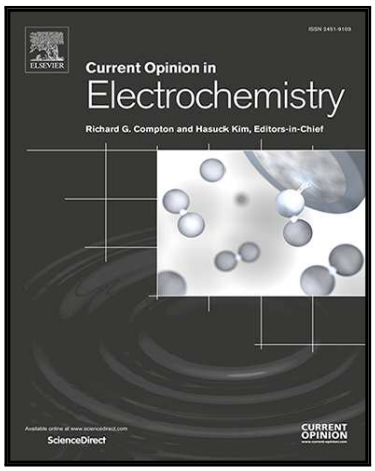


Shewanella oneidensis MR-1 electron acceptor taxis and the perception of electrodes poised at oxidative potentials

Joseph Oram , Lars J.C. Jeuken

PII: S2451-9103(17)30111-4
DOI: 10.1016/j.coelec.2017.07.013
Reference: COELEC 82

To appear in: *Current Opinion in Electrochemistry*

Received date: 3 July 2017
Revised date: 27 July 2017
Accepted date: 29 July 2017

Please cite this article as: Joseph Oram , Lars J.C. Jeuken , Shewanella oneidensis MR-1 electron acceptor taxis and the perception of electrodes poised at oxidative potentials, *Current Opinion in Electrochemistry* (2017), doi: 10.1016/j.coelec.2017.07.013

Highlights

- S. oneidensis senses insoluble electron acceptors, including electrodes.
- Chemotaxis and energy taxis are proposed to be responsible.
- Microbial electrochemical system design requires more insight in taxis.
- New electrochemical setups can increase insights into taxis.

Shewanella oneidensis MR-1 electron acceptor taxis and the perception of electrodes poised at oxidative potentials

*Joseph Oram and Lars J. C. Jeuken**

School of Biomedical Sciences and the Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom

*Corresponding Author: L.J.C.Jeuken@leeds.ac.uk

Abstract

Shewanella oneidensis MR-1 is a facultative anaerobe, capable respiring on an extraordinarily large and diverse array of both intra- and extracellular terminal electron acceptors, including insoluble metal oxides and electrodes. The ability to perform extracellular electron transfer has sparked great interest over the last three decades and MR-1 has become both a model organism for fundamental research into extracellular electron transfer and a candidate microbe for microbial electrochemical systems, including microbial fuel cells. A pre-requisite for colonisation and biofilm formation on electrodes is the migration of bacteria towards the electrode. Here, we review current understanding in the steps involved in MR-1 migration towards insoluble electron acceptors and electrodes. The main experimental techniques used to evaluate taxis are summarised and different mechanisms proposed for MR-1 taxis are contrasted, in particular chemotaxis versus energy taxis.

Introduction and Background

Since the discovery of Extracellular Electron Transfer (EET) capable microbes, such as *Shewanella oneidensis* MR-1 (MR-1) [1], there has been a concerted effort to uncover the underlying mechanisms of EET. Interest in EET has been amplified by potential applications of these bacteria in microbial fuel cells (MFC) and microbial electrosynthesis. MFC provide the vehicle by which electrical energy from electrogenic organisms can be harnessed. MFCs were already studied in the 1960s by NASA as a means to generate electric power from organic waste during long haul space journeys [2]. MFC have enjoyed considerable improvements since then, especially during the last 2 decades, yet are still severely limited in practical applications, particularly those concerning energy production. This is predominantly due to low power output along with high internal resistances and/or prohibitive material costs (required for cathodes of the more efficient MFCs) [2-4]. Typical MFCs for waste water treatment can, at best, produce power densities between 0.1-0.5 W/m^2 [3, 5]. Crucially, MFC power output tends to scale poorly with increasing reactor volumes. MFC power densities with reactor volumes 1 L or greater fall below the required threshold for feasibility in industrial applications such as electricity generation during wastewater remediation [6, 7].

Difficulties and limitations in improving the efficiency of MFCs have in part led to a diversification in the potential applications being explored, leading to an explosion in numbers of related devices collectively known

as microbial electrochemical systems (MES). Potential applications of MES range from bioremediation and waste water treatment to microbial electrosynthesis and biosensing, all of which fundamentally rely on the EET ability of certain microbes.

