Supplementary Materials

Bacterial Competition for the Anode Colonization Under Different External Resistances in Microbial Fuel Cells

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S1. Image analysis with image J

The images were analyzed by using the image J software. The green channel and the red channel were treated separately. In the order to remove the out-of-focus signal recorded for each individual image, different filters were applied. First, a 3D median filter was applied, then the background was subtracted (Figure S1). The green channel and the red channel were then grouped together to form a composite image. Finally, the maximal intensity z-projection was used to quantify the percentage of coverage or to have a general view of the image. The maximal intensity z-projection built an image from the maximal intensity of each pixel as a function of the thickness z. The orthogonal view was used to observe the general structure of the biofilm and to measure the thickness of the biofilm.
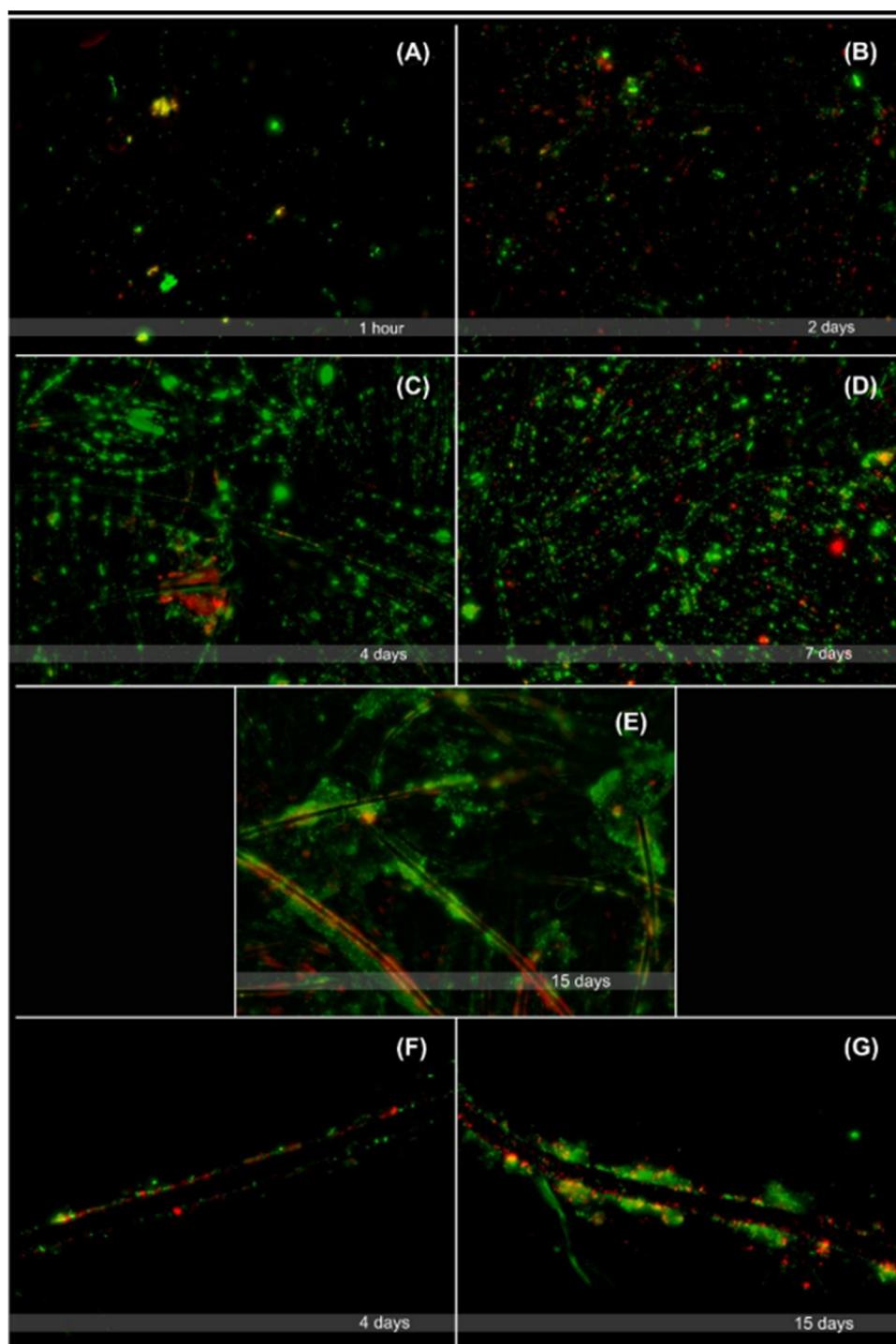
Figure S1. Anodic biofilm dynamics observed in fluorescence microscopy using a focus x200 (A-E) or x500 (F and G). Biofilms were labelled using a LIVE/DEAD Bactlight viability kit. Dead bacteria with damaged membrane are red, whereas bacteria with an undamaged membrane are green. Presented images are biofilms from the MFC0a taken at different times: after 1 hour (A), 2 days (B), 4 days (C and F), 7 days (D) and 15 days (E and G).

