of electroactive biofilms were acquired with a confocal laser scanning
157 system (Leica TCS SP2, Leica Microsystems, Wetzlar, Germany).

158 **2.7. Modelling of the current density distribution on the electrode surface with** 159 **COMSOL Multiphysics®**

160 This type of exemplary modelling approach is more frequently employed to the study of
161 strictly chemical electrode processes and very rarely employed for the study of the
162 interactions of EAB embed within biofilms and electrode materials in METs [13-15].
163 Notwithstanding, the current density produced at each potentiostatically controlled electrode
164 was later modeled with the software COMSOL Multiphysics® in an attempt to graphically
165 illustrate the total PSA contribution on the current density production in METs.

166 To conduct the modelling, in COMSOL Multiphysics® the following parameters were
167 introduced: A) Reactor geometry; B) Calculated conductivity for the electrolyte used; C)
168 Polynomial equations for the anodic kinetic reactions; and D) Polynomial equations for the
169 cathodic kinetic reactions.

170 It is worth noticing that the experiments carried out were originally focused on the anode
171 performance. The potential of the electrode against the counter electrode has therefore not
172 been measured during chronoamperometry and cyclic voltammetry. The value of the cell
173 voltage used in modelling was thus adjusted to 1.1 V to reproduce the results experimentally
174 observed.

175 **2.7.1. Reactor geometry definition in COMSOL Multiphysics®**

176 A cylindrical electrochemical reactor as the one shown in Fig. A2 and Fig. A3 was used in the
177 experiments and its dimensions were used in COMSOL Multiphysics®. Its diameter was 10
178 cm with a height of 6 cm (working volume of 500 ml, i.e.: filled with microbiological medium
179 for bacteriological growth). As depicted in Section 2.3, four graphite plates were used as
180 working electrodes. The reference electrode was placed right in the center of the reactor at a
181 height that did not interfere with the distribution of current and potential. The relative position
182 of all electrodes is depicted in Fig. 1, A3 and A4 (see Appendix B).

183 **2.7.2. Microbiological medium conductivity**

184 With the microbiological medium described in Section 2.2 and the information presented in
185 Table 1 a conductivity of 5.0 S/m at 25°C was calculated. This value has been introduced into
186 COMSOL Multiphysics® and a simulation has been accordingly conducted. Additionally, a

197 simulation at a conductivity value much lower of 0.1 S/m was conducted since this
 198 conductivity value is the one usually ascribed to domestic wastewater. At his point, it is good
 199 to keep in mind that the current applications of METs are thought to be focused on the
 200 treatment of domestic wastewater.

191 **Table 1. Electrolyte conductivity calculation of the growth medium used in this study.**

Compound "i"	[i] / g L ⁻¹	[i] / mmol L ⁻¹	[Cation] / mmol L ⁻¹	[Anion] / mmol L ⁻¹	λ_+ / 10 ⁻⁴ m ² S mol ⁻¹	λ_- / 10 ⁻⁴ m ² S mol ⁻¹	Conductivity / S m ⁻¹
NaCl	17.0	290.9	[Na ⁺] = 290.9	[Cl ⁻] = 290.9	Na ⁺ : 50.1	Cl ⁻ : 76.3	3.7
MgCl ₂ ·6 H ₂ O	4.5	22.2	[Mg ²⁺] = 22.2	[Cl ⁻] = 44.4	½ Mg ²⁺ : 53.0	Cl ⁻ : 76.3	0.6
NH ₄ Cl	1.0	18.7	[NH ₄ ⁺] = 18.7	[Cl ⁻] = 18.7	NH ₄ ⁺ : 73.5	Cl ⁻ : 76.3	0.3
NaHCO ₃	3.5	41.7	[Na ⁺] = 41.7	[HCO ₃ ⁻] = 41.7	Na ⁺ : 50.1	HCO ₃ ⁻ : 44.5	0.4
CH ₃ COONa	1.0	12.2	[Na ⁺] = 12.2	[CH ₃ COO ⁻] = 12.2	Na ⁺ : 50.1	CH ₃ COO ⁻ : 41.0	0.1
Total*							5.0

192 Note: λ_+ stands for molar cation conductivity at 25°C; λ_- stands for molar anion conductivity at 25°C; *Total growth medium conductivity
 193 due to "i" compounds. Due to the high content of NaCl used in this medium preparation, it is evident that the conductivity of the medium it is
 194 independent of the rest of the additional compounds within the medium and mainly attributed to the NaCl content.

195 2.7.3. Anodic reactions' kinetics

196 Anodic kinetic curves were obtained during cyclic voltammetry (CV) under catalytic
 197 conditions (Fig. A5). Both forward and backward sigmodal-like signals were very similar.
 198 Thus, only the backward signal was adjusted to a polynomial fitting. CV shown that the three
 199 electrodes share similar kinetics. Independently of the orientation of the available electrode
 200 surface the current density obtained by each electrode was very similar as well (see Fig. 3A).

201 2.7.4. Cathodic reaction's kinetics

202 The kinetics of the electrolysis of water for hydrogen production at a scan rate of 1 mV/s on a
 203 Pt/Ir counter electrode was experimentally measured in similar three-electrode arrangement
 204 set-up as the one previously described, i.e.: graphite working electrode, Pt/Ir counter electrode
 205 and SCE reference electrode. The medium composition shown in Table 1 was used as
 206 electrolyte.

