Chapter 6

CONCLUSIONS

6.1 Summary

This thesis provides some insights into the biological process of iron mineral reduction mediated by microbially produced extracellular redox-active organic molecules. It demonstrates that a dissimilatory iron reducing bacterium (DIRB), *Shewanella oneidensis* MR-1, can reduce iron at a distance and that reduction is not mediated by a quinone derived from the menaquinone biosynthesis pathway. Whether iron reduction at a distance occurs via electron shuttling or via solubilization of the Fe(III) is yet not known. This research also provides evidence that phenazine antibiotics that are produced by a variety of soil bacteria can function as extracellular electron shuttles mediating iron mineral reduction by *Pseudomonas chlororaphis* and also by *S. oneidensis* MR-1. While *S. oneidensis* achieves faster rates of mineral reduction and growth when phenazines are present, it remains to be proven whether there is a physiological role for phenazines either in iron assimilation or in energy metabolism.

6.2 Environmental implications

My research shows that redox-active secondary metabolites such as phenazines that are produced by bacteria in soils, can mediate iron reduction by DIRB and by bacteria that are not able to respire iron minerals directly. Thus, this finding extends the current paradigm of humics as mediators of iron respiration and affords a mechanistic

basis for DIRB and non-DIRB mediated reduction and solubilization of iron in soils and sediments. It demonstrates that when shuttles are not readily available in their direct environment, DIRB are still able to mediate iron reduction at a distance, providing a means for iron reduction in occluded pores, clays, and particles that are not accessible to a direct contact with the bacterial cell. Whether this process is environmentally significant remains to be determined.

If extracellular electron transfer is shown to be a mechanism of energy generation, its occurrence can potentially be widely extended, since not only iron oxides but any oxidant, that is not easily accessible to the cell but has a more positive redox potential than the shuttle, will reoxidize the shuttle and therefore support this metabolism. Moreover, other bacterial cells may use the reduced shuttle as electron donor, creating syntrophic relationships between different bacteria in a community, as it has been shown to occur in laboratory experiments with AQDS and cysteine (1, 2)

6.3 Questions and specific suggestions for future research

6.3.1 Indirect iron reduction by DIRB

Important remaining questions include the following: What is the nature of the MR-1 mediator? Is it an electron shuttle or an Fe chelator? How far away from the beads can the cells be in order for this process to work? Do many DIRB produce these compounds, or do different DIRB adopt different strategies for reducing minerals in the environment? How specific are these mediators? Can they reduce other metals and radionuclides in the same way? Can bacteria couple growth to the reduction of these

mediators? What are the genes and gene-products that are involved in reducing Fe at a distance, and how are they regulated?

There is an indirect pathway for iron reduction within the beads, but it seems still to require proximity between cells and beads. It would be interesting to establish how far the substrate can be away from the cells and still be reducible by this mechanism. A possible approach to test this would be by synthesizing Fe- beads with different particle size to vary the distance between bacteria and the internal iron. By stripping the iron in the cortex by rapid washes with a dilute reductant previous to the cell exposure, it would be possible to determine if the iron located in the cortex and therefore directly accessible to the cell is critical to initiate or regulate this process.

The method developed for testing the indirect pathway is well characterized, reproducible and can be easily used to answer a variety of questions. A color change from orange to transparent provides a visual indication that can be utilized for a genetic screen to unveil the genes involved in iron reduction at a distance and their regulation. Specific knock-outs can be made to test for the involvement of certain genes like those that encode putrebactin, the siderophore produced by *Shewanella oneidensis* in iron deficient but aerobic conditions. Whether that molecule is produced under anaerobic conditions and whether it has a role in iron reduction is currently unknown.

Finally, an interesting question is what is the composition and role of the exopolymeric substances (EPS) that hold the beads and cells together and whether they are necessary for this process. EPS in *Shewanella oneidensis* has been shown to bind Fe(II). Would this facilitate the reduction of and Fe(III) chelator? Moreover, if this EPS-bound Fe(II) were to be re-oxidized by oxygen, *Shewanella* might be able to reuse it

as an electron acceptor. This process can be tested with the bead system by exposing the cells to a cycle of oxic and anoxic regimes. In the environment this is likely to happen in soils and at redox interfaces.

6.3.2 Phenazines

Phenazines can shuttle electrons to iron minerals in lab experiments. Is this an important role in the environment? Do they facilitate iron acquisition by dissolving iron minerals? What is the relationship with siderophores, the current paradigm for iron acquisition? Could iron reduction by phenazines make iron more available for binding by siderophores? Pyocyanin has been shown to reduce iron contained in the chelator transferrin; can it also reduce iron held by its own or other bacterial siderophores? What are the environmental constraints on phenazines in natural environments? Are they diffusion-limited due to low water content in soils and/or sorption onto soil/clay particles or humic substances? Is the reduction of phenazines mediated by the respiratory chain and is it used for energy generation? Can *P. chlororaphis,* and other bacteria, grow with phenazines as electron acceptors when oxygen or nitrate are no longer available or can they get at least maintenance energy from phenazine reduction? If so, are phenazines used as electron acceptors in biofilms? Otherwise, is their release a form of dumping reducing equivalents? While some phenazines can transfer electron to oxygen and thus generatate toxic oxygen radical species, they are also radical scavengers. Could phenazines be involved in protecting the producer strain from oxidative stress? Can any of these suggested functions (or a combination of them) explain why phenazine-producing *Pseudomonas* are more ecologically competent in the rhizosphere?

6.4 References

1. Kaden, J., A. S. Galushko, and B. Schink. 2002. Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*. *Archives of Microbiology* 178:53-58.

2. Lovley, D., J. Fraga, J. Coates, and E. Blunt-Harris. 1999. Humics as an electron donor for anaerobic respiration. *Environmental Microbiology* 1:89-98.