

## MDAG.com Internet Case Study 36

### Microbial Effects on Minesite-Drainage Chemistry

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“As large animals, we can be forgiven for holding a worldview that celebrates ourselves, but, in truth, this outlook is dead wrong. We have evolved to fit into a bacterial world, and not the reverse. Why this should be is, in part, a question of history, but it is also an issue of diversity and ecosystem function. Animals may be evolution’s icing, but bacteria are the cake.” (Knoll, 2003)

#### Abstract

Our Earth encompasses wonderfully complex combinations of inorganic and organic processes. Thus, questions arise on how organic processes, particularly microbial activities, affect inorganic aspects of minesite-drainage chemistry, like pH, aqueous metal concentrations, and sulphide-mineral oxidation. From one perspective, inorganic minesite drainage should reflect microbial activity to some degree. From another perspective, inorganic drainage chemistry may be mostly independent of microbial activity.

This MDAG.com Internet case study examines both perspectives. This included: the simplistic recognition of predator-prey relationships, the historical context of bacterial contributions back to the 1920's, the exaggeration of bacterial acceleration of iron oxidation a million-fold in the 1970's, and recent DNA and proteomic studies of microbial populations.

We conclude that detailed studies of microbial populations in minesite-drainage chemistry are still rudimentary and sometimes misinterpreted. However, this is not a barrier to environmental assessments and predictions. We note that (1) microbes are always present in our environment, (2) microbial effects on full-scale aqueous chemistry cannot be reliably isolated and quantified at this time, and (3) microbes cannot be eliminated from minesite components. In other words, microbial activity can be considered an ever-present and ubiquitous factor in minesite-drainage chemistry. Microbial effects are already typically included in empirical testwork and are actually difficult to exclude. Where explicitly considered, microbial effects can lead to equilibrium chemistry even in relatively fast-flowing subsurface systems.

## 1. INTRODUCTION

There is no doubt – we live on a biotic planet. Life is an integral part of our Earth, and is found in seemingly unlikely places. Life is found:

- at sub-zero freezing temperatures (e.g., Schmidt et al., 2009; Laybourne-Parry, 2009);
- in high-salinity brines from subsurface aquifer seeps, in deep-sea brine pools, and ancient subglacial brine (e.g., Boetius and Joye, 2009; Oremland et al., 2005);
- at deep-ocean metal-rich “smoker” vents at high pressures and temperatures (e.g., Le Bris et al., 2005; Sarradin et al., 2008);
- on contaminated sites with chlorinated hydrocarbons (e.g., Suthersan and Horst, 2007);
- up to kilometers deep beneath the earth’s surface (e.g., Goldscheider et al., 2006; Lin et al., 2006); and,
- at extreme levels of acidic pH, alkaline pH, and aqueous metals, with some levels having positive effects (e.g., Baker et al. 2006; Bond et al., 2000; Druschel et al., 2004; Edwards et al., 2000; Oremland et al., 2005; Roadcap et al., 2006; Sprocati et al., 2006; Walker et al., 2005).

For minesites in cold climates, these examples alone would cast doubt on the sometimes assumed inactivity of microbes near and below freezing temperatures, and the associated lack of reaction and leaching. In fact, these assumptions for minesites have been known to be wrong for several decades (e.g., Cameron, 1977; Dawson and Morin, 1996; Morin, 2003; Elberling et al., 2000; Elberling and Langdahl, 1998), but they still persist.

Because of the ubiquity of life on this planet, the interplay of organic and inorganic processes should come as no surprise. For example, organic compounds like amino acids have been found with minerals like jarosites (Kotler et al., 2009). Also, the formation of secondary minerals like ferric-iron oxyhydroxides can be determined by interactions of biological activity and aqueous chemistry (Laresse-Casanova et al., 2010).

