Soluble Electron Shuttles Can Mediate Energy Taxis toward Insoluble Electron Acceptors

Rui Li, §, #, James M. Tiedje, ¶, #, Chichia Chiu, †, and R. Mark Worden*, §, #

§Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, Michigan 48824, United States
¶Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, United States
†Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824, United States

Supporting Information

ABSTRACT: Shewanella species grow in widely disparate environments and play key roles in elemental cycling, especially in environments with varied redox conditions. To obtain a system-level understanding of Shewanella’s robustness and versatility, the complex interplay of cellular growth, metabolism, and transport under conditions of limiting carbon sources, energy sources, and electron acceptors must be elucidated. In this paper, population-level taxis of Shewanella oneidensis MR-1 cells in the presence of a rate-limiting, insoluble electron acceptor was investigated. A novel mechanism, mediated energy taxis, is proposed by which Shewanella use riboflavin as both an electron shuttle and an attractant to direct cell movement toward local sources of insoluble electron acceptors. The cells secrete reduced riboflavin, which diffuses to a nearby particle containing an insoluble electron acceptor and is oxidized. The oxidized riboflavin then diffuses away from the particle, establishing a spatial gradient that draws cells toward the particle. Experimental and modeling results are presented to support this mechanism. S. oneidensis MR-1 cells inoculated into a uniform dispersion of MnO2 particles in dilute agar exhibited taxis outward, creating a clear zone within which riboflavin was detected by mass spectrometry. Cells inoculated into dilute agar containing oxidized riboflavin similarly exhibited taxis, rapidly forming an expanding zone of reduced riboflavin. A mathematical model based on the proposed mechanism was able to predict experimental trends, including how concentrations of riboflavin and insoluble electron acceptors (e.g., MnO2) affected tactic cell migration.

INTRODUCTION

Microbial reduction of insoluble electron acceptors is important in microbial physiology, biogeochemical cycles of metals,1 and microbial fuel cells.2 Shewanella species reduce a wide range of terminal electron acceptors, including iron oxides (Fe(OH)3, Fe2O3),3 and manganese dioxide (MnO2).4 Insoluble electron acceptors or their reduction products also play a role in bioremediation of uranium.5,6 The Mtr respiratory pathway has been proposed for metal reduction by Shewanella,7 in which the terminal reductase MtrC either directly reduces an insoluble terminal acceptor8 or reduces a self-secreted electron shuttle molecule9 that transfers the electrons to the terminal acceptors.10–12 Biogenic, nonreductive chelation of metal oxides into soluble organic-metal complexes may also help cells transfer electrons through the cell membrane to insoluble electron acceptors.13,14 When the availability of soluble electron acceptors becomes rate-limiting, many bacteria migrate tactically up a concentration gradient into a region having a higher electron-acceptor concentration. Bacterial taxis is a biased random walk consisting of straight runs interspersed with tumbles. During runs, cells continuously monitor either an extracellular chemoattractant concentration or an intracellular indicator of energy production. When an increase is detected, the run is extended,15 resulting in chemotaxis or energy taxis, respectively.

Shewanella cells exhibit the unusual ability to migrate tactically through dilute suspensions of insoluble electron acceptors in the absence of soluble electron acceptors. Under these conditions, there would presumably be no continuous electron-acceptor gradient on the length scale of a cell’s run, suggesting that a tactic mechanism other than conventional chemotaxis or energy taxis may be involved. Childers et al. suggested that Geobacter, another metal-reducing bacterium, might use chemotaxis toward soluble reduced metal species, such as Mn(II) and Fe(II).16 Bencharit and Ward found that S.
Shewanella MR-1 cells exhibited chemotaxis toward Mn(II) and Fe(II) and might use gradients of these soluble reduction products to locate the insoluble electron acceptors Mn(III/IV) and Fe(III) oxides for dissipatory purposes. They also suggested that the humic acid analog anthraquinone-2,6-disulfonate (AQDS) was another attractant of S. oneidensis MR-1. Because AQDS is an exogenous electron shuttle for Shewanella fuel cells, we hypothesize that Shewanella uses the oxidized form of a self-secreted electron shuttle as a tactic attractant to migrate toward nearby insoluble electron acceptors. Flavins, including FMN and its hydrolysis product riboflavin, have been shown to be secreted by various Shewanella species, even in the presence of oxygen, suggesting that Shewanella might use one or more flavin(s) as an electron-shuttling attractant(s).

