Electrokinesis is a microbial behavior that requires extracellular electron transport


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We report a previously undescribed bacterial behavior termed electrokinesis. This behavior was initially observed as a dramatic increase in cell swimming speed during reduction of solid MnO₂ particles by the dissimilatory metal-reducing bacterium *Shewanella oneidensis* MR-1. The same behavioral response was observed when cells were exposed to small positive applied potentials at the working electrode of a microelectrochemical cell and could be tuned by adjusting the potential on the working electrode. Electrokinesis was found to be different from both chemotaxis and galvanotaxis but was absent in mutants defective in electron transport to solid metal oxides. Using in situ video microscopy and cell tracking algorithms, we have quantified the response for different strains of *Shewanella* and shown that the response correlates with current-generating capacity in microbial fuel cells. The electrokinetic response was only exhibited by a subpopulation of cells closest to the MnO₂ particles or electrodes. In contrast, the addition of 1 mM 9,10-anthraquinone-2,6-disulfonic acid, a soluble electron shuttle, led to increases in motility in the entire population. Electrokinesis is defined as a behavioral response that requires functional extracellular electron transport and that is observed as an increase in cell swimming speeds and lengthened paths of motion that occur in the proximity of a redox active mineral surface or the working electrode of an electrochemical cell.

*S. oneidensis* species are widespread in nature, enjoying a cosmopolitan distribution in marine, freshwater, sedimentary, and soil environments (1). They have attracted considerable attention in recent years because of their ability to reduce an extensive number of different electron acceptors, including the solid (oxy)hydroxides of iron and manganese, such as Fe(OH)₃ and MnO₂, using one or more proposed mechanisms of extracellular electron transport (EET) (2, 3). The EET ability of *Shewanella* species is consistent with their ability to generate electric current in microbial fuel cells (MFCs) in the absence of exogenous electron shuttles (4). Various strategies of extracellular electron transfer have been proposed in metal-reducing microbes, including naturally occurring (2) or biogenic (5–7) soluble mediators that “shuttle” electrons from cells to acceptors, as well as direct transfer using multiheme cytochromes located on the cell exterior (8) and transfer via conductive nanowires (9–11).

*S. oneidensis* MR-1 features several proteins that are involved with the transport of electrons to the exterior of the cell, where they play an important role with regard to the reduction of solid electron acceptors such as metal oxides. These include two outer-membrane decaheme c-type cytochromes (MtrC and OmcA), a membrane-spanning protein (MtrB), and two periplasmic multiheme c-type cytochromes (MtrA and CymA). Deletion of the genes encoding any of these proteins leads to phenotypes that are greatly inhibited with regard to metal-oxide reduction and current production in MFCs (12, 13). The mutation of genes that code for proteins involved in the placement of cytochromes to the outer membrane also results in the loss of metal-reducing phenotypes (13).

The *shewanellae* are highly motile, by virtue of a single polar flagellum, and individual *S. oneidensis* MR-1 cells have been tracked swimming at speeds of up to, and sometimes more than, 100 μm/sec, although the average swimming speed of cells in a population is considerably lower (14). Research has also shown that *S. oneidensis* MR-1 displays chemotactic responses to several soluble electron acceptors, including Fe(III) citrate (15, 16), and that the CheA-3 histidine protein kinase is required for this chemotactic behavior (14). In the absence of an electron acceptor MR-1 cells stop swimming; however, motility can be restored upon the readadding of an electron acceptor.

Here we present data that suggest that the *shewanellae* exhibit a unique motility response; we call it electrokinesis. This response, which involves increased swimming speeds and prolonged runs, was observed when cells were in close proximity to a redox active surface, such as an MnO₂ particle, or the working electrode of an electrochemical cell. Cells in the presence of MnO₂ particles were frequently observed to touch the mineral surface and sometimes pause for up to 1 sec, before swimming away. Video microscopy showed a concomitant reduction of the MnO₂ particles, suggesting that electrokinesis could be associated with the use of insoluble surfaces as electron acceptors. Electrokinesis is not a uniform response that can be observed in all cells, although if an electron shuttle is added the proportion of cells swimming and the average swimming speed of these cells rapidly increased.