207 3. Results and Discussion

208 3.1. Chronoamperometric current density of *Glk. subterraneus* biofilms

209 In Fig.2 current is shown as i) per projected surface area (j_{PSA}), an adequate calculation for
 210 planar graphite plate electrodes with a very well defined geometric surface area. For sake of
 211 completeness current is also shown as ii) absolute current ($i_{absolute}$), i.e., the current without
 212 taking into account the available surface; and iii) current density as per electrochemically
 213 active surface area (j_{EASA}), based on the obtained charge by acetate oxidation during
 214 Chronoamperometry.

215 After inoculation with cells of *Glk. subterraneus*, the biofilm growth began and it was
216 illustrated as an exponential-like current production trend as previously described for this
217 particular EAB [10] (Fig. 2). Interestingly, the biofilm growth was very similar on all WEs as
218 indicated by the chronoamperometric current production trend. No effect was observed in
219 terms of current production among WE1, WE2 and WE3. The current density j_{max} was very
220 closely similar for WE1, WE2 and WE3, with 2.60 ± 0.01 , 2.66 ± 0.07 and 2.57 ± 0.07 A/m²,
221 respectively. Although j_{max} of WE2 was slightly higher with respect to WE1 and WE3, there
222 was no significant difference between these three WEs.

223 During chronoamperometric measurements the actual area of anode that contributed to j_{max}
224 was the total PSA (or apparent geometric area) immersed in the electrolyte regardless the side
225 of the anode that faced the cathode.

226 3.1.1. Accurate calculation of current density regarding the porosity of the material

227 As described in the Experimental section, current density was calculated by two methods. The
228 first one divides the absolute current ($i_{absolute}$) by the geometric projected surface area (j_{PSA}).
229 The second one is an attempt to normalize the current to the real electrochemically active
230 surface area of electrode available for the EAB to form a biofilm (j_{EASA}). In this work, graphite
231 plate electrodes with a very well defined surface area were used. Nevertheless, when
232 calculating j_{EASA} by the method suggested elsewhere it was observed, as expected, that the
233 available electrochemically active surface area (EASA) was higher than the one geometrically
234 calculated. Thus, j_{EASA} was lower than j_{PSA} since the Anson equation (see equation 1)
235 somehow provides an approximation of the real EASA available for the EAB to form a
236 biofilm. It is worth noticing that EASA will always be higher than the geometric surface area
237 consider in the calculation of j_{PSA} due to the inherent complex distribution of the EASA at a
238 microscopic level.

239 -Please insert **Figure 2** here-

240 3.2. Direct electron transfer mechanism (ETM)

241 Cyclic voltammetry (CV) is one of the most employed electrochemical techniques in METs to
242 study at some extent how EAB interact with the electrode material, either anodes or cathodes
243 [16]. CV is used to decipher the electron transfer mechanism (ETM) by which electrons are
244 transferred between EAB and electrodes [17]. Here, turnover CV (*i.e.*, under
245 bioelectrocatalytic substrate consumption conditions) was applied to analyse whether there
246 was any difference in the ETM of microbial biofilms developed on different sides of the
247 anode in METs.

248 Independent biofilms grown on WE1, WE2 and WE3 showed an indistinguishable sigmoidal
249 shape from each other (Fig. 3A). Such shape is typical of EAB able to produce high currents
250 and thick biofilms via a direct ETM [10]. The similarities in the ETM showed by all anodes
251 imply that irrespective of the anode side available for biofilm growth, the same ETM is used
252 at both sides of an anode that faces or opposes a cathode. In Fig. 3A a flat voltammogram
253 indicates a typical control CV with no electroactive biofilm to illustrate that the sigmoidal CV
254 shape is caused by the biofilm under catalytic “turnover conditions”.

255 When determining the formal potential (E_f) of the ETM by calculating the first derivative of
256 CVs (Fig. 3B), the E_f observed for all tested WEs was -474.99 ± 1.29 mV vs. SCE. Such low
257 standard deviation confirmed that the EAB within biofilms used the same ETM. This is easily
258 understood when taking into account that the same scan rate and the same biological
259 conditions were assured for all WEs since all anodes were hosted within the same
260 electrochemical reactor.

261 Cyclic voltammetric measurements thus confirm that the actual area of anode that contributed
262 to the ETM was the total PSA (or apparent geometric area) immersed in the electrolyte
263 regardless the anode side that faced the cathode.

264 -Please insert **Figure 3** here-

265 **3.3. Homogeneous biofilm formation independently of the anode side orientation**

266 Together with the analysis of j_{max} and ETM on WE1, WE2 and WE3, a microscopic analysis
267 with CLSM was performed on all anodes (including WE4) to determine any possible effect on
268 biofilm formation and thickness due to the orientation of the anode.

269 Once again, no appreciable effect was observed whatever the orientation of the anode side
270 with respect to the cathode. Biofilms on WE1, WE2 and WE3 were similarly covering the
271 entire apparent available anode PSA (Fig. 4). With the LIVE/DEAD[®] kit, it was possible to
272 see that WE1, WE2 and WE3 were homogeneously covered by metabolically active cells of
273 *Glk. subterraneus* with similar thickness values of 75 ± 7 , 66 ± 26 and 71 ± 12 μm , for WE1,
274 WE2 and WE3, respectively. CLSM analysis of biofilms not only confirmed a very uniform
275 coverage of anodes at the microscopic level but also at the macroscopic perspective as
276 previously observed by visual inspection of a reddish biofilm (see Fig. A2 in Appendix A).