This paper integrates experimental and modeling studies to establish a novel mechanism (mediated energy taxis) by which Shewanella simultaneously enhances respiration and achieves taxis toward insoluble electron acceptors. In this mechanism, Shewanella secretes a reduced electron shuttle (e.g., riboflavin9) that is oxidized by nearby insoluble electron acceptors. The resulting oxidized shuttle then serves as an attractant to direct energy taxis of Shewanella cells toward the insoluble electron acceptors. Swarm plate experiments were used to demonstrate that S. oneidensis MR-1 is tactic toward oxidized riboflavin and exhibits tactic behavior in the presence of dilute suspensions of insoluble electron acceptors (e.g., MnO2 particles). The migration velocity and cell density of the tactic bands were shown to vary with concentrations of both riboflavin and MnO2 particles. A mathematical model based on the new mechanism was developed and used to reproduce experimentally observed trends in S. oneidensis MR-1’s growth, riboflavin-mediated taxis, and MnO2 reduction.

**MATERIALS AND METHODS**

**Strains and Growth Conditions.** Wild-type S. oneidensis MR-1, its in-frame cheA-3 deletion mutant,20 its SO2240 SO3282 double-deletion mutant (ΔSO2240ΔSO3282),21 S. putrefaciens CN-32, and S. sp. W3-18-1 were tested in this study. The ΔSO2240ΔSO3282 mutant does not express a major and a minor methyl-accepting chemotaxis protein (MCP) involved in energy taxis in S. oneidensis21 and has partially abolished energy taxis capability. Cells were grown either aerobically on LB medium at 30 °C or anaerobically on M1 basal medium at room temperature containing sodium lactate and electron acceptors at concentrations indicated below.

**Taxis Assays.** Swarm plate assays were conducted anaerobically to test S. oneidensis MR-1’s tactic responses. Twenty mL of soft (0.25%) M1 agar containing 40 mM of the nontoxic carbon source, sodium lactate, and an electron acceptor (riboflavin and/or insoluble electron acceptors) at various concentrations was poured into each Petri dish. In all cases, the carbon source was present in excess, and the electron acceptor was the limiting nutrient. Freshly prepared amorphous δMnO2 or amorphous Fe(OH)3 particles were ground into powder by mortar and pestle. Crystalline MnO2 and crystalline Fe2O3 were purchased from Sigma-Aldrich. These metal oxides dispersed readily and remained suspended in the agar. Cells for inoculation were grown aerobically in 5 mL of LB medium at 30 °C overnight to an OD600nm of 1.1, washed by centrifugation, and resuspended in 100 μL of 30 mM HEPES buffer. Riboflavin was added to the cell suspension to a final concentration 200 nM unless otherwise indicated, and then a sterile pipet tip was used to inject 8 μL of the cell suspension into the swarm agar. Adding a trace amount of riboflavin into the cell suspension prior to inoculation was found to increase repeatability of the experiments. However, variation of riboflavin concentrations in the inoculum from 100 nM to 1000 nM did not lead to obvious differences in results for both experiments and mathematical simulations. Inoculated plates were incubated in an anaerobic chamber containing 4% H2 (the balance in N2) at room temperature. A digital camera was used to record the tactic migration patterns, while the swarm plate was mounted on a transilluminator box.

