Catalysis by Bacteria: Opportunists at Work.
A Contentious Story

Abstract

Looks at the ways catalysis can occur through the action of bacteria. With billions of years of evolution, bacteria have taken every opportunity to direct and optimise the reaction coordinate. One model is a staircase with step sizes, shapes, and direction such that there is piecewise control over the whole reaction. Steps of a single size are appropriate; the effect of a series of reactions of smaller activation energies is explored; an optimal number of steps is found, with an individual step being RT.

Specific data on Fe(II) oxidation and inoculation with ochre sludge were mined (from Stevenson, 1991); the data are for a controlled pH of 5.8, oxygen at 0.023atms and without nutrient limitations. It seems that 3 to 5 steps are involved in increasing the oxidation rate 3.3 to 7.6 times.

A catalytic kinetic model involving protein as a surrogate for cells is used to fit the data. Two simultaneous differential equations evolve and are fitted to the initial and final concentrations of Fe(II) and protein. The observed trends are incompletely fit by the model but the parametric values give some insight into bacterial catalysis. The fractional mass increase in oxidation of Fe(II) per µg/litre of cell protein was around 0.7 at low protein concentrations.

This is an adventure: just about everything I have done here has been contentious. By no means am I a biologist, nor a very applied hydrologist. Keeping that in mind, I have been teaching groundwater hydrology for 20 years, but teachers tend to talk about things rather than do. While other people have been doing, I have been exploring and playing with mathematics.

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<td>Ralph &amp; Stevenson</td>
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<td>Trial &amp; Error</td>
<td>Fe(II) =&gt; Fe(III)</td>
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<td>Regular steps</td>
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I have two looks at some data, which, for the most part, comes from David Ralph and Judith Stevenson who worked on this around 1990 - 95. A little story here, because it shows the diverse linkage that exists. Without knowing that David was working in this particular area, only that he had an interest in bacteria, and also knowing Ralf Cord-Ruwisch was in the iron oxidation area, I went to the library and asked for a search on a few words. When the printout arrived, there were two theses on the top of the list, honours theses from Murdoch around 1990. As far as I know, Ralf Cord-Ruwisch didn’t really know about David Ralph, or vice versa, and I certainly didn’t know about either one, and these theses are fantastic bits of work.

My talk is going to look at a theory about organisms as opportunists, at populations of organisms, consortia of organisms. A lot of my background is in physical chemistry, and I looked at it as a physical chemist; I really don’t care if there are organisms there or if we need to identify any. I am only saying, “What would they do?” if we are looking at this idea thermodynamically. That’s all. They
have had billions of years of evolution to work on it. As far as I am concerned, they will get it perfect. I can’t believe that some organism working by trial and error, the most effective tool for learning, won’t have gotten something right after a billion years, or close to right. If it is possible to get it right, they will. What will they do? I put forward the hypothesis that whatever they do, they will do it in regular steps.

I can make some predictions of what I expect in terms of physico-chemistry in regard to bacteria and oxidation, mostly of iron, Fe(II) to Fe(III), but it could be almost any situation. I have some data on Fe(II) to Fe(III) oxidation from Ralph and Stevenson. They did an experiment on the sterilised oxidation of Fe(II); then they added some bore sludge and looked at the oxidation rate as enhanced by the ochre. I don’t know the reasons, but there must be some real objection from any scientist to bore sludge. It’s nasty and they don’t want to deal with it. David seems to be one of the few people in the world who has taken a sterilised environment and added a bit of yuk to it.

Anything that exists in the world, chemicals, molecules, must exist in this environment: they are sitting in a well of energy and they have to have a dike around the outside. If they don’t sit in that well, they will fall out and become something else; they will lose their identity. If they do lose their identity, they no longer exist. In this simplest of models, they must live and have their existence in such an energy environment. This is just a picture, of course, of a situation of extreme complexity.

Further, consider the energy requirements for change, the ‘activation energy’ for reaction. Strictly speaking, this is not thermodynamic energy - this is an extra energy to get out of the well, to get over the dike: from the outside, to get inside, to change things around, make it into something else. It is a requirement for extra energy, an activation energy. We take hydrogen atoms and add them together; they are living in this well with a dike around it. When they come together, there is a problem. We have added both of these dikes up in terms of energy and now we have a double hike there in the middle for the atoms to get together. We get them together eventually and they meld together and are back in another well with a dike. The reaction is complete; the combining process has to go through an ‘added energy’ or ‘activation energy’ state. The energy indicated by the green arrows is thermodynamic energy generated or consumed during the ‘equilibrium reaction.’ The atoms have to have extra energy to get over the hump, as shown by the white arrow. Often, this energy is huge, independent
This has been described variously in history. Henry Erying, in the 1930s, created this Reaction Coordinate. The diagram (previous page, bottom) shows the ‘reaction coordinate,’ the energy states during the reaction process. Note the requirement of an extra energy in pulling the reactants together, the activation energy. For the reaction to occur we must get over the activation energy hump; afterwards, we can ‘roll downhill,’ releasing energy while making products, different compounds. I think the best analogy might be with scrambling eggs. You start out with eggs and they are in one form, but they are not going to remain in that form. You use some energy to scramble the eggs, mix them up, then you fry them so you can eat them. They are still eggs, but in a different form. Scrambled eggs don’t just happen on your plate; you have to work at it. The Erying Coordinate goes from here to there - it is all conceptual; it is not something you can easily plot. It gives you a logical mechanism for getting there.