Both *Shewanella* (primarily MR-1) and *Geobacter* species (sp) are used extensively as model organisms for EET studies and MES in general [8-12]. The two main mechanisms proposed for microbial/bacterial EET are direct electron transfer (DET) and mediated electron transfer (MET). DET, through outer membrane cytochromes, is the predominant method of EET used by *Geobacter* sp within MES [12, 13]. In the case of MR-1, things are less clear. While there is a consensus in the literature that MR-1 is capable of both DET and MET [10, 14], the relative contributions/importance of both to the overall rate of EET in MES is still debated[10, 14, 15].

In contrast to *Geobacter* sp, which forms thick (20-45 μm) and stable electroactive biofilms [16-18], MR-1 forms thinner (1-16 μm) and relatively loosely adherent biofilms and typically populates electrodes only partially [18-21]. *Geobacter* sp also generally perform better regarding maximum current density of MFCs. Higher current densities can be related back to *Geobacter* sp ability to form relatively thick, high-quality electroactive biofilms on electrodes that help to increase EET through DET. The reasons attributed to the popularity of MR-1 as a model organism, in addition to *Geobacter* sp, stem from two main points 1) MR-1 is a facultative anaerobe as opposed to a strict anaerobe like *Geobacter* sp and is therefore much easier to work with, and 2) MR-1 utilises an unparalleled large array of diverse terminal electron acceptors, including Mn(III), Fe(III), Co(III) nitrate, nitrite, fumarate, DMSO, TMAO, thiosulfate, humic acid, and even radioactive uranium isotopes, which opens up other applications such as soil remediation.[22-25]

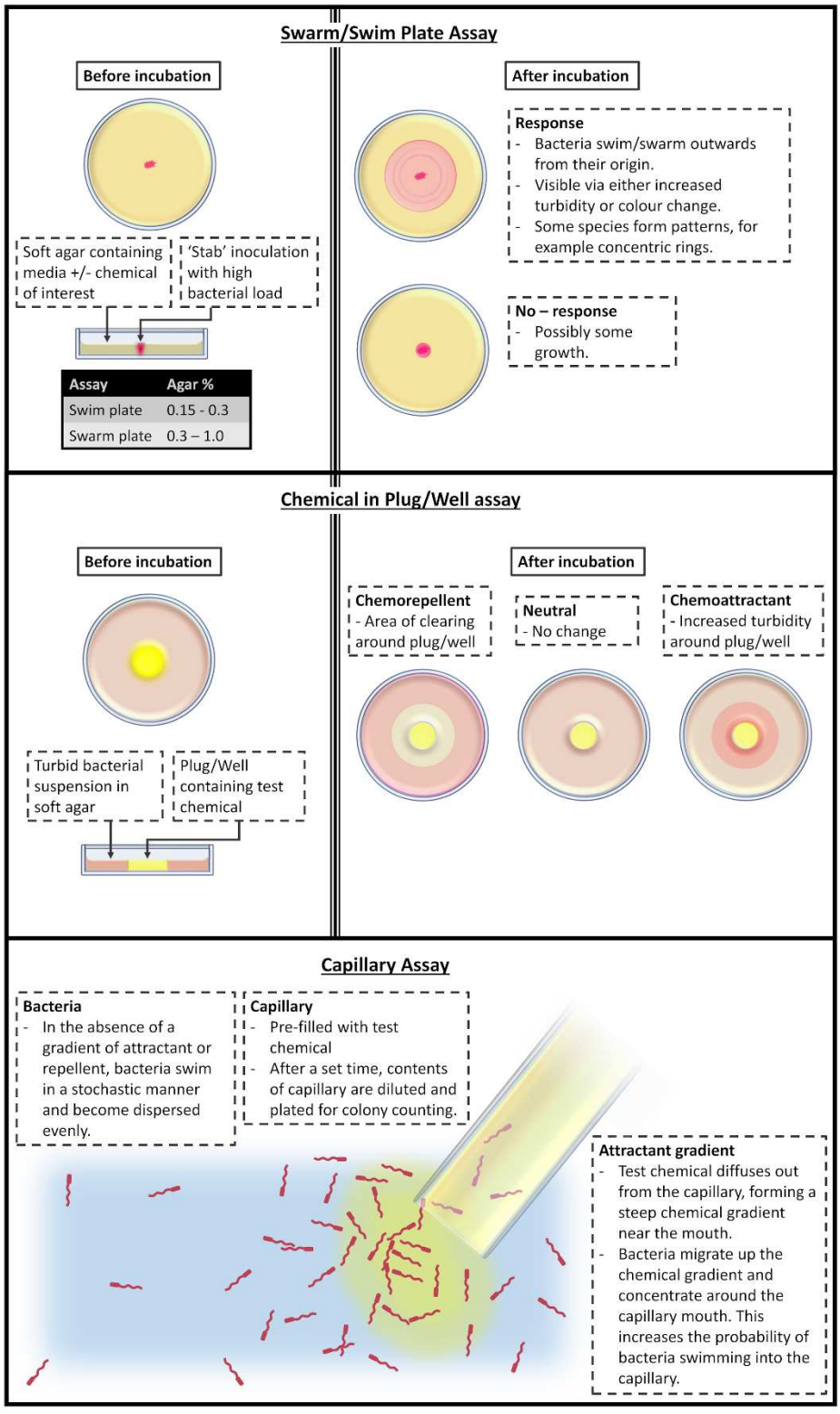
If MR-1 could form more substantial biofilms at the electrode interface, it would likely enhance the performance of MFCs. There have been some encouraging results from recent efforts to improve MR-1 biofilm quality, either through engineering electrode materials to increase biocompatibility with enhanced electrode-cytochrome connectivity and denser cell coverage [26] or engineering the microbes themselves to enhance their biofilm forming capabilities [18]. For example, Liu *et al* [18] over expressed YdeH, a diguanylate cyclase which catalyses the biosynthesis of bis-(30'-50')-cyclic dimeric guanosine monophosphate (c-di-GMP) from guanosine triphosphate. High levels of c-di-GMP promotes the expression of adhesive matrix components which in turn enhance bacterial biofilm formation. Compared to WT MR-1, the strain in which c-di-GMP was overexpressed formed better biofilms with a significantly increased biomass and ~2.8 fold increase in maximum power density [18].