**RESULTS AND DISCUSSION**

**Conceptual Model.** The conceptual model underlying mediated energy taxis (Table of Contents Art) is based on the following premises: 1: Shewanella cells eliminate electrons produced during respiration by secreting reduced riboflavin, which diffuses through the medium, shuttling electrons from the cells to nearby insoluble electron acceptors; 2: the insoluble electron acceptors oxidize the reduced riboflavin in a reaction that consumes the insoluble electron acceptors and generates oxidized riboflavin; 3: the oxidized riboflavin serves as an attractant for Shewanella energy taxis; 4: the insoluble electron acceptors do not diffuse but are small enough and well enough dispersed that a continuum model based on an insoluble electron acceptors “concentration” is sufficient to predict population-level cell behavior.

A possible variant of the conceptual model was also considered: that the species driving taxis is a soluble product of the redox reaction. However, in this scenario, respiratory activity by a tactic band moving in the positive x direction would generate a positive gradient of oxidized substrate and a negative gradient of reduced product. If the reduced product were driving taxis in the direction opposite to its gradient, the product would have to be a chemorepellent. This situation would be inconsistent with the observations that the reduction products of MnO2 reduction, Mn(II) and Mn(III), are chemoattractants for Shewanella. However, inorganic Mn(II) is unstable without chelates due to fast disproportionation and can be reduced by HEPES buffer used in this study. Hence, Mn(III) was considered unlikely to play a prominent role under our experimental conditions.

**Taxis Toward Riboflavin and MnO2.** Because oxidized riboflavin is yellow and reduced riboflavin is colorless, tactic migration of S. oneidensis MR-1 populations created a sharp, circular yellow-to-clear swarm boundary that separated the outer (yellow) region containing oxidized riboflavin from the inner (clear) reduction zone (Figure 1 A). Riboflavin’s demarcation of the swarm boundary was fortuitous, because the S. oneidensis MR-1 cell density in the tactic cell bands was typically too low to be visible. A tactic response was not observed for the nontactic cheA-3 mutant (Figure 1 A),
indicating that cheA-3 is essential for *S. oneidensis* MR-1 taxis under the conditions tested. The ΔSO2240ΔSO3282 double mutant exhibited a swarm boundary that migrated more slowly than that for the wild-type strain (Figure 1 A), consistent with mutant exhibited a swarm boundary that migrated more slowly containing the riboflavin was added to either the inoculum or the agar, whereas dark areas around the inoculation point indicate reduced electron acceptors. Picture C was taken 36 h after inoculation. Brown areas indicate unreduced MnO₂, whereas dark areas around the inoculation point indicate reduced MnO₂.

In swarm plate experiments conducted without riboflavin but with amorphous MnO₂ (ΔMnO₂) particles uniformly dispersed throughout the agar, an expanding visible swarm boundary was again observed (Figure 1 C), similar to that seen with soluble electron acceptors. As before, the cheA-3 mutant did not show a tactic response, and the ΔSO2240ΔSO3282 double-deletion mutant (Figure 1 C) showed slower tactic migration than the wild-type. Over time, a few black particles appeared in the gel (Figure 1 C) which were not reduced by the tactic cell band. These particles were presumably larger MnO₂ particles that grew via Ostwald ripening. We did not attempt to develop a mutant unable to secrete flavins, because flavins serve as essential enzyme cofactors.

Experiments were conducted to determine whether secreted riboflavin may have been involved in mediating ΔMnO₂ particle reduction and cellular taxis in swarm plates in which no riboflavin was added to either the inoculum or the agar containing the ΔMnO₂ particles. Samples were taken before and after inoculation, both inside and outside the swarm boundary. LC/MS/MS analysis detected no riboflavin for samples taken before inoculation and outside the migration band. However, riboflavin was detected in samples taken inside the tactic band at an average concentration of about 100 nM, consistent with previous reports. The MS signal attributed to riboflavin had a mass-to-charge ratio (m/z) of 377.2, and secondary MS analysis of the 377.3 peak yielded an ion with a m/z ratio of 243.1, identical to values reported for riboflavin.