The reaction proceeds by going over this hump. Another analogy: we are going up a mountain. We will step up the mountain and down the mountain to get to the other side. Isn’t it a little easier to go up a mountain if there are steps? Now, these won’t be real steps: they will be energy steps, but the bacteria are going to make some kind of steps. A stepped process would be a good one. Each little step is easier to do, you have more control, you won’t fall so easily, you can stop and rest. You can go in different directions. You can create intermediate chemicals which you can use in your metabolic processes.

This is one of the little things that came out of a biochemistry book I looked into (bottom, right)*. It gives you some idea of metabolic processes in the substrate in oxidation, free energy that you can use. You can see that organisms or the biochemists are thinking of steps. There are steps in useful energy, free energy.

Now I will frighten you with equations. We go through a simple computation and the simplest case of equal steps. If you accept steps and that it is easiest for them to be equal - the reason I am saying equal steps is that if there is one big step, and it is an energy step, it will become dominant and

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The boxes to the left show the sort of energy equation we are looking at. I have written it as enthalpy rather than free energy, but it can be free energy, if you like, as a logical concept. Each of the steps would divide that energy into n parts to make smaller energies with a final composition can give an optimal result, as an increase in the rate of reaction.

I am not going through the details, but we have smaller steps, all of equal energies. $\Delta H$ is divided by n. You add the steps up to find n, you will end up with an increase in the rate given by this equation. It turns out there is an optimal number of steps. With an optimal number of steps and an increase in the rate when you do a reaction with the beasties throwing sludge, you should be able to apply this equation. You can calculate the $\Delta H$ and the $\Delta G$ to see what the energy should be. The result can be checked by measuring the increased reaction rate with increase in temperature. We haven’t done a thermal experiment, but we get a thermal result. The logic is a little difficult; the picture shows the enhanced reaction rate and an optimal value. The different colours are for different values of $\Delta$.

The result is a reaction rate enhancement factor. I just picked some arbitrary numbers. Two competing effects produce the optimum or maximum. One is that you are lowering the energy for each individual step, which makes it easier; but there has to be a large number of steps. Each step should be equal, from the logic of it. Next, we go back and recalculate, based on data; the data comes in, mostly from David Ralph’s and Judith Stevenson’s work.

Ralph and Stevenson did an experiment with inorganic reactions of Fe(II) going to Fe(III), measuring the reaction rate and then putting a bit of sludge in the reaction vessel, keeping everything constant: substrate; nutrients; temperature; pH. The reaction rate increases with the addition of sludge. I put together a model which predicts the change in the iron concentration with time and, secondly, predicts the cell formation rate change. The graphs shows a kinetic model for cell protein; they measured cell protein as a surrogate for cells or cell mass. The model allows that two things run side by side, two differential equations that are reasonably soluble. They proceed to predict how the iron changes with time.

Assumptions

- No substrate limitations--plenty of nutrients
- Nutrient concentrations held constant
- Protein concentrations are indicative of cell concentrations
- Competition, predator/prey, and death effects are small
and the cell protein changes with time, simultaneously. And it somewhat fits the data, and I can go ahead and say how many steps were involved and what activation energy was also involved.

There are several arguments here. Firstly, you are looking at the pressure of oxygen as an important factor. Iron concentration is a factor. Any reaction has to be more or less proportional to the amount of material, the probability of finding it present. The more material, the more reaction is possible; a general chemical principle. Reaction rates increase with the amount of oxygen; we are just oxidising ferrous iron. There are two possibilities of hydroxyl species; ions, perhaps. If you wipe out the term next to the one, the equation becomes appropriate for a sterile situation; the other term is for the activity of the bacteria. The graphs show the concentration of bacteria in terms of their protein. The iron and protein interact to produce the coloured curves. The top graph shows the concentration of iron as a function of time. The bottom graph shows the protein generated by the bacteria. The bacteria are introduced at the start and grow. Only the iron concentrations are plotted here, not the bacteria. The iron is oxidising and going away as a function of time. The curves are drawn for different values of kp; kp is just a parameter, the effectiveness of the bacteria. There is a hint of a twist in the curves. It doesn’t fit a single value, so it is not a good model, but it does give an idea.

At long times, the enhanced reactivity is better than would be expected; a simple, first-order reaction rate constant, itself, is not a good fit. There are population effects - the bugs are more effective than you would expect. The mechanism is more complex, but it does work.

The lower graph shows the actual protein increase. These are similar curves to the above, but it was difficult to get even one value for the protein concentration to fit the model. There is one datum for the protein existing in the bacteria as a function of time. It is not possible to say much with only one data point.