Chemotaxis and energy taxis

Prior to the formation of biofilms on electrodes, bacteria must first locate, migrate towards and then colonise the surface. Understanding these steps would undoubtedly be beneficial for designing improved MES, possibly enabling strategies that speed up the recruitment of bacteria to electrode surfaces. In general, bacteria steer

migration via either chemotaxis or energy taxis. During both chemotaxis and energy taxis, bacteria migrate from areas of low attractant (e.g. electron acceptor) concentration to areas of higher concentration. Bacteria are thought to perceive spatial concentration gradients by sensing temporal gradients whilst moving [27-29]. Energy taxis employs a generic sensor for a metabolic indicator, for example the ΔpH or Δψ component of the proton motive force (pmf), whereas chemotaxis uses sensors for a specific molecule, e.g. a food/carbon source or an electron acceptor [30, 31]. One advantage of energy taxis is that it does not require specific sensors for every molecule of interest, unlike chemotaxis where each chemoattractant will usually require its own complementary sensor.

In aquatic systems, MR-1 is found concentrated in and around pockets containing high concentrations of electron acceptors, such as found in sediments [23, 24], in an otherwise electron acceptor limited environment. Steep redox gradients exist at the interface of these pockets and MR-1 can migrate up redox gradients, either directly or indirectly, to reach and stay close to these pockets. Energy taxis is a likely candidate considering MR-1 can reduce some species non-specifically via outer membrane cytochromes in addition to its many other terminal reductases with specific substrates. An alternative option is that MR-1 senses changes in redox conditions directly, possibly with a sensor, comparable to the Aerotaxis receptor (Aer) of *E. coli*, with an Flavin adenine dinucleotide (FAD) associated, 'velcro like' Per-Arnt-Sim (PAS) domain, for detecting redox status [25]. Importantly, in case of insoluble electron acceptors (including electrodes in MES), there is not necessarily any gradient of soluble molecules to allow either chemotaxis or energy taxis. Therefore, MR-1 must possess another method for locating these electron acceptors. In this review, we discuss studies of MR-1 taxis towards electron acceptors and highlight recent studies which try to address MR-1 migration towards insoluble electron acceptors such as electrodes. Proposed mechanisms for MR-1 electron acceptor taxis and the main techniques used to gather information on MR-1 taxis will also be discussed. Box 1 provides an overview of some of the main techniques.



Box 1. Illustrations of the three predominant techniques used for bacterial taxis studies. Top) Swarm/swim plate assay; depending on the concentration of agar used. Middle) the qualitative version of the chemical in well assay and the almost identical chemical in plug assay. Bottom) the capillary assay.