Supplementing the ΔMnO₂-containing agar with low concentrations of riboflavin prior to inoculation increased the swarm boundary’s average migration rate. During 72 h experiments containing 10 mM ΔMnO₂ particles dispersed in the agar, the migration rate increased from 0.008 cm/h (R² = 0.96) without riboflavin to 0.067 cm/h with 0.002 mM riboflavin in the agar (R² = 1) (Figure 2 A and B). The increase in migration rate with increasing riboflavin concentration suggests that the riboflavin concentration secreted by *Shewanella* cells (~100 nM) is enough to mediate reduction of insoluble electron acceptors and energy taxis but not enough to saturate the cells’ respiratory capacity. Riboflavin supplementation also resulted in formation of a white, insoluble reduction product, possibly Mn(II) carbonate (Figure 2 B and F). In swarm plate experiments conducted using crystalline MnO₂ without added riboflavin, no swarm boundary was observed within 48 h (Figure 2 C). However, supplementing the crystalline MnO₂ with riboflavin did yield tactic behavior (Figure 2 D). In some cases, tactic migration of *Shewanella* cells through agar preloaded with riboflavin and MnO₂ resulted in concentric, stationary circles (Figure 2 E and F) that were stable after exposure to oxygen for 1 week or longer. The rings are presumably composed of insoluble Mn(II) compounds.

*S. putrefaciens* CN-32 and *S. sp.* W3-18-1 were also tested for their responses to riboflavin and MnO₂. These strains have the highest reported Coulombic efficiency among *Shewanella* strains in biofuel cells, indicating they effectively donate electrons to insoluble electron acceptors (i.e., electrodes). In swarm plates with riboflavin and MnO₂, these strains exhibited tactic behavior similar to *S. oneidensis* MR-1.

In swarm plates containing amorphous Fe(OH)₃ reduction zones were observed for the wild-type strain but not for the cheA-3 mutant. However, the double-deletion mutant gave results similar to the wild-type strain (Figure 3 A). As was observed for ΔMnO₂, supplementing Fe(OH)₃-containing agar with riboflavin prior to inoculation increased the swarm boundary’s migration rate. During 72 h experiments containing 10 mM Fe(OH)₃ particles dispersed in the agar, the migration rate increased from 0.013 cm/h without added riboflavin (R² = 0.97) to 0.067 cm/h with 0.002 mM riboflavin in the agar, R² = 1) (Figure 3 B and C), presumably due to a riboflavin-mediated increase in Fe(OH)₃ reduction rate of Fe(OH)₃ with increased riboflavin concentration.

To further confirm that the color change in the reduction zone is not due to some abiotic process, 2 μL samples were
taken from plates with 0.2 mM riboflavin, 0.2 mM FMN, 10 mM δMnO₂, and 10 mM amorphous Fe(OH)₃ by using capillary tubes, and the cell numbers were counted. Within the reduction zones, average cell densities were 663 ± 93 cells/μL on the riboflavin plates, 688 ± 83 cells/μL on the FMN plates, 712 ± 1124 cells/μL on δMnO₂ plates, and 3071 ± 1812 cells/μL on amorphous Fe(OH)₃ plates. No cells were found in samples taken outside the reduction zones.

Mathematical Model of Mediated Energy Taxis by \textit{S. oneidensis} MR-1. The mathematical formulation is based on one developed by Widman et al. to describe \textit{Escherichia coli} chemotaxis in a diffusion gradient chamber, and later modified by Li et al. to describe \textit{S. oneidensis} MR-1 taxis toward soluble electron acceptors. The model consists of coupled, unsteady-state mass balance equations for cells, oxidized riboflavin, reduced riboflavin, and the insoluble electron acceptor.