**Results**

**Behavioral Responses of *S. oneidensis* MR-1 to Manganese and Iron Oxides.** Initial microscopic observations of *S. oneidensis* MR-1 cells mixed with MnO₂ particles showed a population of highly motile cells around the solid phase electron acceptor particles. In contrast, MR-1 cells sealed into capillary tubes in the absence of MnO₂ particles eventually ceased swimming and exhibited only Brownian motion. The motility response, which we call electrokinesis, was localized to the areas surrounding the metal oxide particles and was coincident with the reduction and subsequent dissolution of the particles (the highly motile cells are shown in Movie S1 and Movie S2; reduction of an MnO₂ particle, during a 24-h experiment, is shown in Fig. L4 and Movie S2). The swimming activity was quantified within the first 2 h of each experiment using video-microscopic observations coupled with cellular tracking algorithms that yielded cell positions, trajectories, and 2D maps of swimming speed (*Materials and Methods*). An analysis of the motility response in the presence of MnO₂ particles showed a dramatic increase in cell swimming velocity


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close to the particles (Fig. 1B, Top). Prolonged run lengths were also observed (Fig. 2B). Cells were also frequently observed to touch the MnO\(_2\) particles, sometimes pause for up to 1 sec, then swim away, suggesting that the motility response could be associated with the direct transfer of electrons to the mineral surface. When soluble electron acceptors (10 mM fumarate, 5 mM nitrate, or 10 mM thiosulfate) were added individually to anaerobic capillary tubes containing \textit{S. oneidensis} MR-1 cells and MnO\(_2\) particles, the fast-swimming motility response became uniform, rather than being localized around the MnO\(_2\) particles.

Video-microscopic analysis was used to observe behavioral responses of \textit{S. oneidensis} MR-1 cells in the presence of both MnO\(_2\) and iron (oxy)hydroxide \([\text{Fe(OH)}_x]\) particles. The data showed that kinetic responses to these anaerobic electron acceptors were distinct: in contrast to the MnO\(_2\) particles, which evoked a strong response (i.e., a large number of fast-swimming cells were observed around the particle), \text{Fe(OH)}_x\) particles elicited very subtle responses (i.e., few cells were observed swimming in the proximity of the particles, and these cells were, on average, swimming more slowly). Figure 2A and Movie S3 show the lack of swimming around \text{Fe(OH)}_x\) particles, whereas Movie S4 shows the slow reduction of an \text{Fe(OH)}_x\) particle. Because of this difference, our subsequent studies were performed with MnO\(_2\) particles. The response to MnO\(_2\) resulted in fast-swimming bacteria around the particle for the first 2 h of each experiment, followed by the eventual formation (over the next 24 h) of a biofilm. Cell tracking was not performed after the first 2 h of each experiment because at later times an accumulation of cells around the particles was observed (Movie S2). This accumulation was potentially caused by a chemotactic response to soluble Mn(II) gradients, generated during the reduction of the MnO\(_2\) particles, and was not considered to be part of the electrokinetic response.

One important characteristic of the electrokinetic response was that it involved only a fraction of the population of MR-1 cells. Although the exact proportions of the cell populations that were swimming were difficult to quantify because of the 3D distribution of the bacteria, they seemed to range from zero to \(\approx\)35% of the cells in view near the MnO\(_2\) particles. When cells were observed far from the MnO\(_2\) particles but still inside the anaerobic capillary, they were uniformly nonmotile (Movie S5 is a panoramic view of the bacterial response around an MnO\(_2\) particle). Nonmotile cells are still represented in our graphs as having nonzero velocity because our computer tracking algorithms recorded Brownian motion indiscriminately from swimming. However, because we could distinguish the region around the particles where bacterial swimming speeds were increased, from the region where cells showed Brownian motion alone, we noted “activity radii” around the particles, within which the behavioral change occurred (Table 1).