Oxalate ( $C_2O_4^{2-}$ ) is a relatively simple, primarily organic ligand, which forms aqueous species and solid-phase minerals. Ignoring its presence when significant can cause deviations from calculated aqueous speciation and associated mineral saturation indices based on inorganic information (i.e., no oxalate analyses) with geochemical models like MINTEQ and PHREEQE. These same deviations can arise from the simple adsorption of aqueous ions by bacterial cell walls (e.g., Wightman and Fein, 2004).

Also, arguments are made that minerals like dolomite and barite are biominerals because they can be formed by microbial activity (Lubick, 2002; Senko et al., 2004). Such organic effects do not preclude equilibrium inorganic drainage chemistry (Turner and Fein, 2007), but can cause inorganic equilibrium to reflect site-specific conditions (Morin and Hutt, 2007).

However, our ability to isolate and identify microbial effects reliably, separate from inorganic effects, is limited. For example, even stable isotopes can be inconclusive (Balci et al., 2007; Croal et al., 2004; Kappler and Newman, 2004).

Therefore, a double-sided issue exists about how biological activity affects inorganic geochemistry and minesite-drainage chemistry. On one side, there logically can be some effects, but how are they incorporated into drainage chemistry if significant? This can be addressed

empirically by macroscale testing at minesites (Morin and Hutt, 2007), where local biological, inorganic, and other effects are naturally included. But what about laboratory-based testwork? What about computer-based geochemical models?

On the other hand, does inorganic chemistry inherently reflect organic processes? For example, because jarosite can be “microbially produced” on earth, is it a “clear biomarker” for life on Mars (Norlund et al., 2010)? Answers to these require the discussion of some basic biological factors. These show that some authors seriously misunderstood biological effects at minesites.

We have discussed these issues in previous Internet case studies (Morin and Hutt, 1998 and 2000). However, new information such as DNA and proteomics emphasizes some observations, and a detailed review of historical work shows where some authors went wrong.

## 2. SOME BASIC OBSERVATIONS

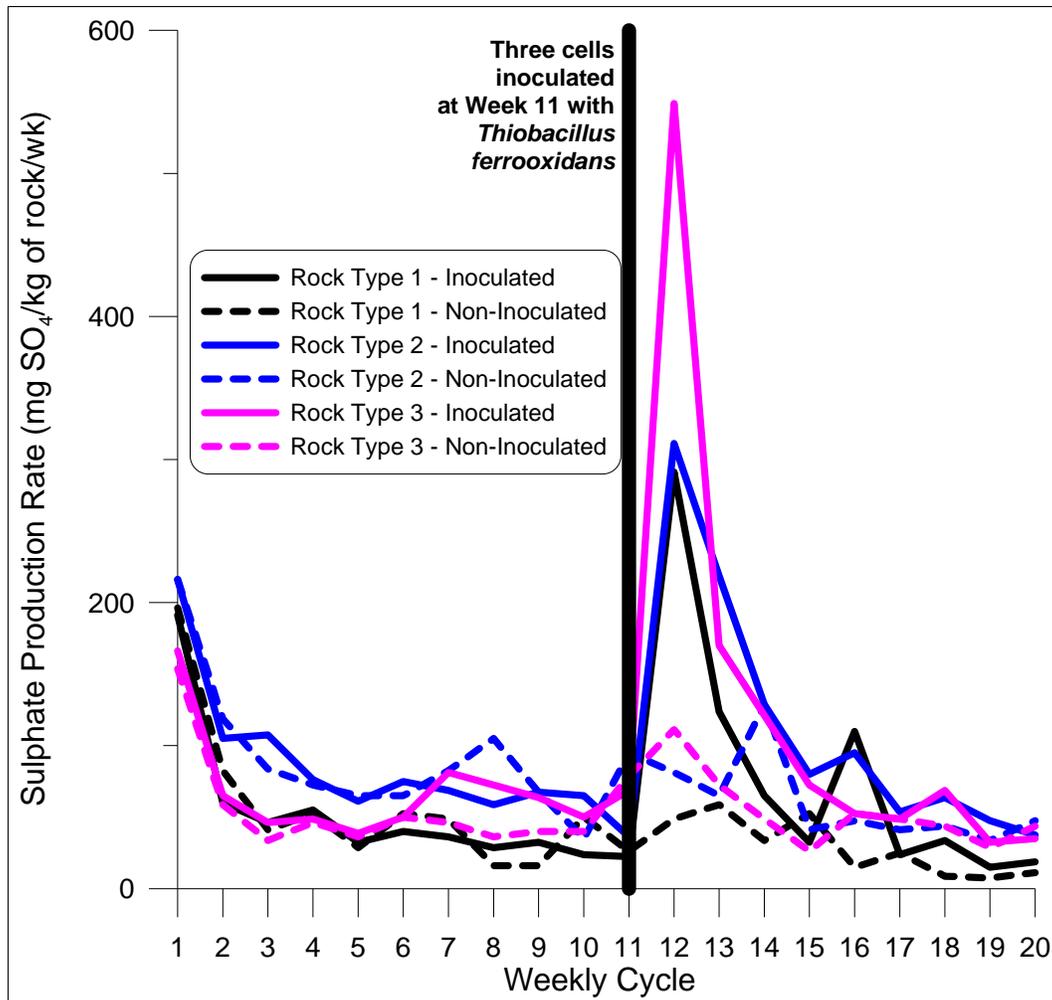
A more appropriate viewpoint for minesite-drainage chemistry is not whether biological effects can be isolated and added to laboratory-based testwork, but whether they can be reliably excluded. At hospitals, surgical operating rooms strive to completely eliminate all harmful microbes, sometimes with poor success. Rarely do geochemical laboratories conduct microbial-decontamination measures to the extent of surgery rooms. Therefore, the presence and contributions of microbes to laboratory tests should be expected.