The cell mass balance is

\[
\frac{\partial u}{\partial t} = \mu \nabla^2 u - \chi_0 \nabla \cdot \left( \frac{K_D}{(K_D + S)^2} \nabla S \right) + \nu u \frac{S}{C_S + S} - k_{D} u
\]

(1)

where \( u \) is the cell density, \( t \) is time, \( \mu \) is the random motility coefficient, \( \chi_0 \) is the taxis coefficient, \( S \) is the concentration of oxidized riboflavin, \( K_D \) is the taxis saturation constant, \( \nu \) is the maximum specific growth rate, \( C_S \) is the half-saturation constant for consumption of the rate-limiting electron acceptor, and \( k_{D} \) is the death rate constant. The first term on the right-hand side of the equation accounts for random motility, and the second term describes consumption of oxidized riboflavin due to cell growth and death, respectively. The electron acceptor was the limiting nutrient under the experimental conditions, so the growth is assumed to be limited by the oxidized riboflavin concentration. The death rate is assumed to be proportional to the cell concentration.

The mass balance for extracellular oxidized riboflavin is

\[
\frac{\partial S}{\partial t} = D_S \nabla^2 S - \frac{u (\nu + k_m)}{Y} \frac{S}{C_S + S} + k_R Q M
\]

(2)

where \( D_S \) is the diffusion coefficient for oxidized riboflavin in the agar, \( Y \) is a yield coefficient (mass of cells produced/amount of oxidized riboflavin consumed), \( k_m \) is the maintenance energy rate constant, \( k_R \) is the rate coefficient for oxidation of reduced riboflavin by MnO₂, \( Q \) is the reduced riboflavin concentration, and \( M \) is the MnO₂ concentration. The second term describes consumption of oxidized riboflavin due to cell growth and maintenance. The last term accounts for generation of oxidized riboflavin as reduced riboflavin is oxidized by MnO₂. Since a detailed mechanism for the reaction between riboflavin and MnO₂ particles is not yet available, we assumed the reaction rate was proportional to the concentrations of both reduced riboflavin and MnO₂.

The mass balance for extracellular reduced riboflavin is

\[
\frac{\partial Q}{\partial t} = D_S \nabla^2 Q + \frac{u (\nu + k_m)}{Y} \frac{S}{C_S + S} + k_R Q M - k_R Q M
\]

(3)

The second term describes reduction of oxidized riboflavin by cellular respiration for growth and maintenance. In the third term, which accounts for riboflavin secretion, \( k_R \) is the riboflavin secretion rate constant. The diffusion coefficients for the oxidized and reduced forms of riboflavin are assumed to be identical. Based on the assumption that cells would not secrete additional riboflavin once the extracellular concentration reached a saturating level, \( k_R \) is set to zero if \( S + Q \) exceeded 4 × 10⁻³ mM.

The MnO₂ balance, which describes consumption via reaction with reduced riboflavin, is

\[
\frac{\partial M}{\partial t} = -k_R Q M
\]

(4)

Zero-flux boundary conditions are applied for all components on all boundaries (Ω):

\[
\left\{ \begin{array}{l}
\nabla \cdot \left[ \frac{K_D}{(K_D + S)^2} \nabla S \right] = 0 \\
\n\nabla S \cdot \mathbf{n} = 0 \\
\n\nabla Q \cdot \mathbf{n} = 0 \\
\n\nM \cdot \mathbf{n} = 0
\end{array} \right\}
\]

(5)

(6)

(7)

(8)

