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1 **Effect of Anode Polarization on Biofilm Formation**
2 **and Electron Transfer in *Shewanella***
3 ***oneidensis*/Graphite Felt Microbial Fuel Cells**

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8 **KEYWORDS.** Microbial Fuel Cell, *Shewanella oneidensis*, biofilm, bacteria/carbon
9 interface, polarization.

10 **ABSTRACT.** In microbial fuel cell, electricity generation is assumed by bacterial
11 degradation of low-grade organics generating electrons that are transferred to an electrode.
12 The nature and efficiency of the electron transfer from the bacteria to the electrodes are
13 determined by several chemical, physical and biological parameters. In particular, the
14 application of a specific potential at the bioanode has been shown to stimulate the
15 formation of an electro-active biofilm but the underlying mechanisms remain poorly
16 understood. In this context, we have here studied the effect of an applied potential on the
17 formation and electroactivity of biofilms established by *Shewanella oneidensis* bacteria on
18 graphite felt electrodes in single- and double-chamber reactor configurations, in oxic
19 conditions. Using amperometry, cyclic voltammetry and OCP/Power/Polarization curves
20 techniques, we showed that a potential ranging between -0.3 V and +0.5 V (vs.
21 Ag/AgCl/KCl sat.) and oppositely applied to a couple of electrodes leads to different
22 electrochemical behaviors, anodic currents and biofilm architectures. In particular, when
23 the bacteria were confined in the anodic compartment of a double-chamber cell, a negative
24 applied potential (-0.3 V) at the bioanode favors a mediated electron transfer correlated
25 with the progressive formation of a biofilm filling the felt porosity and bridging the
26 graphite fibers. In contrast, a positive applied potential (+0.3 V) at the bioanode stimulates
27 a direct electron transfer resulting in the fast-bacterial colonization of the fibers only. These

1 results provide fruitful insights for the understanding of the complex bacteria-electrode
2 interplay in microbial fuel cells.

3

4

INTRODUCTION

Microbial fuel cell (MFC) represents a promising energetical solution allowing the production of electricity from a diversity of organic substrates through direct oxidation by electrochemical active microorganisms under ambient conditions¹. The power density output of an MFC is far lower than that of chemical fuel cell because the latter uses relatively clean energy sources such as hydrogen or methanol without implying other biological system, while MFCs typically use low-grade organics, such as domestic or industrial wastes. However, in return, using MFCs for waste treatment may significantly save energy².

Many parameters influence the performance of MFCs. Besides the electrode materials and the used micro-organisms, there is accumulating evidence that the potential applied at the bioanode impacts on its colonization and therefore the MFC electrochemical performance³⁻⁸. However the intensity and the sign of the potential is often a matter of discussion^{9,10} and highly dependent on the micro-organisms which can be a bacterium or a consortium artificially-build or sampled from real environment^{4,11}, such as domestic or industrial wastewaters¹², garden composts¹³ or marine soils¹⁴. As an example, for *Geobacter sulfurreducens* positive applied potential (ca. + 0.26 V vs. SCE, between 0 to + 0.4 V vs. Ag/AgCl¹⁵ or + 0.51 V vs Ag/AgCl¹⁶ depending on the quoted study) was necessary. For mixed cultures and real inoculum, the behavior is often more complex, due to the multiplicity of bacterium strains, the synergic behavior of these strains and/or the domination of a specific strain. Moreover, the origin of inoculum influences its composition, and therefore its electrochemical response¹⁷.

Most of the time, the potential is empirically defined and, to date, no general law exists to predict the “ideal potential of polarization” with efficiency and accuracy. However, the use of a potential higher than the standard potential of the electron donor that is degraded is necessary¹⁸. Note that potential higher than the equilibrium potential favors electron deficiency at electrode surface, then encouraging oxidation reaction by bacteria¹⁹. In most cases, the polarization as well as the measurements are carried out in strict anoxic condition^{20,21} to limit the competition with oxygen reaction for electron collection and to ensure the viability of anoxic strains such as *G. sulfurreducens*.

1 In this context, the aim of the present work is to study whether the potential applied at a
2 bioelectrode constituted of *S. oneidensis* bacteria in contact with a graphite felt (GF) in oxic
3 conditions affects or not the MFC performances. Two different MFC configurations were
4 employed, single- and dual-chamber MFC configuration^{21,22}, in order to apply opposite
5 polarization at the two graphite felt electrodes considered as a bioanode²³ and a cathode^{24–}
6²⁷. The applied potential was varied from - 0.3 V to + 0.5 V (vs. Ag/AgCl/KCl sat.) at the
7 bioanode and its influence on biofilm formation and MFC electrochemical performances
8 was studied. Electrochemical activity was measured by monitoring electrodes open-circuit
9 potential, current density, MFC open-circuit voltage, and MFC polarization curves
10 (maximal current and power)²⁸. Moreover, the characterization of the biofilm organization
11 after several days of functioning permitted to establish correlations between the
12 electrochemical response and the colonization progress that was found to be sensitive to the
13 sign and intensity of the applied potential.

14 15 MATERIALS AND METHODS

16
17 All chemicals are bioreagent grad, provided by Sigma-Aldrich France and used without
18 further purification. The graphite felt is gratefully delivered by Morgan Carbon company
19 (Luxembourg)²⁹. *S. oneidensis* CRBIP17.141 bacterial strain is prepared and delivered by
20 Biological Resource Center of the Pasteur Institute (France).

21 **Inoculum.** *S. oneidensis* inoculum is prepared following two steps of pre-growth and
22 growth. A fraction of the strain, conserved at -80°C, is inoculated and pre-cultivated into a
23 Luria-Bertani Broth medium stirred at 150 rpm for 24 h at 30°C. Then, 1 mL of the pre-
24 cultivated bacteria is transferred into a 50 mL MR1 growth-medium (see SI for complete
25 description) with 30 mM sodium lactate and sodium fumarate as electron donor (carbon
26 source) and electron acceptor, respectively. Bacterial growth is carried out for 18 h in oxic
27 condition until reaching an optical density (at 600 nm, OD₆₀₀) corresponding to the last
28 quarter of the log-phase (OD₆₀₀ = 1.7) of the bacterial growth. The final inoculum is
29 prepared by transferring the obtained bacterial pellet into fresh MR1 medium supplemented
30 with 30 mM sodium lactate and free of sodium fumarate. The inoculum is stored at 4 °C in

1 sterile oxic condition for 10 min before being used as electrolyte for electrochemical
2 reactor.