“Bacteria have immense population sizes, and they can reproduce rapidly . . . Bacteria also track shifting environments with ease . . .

Bacteria are particularly good at doing nothing. When the surrounding environment is favorable for growth, bacteria multiply rapidly, as they do in your mouth. But when ambient conditions do not favor growth, they are able to persist in a dormant stage, with little expenditure of energy. Actually, most bacteria at most times may exist in a state of metabolic torpor, ready to spring into action the moment that resources become available.” (Knoll, 2003)

As laboratory tests and minesites operate, microbial communities and ecosystems form and stabilize. If this stabilization is disturbed, such as by the addition of more bacteria (e.g., Figures 1a and 1b), transient geochemical changes may be detected. However, the microbial ecosystem should then re-establish some stability and equilibrium.

Such stability in an ecosystem typically includes predator-prey relationships, where species’ populations are controlled by other species. In turn, this means that any biological effects from one species will be kept under some type of control and stability by others. Put more gently, one species may “farm” another for later food, like our raising cattle or fertilizing soil to increase crop production. We humans could add a phenomenal amount of fertilizer to a hectare of farmland and obtain phenomenal productivity. However, this is not a sustainable process – the soil will degrade and become mostly unproductive. So, like other organisms on this planet, we manage our predator-prey relationships and our food production for longer-term sustainability.



**Figure 1a.** Temporary effect on the sulphate production rate (representing the rate of sulphide oxidation) and re-establishment of equilibrium after humidity-cell inoculation with *Thiobacillus ferrooxidans* (from Morin and Hutt, 1997 and 2001, see also Figure 1b).