277 WE4 was not potentiostatically controlled. Consistently, no significant biofilm formation was
278 observed when using CLSM. The thickness value estimated for WE4 (13 ± 1 μm) suggests
279 that the “few” bacterial cells observed by CLSM on the anode surface corresponded to an
280 heterogeneous deposition of bacterial cells likely detached from the other potentiostatically
281 controlled anodes (WE1, WE2 and WE3) on which *Glk. subterraneus* successfully formed an
282 electroactive biofilm.

283 CLSM certified that the actual area of anode that was available for biofilm formation was the
284 total PSA (or apparent geometric area) immersed in the electrolyte regardless the side of the
285 anode that faced the cathode.

286 -Please insert **Figure 4** here-

287 **3.4. Modelling of the current density distribution on anodes with COMSOL** 288 **Multiphysics[®]**

289 The current density produced at each potentiostatically controlled anode was later modeled
290 with the software COMSOL Multiphysics[®] in an attempt to graphically illustrate the anode
291 area contribution on the current density produced in METs (Fig. 5). It was clear that the
292 anodic current densities are very similar on the available surface areas of each electrode
293 independently whether the available surface of the anode faced or opposed the cathode.

294 A common argument regarding the calculation of the current density per available surface
295 area in METs is that at such high conductivity of 5.0 S/m as the one used here all the available
296 anode surface area will very likely contribute to the current density regardless the side of the
297 anode that faced the cathode (see Table 1). Thus, a further simulation was conducted at the
298 typical conductivity found in urban wastewater (0.1 S/m).

299 It was observed that at 0.1 S/m, a decrease of the current density of about 16% can occur on
300 all WEs when compared to the modelling at 5.0 S/m (data not shown). Nonetheless, the
301 current density of WE1, WE2 and WE3 is still independent of the orientation of the electrode
302 surface with respect to the CE.

303 The conducted modelling approach with COMSOL Multiphysics® showed that the actual area
304 of anode that contributes to j_{max} is the total PSA (or apparent geometric area) immersed in the
305 electrolyte independently of the side of the anode that faces the cathode even in a medium
306 with low conductivity such as urban wastewater, a common niche of application for METs.

307 -Please insert **Figure 5** here-

308 **4. Conclusions**

309 The use of a combined approach of electrochemistry, microscopy and modelling for the
310 analysis of electroactive biofilms formed by *Glk. subterraneus* allowed to determine the
311 contribution of each anode side orientation on the current density produced in METs. The use
312 of chronoamperometry showed that no effect was observed whatever the anode orientation
313 (WE1, WE2 and WE3), since j_{max} was similar for all anodes potentiostatically controlled.
314 Turnover cyclic voltammetry of anodes also indicated a common direct electron transfer
315 mechanism that was confirmed after calculation of their shared formal potential from first
316 derivative curves. The analysis of biofilms by confocal laser scanning microscopy showed,
317 once again, no appreciable effect of the anode orientation side with respect to the cathode.
318 Biofilms on WE1, WE2 and WE3 anodes covered the entire apparent available projected
319 surface area with clearly similar thickness values ranging from 66 to 75 μm . A modelling
320 approach supported the experimental observations by confirming that the current densities
321 were very similar whatever the available anode side considered, *i.e.* anode side that faced or
322 opposed the cathode. In conclusion and with a purpose of standardization of j_{max} assessment,
323 certain areas of the anode (hidden edges or faces) with uncertain electrochemical activity
324 should be covered with an electrical insulator to further consider only the flow of current on
325 the side of the anode directly facing the cathode when for example the main goal of the study
326 is to quantify the current density produced by a novel i) electroactive bacterium, ii) electrode
327 material or iii) reactor design.

328 **5. Acknowledgements**

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331 TRI-Genotoul for technical assistance with CLSM.