3 **Single- and dual-chamber reactor set-up and polarization experiment.** In both single-
4 and dual-chamber reactors, GF was used as anode (1 cm^3) and cathode (2 cm^3 , to ensure no
5 current limitation due to the cathodic electro-active surface). A three electrodes
6 configuration was employed for electrodes polarization and for electrochemical
7 characterization using Ag/AgCl/KCl sat. reference electrode.

8 The single-chamber configuration was composed of a 30 mL sealed vessel. The GF anode
9 and cathode were used in 20 mL of the *S. oneidensis* inoculum diluted at 0.7 in OD_{600} ,
10 corresponding to $8 \cdot 10^8 \text{ cfu.mL}^{-1}$. For all experiment, *S. oneidensis* inoculum corresponds to
11 the reactor electrolyte composed of bacteria dispersed in a MR1 medium (growth medium,
12 as previously described) supplemented with 30 mM lactate and free from fumarate,
13 buffered at pH 7.

14 For the dual-chamber configuration, two compartments were separated by an
15 ultrafiltration membrane (pore size: $0.2 \mu\text{m}$). The anodic side was filled with a bacteria
16 solution (as previously described) and the catholyte consisted in a solution of 10 mM
17 $\text{K}_3\text{Fe}(\text{CN})_6$ and 150 mM NaCl. Both GF anode and cathode were placed at 2.5 cm from the
18 separator together with an Ag/AgCl/KCl sat. reference in each compartment (figure S1,
19 Supplementary Information). Lactate was supplemented in the anodic compartment and
20 interval between additions of 30 mM lactate was evaluated by current monitoring;
21 decreasing of current corresponds to a decrease in lactate availability. To ensure sterility,
22 all the reactors components were autoclaved at $120 \text{ }^\circ\text{C}$ and then assembled in sterile
23 conditions. The oxic condition was achieved during the whole experiment with vent-caps
24 and sterile filters (pore size: $0.2 \mu\text{m}$) to ensure atmospheric exchange.

25 To apply fixed potential at electrodes, the anode and the cathode were symmetrically and
26 continuously polarized by applying well-defined potentials against a reference electrode
27 (figure S1, Supplementary Information). The polarization effect was evaluated for anodic
28 potential varying between -0.3 V and $+0.5 \text{ V}$ (vs. Ag/AgCl/KCl sat.) while the potential at
29 the cathode was oppositely poised. Both the polarization step and the electrochemical
30 characterizations were carried out in oxic condition.

1 **Electrochemical and Scanning electron microscopic characterization.** The evolution
2 of the bio-anodic electrode was electrochemically monitored by chronoamperometry at +
3 0.3 V vs. Ag/AgCl/KCl sat. (after stabilization of the measured current, *ca.* 1 h) and cyclic-
4 voltammetry (scan rate: 10 mV.s⁻¹). All the electrochemical characterizations were carried
5 out with a Biologic VSP potentiostat in a 3-electrodes configuration with an Ag/AgCl/KCl
6 saturated (sat.) reference electrode (figure S1, Supplementary Information). Anodic and
7 cathodic open-circuit potential (OCP) were also monitored. In the dual-chamber
8 configuration, polarization and power curves were determined by monitoring anodic and
9 cathodic potential for an incremental series of current *i*, corresponding to equivalent
10 defined densities of current *j* (mA per square centimeter of geometric surface area). Before
11 electrochemical characterization, electrodes were left for stabilization at the OCP for 1 h.

12 After several days of polarization, anode and cathode materials were characterized by
13 scanning electron microscopy (Hitachi S-3400N). To prevent degradation of the biological
14 structures at the surface of the GF fibers, the samples were chemically fixed by their
15 immersion in several successive baths. First, the sample was immersed in a 2.5 %
16 glutaraldehyde and 0.1 M sodium cacodylate solution for 2 h at 4 °C and then washed three
17 times in a bath of 0.2 M cacodylate for 10 min each. Finally, the sample was dehydrated in
18 several baths of ethanol from 30 % to 100 % and dry in air.

19

20

RESULTS AND DISCUSSION

21

22 **Single compartment studies.** A first set of reference experiments was performed in
23 single-chamber reactor in the absence of polarization using a freshly-prepared highly-
24 concentrated suspension of *S. oneidensis* in PBS supplemented with 30 mM sodium lactate,
25 in oxic conditions and using a platinum electrode as working electrode (at *t*₀ when bacteria
26 were transferred in PBS). Cyclovoltamograms showed two sigmoidal waves of oxidation
27 demonstrating the existence of two distinct mechanisms of electron transfer. According to
28 the literature ⁹, they correspond to MET (Mediated Electron Transfer) for potentials below
29 0 V vs. Ag/AgCl/KCl sat. and DET (Direct Electron Transfer) for potentials above 0 V vs.
30 Ag/AgCl/KCl sat. (Figure 1).

31

1 HERE FIGURE 1

2
3 In a second set of experiments, *S. oneidensis* bacteria harvested in the log phase and
4 resuspended in MR1 medium containing 30 mM lactate were used and the anodic and
5 cathodic applied voltage at the carbon felt electrodes were varied from - 0.3 V to + 0.5 V
6 vs. Ag/AgCl/KCl sat. ($E_c = - E_a$).