**Electrokinesis Requires Extracellular Electron Transport.** Our initial series of experiments suggested that electrokinesis could be associated with the use of MnO\(_2\) as an anaerobic electron acceptor because the motility response occurred coincidentally with MnO\(_2\) particle reduction, and because the cells were frequently observed to touch the mineral surface. Several \textit{S. oneidensis} MR-1 mutants, including \(\Delta\text{mtrA}\), \(\Delta\text{mtrB}\), and \(\Delta\text{cymA}\), are unable to reduce MnO\(_2\) during anaerobic respiration (13). These mutants were consequently screened in our assay for electrokinesis in response to the presence of MnO\(_2\) particles. None of the mutants showed an increase in swimming speed or a notable activity radius around the MnO\(_2\) particles (data shown in Table 1 and for the \(\Delta\text{mtrB}\) mutant in Fig. 1B, Bottom). This result suggested that electrokinesis is dependent on EET and, consequently, that other extracellular electron acceptors, such as the graphite electrodes of MFCs, could also elicit electrokinesis.

**Electrokinetic Responses to Graphite Electrodes.** Electrokinesis was observed in \textit{S. oneidensis} MR-1 cells exposed to the working electrode of a miniature electrochemical cell comprising two compartments separated by an ion-exchange membrane (Fig. S1).
These electrode-based experiments offer the advantage of observing cells without interference from soluble metal species generated as a consequence of mineral dissolution (an important distinction because the Mn(II) and Fe(II) gradients generated during the reduction of Mn(III/IV)- and Fe(III)-containing minerals, respectively, have been suggested to elicit chemotactic responses in S. oneidensis MR-1 (16)]. Observations of MR-1 motility behavior in close proximity to a graphite electrode maintained at a positive potential (+600 mV vs. graphite reference electrode) identified behavior similar to that observed during MnO₂ particle reduction (Movie S6). MR-1 cells exhibited strong electrokinetics around the electrode with +600 mV applied potential (Fig. 2D, Fig. 3A, Top, and B, and Table 1), less response with +300 mV applied potential, and very limited response at negative or zero applied potentials (Fig. 2C, Fig. 3B, and Table 1). As with the MnO₂ particle reduction, fast swimming was confined to a small percentage of the cells in the vicinity of the electrode. Analysis of the ΔmtrB, ΔmtrA, and ΔcymA mutants again showed that electrokinetics in response to the working electrode potential was dependent on EET functionality (Table 1 and for the ΔmtrB mutant in Fig. 3A, Bottom, and B).

**Electrokinesis Occurs Independent of Electron Shuttles.** Our experiments with both MnO₂ particles and graphite electrodes showed that the electrokinetic response was always confined to a small percentage of the cell population. This restriction might not be expected if EET under these conditions was dependent on the presence of a soluble electron shuttle (which would make an electron acceptor available to all of the cells). The addition of 1 mM of an extracellular electron shuttle, 9,10-anthraquinone-2,6-disulfonic acid (AQDS), to a culture of MR-1 cells mixed with MnO₂ particles, induced the fast-swimming motility response in more than 90% of the cells (the average swimming speed of tracked cells increased from 9.1 ± 2.1 μm/sec to 23.7 ± 1.4 μm/sec), along with a more rapid reduction of the MnO₂ particles. An analysis of the ΔmtrA and ΔmtrB EET mutants showed that these mutants responded similarly to wild-type in the presence of both MnO₂ particles and AQDS (Table 1), even though a previous report indicated that a different mtrB mutant (DKN248) could not reduce AQDS (17). The reason for the differences in the mtrB mutants is unclear.

**Electrokinesis Is Distinct from Chemotaxis and Galvanotaxis.** The increase in cell swimming speed and the associated prolonged runs observed during electrokinetics suggested that this behavioral response was different from chemotaxis (which involves changes in the reversal frequency of cell swimming). To determine whether chemotaxis did play a role in the motility response to both the MnO₂ particles and the graphite electrode of our electrochemical cell, we tested the behavior of a S. oneidensis ΔcheA-3 mutant. This mutant lacks a CheA histidine protein kinase that is essential for chemotactic signal transduction in S. oneidensis MR-1 (14). With changing applied potential the ΔcheA-3 mutant responded similarly to MR-1. However, in the presence of MnO₂ particles the ΔcheA-3 mutant cells were mostly nonmotile. The lack of a localized swimming population around the MnO₂ particles was potentially due to the smooth-swimming (nonstopping)