332 **6. References**

- 333 [1] S. Patil, C. Hägerhäll, L. Gorton, Electron transfer mechanisms between microorganisms and
334 electrodes in bioelectrochemical systems, *Bioanalytical Reviews*, 4 (2012) 159-192.
- 335 [2] A. Aguirre-Sierra, T. Bacchetti-De Gregoris, A. Berna, J.J. Salas, C. Aragon, A. Esteve-Nunez,
336 Microbial electrochemical systems outperform fixed-bed biofilters in cleaning up urban wastewater,
337 *Environmental Science: Water Research & Technology*, 2 (2016) 984-993.
- 338 [3] Z. Borjas, A. Esteve-Núñez, J.M. Ortiz, Strategies for merging microbial fuel cell technologies in
339 water desalination processes: Start-up protocol and desalination efficiency assessment, *Journal of*
340 *Power Sources*, (2017).
- 341 [4] C.W. Marshall, E.V. LaBelle, H.D. May, Production of fuels and chemicals from waste by
342 microbiomes, *Current Opinion in Biotechnology*, 24 (2013) 391-397.
- 343 [5] B.E. Logan, Essential Data and Techniques for Conducting Microbial Fuel Cell and other Types of
344 Bioelectrochemical System Experiments, *ChemSusChem*, 5 (2012) 988-994.
- 345 [6] C. Dumas, R.g. Basseguy, A. Bergel, DSA to grow electrochemically active biofilms of *Geobacter*
346 *sulfurreducens*, *Electrochimica Acta*, 53 (2008) 3200-3209.
- 347 [7] A.J. Bard, L.R. Faulkner, *Electrochemical Instrumentation, Electrochemical Methods:*
348 *Fundamentals and Applications*, Wiley2000.
- 349 [8] D. Pletcher, *Experimental electrochemistry, A First Course in Electrode Processes*, Royal Society
350 of Chemistry2009.
- 351 [9] C.I. Torres, R. Krajmalnik-Brown, P. Parameswaran, A.K. Marcus, G. Wanger, Y.A. Gorby, B.E.
352 Rittmann, Selecting anode-respiring bacteria based on anode potential: Phylogenetic,
353 electrochemical, and microscopic characterization, *Environmental Science and Technology*, 43 (2009)
354 9519-9524.
- 355 [10] A.A. Carmona-Martínez, M. Pierra, E. Trably, N. Bernet, High current density via direct electron
356 transfer by the halophilic anode respiring bacterium *Geoalkalibacter subterraneus*, *Physical*
357 *Chemistry Chemical Physics*, 15 (2013) 19699-19707.
- 358 [11] N.S. Malvankar, D.R. Lovley, Microbial nanowires for bioenergy applications, *Current Opinion in*
359 *Biotechnology*, 27 (2014) 88-95.
- 360 [12] M. Sharma, S. Bajracharya, S. Gildemyn, S.A. Patil, Y. Alvarez-Gallego, D. Pant, K. Rabaey, X.
361 Dominguez-Benetton, A critical revisit of the key parameters used to describe microbial
362 electrochemical systems, *Electrochimica Acta*, (2014).
- 363 [13] C. Picioreanu, M.C.M. van Loosdrecht, T.P. Curtis, K. Scott, Model based evaluation of the effect
364 of pH and electrode geometry on microbial fuel cell performance, *Bioelectrochemistry*, 78 (2010) 8-
365 24.
- 366 [14] E. Dalak, *Evaluation of Unified Numerical and Experimental Methods for Improving Microbial*
367 *Electrochemical Technologies (MXCs)*, University of Toulouse III - Paul Sabatier, Toulouse, 2012, pp.
368 91.
- 369 [15] R. Lacroix, S.D. Silva, M.V. Gaig, R. Rousseau, M.-L. Delia, A. Bergel, Modelling potential/current
370 distribution in microbial electrochemical systems shows how the optimal bioanode architecture
371 depends on electrolyte conductivity, *Physical Chemistry Chemical Physics*, 16 (2014) 22892-22902.
- 372 [16] F. Harnisch, S. Freguia, A Basic Tutorial on Cyclic Voltammetry for the Investigation of
373 Electroactive Microbial Biofilms, *Chemistry – An Asian Journal*, 7 (2012) 466-475.
- 374 [17] C.I. Torres, A.K. Marcus, H.S. Lee, P. Parameswaran, R. Krajmalnik-Brown, B.E. Rittmann, A
375 kinetic perspective on extracellular electron transfer by anode-respiring bacteria, *FEMS Microbiology*
376 *Reviews*, 34 (2010) 3-17.

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378

379 **Figure captions:**

380 **Figure 1. (A) Photograph and (B) Top view of the electrode arrangement within the**
381 **electrochemical reactor showing the relative orientation of the four anodes (WE1-4).** The
382 availability or electrical insulation of the anode surface is indicated by the green “tick” or red
383 “cross” symbol, respectively. Anode WE4 was not potentiostatically controlled.