Because of the inherent complexity of this system, an exhaustive effort was not made to independently evaluate all constants. \( D_S \) was evaluated using a diffusion gradient chamber. Values for \( \mu \) and \( \chi_0 \) for \textit{S. oneidensis} MR-1 were obtained from the literature. \( K_D \) and \( C_S \) were estimated to be around 0.0001 mM, the reported concentration of \textit{Shewanella}-secreted riboflavin. A trial-and-error approach was used to determine values for the other parameters that gave reasonable agreement between the model and experimental results. A single set of parameter values, listed in Table 1, was used for all simulations. Initial conditions for \( Q \) and \( M \) were \( Q(x,y) = 0 \) and \( M(x,y) = M_0 \) for all \( x \) and \( y \). Initial conditions for \( u \) and \( S \) were \( u(x,y) = u_0 \) and \( S(x,y) = S_0 \) for \( x, y \) within the inoculation zone, and \( u(x,y) = 0 \) and \( S(x,y) = 0 \) for \( x, y \) outside the inoculation zone. The values of \( M_0 \), \( u_0 \), and \( S_0 \) were experimentally determined. An alternating direction implicit (ADI) algorithm described previously was used to numerically integrate the system of coupled, partial differential equations.

Experimental results and model predictions depicting energy taxis by \textit{S. oneidensis} MR-1 in response to oxidized riboflavin are compared for different times and initial riboflavin concentrations in Figure 4 (top) and Figure 5 (top). Two trends
Table 1. Constants Used in Simulations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_0$</td>
<td>$2.5 \times 10^{-4} \text{ cm}^2 \cdot \text{h}^{-1}$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>0.0005 cm$^2$ · h$^{-1}$</td>
</tr>
<tr>
<td>$x_0$</td>
<td>0.075 cm$^2$ · h$^{-1}$</td>
</tr>
<tr>
<td>$K_D$</td>
<td>$5 \times 10^{-7}$ mM</td>
</tr>
<tr>
<td>$\nu$</td>
<td>0.2 h$^{-1}$</td>
</tr>
<tr>
<td>$C_s$</td>
<td>$1 \times 10^{-3}$ mM</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.1 mg cell/mmol oxidized riboflavin</td>
</tr>
<tr>
<td>$k_d$</td>
<td>0.12 h$^{-1}$</td>
</tr>
<tr>
<td>$k_a$</td>
<td>0.12 h$^{-1}$</td>
</tr>
<tr>
<td>$k_s$</td>
<td>200 mM$^{-1}$ · h$^{-1}$</td>
</tr>
<tr>
<td>$k_t$</td>
<td>$2.5 \times 10^{-4}$ mM riboflavin · (mg cell)$^{-1}$ · h$^{-1}$ if $(S + Q) \leq 7.5 \times 10^{-4}$ mM</td>
</tr>
<tr>
<td></td>
<td>0 if $(S + Q) &gt; 7.5 \times 10^{-4}$ mM</td>
</tr>
</tbody>
</table>

Figure 4. Swarm plates results depicting *S. oneidensis* MR-1 taxis to 0.2 mM oxidized riboflavin (top two rows) and 5 mM MnO$_2$ (bottom two rows) at various times after inoculation. In both photographs of experiments (rows 1 and 3) and simulations (rows 2 and 4), brighter areas indicate unreduced electron acceptors, and a darker area indicates reduced electron acceptors. The time shown in the upper left-hand corner of experimental results corresponds to the time after inoculation.

Simulations were also performed using model parameters that eliminated chemotaxis, random movement, or flavin secretion to explore the influence of these processes on predicted cell growth and migration patterns. Eliminating chemotaxis or random movement significantly reduced the predicted migration rates (SI Figure S2 A, B, and C), consistent with experimental data. Eliminating flavin secretion significantly decreased both migration rate and MnO$_2$ reduction rate, even when riboflavin initial concentration at the inoculation point was set to 100 nM (SI Figure S2 D).

The mathematical model’s predictions showed reasonable quantitative agreement with the experimental results across the wide range of experimental conditions tested. Following validation, the model was used to predict variables that are difficult to measure in order to elucidate mechanisms underlying tactic band formation via mediated energy taxis. SI Figure S3 shows predicted concentration profiles across a *Shewanella* tactic band, with variables scaled so that all five profiles can be viewed on one plot. The shapes of these profiles are intuitively reasonable and illustrate several aspects of the assumed mechanism. The chemotactic band of cells migrates outward from the inoculation point into MnO$_2$-rich regions. Reduced riboflavin that is secreted by the cell band is rapidly oxidized by MnO$_2$ outside the cell band but accumulates inside the cell band, where the MnO$_2$ has been depleted, resulting in a band of reduced riboflavin that tracks the cell band. Reduction of MnO$_2$ in the vicinity of the cell band results in symmetrical patterns indicating Mn(II) production at the expense of MnO$_2$. The sharp gradient of oxidized riboflavin within the cell band drives energy taxis that sustains the steep cell band.