**Table 1. Comparison of electrokinetic response to MFC current production in Shewanella strains and mutants**

<table>
<thead>
<tr>
<th>Shewanella strains/mutants</th>
<th>Electron acceptors (activity radii, μm)</th>
<th>Electrode (activity radii, μm)</th>
<th>Current production (relative to MR-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Electron acceptors (activity radii, μm)</td>
<td>Electrode (activity radii, μm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>MnO₂</td>
<td>Fe(OH)₃</td>
</tr>
<tr>
<td>S. oneidensis MR-1</td>
<td>nr</td>
<td>&lt;200</td>
<td>nr</td>
</tr>
<tr>
<td>MR-1 ΔcheA3</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>MR-1 ΔcymA</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>MR-1 ΔmtrA</td>
<td>nr</td>
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<tr>
<td>MR-1 ΔmtrB</td>
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<td>nr</td>
<td>nr</td>
</tr>
<tr>
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<td>nr</td>
<td>&lt;450</td>
<td>nr</td>
</tr>
<tr>
<td>SB2B</td>
<td>nr</td>
<td>&lt;250</td>
<td>nr</td>
</tr>
<tr>
<td>S. putrefaciens</td>
<td>nr</td>
<td>&lt;250</td>
<td>nr</td>
</tr>
<tr>
<td>CN32</td>
<td>nr</td>
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</tbody>
</table>

nr, no or negligible response; nd, not determined; ++, current production of wild-type MR-1.
phenotype of the mutant. However, the results of the electrode experiments indicate that the CheA-3 chemotactic signal transduction pathway is not required for electrokinesis.

Previous studies have demonstrated that cells respond to strong electric fields according to their surface charge, in a direction aligned with the electric field. These studies have shown that microorganisms can respond to electric fields by swimming in the direction of the electric field, a behavior known as galvanotaxis. In our study, we aimed to investigate the response of Shewanella species to electric fields, with a focus on electrokinesis.

Electrokinesis in Other Shewanella Species. Electrokinetic responses were studied for two other Shewanella strains using both the MnO$_2$ particle and electrochemical cell electrode assays. S. amazonensis and S. putrefaciens CN32, which can generate comparable current to MR-1 in an MFC (Table 1), was found to exhibit strong electrokinesis around the MnO$_2$ particles, with a wider activity radius than MR-1 (Fig. 1B and Table 1). Around the electrode, the activity radius of SB2B was similar to that of MR-1 (Fig. 3A and Table 1), as was the average swimming velocity (Fig. 3B). Conversely, S. putrefaciens CN32, which generates less current than MR-1 in a MFC (Table 1), exhibited a spatially more confined motility response than MR-1 around both the MnO$_2$ particle (Fig. 1B and Table 1) and the electrode (Fig. 3A and Table 1). The average swimming speed of CN32 cells in response to +600 and +300 mV applied potentials was also lower than that of MR-1 and SB2B cells (Fig. 3B).

Kinetic Relaxation Time. With the electrode it was possible to perform experiments that could not be done with metal oxides, namely to vary the surface potential in an unobtrusive way and watch the response of the cells. Each evaluated wild-type strain showed a decrease in swimming speed when the applied potential was varied from +600 to –600 mV, although CN32 swimming speeds varied less than those of MR-1 and SB2B cells (Fig. 3B). In all cases the reduced speed was observed to occur within minutes after the potential was lowered. We refer to the period that cells continued to swim after the potential was decreased as the relaxation time. Strains SB2B and MR-1 were the most persistent swimmers after potentials were dropped (Fig. S2) i.e., they had the longest relaxation times. When the potentials were varied in the positive direction, up to +600 mV, swimming speeds increased to previously observed levels.