7 Chronoamperometry experiments were performed over 19 days (Figure 2). In the
8 presence of a negative polarization, the current was 60 μ A after 1 day, dropped down to
9 less than 10 μ A after 4 days and then continued to decrease despite the regular lactate
10 feeding. In the absence of polarization, the initial current was smaller (*ca.* 35 μ A) and also
11 underwent a rapid decrease but then increased again until it reached *ca.* 20 μ A after 19
12 days. When a +0.3 V vs. Ag/AgCl/KCl sat. polarization was applied, the initial decrease
13 phase was also observed and the current values varied around 50 μ A average value. Finally,
14 for a + 0.5 V vs. Ag/AgCl/KCl sat. potential, the current was stable over the first days of
15 the experiment and then increases progressively to reach *ca.* 120 μ A. Nevertheless, we
16 observed a reproducible dispersion of current values at a given time points, suggesting that
17 such high potentials may impact the bacterial activity and/or the stability of the bacteria-
18 electrode interface.

19
20 HERE FIGURE 2

21
22 Cyclovoltamograms were recorded at different times of the experiments (Figure 3).
23 After one day, regardless of the applied potential, cyclovoltamograms exhibited a pair of
24 reversible faradaic peaks centered at - 0.4 V vs. Ag/AgCl/KCl sat. corresponding to
25 riboflavin. An endogenous oxidation wave of similar shape and intensity was observed in
26 the high positive and negative potential regions (Figure 3a) responsible for an extracellular
27 electron transfer. After 4 days, the oxidation wave has increased in intensity for a + 0.5 V
28 vs. Ag/AgCl/KCl sat. and a + 0.3 V vs. Ag/AgCl/KCl sat. polarization, in agreement with
29 the chronoamperometry measurements (Figure 3b). In parallel, in the negative potential
30 range, the signal becomes more complex, with an apparent splitting of the riboflavin peak.
31 Such a splitting is confirmed at day 8 for all polarization conditions (Figure 3c). In parallel,

1 the oxidative wave has gained in intensity for systems under a positive polarization and
2 especially at + 0.5 V vs. Ag/AgCl/KCl sat.. These two observations are still valid at day 19
3 although, for a + 0.5 V vs. Ag/AgCl/KCl sat. polarization, the two peaks appear to have
4 merged (Figure 3d), resulting in a single signal in the same potential region but much
5 broader than at day 1 (Figure 3a).

6
7 HERE FIGURE 3

8
9 These sets of experiments confirm the existence of different electron transfer mechanism
10 (DET and/or MET/riboflavin), depending on the applied potential. On the one hand, the
11 continuous increase in the intensity of the signal in the positive potential range suggests
12 that the DET mechanism becomes more and more effective with time, especially under
13 highly positive polarization conditions. This should reflect that an increasing number of
14 bacteria are in direct contact with the graphite felt electrodes (*i.e.* anode colonization)
15 and/or that the electron transfer at the bacteria/electrode interface becomes facilitated. For a
16 non-polarized electrode or a polarization at -0.3 V vs. Ag/AgCl/KCl sat. the increasing of
17 the wave intensity is less marked. On the other hand, the signals in the negative potential
18 range do not evolve much in intensity but rather in shape. This can indicate the production
19 of other mediators by the bacteria and/or a modification of the mediator/graphite interface.
20 In particular, the splitting of the riboflavin peak may correspond to the co-existence of
21 molecules originating from bacteria in solution and within the deposited biofilm.

22 To clarify these points, SEM imaging of the samples were performed at the end of the
23 chronoamperometry experiments (Figure 4). The lowest density of bacteria is observed at a
24 negative potential ($E_a = -0.3$ V vs. Ag/AgCl/KCl sat.). These bacteria are embedded in a
25 dense biofilm. Under non-polarized condition or positive applied potential, a similar
26 situation is observed but the cell density seems higher, although biological analyses would
27 be necessary to quantitatively ascertain this point. While the specific case of negative
28 polarization fits well with the measurement of a very low current value, it is clear that the
29 biofilm morphology, as it is observed here, cannot explain the differences in the
30 electrochemical behavior for the other polarization conditions. Hence, the applied potential
31 should have a deep impact on the internal structure and organization of the biofilm,

1 influencing the diffusion of the mediator and/or the connectivity of the conductive
2 elements.

3
4 HERE FIGURE 4

5
6 **Double compartment studies.** In a step forward the development of a complete MFC, a
7 dual compartment reactor was set up using an ultrafiltration membrane (pore size = 0.2 μm)
8 as a separator to prevent bacterial diffusion from the anodic side to the cathodic
9 compartment. A potential of either + 0.3 V or - 0.3 V was applied at the GF anode with an
10 oppositely polarized cathode. The electrochemical behavior under symmetrical polarization
11 was monitored by chronoamperometry and the evolution of the anode potential,
12 polarization and the power curves as function of time were established

13 Figure 5 displays the evolution of the polarization and power curves over 19 days using a
14 symmetrical polarization when the anode was polarized at + 0.3 V vs. Ag/AgCl/KCl sat.
15 (vs. $E_c = - 0.3$ V vs. Ag/AgCl/KCl sat.). At day 2, the potential of the bioanode is equal to -
16 0.4 V vs. Ag/AgCl/KCl sat. and remains stable until the end of the experiment (Figure 5a).
17 The anodic current density at $E_a = 0$ V vs. Ag/AgCl/KCl sat. evolves from 70 (day 2) to 30
18 $\text{mA}\cdot\text{m}^{-2}$ (day 19). The evolution of the MFC polarization curves (Figure 5b) is a result of
19 the anodic and cathodic I - V profiles. MFC OCV remains stable at *ca.* 0.65 V, indicating
20 that the electroactivity established at the bioanode is stable. The internal resistance
21 (polarization curve slope, $R_{\text{INT}} = \Delta E/j$) increases with time. Since no detrimental clogging
22 of the separator was observed after 19 days, such an increase indicates a modification of
23 electron transfer at the surface of the bioanode. This is confirmed by evolution of the
24 current density (the maximum current density decreases from *ca.* 100 to 35 $\text{mA}\cdot\text{m}^{-2}$) and
25 the power curves (Figure 5c) where a loss in power from *ca.* 20 (day 2) to *ca.* 7 $\text{mW}\cdot\text{m}^{-2}$
26 (day 19) was observed.

