Abstract

Typically the iron bacteria are described as ferrous iron (Fe^{2+}) oxidisers (with oxygen) to ferric iron (Fe^{3+}). Exposure of ferrous iron and sulphide containing groundwater to atmospheric oxygen forms the well known phenomena of ferric iron and sulphur precipitation which typically, but not necessarily, involves iron and sulphide oxidising bacteria. In this process ferrous iron serves as the electron donor and molecular oxygen as the electron acceptor. The flow of electrons from ferrous iron to oxygen enables the respiration process of the bacteria and allows their growth. Also insoluble iron species allow an electron flow that bacteria can use for their respiration and growth. Two such examples are covered in this paper:

1. Bacterially influenced corrosion of steel. The iron in steel (Fe^0) is even more reduced than ferrous iron and hence offers a more attractive electron donor for bacteria. Sulphate-Reducing bacteria can utilise the electrons from metallic iron using sulphate, sulphite or elemental sulphur as electron acceptor. Again, this electron flow from metallic iron to sulphate provides the bacteria with a suitable energy source for growth. And like usual this electron flow is used for respiration, here an anaerobic respiration. Sulphate-Reducing bacteria (SRB) play an important role in the corrosion of steel in anaerobic environments, such as oilfields, marine sediments, treatment tanks etc. This paper covers the principle and examples of steel corrosion by SRB.

2. Bacterial reduction of ferric iron to ferrous iron. Also the most oxidized form of iron, ferric iron can be used by bacteria for growth. However, as it is in its most oxidised form it can not act as electron donor but as electron acceptor. Analogous to the oxygen respiration using O_2 as electron acceptor for electrons derived from the oxidation of organic material, iron reducing bacteria can ‘respire’ ferric iron. This reaction occurs typically in anaerobic ecosystems with organic compounds (e.g. organic acids) as the electron donor. The role and economic potential of iron reducing bacteria in groundwater are discussed in this paper.

Both the above cases of iron metabolism involve insoluble species of iron. As bacteria by definition can only metabolise soluble material, specific mechanisms need to be in place that enable the electron transfer. This paper discusses possibilities and implications of such electron flow by also covering other species relevant to groundwater chemistry (e.g. elemental sulphur and chlorinated solvents).

As a microbiologist, I have an interest in microbial ecology and the role of bacteria in industrial environments such as oil fields. This includes nutrient removal, ammonium oxidation and nitrate reduction, typically that by bacteria that live in groundwater. Bioreactors are used for the study of nutrient removal and bioleaching particularly when they are controlled by a computer. We model the reactions and make precise predictions how their internal conditions are affected by bacteria, and maybe understand and optimise the processes.

Today I will focus on two aspects that relate to iron metabolism and two iron species that are more difficult than other species because they are very insoluble, and yet bacteria make a living by interacting with them. I used to consult with petroleum companies about sulphate-reducing bacteria eating their metal pipelines; I spent several months working on Geobacter, an exciting bacteria.
In my view bacteria make a living using electron donors and electron acceptors. Microbes don’t grow on a food, they don’t grow on nutrients, what they all need is a donor and acceptor. They catalyse redox reactions. Oxygen, for example, is an acceptor and iron species are electron donors. Anytime you observe some life activity, you should be able to say, “What is happening with the electron flow?” You need to have a suitable electron donor and acceptor with a redox potential between them that is large enough that it supplies sufficient free energy for the organisms to live. The electron flow is the common energy source. With that view, it soon becomes clear that since you need to generate an electron flow with insoluble species, the bacteria need to do some tricks.

A typical electron flow in a biochemical textbook is in a very simplified form: since the bacterial cell needs an electron donor and an electron acceptor. The electron donor is, perhaps, ammonium and the electron acceptor is, say, oxygen. The bacterial cell oxidises ammonium to nitrate. Equally, it could use sulphide as the electron donor and oxygen as the electron acceptor. Sulphate can be an electron acceptor. Not all combinations are possible. For example, we won’t find bacteria that oxidise sulphide by using sulphate as the electron acceptor, because the redox potential difference isn’t enough to allow such processes.