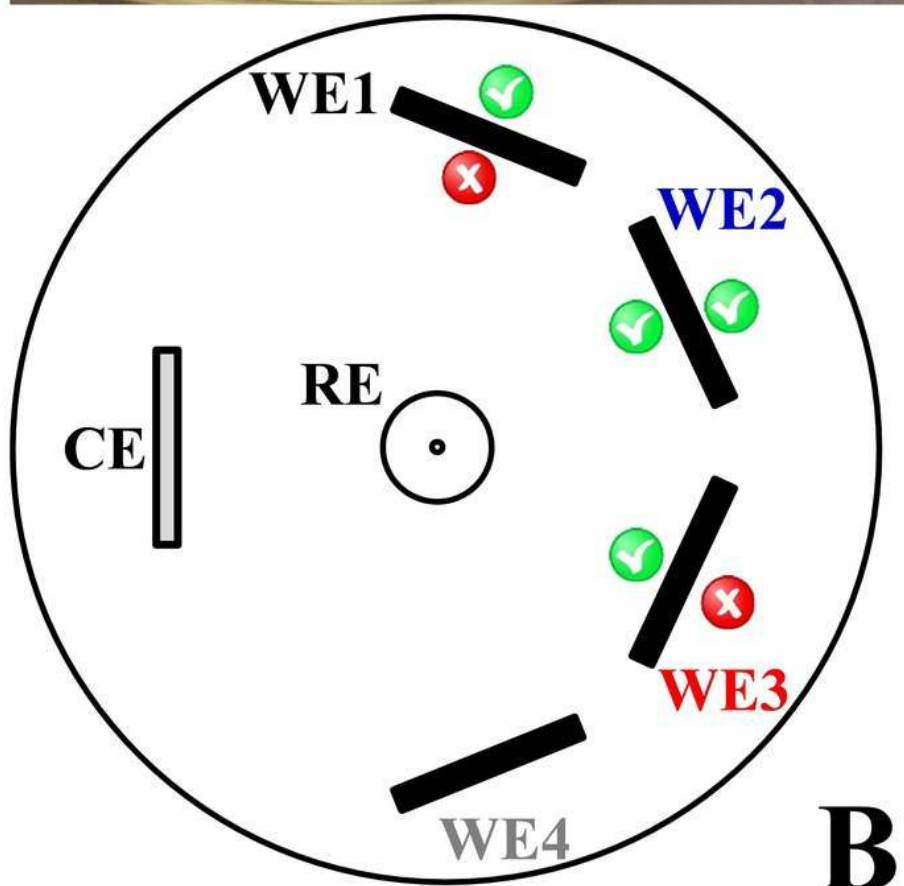
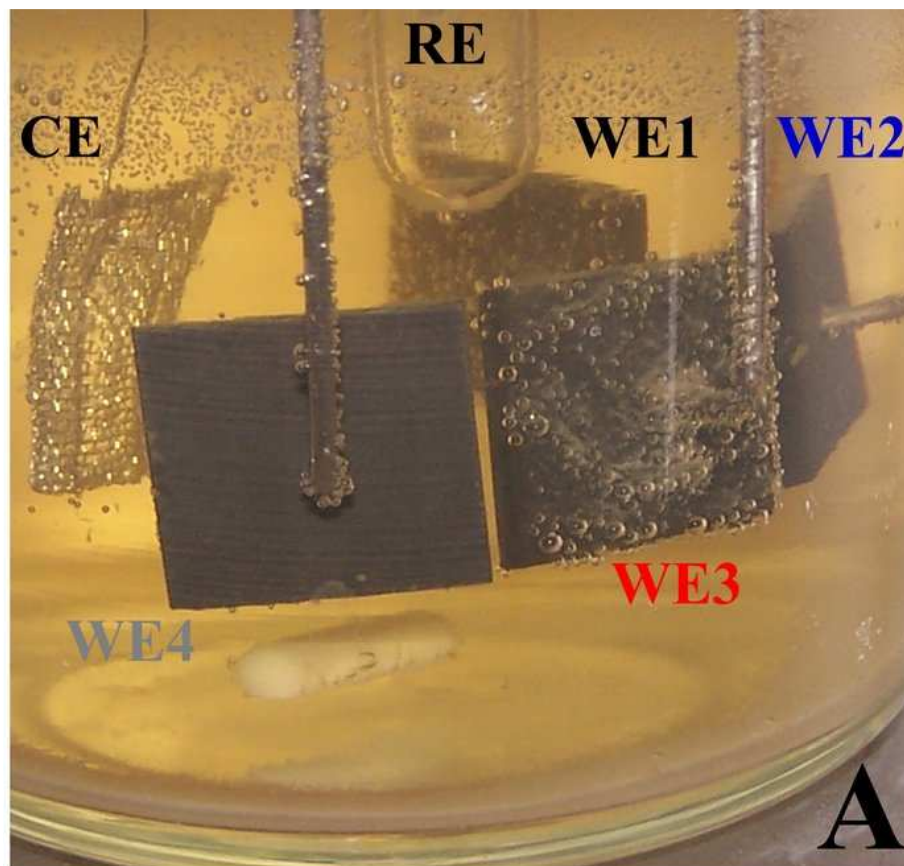
384 **Figure 2. Chronoamperometric current production by biofilms grown on WE1, WE2**
385 **and WE3 anodes.** A) Absolute current without dividing by the available surface area, B)
386 Current density by geometric surface and C) Current density calculated by the real
387 electrochemically active surface area. Thick and thin lines indicate duplicates of the same
388 experimental condition conducted independently in chronological order. ↓CV: control cyclic
389 voltammetry (CV) without biofilm; t-CV↑: turnover-CV of biofilm under catalytically active
390 acetate conversion; CLSM: stop of experiment and biofilm preparation for CLSM analysis.
391 WE4 was not potentiostatically controlled. Thus, it is not depicted.

392 **Figure 3. A) Turnover cyclic voltammetry (i.e.: under catalytically active acetate**
393 **conversion) of mature biofilms grown on anodes WE1 (black), WE2 (blue) and WE3**
394 **(red) and B) their respective first derivative curve.** In A: flat CV indicates control CV
395 without biofilm. WE4 was not potentiostatically controlled. Thus, it is not depicted.