27
28 HERE FIGURE 5

29
30 The same experiment was performed with a - 0.3 V vs. Ag/AgCl/KCl sat. negative
31 potential applied to the bioanode. Figure 6 summarizes the evolution of the characteristic

1 polarization and power curves. At the open-circuit, the potential of the bioanode (figure 6a)
2 regularly decreases from - 0.25 V (day 2) to - 0.6 V vs. Ag/AgCl/KCl sat. (day 15). The
3 MFC OCV shown in figure 6b increases from 0.5 V (day 2) to 0.85 V (day 15).
4 Additionally, the maximal power density increases from 12 to 20 mW.m⁻² (figure 6c). The
5 initial maximal density of current is about 100 mA.m⁻² and decreases to 60 mA.m⁻² at day 5
6 and then increases again to *ca.* 70 mA.m⁻² (17% recovered) and remains stable until day 15.
7 Noticeably, a loss of electrochemical performances was observed after 19 days that can be
8 explained by the depletion in lactate due to the absence of feeding after day 13.

9
10 HERE FIGURE 6

11
12 These results indicate two different behaviors as function of the polarization condition
13 applied. Figure 7 summarizes the different situations: for both negative (- 0.3 V) and
14 positive (+ 0.3 V) polarization, the maximal MFC current decreases in the first 5 days from
15 100 to about 60 mA.m⁻². Then, the value remains stable or slowly decreases. For an applied
16 potential of + 0.3 V vs. Ag/AgCl/KCl sat., the MFC OCV remains constant at + 0.65 V
17 while the power density decreases with time from *ca.* 20 mW.m⁻² to 5 mW.m⁻². This can be
18 linked to a modification of the electron transfer from bacteria to graphite electrodes,
19 correlated with the increase of the ohmic losses of the MFC. On the contrary, for applied
20 potential of - 0.3 V vs. Ag/AgCl/KCl sat., an increase of the MFC OCV, from 0.55 V to
21 0.85 V, and power density, from 12 mW.m⁻² to 20 mW.m⁻², are observed, keeping day 19
22 data aside. The progressive increase of MFC OCV suggests a longer phase of stabilization
23 for the bacteria in contact with the graphite anode compared to the positive applied
24 potential. Additionally, the increase in power density can be linked to a better transfer of
25 electron from the bacteria to the GF fibers.

26
27 HERE FIGURE 7

28
29 The microstructure of the electrode after 19 days was studied by scanning electron
30 microscopy (figure 8). For a potential applied at the bioanode of + 0.3 V vs. Ag/AgCl/KCl

1 sat., the bacteria can be distinctly observed at the surface of fibers and form a uniform
2 layer. This bacterial layer is embedded in a thin and dense biofilm which forms a sheath
3 wrapped around the fibers. In contrast, for potential applied at the bioanode of - 0.3 V vs.
4 Ag/AgCl/KCl sat., a large quantity of biomass (EPS, bacteria, ...) occupies the porosity
5 formed by the graphite fiber network. The fibers themselves are embedded in a thick and
6 porous biofilm structure composed of a high density of bacteria and EPS.

7
8 HERE FIGURE 8
9

10 These observations can be related to the results of the single compartment experiments.
11 Under positive polarization, DET becomes rapidly prevalent over MET, which can be
12 correlated with an efficient colonization of the fiber surface by bacteria. In these conditions,
13 as the biofilm thickens, the electron transfer may become less favorable, leading to a
14 decrease in the electrochemical performance as observed for MFC power density. Yet, the
15 MFC OCV remains stable meaning that the efficiency of the redox reaction and electron
16 transfer at the fiber surface remains the same. In contrast, under negative polarization, both
17 DET and MET are involved in the electron transfer, so that bacteria growth is favored both
18 in solution, *i.e.* in the felt porosity, and on the fiber surface. In these conditions, the increase
19 in the power density with time can be related to the simultaneous development of both
20 populations. Moreover, the ability of the biofilm to bridge graphite fibers may provide
21 additional conduction pathways. Yet it is interesting to point out that such bridging
22 structures were not observed in single compartment experiments. This difference can be
23 explained considering that, in the latter situation, the cathode is also in contact with the
24 bacterial suspension and can interfere with its behavior. As a matter of fact, in these
25 conditions, colonization of the graphite felt used as the counter-electrode could be observed
26 by SEM (figure S3, Supplementary Information). On the contrary, in the double
27 compartment configuration, the oppositely polarized electrode is isolated from the bacterial
28 anolyte by the ultrafiltration membrane so that bacterial behavior must be influenced only
29 by the anodic potential of polarization.

30 31 CONCLUSION

1 In this work, the impact of polarization on the colonization of graphite felts used as
2 bioanodes by wild type *S. oneidensis* in oxic conditions and its influence on the
3 electrochemical performances of single- and double-compartment fuel cells were studied.
4 In single-compartment MFC configurations, both anodic current and electrode colonization
5 are directly correlated to positive polarization (+ 0.3 and + 0.5 V vs. Ag/AgCl/KCl sat.).
6 Under non-polarized condition, a lower and less reproducible current is measured but it
7 remains higher than the current measured under negative polarization. These results suggest
8 that positive polarizations favor the felt colonization but also impact the properties of the
9 resulting biofilm. However, we also observed an unintended colonization of the counter
10 electrode, which suggests a combined effect of both fixed anode and cathode potentials. To
11 elucidate this phenomenon and prevent the cathode colonization by bacteria, dual-
12 compartments MFC experiments were carried out in the same polarization conditions. Two
13 distinct behaviors are observed: (i) under positive polarization, MFC performance
14 (polarization and power curves), anodic OCP and MFC OCV are already at their maximal
15 value after a short time of polarization; however, MFC performances decrease rapidly with
16 time. The biofilm appears thin and dense around the felt fibers. (ii) Under negative
17 polarization, the electrochemical parameters progressively increase to reach their maximal
18 values after more than 20 days. In this condition, the biofilm is thick, porous and fills the
19 porosity of the felt. These two behaviors seem in agreement with the DET/MET hypothesis.
20 At + 0.3 V vs. Ag/AgCl/KCl sat., the electrode colonization is controlled by DET leading
21 to the accumulation of bacteria on the fiber surface. On the contrary, at - 0.3 V vs.
22 Ag/AgCl/KCl sat., the MET mechanism is more effective, driving the development of
23 bacteria in the liquid phase, *i.e.* colonization of the felt porosity. Yet, biofilm formation of
24 the fiber surface is also observed, suggesting that DET can also occur. Whether a negative
25 potential favor MET, hinder DET or both remains an open question.