I will quickly list a group of the major electron acceptors: once oxygen (at the top of the list) is not available in groundwater, typically some of the other acceptors are still available. Bacteria that used to use oxygen now do other things, perhaps use nitrate and form N₂. Some bacteria take the nitrate and form ammonium, which is not a desired reaction in groundwater. Iron reduction is something that I will discuss later. It is possible the bacteria might use CO₂ to produce methane when there are sufficient organics, in bogs and swamps. Given substrates: each possible chemical (redox) step is a niche inviting bacterial species to exploit it.

You ask, are bacteria going to reduce ferric iron or nitrate or sulphate or what? There is a fairly clear recipe. If you order the electron acceptors according to their redox potential, it provides you with the available energy. It is not intelligence by the bacteria; there are physical and chemical mechanisms that predict that the bacteria will first use oxygen. But if the oxygen is gone, they will use nitrate. When nitrate is gone, they will use sulphate, and so on. For example, sulphate in the groundwater environment typically suppresses the microbial conversion of CO₂ to methane. Methane emerges only when sulphate is gone. Also, in marine mangrove areas, there is not much methane production because the bacteria prefer to use marine sulphate as an acceptor, which gives them more energy. In the same way, the presence of nitrate suppresses the reduction of sulphate. One should be able to control the environment by using this knowledge and gain an advantage from the bacteria.
Once an electron acceptor is used, it is turned into an electron donor. There are inorganic species on both sides of the table above. To confuse us more, the bacteria can have an electron donating species and link it with an electronic accepting species; in theory, if you use that matrix, you can come up with a large number of possible reactions. There are even more electron donors and acceptors that could be included (at right), including manganese, uranium and other metals. Not all of them can be linked. As soon as something is oxidised, for example, ammonium is oxidised to nitrite, the nitrate can again be reduced, and in some cases, go back to ammonia. It can be confusing: bacteria convert A to B, and then B back to A. It is not quite that without compartmentalization, because that would not give any energy. They convert A to B with the help of an external electron donor, and then they convert B back to A with the help of an external electron acceptor. So it is all driven by extra, external components.

If a bug is to make use of these processes, it must have both an electron donor and an electron acceptor. Often these are not present in the same environment. Typically, wherever there is oxygen, there isn’t much ammonium or much sulphide. So, where life happens, in many spots on the globe, including in the plaque of your teeth, the sediments of rivers, or biofilms in pipelines, where there is a sharp boundary layer, where the oxidised and reduced species are present; that is where the bugs make a living. At the Black Sea, there is a very sharp interface of the “black” life, the lower water levels which are oxygen-free, with sulphide and ferrous iron, and the oxidised life. This sharp interface is where the energy is, because electron donors and acceptors are available, driving life.

We will leave the typical electron donors and acceptors and just pick the most difficult ones relating to iron: they corrode iron, metallic iron, which relates to the Titanic, and the reduction of ferric iron back to ferrous iron. You might wish that once insoluble ferric iron is formed in certain bore wells that there is some magic trick that might bring it back to the soluble ferrous form. Corrosion of iron involves an electron flow from metallic iron to bacteria, otherwise, they can’t live. Life, as we know it, is based on this. We call it ‘respiration’ when we talk about us. I present this as ‘where the electron flow is happening.’

The main bacteria involved in corrosion are sulphate-reducing bacteria and they play a major role in the oil industry. I don’t believe anyone has measured them nibbling away at steel constructions that are in aerated water. Sulphate-reducing bacteria are anaerobes that live underground in the oil and in oil tanks that are free of oxygen. Typically, one wouldn’t find them in rusting environments, but if the oxygen doesn’t penetrate the rusticles, I would think there are some oxygen-free spots for the bacteria to live.
Here is an image of a pipeline which has the characteristic pitting corrosion caused by the sulphate-reducing bacteria. Oil people usually ignore this, but when a pipeline that was constructed to have a 20-year lifetime has pitting holes through it within two years, they are concerned and want to find out how this happens and how it can be controlled. This is the outside picture of the pipe. The bacteria are thermophiles and are very good at eating the pipeline from the inside where there is a mixture of warmer water and oil.

This is a photograph of *Desulfovibrio* (second from top), one of the species of sulphate-reducing bacteria. The question is, how do bacteria suck electrons from metallic iron? Metallic iron has an oxidation state of zero; as such, it has a lot of energy in it. We stick electrons into iron ore to make it metallic; it has more electrons than it normally has. It tends to give them away and there are bacteria that tend to like electrons. What is the mechanism by which bacteria get electrons?