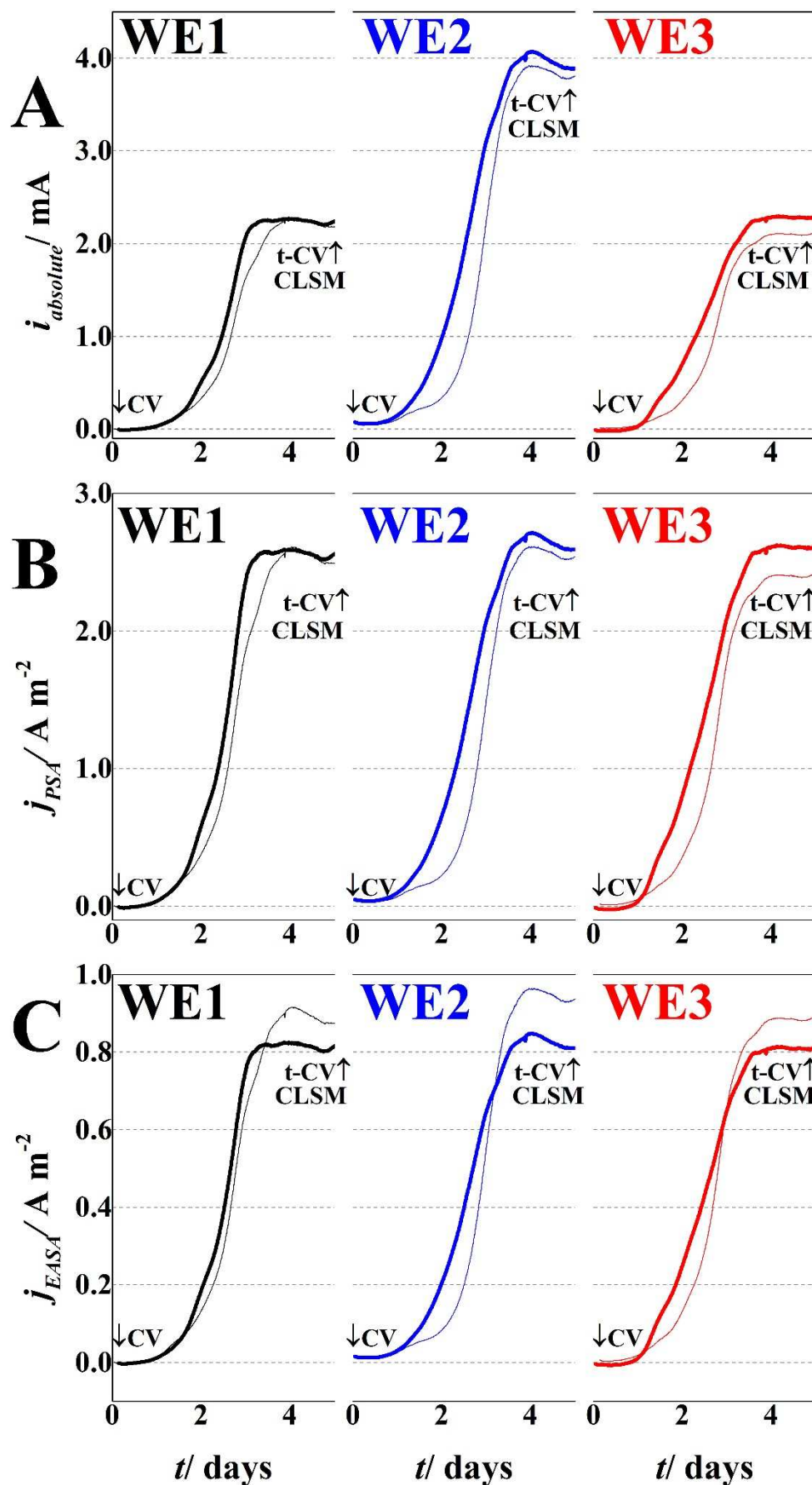
396 **Figure 4. CLSM maximum intensity projections of mature biofilms on anodes.** It is
397 shown that independently of the anode side available for biofilm formation its surface is
398 uniformly covered by *Glk. subterraneus* cells. A projection of WE4 is shown to exemplify
399 that no significant bacterial cell deposition occurred due to the non “potentiostatically-
400 controlled” condition of this anode.

401 **Figure 5. Modelling with COMSOL Multiphysics® of current density distribution on**
402 **anodes.**