Table S1. Anodic potential in mV for the 8 MFCs and at different time. The results are expressed *versus* Ag/AgCl

Experiment a

Time	MFC0a	MFC330a	MFC1000a	MFCinfa
2	-40.25	-60.25	64.75	-490.25
4	-200.25	-435.25	-504.25	-577.25
7	-430.50	-479.25	-460.25	-511.00
15	-266.25	-458.25	-435.25	-461.25

Experiment b

Time	MFC0b	MFC330b	MFC1000b	MFCinfb
2	36.75	56.75	19.75	-254.25
4	-28.73	-70.41	-82.34	-420.76
10	-299.25	-367.25	-385.25	-437.25
15	-488.25	-475.25	-496.25	-526.25

S2. Evolution of the biofilm diversity

A decrease of the biofilm diversity was an indicator of specialization of an environment. The first group, A, represents the microbial communities in the initial state. Two other groups were defined as a function of the dynamic communities previously described in the paragraph 3.3.3 above: a group, B, consisting of anodic biofilms from day 1 to day 4 and a group, C, consisting of anodic communities from the 4th to the 15th day. For these three groups, the number of genus was calculated as an indicator of the richness of the microbial community and the Shannon index was also calculated as an indicator of richness and evenness of the microbial diversity. A strong decrease of the number of genus was observed after one day. The median of the genus number was 232.88 in the group A and only 95.20 and 114.91 respectively in the group B and C (Figure 3.10). So an estimated difference of 126.4 ± 12.5 genus between the group A and B, and of 115.8 ± 9.1 genus between the group A and C was observed. No difference was observed before and after 4 days (group B and C), with an estimated difference between both groups of 10.7 ± 15.2 genus. The Shannon index decreased after one day. The median was 2.836 in the group A and 1.526 in the group B, presenting difference with a p value = $2.0 \cdot 10^{-6}$ (Table S2). Although the number of genus did not increase after 4 days, the Shannon index increased to 2.168 after 4 days. In conclusion, the diversity decreased strongly in the anodic biofilms during the first days in terms of richness and evenness and then the richness stayed low while the evenness increased again.

Table S2. Statistical paired Student test from diversity data. The groups correspond to means of samples from each MFC before day 1 (Group A), after one day and before 4 days (Group B), and after 4 days (Group C). Normality conditions were previously tested using a Shapiro-Wilk test. The difference between paired data was tested between the three groups. P value, estimated difference and the interval of confidence at 80% of this estimated difference are presented in this table.