I set up a battery and fed the sulphur-reducing bacteria with current from a battery. The mechanism of how the bacteria react as a slime on steel is shown in the diagram right. The steel corrodes as it is provided some electrons. The electrons form a hydrogen film that is hardly detectable on the steel surface. The hydrogen keeps the surface polarized and bacteria are very good at using hydrogen as an electron donor. Hydrogen is the most abundant nutrient for bacteria. It is very small and very rapid in its diffusion, and it is pure energy provided there is an electron acceptor with it. These bacteria use sulphate as an electron acceptor, generating sulphide, and make a rich and easy living. As they remove the electrons a positive charge is left on the metal, and that positive charge needs to be equilibrated, preferably by releasing ferrous iron into the environment. The sulphide reacts with the ferrous iron and forms iron sulphides which stimulate corrosion further. There is weight loss and a corrosion rate about 50 to 100 times faster than without the bacteria.

### Cathodic protection good or bad?

- **What is it?**
  Use electricity to allow electron flow without iron dissolution
- **Effects:**
  - Easy supply of electrons for SRB
  - Increased amounts of catalytic bacteria (can mean enhanced corrosion upon interruption of cathodic protection)
  - Depletion of suitable oxidants (e.g. sulphate) can mean stabilisation of environment
  - Depends on whether system is limited by availability of electron donor or electron acceptor.

Cathodic protection is a nice term but I am not sure what you accomplish by cathodically protecting the piece of metal, which means you pump in extra electrons. One thing you do accomplish is to feed the iron with an extra electrons.
This doesn’t belong in the talk, but is part of my background. ‘Pig’ here does not mean the agricultural animal, it is the pigs that petroleum companies use to clean pipes, basically like a toilet brush. They shoot the pig through the pipe with the flow. On one of my trips I took samples of SRB numbers before, during and after the pig was shot through the pipe. The SRB numbers in suspension shot up very high. This allowed me to calculate that most of the SRBs were sitting on the walls, and also to estimate what the average densities were. The bugs cause the damage because they sit on some spots and drill holes rather than cause uniform corrosion, as one would prefer.

Once ferric iron is formed it is rather insoluble except at really low pH values. There are bacteria that were ‘hesitantly’ discovered by Lovelly. He worked for the Geological Survey in the United States, and had trouble with all the geologists who disbelieved that bacteria could convert insoluble ferric iron to ferrous. When I did my sabbatical with him he had five people: now he has fifty people. Look on the internet for geobacter.com; many programs are now underway, studying the activities of Geobacter.

How do bacteria interact with an electron flow and with ferric iron? The electron donors that Geobacters use can be hydrogen, acetate, and they have now found one Geobacter that uses sugar. Geobacters use small organic molecules that are typically abundant in life environments. In one reaction ferric iron accepts electrons from a donor and turns into ferrous iron. The reaction causes a change in redox potential. Interestingly, the bacteria can do many reductions, not only ferric iron. My own impression is that they are ‘everything’-reducers: you throw anything at them and they can reduce it. They have some mechanism to pass electrons to insoluble species that can be reduced, including elemental sulphur. Insoluble ferric sediments, magnetite, is the end product. They convert a brown sediment into a black sediment. Go to the Swan River and look at the colour of the sediment. The top of the sediment looks oxidised, brown with a high positive redox potential; lower down is black with a negative redox potential. This is partly due to sulphide which colours the sediment black, and also due to the iron bacteria that convert ferric iron to magnetite, which is black.

You can see a convincing demonstration in the lab. One of these two test tubes (top of next page) has iron reducing bacteria; the other has none. The magnet pulls the magnetite they have formed to the side of the tube. It makes one wonder how people have overlooked this. In the test tube, you can see macroscopically that they have produced a magnetic end product. How do they transfer the electrons to the iron? I can tell you that even Lovelly hasn’t got the answer yet. Many people have had a shot
at it, but there is disagreement in the literature. It is not clear how the bacteria manage to transfer the electrons to the insoluble ferric iron. There were ideas, for example, that they solubilize by using complexing agents, maybe they physically attach as in bioleaching environments and sort of zap the electrons over with a little wire, or something. There are lots of resources going to try and understand this phenomenon.