26
27

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14 **ABBREVIATIONS**

15 GF: graphite felt, Ea: anodic potential, Ec: cathodic potential, OD₆₀₀: Optical Density at

16 600 nm, MFC: microbial fuel cell, OCV: open-circuit voltage, OCP: open-circuit potential,

17 SEM: scanning electron microscopy, cfu.mL⁻¹: colony-forming unit per milliliter, I-V

18 curve: current-tension curve.

19

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FIGURES CAPTIONS

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23 **Figure 1.** Cyclic voltammetry at $10 \text{ mV}\cdot\text{s}^{-1}$ of a highly concentrated suspension of *S.*
24 *oneidensis* in a PBS buffer supplemented with 30 mM lactate in oxic conditions. The
25 potential range corresponding to mediated electron transfer (MET, red zone) and direct
26 electron transfer (DET, blue zone) and the corresponding scheme are adapted from Roy *et*
27 *al.* ⁹.

1 **Figure 2.** Chronoamperometry measurements for *S. oneidensis*/graphite felt bioanodes in
2 single-compartment reactors with an applied potential of (orange circles and squares) - 0.3
3 V, (blue circles and squares) 0 V, (green circles and squares) + 0.3 V and (dark circles and
4 squares) + 0.5 V

5 **Figure 3.** Cyclovoltammograms at $10 \text{ mV}\cdot\text{s}^{-1}$ for *S. oneidensis*/graphite felt bioanodes in
6 single-compartment reactors with an applied potential of (orange line) - 0.3 V, (blue line)
7 0 V, (green line) + 0.3 V and (dark lines) + 0.5 V after (a) 1 day, (b) 4 days, (c) 8 days and
8 (d) 19 days of polarization. Reference signal of GF/MR1 without bacteria and reference
9 signal of riboflavin on figure S2 (Supplementary Information).

10 **Figure 4.** SEM observations of *S. oneidensis*/graphite felt bioanodes in single-
11 compartment reactors. Representative morphology of colonized felts (a) and higher
12 magnification images for bioanodes polarized at (b) + 0.5 V, (c) + 0.3 V, (d) non polarized,
13 (e) - 0.3 V, after 19 days in working condition.

14 **Figure 5.** Electrochemical characterization of *S. oneidensis*/graphite felt//
15 $\text{K}_3\text{Fe}(\text{CN})_6$ /graphite felt double-compartment reactor with a bioanode polarized at + 0.3 V
16 after (orange circle) 2 days, (grey circle) 5 days, (green circle) 6 days, (blue circle) 15 days
17 and (dark circle) 19 days: (a) anodic I-V, (b) polarization and (c) power curves.

18 **Figure 6.** Electrochemical characterization of *S. oneidensis*/graphite felt//
19 $\text{K}_3\text{Fe}(\text{CN})_6$ /graphite felt double-compartment reactor with a bioanode polarized at - 0.3 V
20 after (orange circle) 2 days, (grey circle) 5 days, (green circle) 6 days, (blue circle) 15 days
21 and (dark circle) 19 days: (a) anodic I-V, (b) polarization and (c) power curves.

22 **Figure 7.** Temporal evolution of (dark circle) open-circuit voltage (OCV), (red circle)
23 maximal power density and (blue circle) maximal current density for a *S.*
24 *oneidensis*/graphite felt// $\text{K}_3\text{Fe}(\text{CN})_6$ /graphite felt double-compartment reactor with a
25 bioanode polarized at (a) + 0.3 V and (b) -0.3 V.

26 **Figure 8.** SEM images of the anodic graphite felt interior after 19 days of function for a *S.*
27 *oneidensis*/graphite felt// $\text{K}_3\text{Fe}(\text{CN})_6$ /graphite felt double-compartment reactor with a
28 bioanode polarized at (a) + 0.3 V and (b) -0.3 V.

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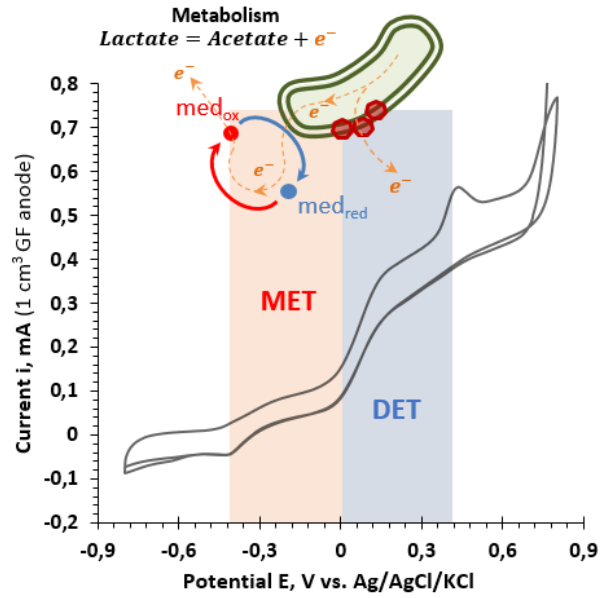
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FIGURES