Collectively, the experimental and modeling results presented here establish how mediated energy taxis enables *Shewanella* to migrate tactically in environments containing insoluble electron acceptors as the sole electron acceptor, even when neither the insoluble electron acceptors nor its reduction product is a chemoattractant. The observation that the tactic wave solubilizes MnO$_2$ indicates a link between taxis and respiration. Riboflavin’s ability to cycle between a reduced form that shuttles electrons from the cells to MnO$_2$ particles and an oxidized form that serves as a tactic attractant enables it to establish that link.

Energy taxis differs from metabolism-independent chemotaxis, in that chemotaxis requires a sensor specific for the chemoattractant, whereas energy taxis uses a generic sensor for some energy-related property. For example, receptor SO2204, which plays a major role in *S. oneidensis* MR-1’s energy taxis toward electron acceptors, is believed to participate in detecting a transmembrane $\Delta$pH. This feature of energy taxis avoids a potential problem inherent in classical chemotaxis, in which chemicals having a structure similar to a metabolically important chemoattractant trigger futile chemotaxis. In addition, this feature allows a single sensor system to direct taxis toward multiple insoluble electron acceptors, consistent with Baraquet et al.’s observation that among 27...
MCP-encoding genes of *S. oneidensis* MR-1 only five influenced taxis.21 The novel mediated energy taxis mechanism presented here is supported by several observations in the literature. Harris et al. reported that *S. oneidensis* MR-1 cells exhibited an increased run length in the presence of insoluble electron acceptors.36 Such behavior is expected, because the insoluble electron acceptors would oxidize riboflavin secreted by the cells, generating oxidized riboflavin that would activate energy taxis and thereby increase run length. Harris et al. reported that ΔcheA-3 mutant was nearly nonchemotactic in the presence of MnO2,36 consistent with our findings. This lack of a tactic response suggests that the cheA protein’s activity is downstream of the energy sensor in the chemotaxis signaling pathway. Harris et al.36 reported an increase in *Shewanella* swimming speed in the presence of insoluble electron acceptors (positively poised electrodes and MnO2). They suggested the effect was independent of electron shuttles because the shuttles were present throughout the solution, while the response was only observed near the insoluble electron acceptors. Instead, they attributed the behavior to electrokinesis, an enhanced motility near insoluble electron acceptors in the absence of shuttle activity. However, shuttle activity cannot be ruled out, because, even though the reduced form of the electron shuttle may be present throughout the solution, the oxidized form is likely to be present only near the insoluble electron acceptors. This oxidized riboflavin would represent a previously unrecognized electron sink that could enhance energy production via respiration and explain the increase in swimming speed. Our results indicate that the riboflavin-mediated energy taxis can be significant even at very low riboflavin concentrations, making its presence and influence easy to overlook. The proposed mechanism is also reasonable from the standpoint of thermodynamics. Riboflavin has a redox potential of ~210 mV vs SHE.11 Substances having redox potentials more positive than this value would be suitable insoluble electron acceptors for riboflavin-mediated energy taxis. Moreover, the larger the potential difference between the insoluble electron acceptors and riboflavin, the greater would be (1) the driving force for electron transfer, (2) the oxidized fraction of riboflavin on the insoluble electron acceptors’ surface, (3) the driving force for diffusion of reduced riboflavin from the cells to the insoluble electron acceptors, and (4) the driving force for diffusion of oxidized riboflavin from the insoluble electron acceptors to the cells. These trends are consistent with Harris et al.’s observation that electrodes with higher potential and metal oxides with higher redox potential range triggered a stronger response.36