Excretion of electron-shuttling species is very topical. It has been shown that some species in the environment, such as humic acids, which are typically present in groundwater environments, are suitable electron mediators. It has been shown that if you give the bacteria and the ferric iron humic acid, immediately the electron flow becomes quicker. The bacteria can just reduce the oxidised humic acids, and the humic acids, once reduced, can chemically react with ferric iron. At one stage, I thought this was the answer, but no, the bacteria can do without the reduced humic acids.

### How do IRB transfer electrons to insoluble iron?

<table>
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<th>Ideas:</th>
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<tr>
<td>• solubilise iron (complexing, acid dissolving, etc.)</td>
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<tr>
<td>• physically attach to ferric iron</td>
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<tr>
<td>• excrete electron shuttling species (mediators)</td>
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<tr>
<td>• use e-carriers present in environment</td>
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<tr>
<td>• humic acids (quinone, analogue to NADH)</td>
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The species that do this are typically aromatic. There are simple differences in oxidised and reduced aromatic species. Consider hydroquinone and quinone; The latter carries two electrons and the bacteria can use them. Just out of interest, in terms of the origin of life, this is the type of compound we have in our cells that mediate the electron flow in mitochondria from A to B. The humic acids and the electron carriers we have in our internal body are chemically quite similar to these compounds.

If you have chlorinated aromatics in the soil, Geobacter can reductively dechlorinate, which means they respire the chlorine. They use the compound containing chlorine as an electron acceptor and reduce it, passing on and gaining from the electron transfer via chlorine respiration. I am involved with a Sydney site at the moment where there are 50 to 70-year-old solvents leaking. The DEP asked them to clean it up. Geobacter can deal with these chemicals. If Geobacter is present with other bacteria, it can pass on electrons. Again, it is not clear how that is done with both bacteria coexisting.

Just yesterday I looked at the Web to see if there was anything interesting about Geobacter. I will show you some of the headlines I found.

First headline: Bioelectricity. If Geobacter can transfer electrons to inorganic surfaces, why not to a carbon anode and generate electricity? Obviously if an electron is transferred, one should
be able to use this electron, provided it has enough oomph through a little bug. With outback batteries, the driving force would be organic waste. With the waste being oxidised to CO\textsubscript{2}, the electrons are transferred to a carbon electrode and make electricity. \textit{Rhodoferax} is the species suggested for this purpose. It is a Geobacter that can use sugar. The process shown is not mine; it comes from Lovelly’s lab at the University of Massachusetts.

\textbf{Geobacter Headlines (Geobacter.com)}:

\textbf{Bioelectricity}

If Geobacter can transfer electrons to almost anything, why not to a carbon anode?
Electricity generation by Geobacter
Outback batteries
Driving force: Organic wastes
Key: Sugar degrading Geobacter type (\textit{Rhodoferax})

Fuel cells have been demonstrated before where there is a bacterium and a mediator, but the cells are rather ineffective. Lovelly has the bacterium sitting right on the surface; Images show that the anode is coated with a film of Geobacter. Perhaps the electron flow is generated from one half cell to the other. There are photos of a red LED glowing, due to the redox difference between an electrode in a tank of Boston sludge plus a bug and one in a tank of clean water.

Next headline: Uranium.

\textbf{Geobacter Headlines 2: Cleanup of Uranium}

Geobacters capability of reducing other metal species includes uranium.

By reducing U(VI) to U(IV) which is less soluble, limitation of contamination has been applied in situ (2003) Tod Anderson

In nature, uranium is often present. Uranium (VI) is the more oxidised form than uranium (IV). The Geobacter don’t mind if you don’t give them ferric iron, they will take uranium and reduce it.
doesn’t remove the radioactivity or the uranium, but it makes the uranium less soluble, and it sticks in the soil and is less likely to pollute groundwater.

Here is another headline from last year: Hottest bug found so far.

**Geobacter Headlines 3: Hottest Bug Strain 121**

Geobacters’ presence of deep ocean vents (black smokers) has been shown.

- Temperature tolerance to 121 degrees C (autoclave).
- Interesting Genome.
- Suggestions of iron reducing Archeae to be one of the oldest lifeforms rather than sulphur reducers.
- Very old magnetite formations are seen to support this view.