Shannon index

	Group A/B	Group A/C	Group C/B
p value	5.0E-02	6.2E-01	1.7E-02
estimated difference	0.658	0.125	0.533
	[0.264 - 1.052]	[-0.221 - 0.471]	[0.292 - 0.774]

Number of Genus

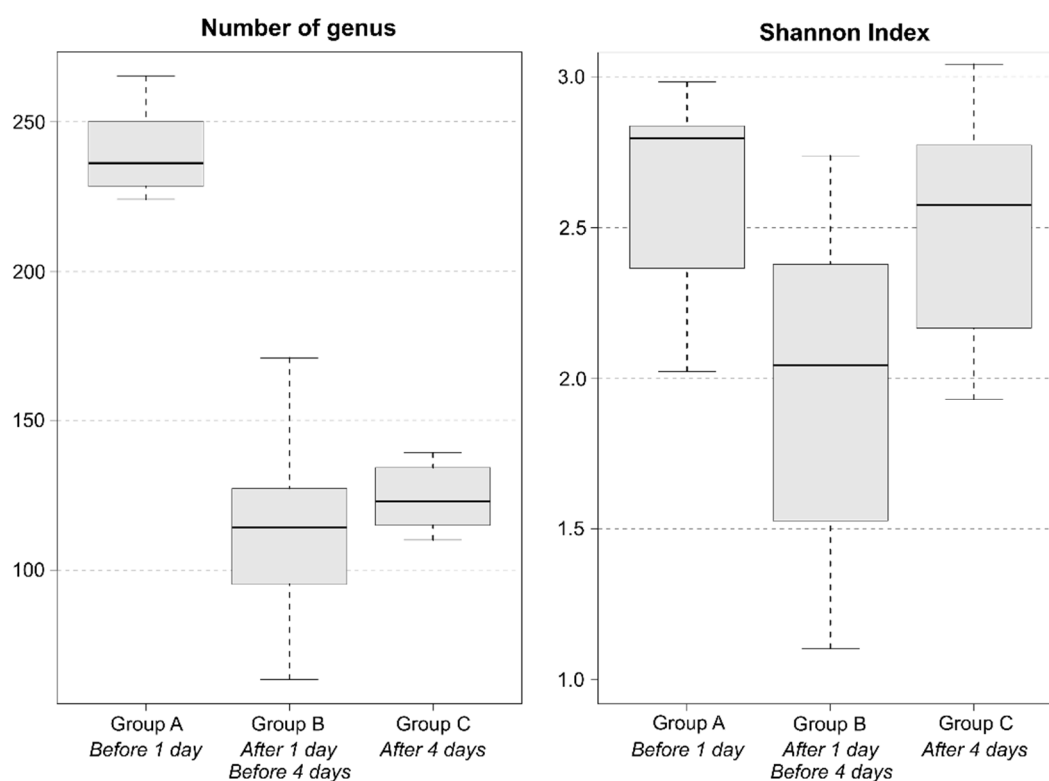|	Group A/B	Group A/C	Group C/B
p value	2.0E-06	3.9E-07	3.8E-01
estimated difference	126.4	115.8	10.7
	[113.9 - 138.9]	[106.7 - 124.8]	[-5.5 - 26.8]