The first-generation mathematical model was designed to illustrate the mechanism of mediated energy taxis and to reproduce experimental trends with minimal complexity. The effects of riboflavin and FMN were lumped together, because FMN has the same electron carrying capacity as riboflavin,10,11 can hydrolyze to form riboflavin,19 and has been reported to be undetectable in *Shewanella* cultures under some conditions.28 However, additional balances could easily be added to describe multiple shuttles. The derivation of the chemotactic velocity expression (shown in the SI) assumes a constant swimming speed. While an increase in swimming speed has been reported as *Shewanella* cells approach insoluble electron acceptors,36 this effect has been reported to be insignificant in modeling energy taxis.37 As additional data that relate swimming speed to oxidized shuttle concentration and/or some intracellular measure of energy level become available, more detailed energy-taxis models could be developed. Influences of additional environmental factors on swimming speed could be also incorporated.38–40 These and other refinements would improve the model’s accuracy and facilitate its use in testing hypotheses about mediated energy taxis and designing bioremediation systems for toxic metals that undergo microbial reduction.

Finally, this study provides new insight into the relative competitive advantages of *Shewanella*’s mechanism to find and utilize insoluble electron acceptors (mediated energy taxis) vs *Geobacter*’s mechanism (chemotaxis toward soluble products of metal-reduction reactions). Previous discussion of this topic16 recognized that *Shewanella*’s secretion of shuttles allows it to discard excess electrons but overlooked what may be its
primary benefit: enabling taxis toward insoluble electron acceptors. One potential advantage of mediated energy taxis is that it would likely work for virtually any insoluble electron acceptor that can oxidize riboflavin, whereas chemotaxis would only work when the reduced form of the electron acceptor is both soluble and a chemotactant. A second advantage is that mediated energy taxis would require a smaller inventory of sensor proteins. Shewanella would only need a sensor for the oxidized form of its secreted shuttle(s), whereas Geobacter would typically require a different receptor for each reduced metal species. A third advantage is that Shewanella’s use of diffusional shuttles eliminates the need for direct contact between the cells and the insoluble electron acceptor and thus may be better suited than Geobacter’s mechanism when insoluble electron acceptors are embedded in microporous matrices. A fourth advantage is that secretion of compounds that serve as attractants can lead to microbial pattern formation and quorum sensing, which can provide additional competitive advantages, including mitigation of damage from hazardous materials. Collectively, these potential advantages of mediated energy taxis help justify the energetic cost of Shewanella’s electron-shuttle secretion in environments containing insoluble electron acceptors.

**ASSOCIATED CONTENT**

- Supporting Information
  Figures S1–S3 and text. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

Corresponding Author
*Phone: 517-353-9015. Fax: 517-432-1105. E-mail: worden@egr.msu.edu.

Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank Dr. Cécile Jourlin-Castelli at Institut de Microbiologie de la Méditerranée, Centre National de la Recherche Scientifique for the kind donation of *S. oneidensis* MR-1 SO2240 SO3282 double-deletion mutant. We thank Dr. Mandy Ward at University of Southern California for her kind donation of *S. oneidensis* MR-1 choA-3 mutant. We thank Dr. Daniel Jones and Ramin Vismeh at Michigan State University for their kind donation of *S. oneidensis* MR-3 mutant. We thank Dr. Mandy Ward at University of Southern California for her kind donation of *S. oneidensis* MR-1 choA-3 mutant. We thank Dr. Daniel Jones and Ramin Vismeh at Michigan State University for their kind donation of *S. oneidensis* MR-1 choA-3 mutant.

**REFERENCES**


(34) Alexandre, G. Coupling metabolism and chemotaxis-dependent behaviours by energy taxis receptors. *Microbiology* 2010, 156 (Pt 8), 2283−93.