Electron acceptor taxis of MR-1

Early studies around MR-1 taxis towards electron acceptors were carried out by Nealson *et al* [24, 32], who used multiple techniques, including chemical in well plate assays, capillary assays and an in house developed spectrophotometric method. Most of the known electron acceptors for MR-1 were observed to act as tactic attractants. The strengths of the attractants towards MR-1 appeared to be related to their redox potential, with oxygen and nitrate/nitrite (high redox potential) showing the largest attractant response whilst DMSO and thiosulphate (low redox potential) presented much weaker responses. In stark contrast with the electron acceptors tested, no carbon source, including lactate, achieved any response under aerobic conditions and only formate could elicit a tactic response under anaerobic conditions [24, 32].

In competition assays, strong attractants (O_2, nitrate, and nitrite) inhibited taxis to all other electron acceptors. Weaker attractants such as fumarate and trimethylamine *N*-oxide (TMAO) had no effect on nitrate or nitrite. Dimethyl sulfoxide (DMSO) and elemental sulphur were the weakest of all the attractants, yet inhibited taxis towards all other electron acceptors including the stronger attractants, nitrate and nitrite. Furthermore, assays with mutants that are deficient in nitrate or nitrite reduction still demonstrated wild type levels of taxis towards nitrate and nitrite. Put together, these results obtained by Nealson *et al* strongly support the conclusion that MR-1 does not utilise energy taxis and appears to use a mechanism more reminiscent of chemotaxis where migration towards the attractant is not related to its metabolism [24]. Results from a study by Bencharit *et al* [23], using the chemical in plug and swarm plate assays, looking at MR-1 taxis responses towards metals and other anaerobic electron acceptors, were in general agreement with Nealson *et al*. In addition, Bencharit *et al* demonstrated tactic responses from MR-1 to soluble forms of Fe(III) and Mn(III) using the swarm plate assay which the authors suggest is more suited towards detecting energy taxis responses than chemical in well/plug plate assays. Bencharit *et al* concludes that MR-1 is capable of both energy taxis and chemotaxis, with Mn(III) and Fe(III) responses more likely a result of energy taxis whereas tactic responses towards Mn(II) and Fe(II) could be the result of chemotaxis [23].

Contrary to the conclusion above, later a comprehensive study by Baraquet *et al* [25] provided very compelling evidence supportive of an energy taxis mechanism. The mutants ΔtorA and ΔdmsA, deficient in terminal reductases TorA and DmsA, were unable to respond to the substrates TMAO and DMSO, respectively. Inhibition of molybdoenzymes which includes the reductases TorA, DmsA, and NapA, by pre-growing MR-1 with excess tungsten, resulted in the inhibition of taxis towards TMAO, DMSO and nitrate, but had no effect on taxis towards electron acceptors reduced by non-molybdoenzyme reductases such as nitrite and fumarate. These results demonstrate the requirement of terminal reductases for taxis towards the corresponding electron acceptors. As mentioned, energy taxis requires sensing of at least one of the two pmf components. To distinguish which of the two components is required for MR-1 energy taxis, the authors used nigericin to neutralise proton gradients and valinomycin to disrupt the membrane potential. Only the addition of nigericin had a detrimental effect on taxis towards electron acceptors, indicating ΔpH is the required component for

MR-1 energy taxis towards electron acceptors [25]. Separate results from Li *et al* [33], using a custom-made diffusion gradient chamber to evaluate MR-1 electron acceptor taxis, corroborate the results and conclusions of Baraquet *et al.* A subsequent study by Li *et al* [34] sheds insight onto how an energy taxis type mechanism, which requires a gradient of soluble substrate, could help MR-1 locate insoluble electron acceptors. Once stabbed into swarm plates with embedded amorphous MnO_2 or $Fe(OH)_3$ particles, MR-1 migrates outward, with a rate dependant on the concentration of particles, in a similar manner to swarm plates with soluble electron acceptors. On the addition of riboflavin, the MR-1 tactic band migration rate increased dramatically, suggesting that flavins, secreted by MR-1 at significant concentrations (~100 nM), play a part in the taxis towards insoluble metal oxides. The authors propose a mediated energy taxis mechanism, whereby MR-1 secretes reduced flavins which diffuse outward. On contact with insoluble electron acceptors, the flavin becomes re-oxidised and thus forms a concentration gradient between the MR-1 and the insoluble electron acceptor (or electrode). MR-1 can then use energy taxis to migrate up the concentration gradient of oxidised flavin.

