# FINAL REPORT—ONR YIP

**Grant number: :** N00014-02-1-0397

**Principal Investigator:** Dr. Dianne K. Newman

**Grant Title:** Direct and indirect mechanisms of iron reduction by *Shewanella oneidensis*

**Award Period:** May 1, 2002 – April 30, 2005

**Objective:**

1. To understand how *S. oneidensis* strain MR-1 regulates its two iron-reducing pathways (e.g. iron reduction by direct contact vs. at a distance)
2. To understand how these pathways work at the molecular level

**Approach:**

Through physiological, genetic, bioinformatic and biochemical approaches, we explored the mechanisms of iron reduction by *S. oneidensis*. We made direct, in-frame "knock-out" mutations of key genes involved in the sensing of aerobic/anaerobic conditions (*e.g. arcA*) and tested their phenotype with respect to iron reduction; in addition, we determined the ArcA regulon using a bioinformatics approach (Gralnick *et al.,* Mol. Micro, 2005). To explore how *S. oneidensis* regulates the expression of genes required for the "indirect pathway", we created a new method for measuring iron reduction at a distance using $Fe(OH)_3$-containing nano-porous glass beads ("Fe beads"). Using Fe-beads, we determine the conditions under which iron is reduced at a distance. In addition, we perfomed experiments using Electrochemical Impedance Spectroscopy (EIS) to study the effect of a dual-species biofilm on the corrosion of carbon steel.

**Accomplishments:**

1.) We showed that the ArcA regulon in *S. oneidensis* is completely different from that in *E.coli.* Moreover, *S. oneidensis* does not contain a robust ArcB homolog, which tells us that the manner in which it senses the redox state of its environment is different than the *E. coli* system.

2.) We discovered that an operon encoding a DMSO reductase is regulated by ArcA, whereas the genes encoding the mineral reductase systems are not. Surprisingly, the DMSO reductase is an extracellular enzymatic complex, similar in localization to the mineral reductase system. This suggests that *S. onediensis* perceiveds DMSO in the environment effectively as a solid (this makes sense, as in aquatic systems, algae are the primary producers of DMSO, and it tends to adsorb to their calcite frustules).

3.) We developed a novel system for measuring Fe-reduction at a distance (the "Fe-bead" method), which is an improvement over previous techniques to measure extracellular electron shuttling.

4.) We found that extracellular electron shuttling by *S. oneidensis* does occur, particularly under conditions relevant for biofilms. The genes required for this process overlap significantly with those required for Fe reduction mediated by "direct contact". This implies that the "direct"/"indirect" distinction may in fact be artificial, and motivates future studies aimed to understand the nature of the mineral reduction event at molecular resolution (*e.g.* structural studies of the decaheme *c*-type cytochromes believed to transfer electrons to Fe oxides "directly"). Our evidence suggests that these same proteins are required for electron shuttle reduction (both native shuttles, and artificial shuttles such as AQDS), raising the question of whether "direct" electron transfer occurs at all.
5.) We completed a study of a 2-species biofilm (*S. oneidensis*—an Fe reducer—and *D. desulfuricans*—a sulfate reducer) growing on carbon steel. Surprisingly, *S. oneidensis* inhibited the corrosive effect of *D. desulfuricans*.

**Conclusions/Significance**: Four important findings have come out of our work. The first is that the pathways for iron reduction that conventionally have been viewed as different, may in fact overlap extensively. The case for "direct" contact between microbes and minerals will not be proven until biochemical experiments demonstrate that electrons can transfer from outermembrane proteins to iron oxides directly; until that is shown, the involvement of an electron shuttle intermediate cannot be ruled out. We have demonstrated that in a biofilm context, the existence of such shuttles is necessary for electron transfer at a distance. The second major finding is that the regulatory network of anaerobic respiration in *Shewanella* is completely different than that in *E. coli.* The third finding, stemming from the 2$^{nd}$, is that DMSO respiration is regulated by ArcA in *Shewanella* and the topology of proteins required for this process is remarkably similar to that of the proteins required for mineral respiration. This implies that in the environment (particularly, in cold marine environments), *Shewanella* may perceive DMSO as a solid. Future work to establish the conservation of this strategy in nature, and the importance of this pathway to DMSO cycling (which has profound impacts for global climate) will be conducted in the laboratory of Prof. Jeff Gralnick (the postdoc who performed this work and who is now an Assit. Professor at the University of Minnesota). The fourth and final finding that an Fe-reducing bacterium can inhibit corrosion when a corrosion-enhancing bacterium is present warrants future study with respect to its potential applicability to the design of biological corrosion-control measures.

**Patent Information**: -NA-

**Award Information**: DKN named Howard Hughes Medical Investigator in 2005

**Refereed Publications**

J.A. Gralnick, H. Vali, D. Lies and D.K. Newman (2006) Extracellular respiration of dimethyl sulfoxide by *Shewanella oneidensis* strain MR-1, *PNAS, in press*

D.K. Newman and J.A. Gralnick (2005) "What genetics offers geobiology", In: Molecular Geomicrobiology, Eds. J.F. Banfield and K.H. Nealson, Mineralogical Society of America, Washington, D.C., Reviews in Mineralogy & Geochemistry, 59:9-26.

J.A. Gralnick, C.T. Brown and D.K. Newman (2005) Anaerobic respiration by an atypical Arc system in *Shewanella oneidensis*, *Molecular Microbiology*, 56(7): 1347-1357.

D.P. Lies, M.E. Hernandez, A. Kappler, R.E. Mielke, J.A. Gralnick, and D.K. Newman (2005) Shewanella oneidensis MR-1 uses overlapping pathways for iron reduction at a distance and by direct contact under conditions relevant for biofilms, *Appl. Environ. Microbiol.*, 71(8): 4414-4426.

A.K. Lee, M. Buehler, and D.K. Newman (2005) Influence of a dual-species biofilm on the corrosion of mild steel, *Corrosion Science*, 48(1):165-178.

NOTE: Four other manuscripts containing work that was supported by this award will be submitted this year.

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**14. ABSTRACT**
We found that the direct and indirect pathways for Fe reduction by Shewanella oneidensis have signficant overlap. We showed that outermembrane proteins that were previously thought to contact Fe "directly" also play a key role in reducing electron shuttling molecules like AQDS. Thus, a challenge for future research will be to demonstrate how electron transfer between these proteins and minerals works in detail. In addition, we defined the regulon of the Arc control system in S. oneidensis, and showed that it positively regulates the expression of a cluster of genes encoding a DMSO reductase. This reductase is similar to that used for Fe respiration with respect to its localization to the external face of the outer membrane. This underscores the metabolic versatility of Shewanella, and suggests that in addition to minerals, Shewanella may perceive DMSO as an "insoluble" substrate. This opens up an exciting new area for future research: how do microbes affect the DMSO cycle in the marine environment? Several publications resulted from this award, including journal articles in Applied and Enviornmental Microbiology, Molecular Microbiology, Corrosion Science, and PNAS (hopefully--the manuscript is in review!). A book chapter on "What genetics offers geobiology" was also written. 2 students, 2 postdocs, and 2 undergrads were supported in part by this award.

**15. SUBJECT TERMS**
Shewanella oneidensis, Fe reduction, direct contact, DMSO, corrosion

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