Protein Production
• Integrated datum --> 1 protein measurement
• Little statistical validity
• suggests best value of kp is around 0.7
There is about a 70% increase in the reaction rate with the addition of sludge with a bacterial protein mass of 1 µg in a litre of solution.
It suggests that the best value of kp is about 0.7, which means one microgram/litre of sludge in the experiment creates about a 70% increase in the observed rate. One microgram of sludge protein per litre of solution creates a 70% increase in the oxidation rate.

In summary, bacteria are effective catalysts; the specifics and the mechanisms remain obscure. I have a longer, more extensive paper on this.

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**Q:** I am curious about the 70% reaction rate increase. It is pretty amazing, considering the small bacterial concentration. Isn’t there a bit of a circular argument? Don’t the bacteria catalyse the reaction and grow as a result of the reaction?

**A:** As you suggest, the effect is circular, interdependent and exponential. Firstly, the abiotic iron oxidation rate is used as a reference. The parameter kp is an indicator of biological activity, the percent of increase per microgram of protein per litre; it is about 0.4 at low concentrations of microbes but increases to 0.7. The effect is self-infecting, self-inflicted, recursive, if you like. It represents bacterial reproduction or perhaps new consortia forming; the net effect is an ever more effective reaction with time, more than is cared for with the mathematically linear set of equations I have presented. Our thinking and your thinking is linear; here we see something that doesn’t add up - it is synergistic.

David Ralph: It is a problem that we haven’t redone the experiments at different temperatures. Also, the protein estimation in the sludge samples was difficult--which explains the paucity of data--only one point. Your model with the steps in the activated complex concerns me. I am quite comfortable with the idea that the reaction progress shuttles the electrons between the donor and the acceptor, which may take place through a series of steps, intermediates or mediators. However, I see the effect more as a series of steps, each with their own activation energy rather than a cascade as you present it. I see the effect as a series of activation energies, each with their own activation energy rather than a cascade as it is presented. The presentation

**A:** I see each step as having its own little ‘hump’ in it, otherwise the ‘partially reacted reactants’ would not sit on the steps; the steps have to have a little ‘hump’, an energy well. What you say is correct, but I think the difference in our opinions is that you are always thinking of going down. The reality is, that doesn’t happen. Every time the reaction proceeds and the reactants make their way along the reaction coordinate, they go through a large activation energy in-between. The large energy in-between is what I worry about. An efficient mechanism would divide the energy into steps: that is one point. One other point is that the chemical species can go up and down. They might go up and down several times, as well. When they do that, the most effective way is with equal-sized, controlled steps.

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**Conclusions**

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<tr>
<th>Arrhenius/Erying</th>
<th>Fe/Protein Model</th>
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<tr>
<td>• Time-tried, general</td>
<td>• Intermediate protein levels needed</td>
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<tr>
<td>• Equal steps optimal</td>
<td>• Complete data sets</td>
</tr>
<tr>
<td>• ΔG or ΔH</td>
<td>• High order at long times</td>
</tr>
<tr>
<td>• Optimum number</td>
<td>• pH, O₂, and T need to be considered</td>
</tr>
<tr>
<td>• May estimate activation energy</td>
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**Bacteria are Effective Catalysts--specifics and mechanisms remain obscure!**

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David Ralph: It is a problem that we haven’t redone the experiments at different temperatures. Also, the protein estimation in the sludge samples was difficult--which explains the paucity of data--only one point. Your model with the steps in the activated complex concerns me. I am quite comfortable with the idea that the reaction progress shuttles the electrons between the donor and the acceptor, which may take place through a series of steps, intermediates or mediators. However, I see the effect more as a series of steps, each with their own activation energy rather than a cascade as you present it. You seem to come up with a number that is close to the number of components in the electron chain - a rather unlikely juxtaposition. I have some lack of comfort with the way in which you represent each of the individual steps, stepping up the activated complex. I am comfortable with the idea that the electron energies are staged - they first go to a slight state of activation, then are handed along to another activation state, shuffling right through the way to oxygen. I am not yet at the stage where I can marshal my arguments.

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Ralf Cord-Ruwisch: It seems you have made first order assumptions throughout your formulations, with arbitrary kinetic factors. From a biologist’s point of view, how can you justify this? Don’t you have limitations to growth that must be cared for?

Bill: You are talking about a Monod technique of assessing limitations to growth, usually producing a sigmoid growth curve. In the experiments of Ralph and Stevenson, I believe the data were collected at low concentrations, without limitations. The chemical reaction model that is put forward is all done at low concentrations or significant excesses of substrate or reactants so that there is no limitation, except for Fe(II), which has its own balance and may be depleted. This general model, with simultaneous interactive differential equations, can and will produce limitations, through substrate depletion. In the experiments and the fitting process with limitation, we would see a tapering off in the reaction rate. In the actual experiments, the rate actually increased with time. David, do you know if you ever got to what you would consider high concentrations in the substrate or the protein?

David Ralph: Not in these experiments. Our major drive was to detect the acceleration that you get from the sludge. Could there be a sufficient increase in the kinetic rate to precipitate the amount of ochre in the area of a bore so as to significantly affect the pumping rate within a couple of weeks? The sterile background reaction rate found in the literature couldn’t cause such clogging. There had to be some form of acceleration.