GEOMICROBIOLOGY AND MICROBIAL GEOCHEMISTRY: A VIEW FROM THE PAST

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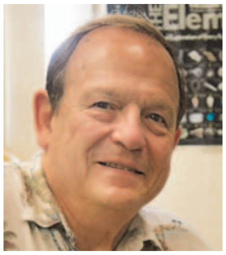

It might be a useful exercise to begin this overview of geomicrobiology and microbial geochemistry, or GMG (aka geobiology), to pose the question, "What has GMG done for me (or anyone else)?" The answer from my perspective is, "A huge amount of good!" Forty years ago there was no GMG. None of the techniques and approaches that we now take for granted were available, and most of what is highlighted in this issue was not even imagined. As you peruse the articles, you will find in each the excitement of making discoveries at the interface between various aspects of microbiology and geochemistry. A new type of young GMG scientist has emerged, unafraid of interdisciplinary work and open to collaboration with previously "unavailable" colleagues. For every one of the articles here (each packed with "good things") several others from this burgeoning field could have been added. Now for overview!

In 1977, after a few years as an assistant professor at Scripps Institution of Oceanography (University of San Diego, California, USA) (SIO), I presented a seminar on the subject of bacterial regulation of bioluminescence. After the talk, Ed Goldberg, a crusty (to be polite) marine geochemist came up to me and said, "Very nice and interesting work: why don't you do something important?" Given that all the advice I had received from Ed in my first four years at SIO had been good, I responded, "What would you suggest?" Ed's answer was, as always, a bit irreverent, but to paraphrase, he said that the geochemistry of neither iron nor manganese in the ocean was well understood, and he suspected it was because of bacterial activities. This conversation, along with discussions with Gustav Arrhenius (a geologist at SIO) and a chance meeting in Israel with a group of microbiologists that included Bo Jorgensen, Yehuda Cohen, and Wolfgang Krumbein (who all have excelled in various aspects of GMG), convinced me to join in the geobiological fun. I set up a series of enrichment cultures for manganese-oxidizing bacteria ("The Nealson Museum") and continued working on bioluminescence until I received tenure (sage advice from Professor Arrhenius). In late 1978, the enrichments were opened and the fun began—and has continued until today.

I mention this scenario because so many early developments in GMG arose through similar interactions between microbiologists, geologists, and geochemists. In a young field, colocation of potential colleagues can be of great importance, and being on the same campus with several marine geochemists and geologists was key to my own progress in geobiology. It was surprisingly easy to find students who wanted to work at the "microbe–mineral interface," as it would soon be called: one of whom has written a textbook on marine sediment geochemistry (Burdige 2006); another who is a coauthor of an article in this issue (Hansel et al. 2015 this issue).

But the transition was difficult: most microbiologists had not been properly trained for geobiology. In general, it was far easier and safer to do reductionist studies of an organism that had a name and grew well in culture than to isolate an unknown microbe that was difficult, if not impossible, to grow and that was equally difficult to identify. To put it bluntly, microbiology was not up to the task. The revelation of the so-called "great plate count anomaly" made it clear that much of our microbiological world resided in the area of the unknown: in most environments, only 1% or less of the organisms seen in a given sample were able to be cultivated (Whitman et al. 1998). This wasn't news to most of us, it was just the first time anyone had openly admitted it—microbial ecology was being done with only a few percent of the microbes. New techniques were needed!

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What was not clear in the mid-1970s was that a great explosion (in techniques and approaches) was about to occur. There were changes taking place that had the potential to deliver us from the "dark ages" of environmental microbiology, previously characterized by limited abilities to identify microbes and by a lack of knowledge of the basics. The coming changes (outlined below) would be major enablers of the progress in GMG.

WHERE DID GMG COME FROM?

New fields arise and grow for several reasons, but I like to view it as a three-phase, interactive process, with positive feedbacks between all phases. In the case of GMG, those asking the questions had neither the academic training nor the technology to answer them. The questions come from one field (phase I), and the answers come from bringing new skills and techniques to what becomes a new field (phase II). In the case noted above, as is often the case with geobiology, the question was one of kinetics—rates of metal oxidation and reduction far too fast to be explained by geochemistry. Altering kinetics is perhaps a microbes' greatest forte—using enzyme catalysts that are both specific and very effective. Phase III is the resulting technology and methods that are developed in response to the newly generated questions, allowing more questions to be asked and, in turn, answered. To summarize this:

PHASE I: One field, geochemistry in this case, identifies problems for which it is unprepared to solve, either with regard to academic training (ideas), or experimental methodology, or through experimental approaches.

PHASE II: A second field, microbiology in this case, which was not aware of the questions, embarks on a new kind of science using the known array of ideas and techniques. Some answers are provided; these generate other questions; and the need for new methods and new technologies (and sometimes new approaches) is thereby created.