Discussion

Electrokinesis: A Unique Behavioral Response? The behavioral response that we have termed electrokinesis was first observed as an excited motility response around MnO$_2$ particles (which have a positive redox potential in the range of +200–400 mV) under conditions in which the mineral was the only available electron acceptor. The response was also coincident with reduction of the electron acceptor. The more limited response to Fe(OH)$_3$ particles was also lower than that of MR-1 and SB2B cells (Fig. 3B).
our experiments have not ruled out the possibility that the response could be based on the rate of flow of electrons to the minerals (because of their different surface characteristics and crystalline or amorphous structures) and thus have a kinetic basis.

Our experiments do show that electrokinetics is closely connected to the flow of electrons via ET because neither the ∆cymA, ∆mtrA, nor the ∆mtrB mutants showed electrokinetics in response to either MnO₂ particles or positive applied potentials in our electrochemical cell. Even more interesting was the observation that the motility response observed for the different Shewanella strains correlated not only with the redox potential of the electron acceptors used in the different experiments, but also with the current-generating ability of the strains, again suggesting that this unique response could have a kinetic basis. We do note that the electrokinetic response is similar to the chemokinetic response observed by Zhulin et al. (22), during studies on behavioral responses to oxygen in Rhizobium meliloti. These authors showed that R. meliloti responds to oxygen by increasing the swimming speed of cells and correlated this response with changes in proton motive force. Our preliminary studies with S. oneidensis MR-1 do not indicate a simple relationship between cell swimming speed and the redox potential of an electron acceptor. Swimming speeds of cells under anoxic conditions in the presence of nitrate [during NO²⁻ reduction to NO²⁻ (E° = -430 mV)] and thiosulfate [during thiosulfate disproportionation (E° = -400 mV)] were very similar (29.2 ± 11.2 µm/sec and 27.6 ± 18.4 µm/sec, respectively), whereas the swimming speed of cells in the presence of fumarate [during fumarate reduction to succinate (E° = +30 mV)] was higher (38.4 ± 6.7 µm/sec). In future experiments we will compare the reduction potential of different electron acceptors, the rates of electron acceptor reduction, and swimming speeds. These data will allow us to more clearly demonstrate whether S. oneidensis MR-1 shows chemokinetic responses to anaerobic electron acceptors and thus whether electrokinetics is a form of chemokinetics.

An important distinction between the behavioral responses to the soluble and insoluble electron acceptors used in this study is that whereas the soluble electron acceptors elicit a general response, the insoluble electron acceptors elicit only a localized response. These differences suggest that sensing, perception, and response may not be involved in electrokinetics. In addition, the ∆cheA-3 mutant, which is unable to sense soluble anaerobic electron acceptors or electrokinetics of the applied potentials. However, our studies using the ∆cheA-3 mutant did generate some contradictory data because the mutant cells did not localize around the MnO₂ particles. However, this lack of localization could have been due to the smooth-swimming phenotype of the mutant (i.e., a cheA-3 mutant cell would continually swim away from any touched particle). Galvanotaxis is a previously reported phenomenon (18–20) in which cells move toward strong electric fields (4 V/cm) with defined trajectories. The response is based on the surface charge of the cells. In contrast, our study used applied potentials to mimic the electron accepting conditions that cells may encounter in the environment. Under these conditions, the swimming velocity of MR-1 was enhanced in all directions of motion. Additionally, the motility response of MR-1 was dependent on the applied potential of the electrochemical cell. An applied potential of 0 mV (vs. graphite reference electrode) induced little cell response and short paths of motion, whereas an applied potential of +600 mV, equivalent to 0.09 V/cm (vs. graphite reference electrode), induced high-velocity swimming and extended paths of motion (Fig. 2 C and D, Fig. 3 B, and Movie S6). Higher applied potentials of +1.2 V (or more) interrupted all swimming: the cells followed a slow uniform migration more similar to the “defined trajectories” described by galvanotaxis. These results indicate that electrokinetics is distinct from galvanotaxis because the electric fields produced in our electrochemical system are very low, and there was no observed bacterial “front” that was attracted to the electrode surface.