A recent study by Kim *et al* [35] investigated the effects of flavin and oxygen on MR-1 migration. Using a microfluidics device and video microscopy, the authors performed cell tracking experiments with gradients of oxygen and riboflavin. The results show that MR-1 migrates up concentration gradients of both oxygen and flavin. Going up the concentration gradients, MR-1 swimming speed increases in combination with an increase in the frequency of direction changes. Reportedly, MR-1 displayed reversal and forward-backward-flick behaviours, both of which are indicative of unipolar flagellates [36, 37], to change its direction. Interestingly, the velocity of MR-1 in the direction of riboflavin gradients is significantly increased under anaerobic conditions. Together, these findings give further support to an energy taxis mechanism, specifically the mechanism proposed by Li *et al* [34] of mediated energy taxis with flavins as the mediator.

Prior to experiments of Kim *et al* [35], who tracked cells by video microscopy, Nealson *et al* [38, 39] used a similar cell tracking technique to investigate MR-1 taxis towards insoluble electron acceptors including MnO_2 particles and electrodes, which supported previous findings that MR-1 is capable of tactic responses towards insoluble metal oxide electron acceptors[23, 34]. A strong positive response was demonstrated towards MnO_2 particles, which is in keeping with swarm plate assays (embedded with MnO_2) by Worden *et al* [34]. MR-1 bacteria that are close to MnO_2 particles display an increase in swimming speed and a concomitant/associated increase in reversal frequency, similar to that observed later by Kim *et al* [35] for flavin and O_2 gradients. In contrast to the flavin mediated energy taxis proposed by Li *et al* [34], Nealson *et al* suggests gradients of Mn(II), formed by MR-1 reducing MnO_2 at the particle surface, could facilitate chemotaxis. A similar, albeit to a much lesser extent, response was observed with $Fe(OH)_3$ particles, indicating the response is not isolated to MnO_2. In addition to using insoluble metal oxides, Nealson *et al* also tested carbon fibre micro electrodes, poised at +600mV (vs Ag/AgCl), to mimic the redox potential of MnO_2 particle surfaces. The response of MR-1 to oxidative electrode potentials was almost identical to that of the metal oxides. Of the potentials tested, no

swimming response was observed at potentials below +500 mV, with optimal responses seen between +550 to +800 mV (vs Ag/AgCl). Manual tracking of cells close to the metal oxide particles and poised electrode surface showed that those cells that made contact would swim faster with a significantly higher reversal frequency then non-contacting cells. Mutant strains deficient in any of the genes required for EET (e.g *cymA*), abolished the responses observed with the WT MR-1 to MnO_2, $Fe(OH)_3$, and poised electrodes, demonstrating the requirement of EET for MR-1 migration to insoluble electron acceptors. As expected, a chemotaxis deficient ΔcheA-3 strain, incapable of reversing the direction of its flagella motor and therefore unable to reverse its swimming direction, was also unable to congregate around the insoluble electron acceptors. This led Nealson *et al* to propose a hypothetical model, termed 'congregation', to explain how MR-1 accumulates around insoluble electron acceptors. Congregation is first initiated when MR-1 randomly encounters an insoluble electron acceptor, allowing transfer of electrons through the MtrC/OmcA pathway from the bacteria to the electron acceptor. This event causes a change in swimming behaviour whereby speed and reversal frequency is increased which allows the bacteria to keep within proximity of the insoluble electron acceptor. Over time this 'congregation' can lead to attachment and biofilm formation [38].

Discussion

A large proportion of studies into MR-1 taxis towards electron acceptors have used agar plate assays, namely chemical in plug/well and swarm plate, as their main technique [22-25, 34]. The attraction of using agar plate based assays comes from their relative simplicity and good sensitivity to both attractants and repellents, as opposed to capillary assays which tend to have poor repellent sensitivity [40]. Unfortunately, no technique is perfect and over the last decade there have been numerous reports of discrepancies between agar plate based assays and other techniques [24, 25, 33, 41], most notably the well-established quantitative capillary assay, developed by Alder *et al* [42]. These discrepancies led Li *et al* [41] to assess the validity of the chemical in plug assay using non-chemotactic/non-motile mutants of MR-1 and *Helicobacter pylori* and found the assay susceptible to false positive responses from both species under certain conditions [41]. This unreliability is one of the reasons that most studies using agar plate assays also use a secondary technique, such as the capillary assay [24], microfluidics chemotaxis chamber [25] or a in house custom made device [24, 33]. On top of the mentioned issues with agar plate assays, other techniques commonly used in taxis studies have also been reported as potentially unreliable. For example, Li *et al* [33] had to discard results obtained by the capillary assay, because dubious values were obtained for chemotaxis constants.