PHASE III: Developments in both technology and methodology provides higher resolution and new abilities, driving the science forward, and making it possible to ask (and answer) new questions.

Other drivers included the discovery of things so new and unexpected that many recruits (young and old!) were attracted to the interdisciplinary world of environmental microbes in ways that couldn't have been predicted. A few examples of such discoveries make the point:

1. Extremophilic microbes that live under conditions of temperature, pH, salinity (and so on) previously thought to be impossible.
2. The hydrothermal vent communities with symbiotic chemolithotrophic bacteria forming the base of food chains in the deep dark ocean.
3. Quorum sensing, and the ability of microbes to communicate with one another.
4. The formation, properties, and ubiquity of bacterial biofilms.
5. Anaerobic methane oxidation, thought to be impossible.
6. The longevity of microbial DNA.
7. The extent (and strangeness) of the microbial world in the deep subsurface.
8. The ability of bacteria to use solid substrates (metal oxides or even electrodes) both as electron acceptors and/or donors.

Another, albeit indirect, driver of the geobiological revolution was the development of new methods for **molecular taxonomy and phylogeny**. As discussed by Dick and Lam (2015) in this issue, Carl Woese (in the late 1970s) devised, revised, and popularized the beginnings of what we now call molecular taxonomy/phylogeny, resulting in the widespread use of the 16S rRNA gene sequence as the universal microbial "dog tag" (Woese and Fox 1977), and the establishment of the three domains of life (and the establishment of the domain Archaea) (Woese et al. 1990; Woese 2004). The many Woese "disciples" (Norm Pace, Steve Giovanoni, Ed Delong, and others) have forever changed the landscape of microbial taxonomy, using fluorescence in situ hybridization (FISH) probes of rRNA to locate, identify, and quantify microbes in situ. Furthermore, as more samples were examined, it became clear that many of the numerically dominant members in most environments were not yet successfully cultivated. Yikes! ... we were surrounded by uncultivated microbes of unknown function!

GENOMICS AND THE "OMICS" REVOLUTION(S)

As sequencing approaches continued to improve, it became clear that microbial genomics would be possible, and several major efforts were put forward to sequence genomes of both archaea (because they were very small), and bacteria (particularly pathogens). The first geobiologically relevant bacteria to have their annotated genomes displayed to the public were from two metal oxide–respiring microbes, *Shewanella* (Heidelberg et al. 2002) and *Geobacter* (Methé et al 2003). This involved several years of work, and cost more than a million dollars each! Compare that to today's technology, scarcely more than a decade later, and it is mind-boggling that microbial genomes can now be sequenced for thousands of dollars and done only in days!

The perspective of seeing an organism's "operating system" laid out gene-by-gene was remarkable to us old-timers, and the follow-on studies of transcriptomics, proteomics, and now, metabolomics have impacted all fields of microbiology. That this can be done in mixed cultures to the "meta" levels of all parts of "omics" simply could not have been imagined only a few years ago (Dick and Lam 2015).

Other new technologies and approaches have also been drivers of the GMG revolution and have been combined with modern molecular taxonomy/phylogeny and the "omics" approaches. Some of these are outlined below:

1. Development of stable isotope fractionation technologies for a wide range of nontraditional isotopes including metals.
2. Development of stable isotope "probes" for the identification of active members of microbial communities.
3. Nano-SIMS technology for the analysis of single-cell activities.
4. Microelectrode approaches for the study of community properties and activities.
5. Development of many variations of FISH probes and technologies.
6. Incorporation of synchrotron methods for analysis and imaging.
7. Development of deep drilling technologies and improvement of drilling ships for sample acquisition and analysis.

CHALLENGES AND PERSPECTIVES

There are many problems (old and new) for which answers are being obtained, with evolving technologies providing new levels of resolution and understanding. But what are the important challenges in GMG? There are several large gaps in our knowledge that could stall or stop progress in the field. Five questions that need answers are the following:

1. **What are all these "noncultivable" microbes doing?** Are there metabolic niches (and new metabolisms) that we don't understand, or are they simply surviving and waiting for a different or better environment to come along?
2. **What is going on in the deep subsurface of our planet?** Work in the last decade has suggested that a huge biomass of microbes resides in the deep subsurface, surviving under conditions of severe nutrient limitation.
3. **What are the role(s) of microbes as members of the microbiomes?** Probably every plant and animal (including humans) contains specific bacterial cohabitants (in our case, several hundred bacteria for each of our own cells) called the microbiome. The same is almost certainly true of every GMG-relevant environment, with its complex microbial communities. But what are they doing?
4. **What are the factors that lead to stable microbial communities, from biofilms to sediment populations?**
5. **What are all the genes of unidentified function doing in microbes?**

Each of these questions will, of course, generate many more specific questions that will keep GMG scientists occupied for many years to come.

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