The possibility that an extracellular electron shuttle plays a role in electrokinetics has not been ruled out. However, the effects of 1 mM AQDS (E° = -184 mV) on MR-1 (in increasing the number of cells swimming and in lengthening the relaxation time) suggest that, if the cells in our experiments were producing a shuttle, the concentration of this shuttle would be low. Many of the motile cells in both the MnO₂ and electrode experiments were, however, observed to transiently touch the surface of the electron acceptor but not to attach to the mineral or graphite surfaces (Movie S1 and Movie S6). Cells displaying this behavior could hypothetically be storing electrons on some biologic “capacitor,” as suggested by Esteve-Núñez et al. (23), then rapidly discharging these electrons to the electron acceptor during these brief interaction events, and thus energizing the cell. If there is a kind of biologic capacitor, this might account for the different relaxation times observed for the different Shewanella strains. To this end, electrokinetics may require a combination of specialized electron storage/donating mechanisms by cells and efficient electron acceptor capacity by minerals. Our future research will explore whether electrokinetics has a thermodynamic or kinetic basis and will perform additional studies to determine whether the cells are indeed acting as biologic “capacitors.”

Materials and Methods

Culture and Strains. S. oneidensis MR-1, S. amazonensis SB2B, S. putrefaciens CN32, and several mutants, originating from S. oneidensis MR-1, were tested in this study. Construction of the ∆mtrB (SO_1776) mutant was described previously (24, 14). The mutant is defective in metal oxide [Fe(III) oxide and MnO₂] reduction (12, 25) and current production in an MFC (13). To study the effects of interrupting electron flow from the cytoplasm to several terminal electron acceptors during anaerobic respiration, a cytochrome (cymA) mutant (ORF SO_1691) that lacks a cytoplasmic membrane-bound, tetraheme c-type cytochrome, was tested (26–28). Additional screening of an mtrA (ORF SO_1777) mutant, deficient in periplasmic decaheme c-type cytochromes involved in Mn(V) and Fe(III) reduction, allowed insight into the effects of cells deficient in terminal reductases (29–31). A mutant with a deletion in the central chemotaxis signal translocation pathway gene cheA-3 (ORF SO_3207) was selected for its defective chemotaxis response (14).

All strains and mutants were grown aerobically on Shewanella Federation minimal medium with lactate (20 mM) for 48 h at 30°C (15). Five-milliliter aliquots of cells were harvested at optical density of 0.2–0.3 at 600 nm, then added to a buffered (50 mM Pipes) mixture of electron donor and acceptor. For any given chemical experiment the electron acceptor was either 10 mM fumarate, 10 mM thiosulfate, 5 mM nitrate, 1 mM AQDS, or 0.3 mM MnO₂. Subsamples containing cells and a mix of electron donor/acceptors were taken for the experiments and inoculated into anaerobic capillary tubes for in situ reduction experiments.

In Situ Metal Oxide Particle Reduction Experiments. Experiments with metal oxide reduction and other chemical electron acceptors were conducted in sealed, anaerobic, transparent capillary tubes (0.02 mm × 0.05 mm × 50 mm). The reduction process was observed under phase contrast (100×) using a Carl Zeiss Optical Microscope equipped with a Nikon digital camera, which captures video for desired intervals (usually 1–60 min).

In Situ Electrochemical Cell Experiments. Electrochemical experiments were conducted using a microelectrochemical device constructed from graphite fibers, transparent capillary tubes, and Nafion ion-exchange membranes (Fig. S1). Based on an MFC model (32), this system incorporates a graphite electrode into a thin glass, anaerobic capillary, which allows 100x light microscopy observation of cells during various applied potentials. The electrochemical cell was sterilized with 70% ethanol before the inoculation of bacterial cultures and then sealed with silicon vacuum grease during the experiments. The microbial suspension (without electron acceptor) was added directly to the apparatus within 1 h 15 min of being harvested from the aerobic culture to achieve equivalent dissolved oxygen content in solution. After device inoculation the system was allowed to settle for 25–30 min, and video data were then recorded. A potentiostat (Gamry Reference 600) was used to apply potentials to the graphite fiber working electrode internal to the electrochemical cell. Potentials were applied sequentially to the graphite fiber reference electrode in the cathode compartment. The cathode electrode was the counter electrode. Potentials were applied sequentially (vs. a graphite reference electrode) for the following time intervals after bacteria were
sealed into the system and allowed to establish equilibrium: (i) ≤600 mV applied for 30 min; (ii) ≤200 mV applied for 14.5 min; (iii) ≤150 mV applied for 10 min; (iv) 0 mV applied for 10 min; (v) ≤10 mV applied for 1 min; (vi) 30 mV applied for 1 min; (vii) ≤60 mV applied for 1 min; (viii) ≤150 mV applied for 10 min; (ix) ≤300 mV applied for 10 min; (x) ≤600 mV applied for 10 min; (xi) ≤600 mV applied for 30 min; (xii) 0 mV applied for 10 min; (xiii) ≤150 mV applied for 10 min; and (xiv) ≤300 mV applied for 10 min.