403

404 **Figures:**405 **Figure 1:**

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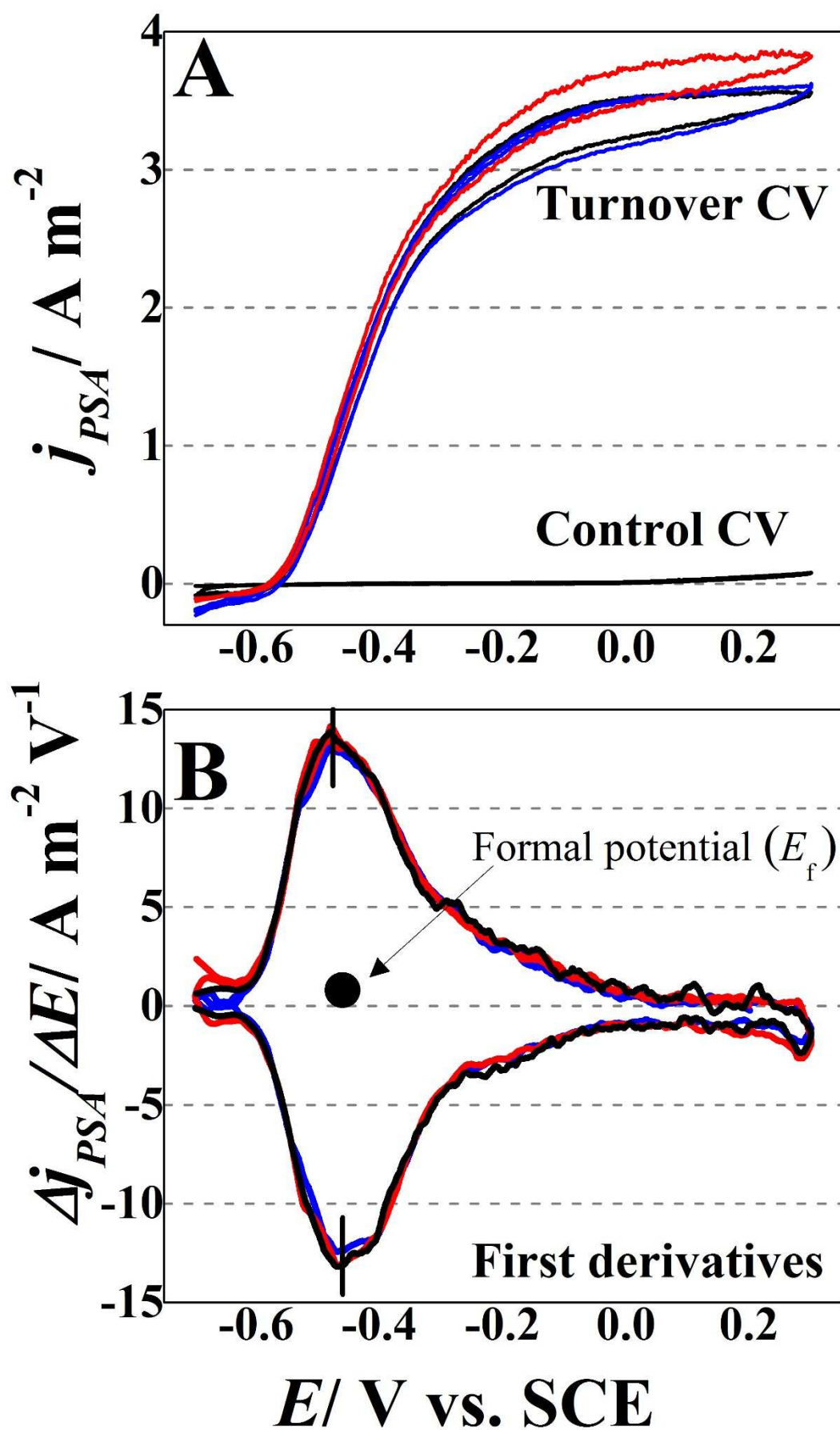
407 **Figure 2:**

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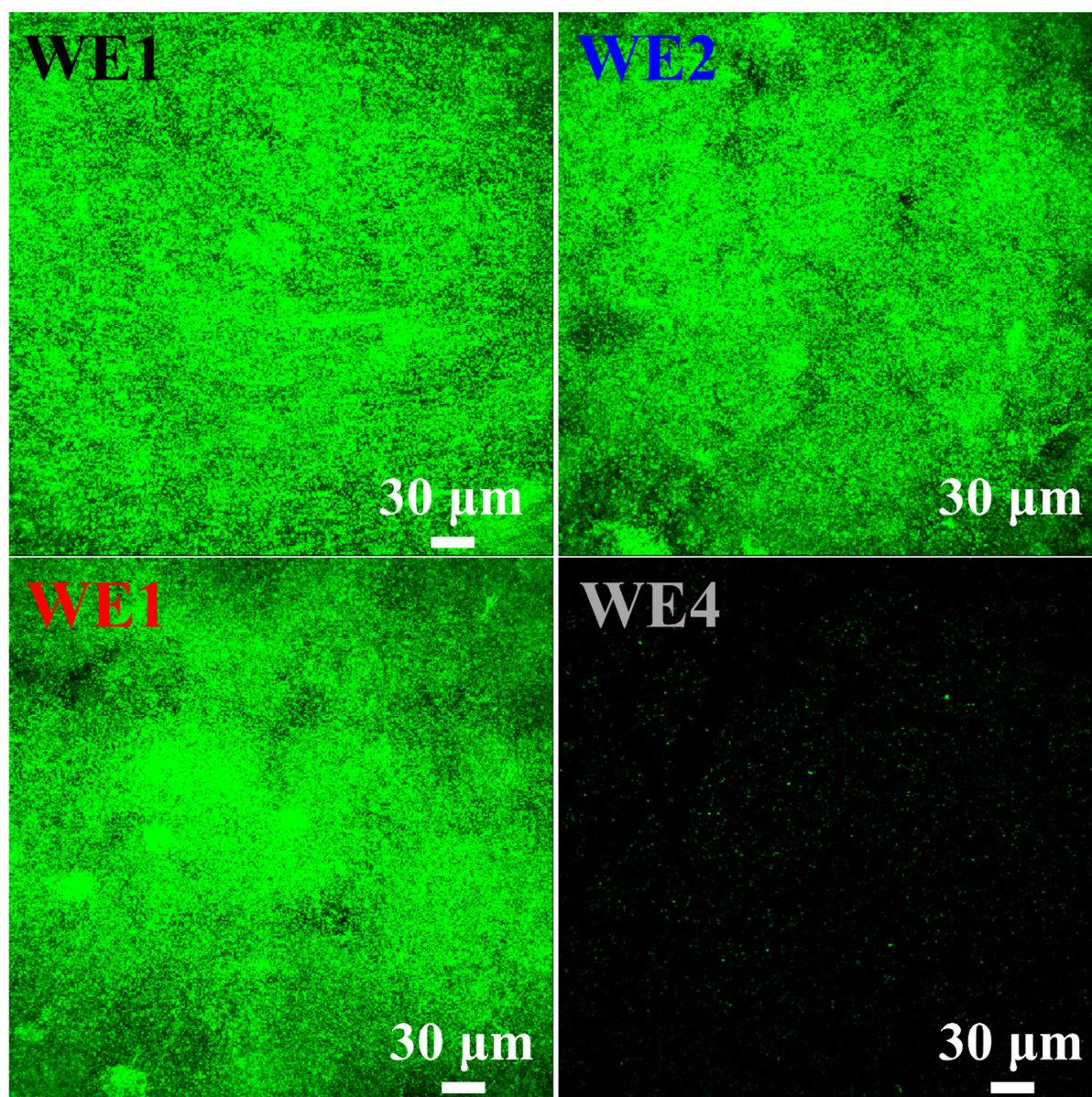
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Carmona Martinez, A., Lacroix, R., Trably, E., Da Silva, S., Bernet, N. (Auteur de correspondance) (2018). On the actual anode area that contributes to the current density produced by electroactive biofilms. *Electrochimica Acta*, 259, 395-401. , DOI : 10.1016/j.electacta.2017.10.200