FIGURE 1

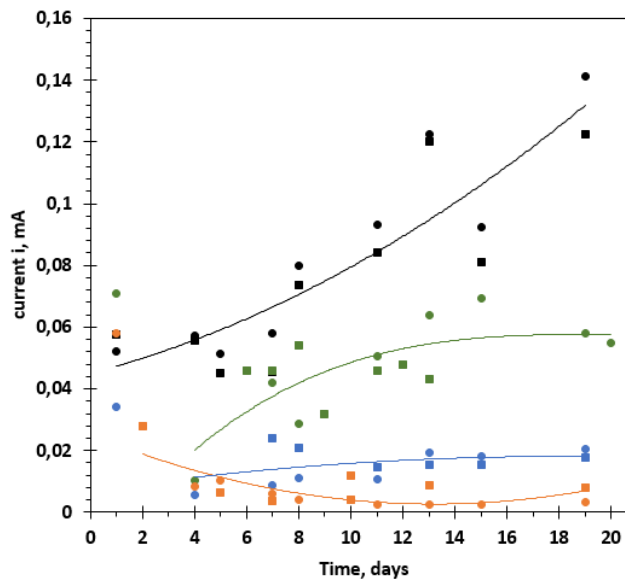


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FIGURE 2



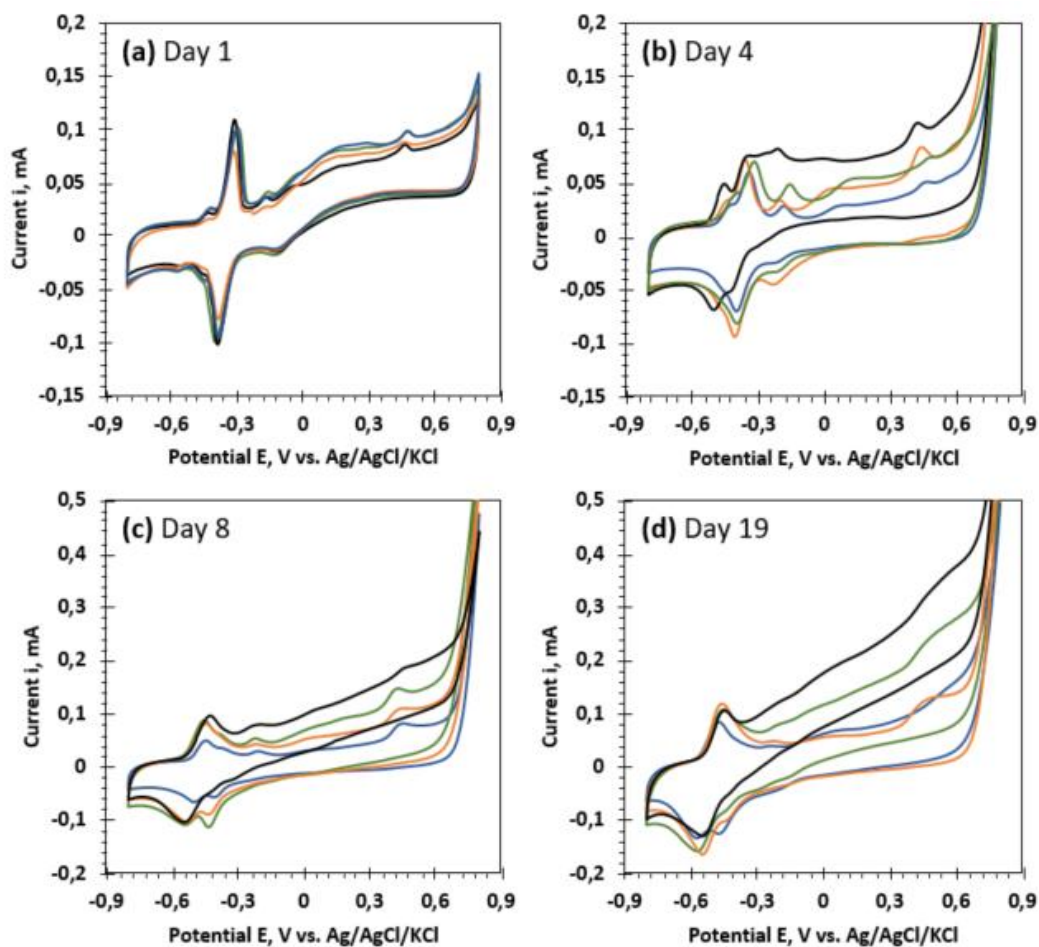
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FIGURE 3



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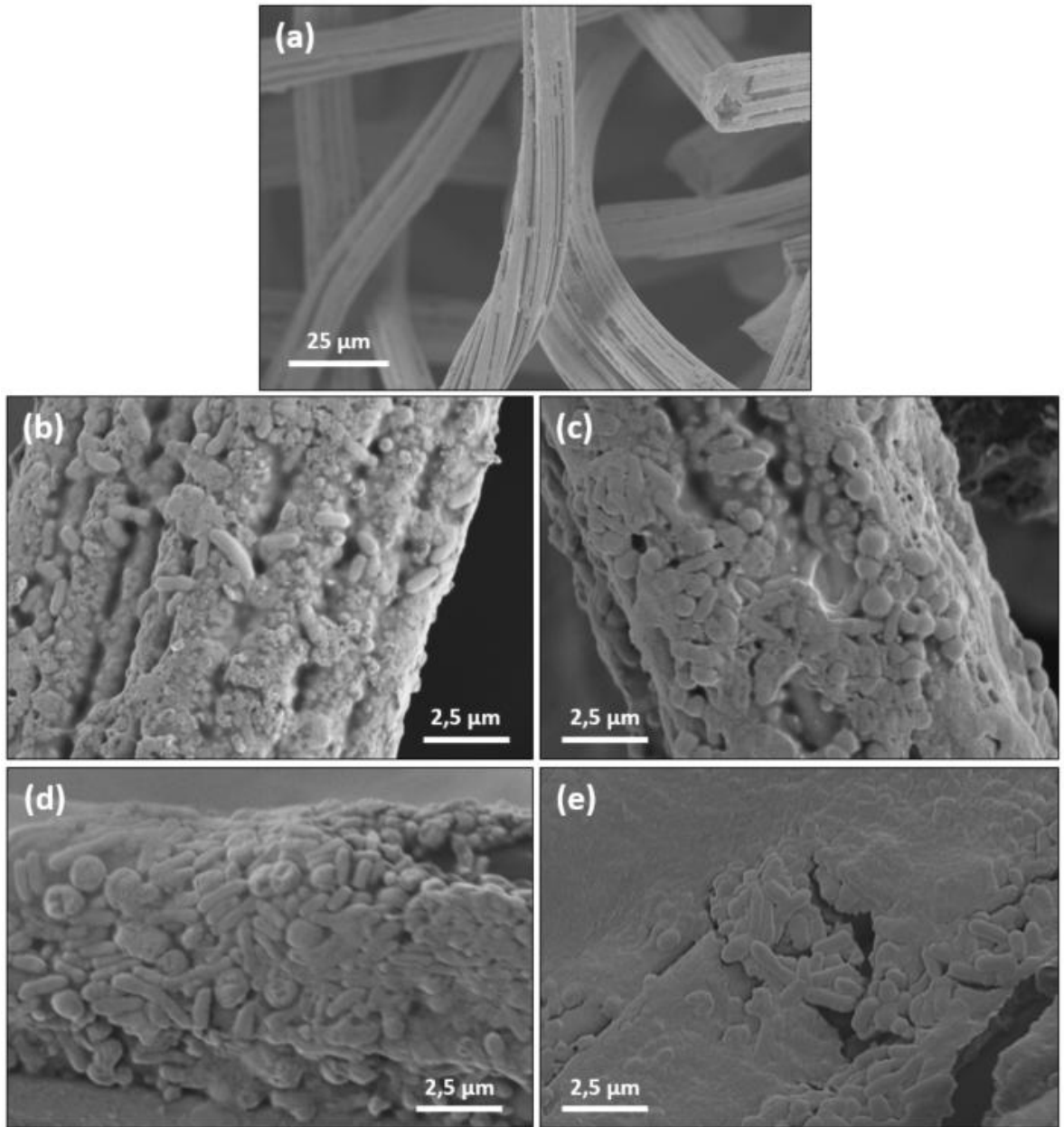
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FIGURE 4