Video data were collected during all cycles, with the field of view focused on the edge of the working electrode. Video data were captured during several key phases with each of the applied potentials shown above. The first video recording captured the open circuit phase, which occurred before the initial application of any potential. Then after applied potential, video was continuously recorded for the next 1 min (for all applied potentials x–xii), then recorded at 5 min, 10 min (for all potential intervals excluding y–xii), and 30 min (for potential intervals i / x). Only the results from the video data collected during the potential intervals/i, ii, iv, x, and xi (from list) have been included in this study.

Tracking Cell Movements. Cells were monitored near metal oxides and working electrodes in electrochemical cells, using 100x light microscopy (33). The locations of individual cells and the subsequent linking of these locations to form trajectories are based on the particle tracking algorithms of Crocker et al. (34). Briefly, individual frames were captured digitally at ≥29 frames per second and passed through a particle detection filter to generate high-contrast images of bacteria. Each image was reduced to a matrix of numeric pixel values. A peak finding algorithm located all of the intensity peaks (bacteria) that were above a given set threshold. The peak locations were linked to form trajectories by seeking the most probable set of inter-frame associations. Motility indicators, such as the average cellular velocity, were linked to form trajectories by seeking the most probable set of inter-frame associations. Motility indicators, such as the average cellular velocity, were subsequently computed from the complete trajectories. We used a MATLAB (The MathWorks) implementation of the tracking algorithms (http://physics.georgetown.edu/matlab/). The computed trajectories were then checked manually by visual inspection and compared with video tracked by hand, by frame. The algorithm parameters (expected cell size, minimum spot intensity, and maximum distance traveled between frames) were adjusted to obtain tracking with an acceptable level of accuracy. For this study, the accessed with a threshold-based post filter to generate high-intensity combinations of motility, shown in Fig. 1

From our analysis of Shewanella motility, it is worth noting that individual cells are capable of swimming significantly faster than the average velocity, because the average velocity takes into account all bacteria in each frame, including many slow-moving or stationary cells. For example, in Fig. S3A and B feature velocity vs. time traces for all of the cells tracked during a 10 sec interval, at 0 mV and ≥600 mV working electrode bias, respectively. Although an increase in high-velocity events at >600 mV is clearly evident, there are many slow-moving ones (below 10 μm/s) under both bias conditions. The term activity radius was evoked to quantify the extent of the bacterial “swarm” surrounding an electron acceptor during the initial 0.5–1.5 h after exposure, which was estimated using the following procedure. First the parameters of motility shown in Fig. 1B and Fig. S4A were assembled from the output of multiple consecutive video data samples. From each experiment, the overall swimming activity within the video frame, equivalent to 48 μm x 64 μm field of view, was most accurately characterized by the sum of all video data collected during the potential intervals immediately adjacent to the solid acceptor, then by adjusting the microscopic stage in the positive x direction by >6 μm, the next field of view just to the right of the previous video frame was captured and analyzed. This step was repeated until all possible elevated activity was evaluated. From analyses of these videos and video data outputs the activity radius was said to be the distance (in micrometers) from the solid acceptor to the right edge of the frame where the swimming activity fell below ≤25% of the overall swimming activity level detected in the first video data in the series. Alternatively, the activity radius was defined as the distance from the solid acceptor to the right edge of the video frame where the motility level decreased below the level detected in a nonstimulated, nonswimming sample captured within the same experiment and time constraints.

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