The mentioned issues with the standard tools for probing chemotaxis has encouraged researchers to turn to other more direct techniques, like video microscopy with cell tracking [35, 38, 39]. This technique can monitor both population responses and single cell behaviours in a quantitative manner, making it a versatile technique. Once bacterial positions have been located in the image stack making up the video, bacterial traces are obtained either manually, which is inherently tedious and time consuming, or by computational methods,

usually in the form of purpose built in-house programs assembled in MATLAB or similar environments. Manual tracking is typically more accurate but not practical for large numbers of frames and/or bacteria. The computational method, although fast and convenient can suffer from artefacts, especially if the average path length of the bacteria between frames is close to or longer than the average distance between individual bacterial cells (i.e., when the cells are closely packed together and/or are moving rapidly).

The use of electrodes to couple electrochemistry with video microscopy allows for more control over tracking experiments and can provide valuable insight into MR-1 taxis. With this type of set up, MR-1 attachment or swimming behaviour can be correlated with the electron transfer rate to insoluble electron acceptors in real time. If a soluble redox mediator was to be investigated, the redox state of the mediator can be rapidly altered close to the electrode, allowing for a clear distinction between MR-1 responses to oxidised and reduced forms of a chemical [43]. Nealson et al [38, 39] noticed that MR-1 demonstrated a relaxation between switching potentials. This effect would have been difficult to pick up without having the ability to electrochemically control and rapidly alter the redox state of the mediator (or soluble electron acceptor).

Although MR-1 taxis has been researched extensively, the issue off how MR-1 locates and migrates towards electron acceptors, especially insoluble electron acceptors such as electrodes, has not been fully resolved. The observation that MR-1 flocks around insoluble electron acceptors and electrodes at oxidative potentials in the absence of added mediators can make it tempting to speculate that MR-1 may sense electric fields. However, typical ionic strengths of the electrolytes used in MES are around 0.1 M. Under these conditions, the Debye length is < 1nm preventing any meaningful electric fields from extending into the media.

Originally Nealson *et al* provided convincing results supportive of a receptor based chemotaxis mechanism for soluble electron acceptors. Since then, however, there have been multiple reports containing equally convincing results supporting an energy taxis or mediated energy taxis type mechanism for both soluble and insoluble electron acceptors. For insoluble electron acceptors, it is proposed MR-1 forms its own redox type gradient using self-secreted flavins. Using video microscopy cell tracking with both insoluble metal oxides and electrodes, Nealson *et al* has proposed a separate model for MR-1 taxis toward insoluble electron acceptors, termed 'congregation' which relies on initial chance encounters of direct contact between MR-1 and acceptor. Out of the two proposed models, for MR-1 migration towards insoluble electron acceptors, mediated energy taxis, in the authors opinion, provides a more complete and appealing explanation to the majority of results reported by the studies looked at here. Future studies should use multiple distinct techniques along with non-motile/non-chemotactic and EET deficient mutants as controls to prevent issues like false positives and overcome limitations of the individual techniques.

Acknowledgements

JO and LJCJ received funding from the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013)/ERC Grant no. 280518.

References

[1] Myers CR, Nealson KH. Bacerial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science. 1988;240:1319-1321.

[2] Santoro C, Arbizzani C, Erable B, Ieropoulos I. Microbial fuel cells: From fundamentals to applications. A review. J Power Sources. 2017;356:225-244.

[3] Logan BE, Rabaey K. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. Science. 2012;337:686-690.

[4] Yang WY, Lee KK, Choi S. A laminar-flow based microbial fuel cell array. Sensor Actuat B-Chem. 2017;243:292-297.

[5] Vologni V, Kakarla R, Angelidaki I, Min B. Increased power generation from primary sludge by a submersible microbial fuel cell and optimum operational conditions. Bioprocess and Biosystems Engineering. 2013;36:635-642.

[6] Pham TH, Aelterman P, Verstraete W. Bioanode performance in bioelectrochemical systems: recent improvements and prospects. Trends Biotechnol. 2009;27:168-178.