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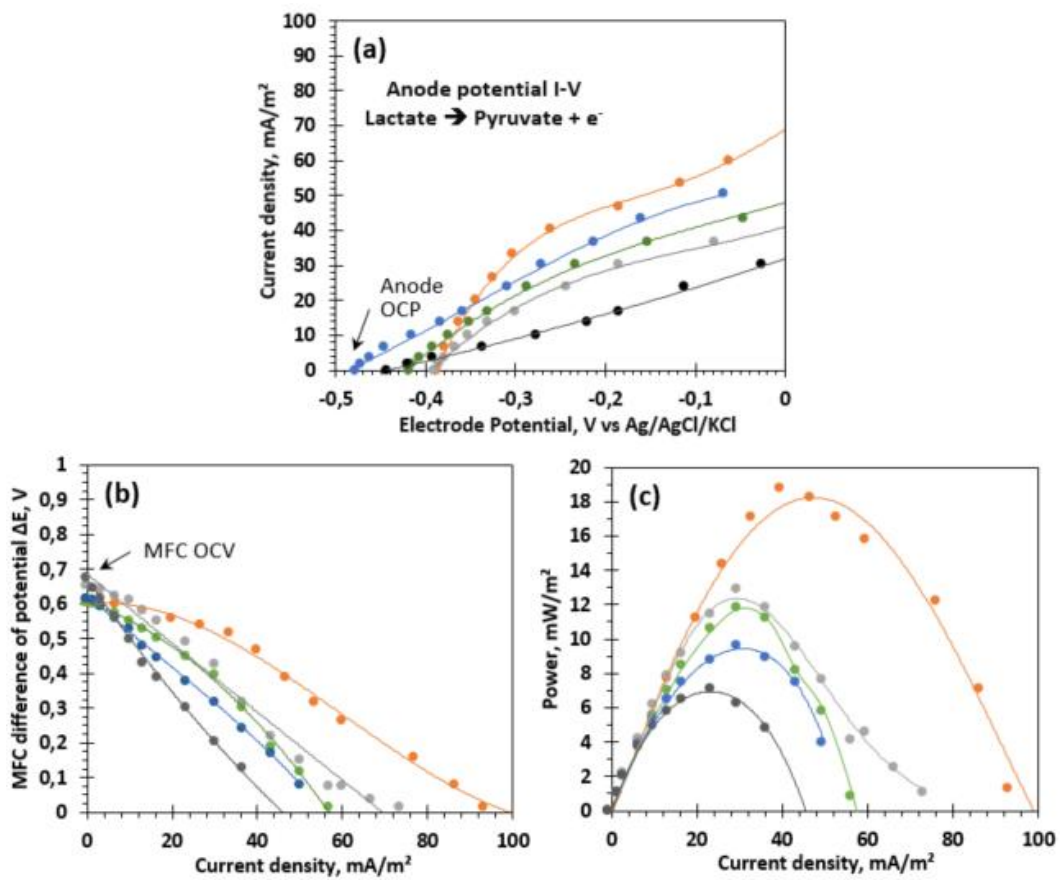
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FIGURE 5