Speculation about the origin of life has revolved around sulphur metabolism, the ocean deep vents. I think that previously 112°C was supposed to be the hottest an organism could survive. If you put *Pyrodictium* in the autoclave for 20 hours, you find it has multiplied during that time. I don’t know why I haven’t used that in my lectures to cheer up the students; it pushes the edge as to the tolerance of life. They call this Strain ‘121,’ which means, to anyone who works with thermophiles, the magic temperature used in autoclaves by microbiologists to kill all bugs. It comes from hydrothermal vents, which not only have organisms that use sulphur metabolism and methane but also iron species. The strain is an archaea, so it is close to other sulphur organisms. One might question why they might be regarded as one of the oldest lifeforms. Magnetite formations are geologically very old and are now proposed to be old biological magnetite. I am not expert enough to comment on this. That is what the strain looks like, fairly round with flagella on one end (left).

Three pictures summarise the bacterial activity involving sulphur and iron. The first picture (left) is a little sulphur cycle. The sulphur-oxidising bacteria use oxygen and produce sulphate; below are the sulphate-reducing bacteria bringing it back to sulphide. This is not perpetual motion, going backwards and forwards. It is driven by the lower bacteria getting some organic substance, and the upper ones getting some oxygen. Overall, it is actually driven by oxygen and organics.

If we switch the chemicals and put iron here, we have the iron cycle in a miniature form, as in the second picture (bottom, left).
The third picture (right) shows the combined effect with metallic iron corrosion with the ferric iron reduction. It involves metallic iron, an electron donor, and a sulphate-reducing bacterium, an acceptor. The bacterium lives on it and produces sulphide. The sulphide is renewed by a sulphide-oxidising bacterium. The ferrous iron can be used by iron bacteria, oxidising into ferric iron without oxygen, as a rusticles bug. The bacterium would be a Geobacter species.

Questions remain. The question that I asked Roy is this: the ferrous iron-oxidising bugs in the second picture get one electron-worth of energy, not as much energy as bugs would get from elemental iron to ferrous ion (Fe → Fe(II)), which is 2 electrons-worth. Even better bugs might get the whole 3 electrons-worth (Fe → Fe(III)) because they get iron from 0 to +2, so the whole energy might be provided in a rusting piece of iron...there is more energy in the step from metallic iron to ferric than from ferrous iron to ferric. I would think that in a rusticle, most biomass forms from energy supplied by the metallic iron corrosion part, and my question is whether this occurs in rusticles.

Q: Was 121° the highest temperature the bacteria could survive?

A: They tested 130°: it survived 130° for 3 or 4 hours, but it doesn’t grow much. This bug was only discovered last October. To me, 121° and growing is a bigger step than surviving. Maybe in transfer between planets you are more interested in what they can tolerate.

Q: What is the name of the bug in that slide (page 90)?

A: This one? It is a sulphate-reducing bacterium, Desulfovibrio. Next, I expect someone to say, “We don’t think this bacterium converts the sulphide completely back to sulphate, maybe just to elemental sulphur. This bacterium then has no problem using elemental sulphur, so it will, naturally, produce sulphide. (Indeed, it is hard to see where the energy advantage lies.)

(The following are some comments from Roy)

That particular cycle, Fe → Fe(III), I would suggest, would be done by a consortium of more than one species. Sulphate-reducing bacteria love to work with iron bacteria. We can speculate that that was done by such a consortium. A general comment was, we talked a lot about sulphate-reducing bacteria in the oil industry. In Canada, the oil industry is becoming paranoid about corrosion from acid-producing bacteria. Over the years, I’ve been trying to define what an acid-producing bacteria really is. Once the pH threshold is past, how does it react to the SRB? A general comment on that is, when you look at the redox gradient, when you get down below minus 150 mV, the methane producers dominate (this is for the acetate). As soon as you get above minus 150 mV, the SRBs become dominant. When you go above that (there are two groups, one above the other) you then start to see the methane degrading.

About the electrical battery. If you go the reverse way and put electricity in on broad, mild steel, and this is what we did as part of learning how to grow rusticles; we found that we could use the consortium and drill through mild steel at 0.4 mm an hour. The bacteria are obviously, radically, removing the iron. Anodes they love, cathodes they hate. Whenever we used combinations of various organisms in rusticles they just went straight through it. I tend to work with communities rather than individual species. You can find much of this information on my website at dbi.com.ca.