[7] Pant D, Van Bogaert G, Diels L, Vanbroekhoven K. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol. 2010;101:1533-1543.

[8] Tan Y, Adhikari RY, Malvankar NS, Ward JE, Nevin KP, Woodard TL, et al. The low conductivity of *Geobacter uraniireducens* pili suggests a diversity of extracellular electron transfer mechanisms in the genus Geobacter. Frontiers in Microbiol. 2016;7:980.

[9] Firer-Sherwood MA, Bewley KD, Mock J-Y, Elliott SJ. Tools for resolving complexity in the electron transfer networks of multiheme cytochromes c. Metallomics. 2011;3:344-348.

[10] Carmona-Martinez AA, Harnisch F, Fitzgerald LA, Biffinger JC, Ringeisen BR, Schroeder U. Cyclic voltammetric analysis of the electron transfer of *Shewanella oneidensis* MR-1 and nanofilament and cytochrome knock-out mutants. Bioelectrochem. 2011;81:74-80.

[11] Clarke TA, Edwards MJ, Gates AJ, Hall A, White GF, Bradley J, et al. Structure of a bacterial cell surface decaheme electron conduit. Proc Nat Acad Sci USA. 2011;108:9384-9389.

[12] Dantas JM, Morgado L, Aklujkar M, Bruix M, Londer YY, Schiffer M, et al. Rational engineering of *Geobacter sulfurreducens* electron transfer components: a foundation for building improved Geobacter-based bioelectrochemical technologies. Frontiers in Microbiol. 2015;6:752.

[13] Korth B, Rosa LFM, Harnisch F, Picioreanu C. A framework for modeling electroactive microbial biofilms performing direct electron transfer. Bioelectrochem. 2015;106:194-206.

[14] Roy JN, Babanova S, Garcia KE, Cornejo J, Ista LK, Atanassov P. Catalytic biofilm formation by *Shewanella oneidensis* MR-1 and anode characterization by expanded uncertainty. Electrochim Acta. 2014;126:3-10.

*** In this work, we show that elecrochemical behaviour previously assigned to direct electron transfer to MR-1 might instead be due to mediated electron transfer via extarcellular iron**

[15] Oram J, Jeuken LJC. A re-evaluation of electron-transfer mechanisms in microbial electrochemistry: shewanella releases iron that mediates extracellular electron transfer. Chemelectrochem. 2016;3:829-835.

** **In this work, Liu et al show that mutants of MR-1 that are engineered to form improved biofilm also generate higher power outputs in microbial fuel cells**
[16] Liu Y, Bond DR. Long-distance electron transfer by *G. sulfurreducens* biofilms results in accumulation of reduced *c*-type cytochromes. Chemsuschem. 2012;5:1047-1053.

[17] Sun D, Chen J, Huang HB, Liu WF, Ye YL, Cheng SA. The effect of biofilm thickness on electrochemical activity of *Geobacter sulfurreducens*. Int J Hydrogen Energ. 2016;41:16523-16528.

[18] Liu T, Yu Y-Y, Deng X-P, Ng CK, Cao B, Wang J-Y, et al. Enhanced Shewanella biofilm promotes bioelectricity generation. Biotechnol Bioeng. 2015;112:2051-2059.

[19] Kitayama M, Koga R, Kasai T, Kouzuma A, Watanabe K. Structures, compositions and activities of live *Shewanella* biofilms formed on graphite electrodes in electrochemical flow cells. Appl Environ Microbiol. 2017;In press, DOI:10.1128/AEM.00903-17.

[20] Okamoto A, Hashimoto K, Nakamura R. Long-range electron conduction of *Shewanella* biofilms mediated by outer membrane *c*-type cytochromes. Bioelectrochem. 2012;85:61-65.

[21] Lin T, Bai X, Hu Y, Li B, Yuan Y-J, Song H, et al. Synthetic *Saccharomyces cerevisiae-Shewanella oneidensis* consortium enables glucose-fed high-performance microbial fuel cell. Aiche Journal. 2017;63:1830-1838.

[22] Armitano J, Baraquet C, Michotey V, Mejean V, Jourlin-Castelli C. The chemical-in-mu well: a high-throughput technique for identifying solutes eliciting a chemotactic response in motile bacteria. Res Microbiol. 2011;162:934-938.