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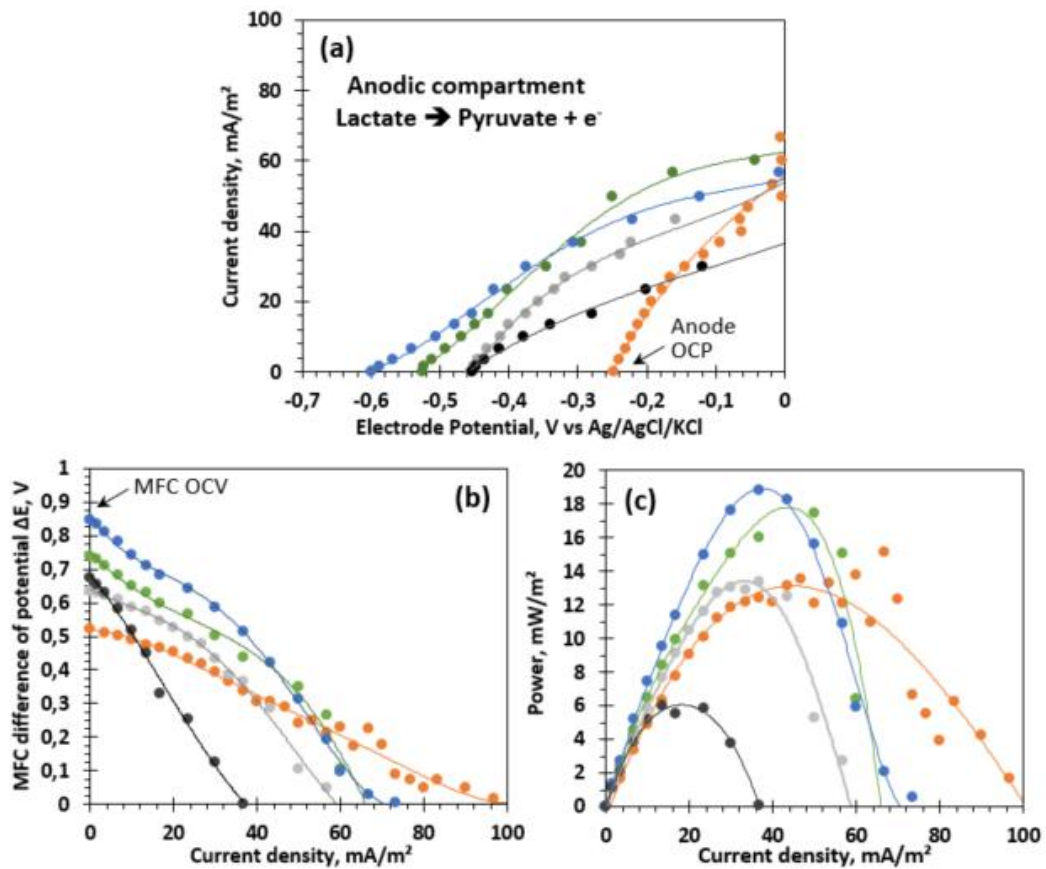
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FIGURE 6



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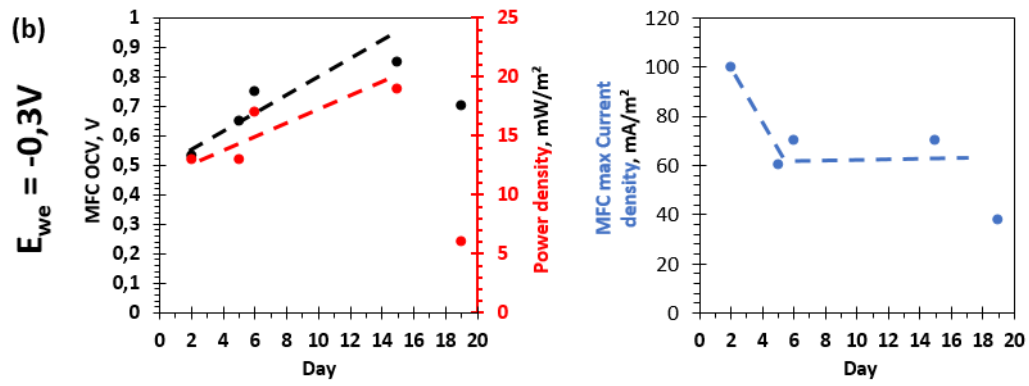
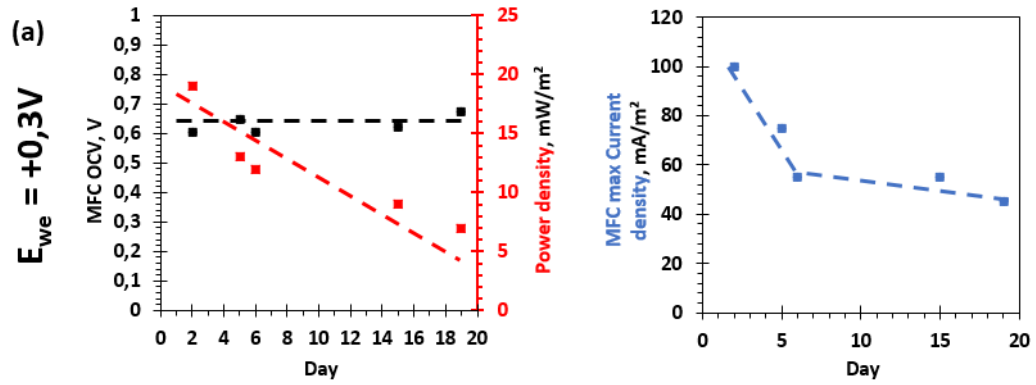
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FIGURE 7

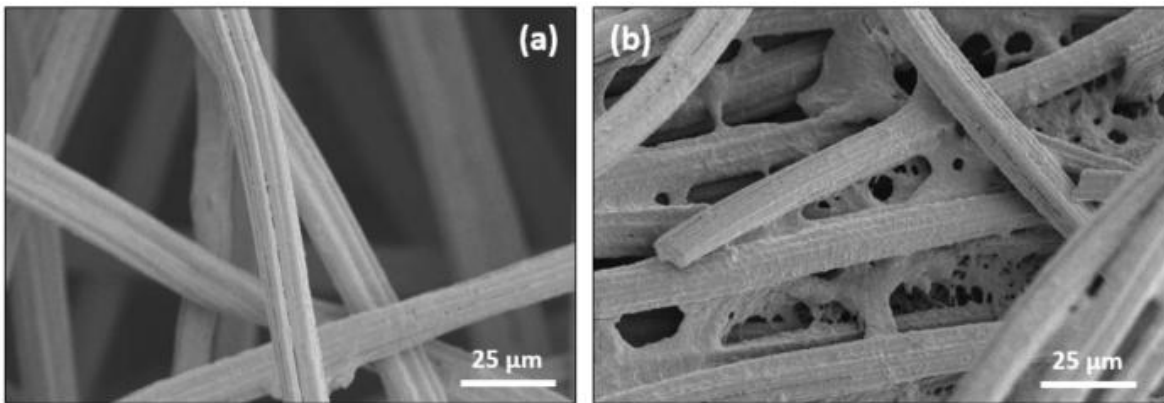


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FIGURE 8



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