409 Figure 3:

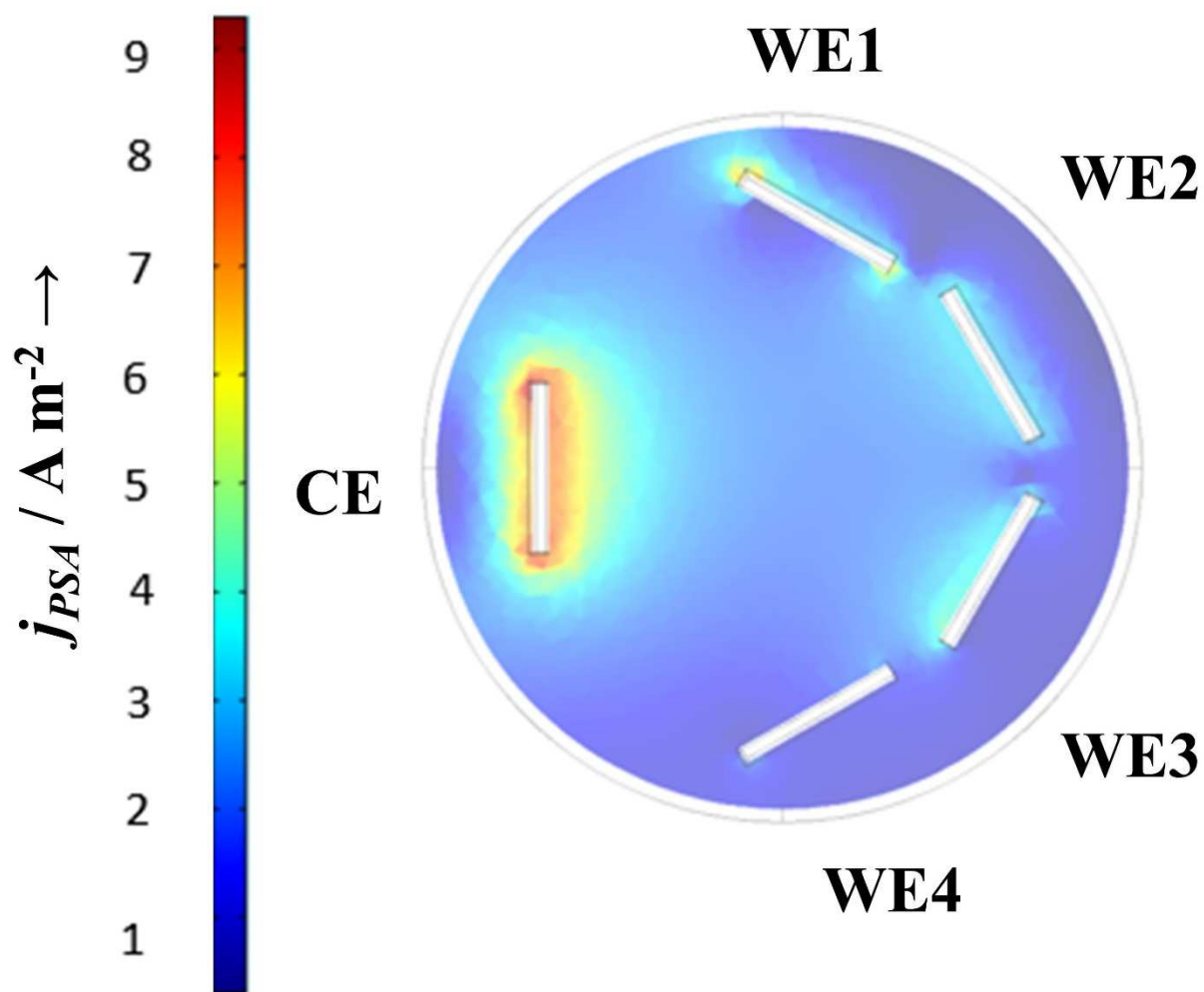


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411 **Figure 4:**

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414 **Figure 5:**

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Highlights

- Consensus on the anode area contributing to microbial current production is proposed.
- Electrochemistry and microscopy confirm the anode area actively producing current.
- Total immersed area in the electrolyte should be used to calculate current density.
- Sides hidden from cathode shall be electrically insulated to avoid overestimation.

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