[23] Bencharit S, Ward MJ. Chemotactic responses to metals and anaerobic electron acceptors in *Shewanella oneidensis* MR-1. J Bact. 2005;187:5049-5053.

[24] Nealson KH, Moser DP, Saffarini DA. Anaerobic electron-acceptor chemotaxis in *Shewanella putrefaciens*. Appl Environ Microbiol. 1995;61:1551-1554.

[25] Baraquet C, Theraulaz L, Iobbi-Nivol C, Mejean V, Jourlin-Castelli C. Unexpected chemoreceptors mediate energy taxis towards electron acceptors in Shewanella oneidensis. Mol Microbiol. 2009;73:278-290.

[26] Deng L, Guo S, Liu Z, Zhou M, Li D, Liu L, et al. To boost c-type cytochrome wire efficiency of electrogenic bacteria with Fe_3O_4/Au nanocomposites. Chem Commun. 2010;46:7172-7174.

[27] Tranquillo RT. Theries and models of gradient perception. Armitage, J P And J M Lackie1990. p. 35-76.

[28] Macnab RM, Koshland DE. Gradient-sensing mechanism in bacterial chemotaxis. Proc Nat Acad Sci USA. 1972;69:2509-2512.

[29] Vladimirov N, Sourjik V. Chemotaxis: how bacteria use memory. Biol Chem. 2009;390:1097-1104.

[30] Schweinitzer T, Josenhans C. Bacterial energy taxis: a global strategy? Arch Microbiol. 2010;192:507-520.

[31] Alexandre G. Coupling metabolism and chemotaxis-dependent behaviours by energy taxis receptors. Microbiol-Sgm. 2010;156:2283-2293.

[32] Nealson K, Saffarini D, Moser D, Smith MJ. A spectroscopic method for monitoring tactic responses of bacteria under anaerobic conditions. J Microbiol Methods. 1994;20:211-218.

[33] Li R, Auchtung JM, Tiedje JM, Worden RM. *Shewanella oneidensis* MR-1 chemotaxis in a diffusion gradient chamber. Environ Sci Technol. 2011;45:1014-1020.

* **In this work it is shown that soluble electron suttles can be used by bacteria to guide them to insoluble electron acceptors via taxis**
[34] Li R, Tiedje JM, Chiu C, Worden RM. Soluble electron shuttles can mediate energy taxis toward insoluble electron acceptors. Environ Sci Technol. 2012;46:2813-2820.

** **In this work Kim et al uses a newly developed microfluidic platform to study taxis of MR-1 and finds that taxis is enhanced when additional riboflavin is added**
[35] Kim BJ, Chu I, Jusuf S, Kuo T, TerAvest MA, Angenent LT, et al. Oxygen tension and riboflavin gradients cooperatively regulate the migration of *Shewanella oneidensis* MR-1 revealed by a hydrogel-based microfluidic device. Frontiers in Microbiol. 2016;7:1438.

[36] Stocker R. Reverse and flick: Hybrid locomotion in bacteria. Proc Nat Acad Sci USA. 2011;108:2635-2636.

[37] Xie L, Altindal T, Chattopadhyay S, Wu X-L. Bacterial flagellum as a propeller and as a rudder for efficient chemotaxis. Proc Nat Acad Sci USA. 2011;108:2246-2251.

[38] Harris HW, El-Naggar MY, Nealson KH. *Shewanella oneidensis* MR-1 chemotaxis proteins and electron-transport chain components essential for congregation near insoluble electron acceptors. Biochem Soc Trans. 2012;40:1167-1177.

[39] Harris HW, El-Naggar MY, Bretschger O, Ward MJ, Romine MF, Obraztsova AY, et al. Electrokinesis is a microbial behavior that requires extracellular electron transport. Proc Nat Acad Sci USA. 2010;107:326-331.

[40] Tso WW, Adler J. Negative chemotaxis in *Escherichia coli*. J Bact. 1974;118:560-576.

[41] Li J, Go AC, Ward MJ, Ottemann KM. The chemical-in-plug bacterial chemotaxis assay is prone to false positive responses. BMC research notes. 2010;3:77.

[42] Adler J. Method for measuring chemotaxis and use of method to determine optimum conditions for chemotaxis by *Escherichia coli*. J Gen MicroBiol. 1973;74:77-91.

[43] Bryant C, Atha D, Reipa V. Electrochemical potential gradient as a quantitative in vitro test platform for cellular oxidative stress. Antioxidants. 2016;5:23.