Figure S2. Evolution of the biofilm diversity. Boxplot of the means of genus number and Shannon index in function of time. Three groups were formed in function of time from biofilm samples: samples before day 1 (Group A), samples from between day 1 and day 4 (Group B), and samples after 4 days (Group C). Means were calculated for each MFC. Resulting data were three groups of 8 values.
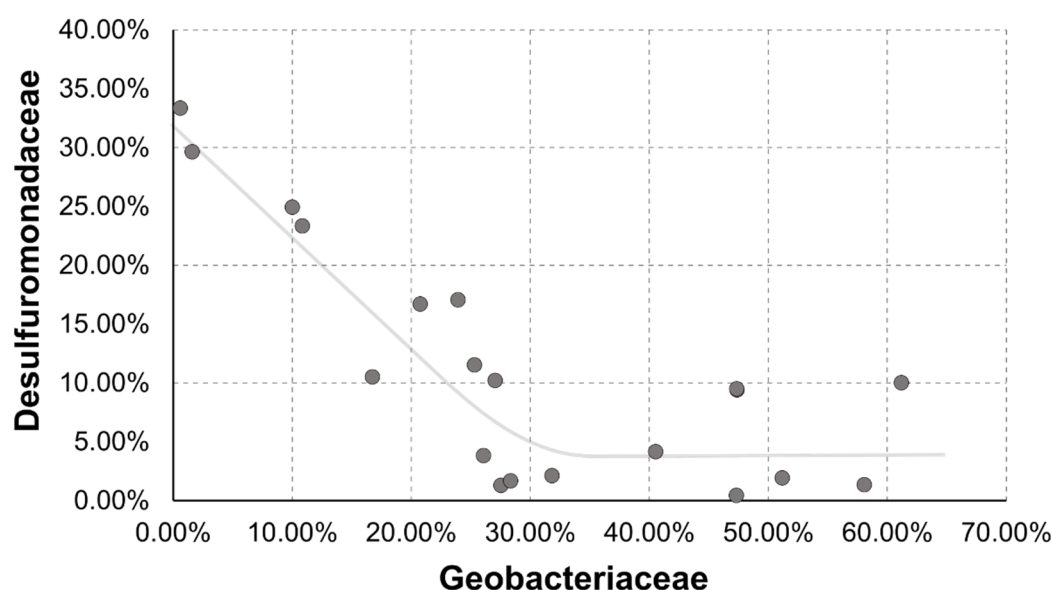
Figure S3. Bacterial competition between specific EAB. The relative abundance of *Desulfuromonadaceae* is drawn in function of the relative abundance of *Geobacteriaceae*. Only the 20 samples where the relative abundance of specific adapted EAB were higher.
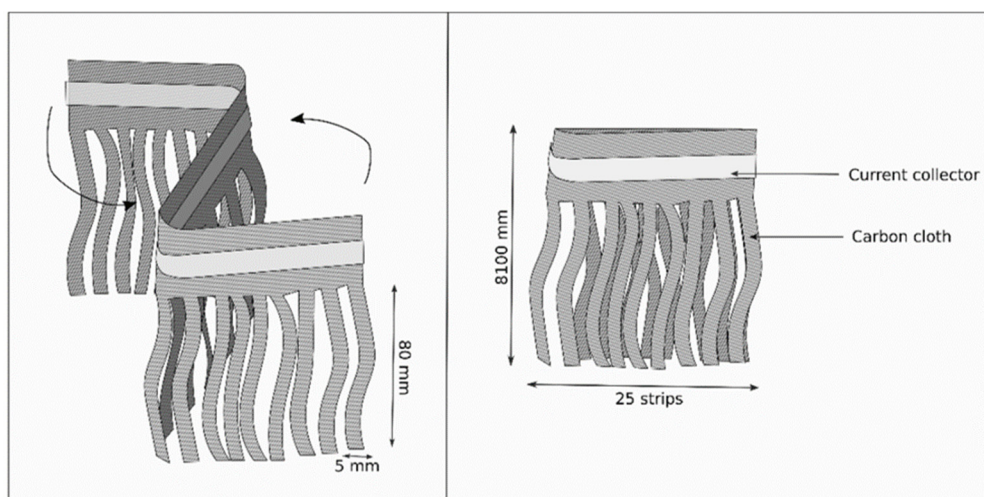
S3. MFC setup and sampling

MFC bottles MFCs with a "classical" configuration were used (Figure S1). The cathode was an air cathode created by following the procedure of Cheng et al. [124]. It contains one catalytic slide (the inner side) and one diffusion side (the outer side). The catalytic side is composed of carbon powder with 5% platinum powder. The diffusion side is composed of 4 layers of PTFE (Polytetrafluoroethylene).

Figure S4. MFC bottle with an air cathode.

The anode consisted of one 10 x 15 cm piece of carbon cloth. The anode was constituted of 25 carbon cloth strips of 80 x 5 mm. A titanium strip was use as the collector. (Figure S5).

The experiment was done in duplicate (a and b) separated by a period of 3 months. 2x4 MFCs were started with different external resistances: 1000 ohms (M-1000-a and M-1000-b), 330 ohms (M-330-a, M-330-b), without resistance (M-0-a and M-0-b) and two with an open circuit simulating an infinite resistant (M-inf-a, M-inf-b) (Figure S6).