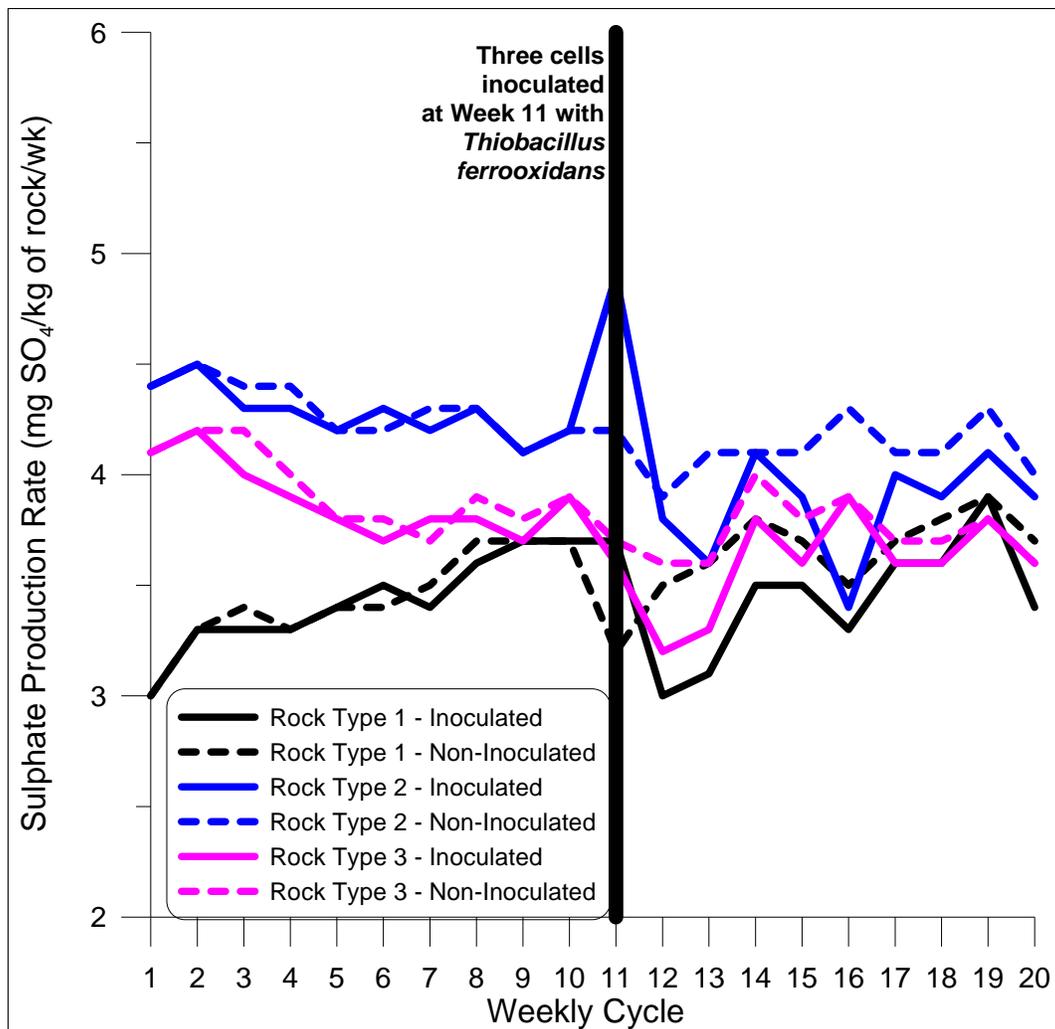


Figure 1b. Temporary effect on aqueous pH and re-establishment of equilibrium after humidity-cell inoculation with *Thiobacillus ferrooxidans* (from Morin and Hutt, 1997 and 2001; see also Figure 1a).

### 3. A SHORT HISTORY OF MICROBES IN MINESITE-DRAINAGE CHEMISTRY

The chemoautotrophic or chemolithotrophic mode of bacterial activity was reportedly discovered in 1890. This mode allows some bacteria and archaea to obtain carbon from carbon dioxide and energy from the oxidation of inorganic species and compounds, like sulphide and ferrous iron (Paine 1987). Unfortunately for minesite-drainage chemistry, the study of sulphur-iron organic-inorganic cycling became focussed on acid mine drainage (AMD). Similar cycles under other pH ranges and for other elements have thus languished, but are likely as important. Methylation of elements like mercury, selenium, arsenic, antimony, and bismuth is an exception (e.g., Domagalski 2001; Celo et al. 2006; Gray et al., 2006; Nimick et al. 2007; Suchanek et al., 2009; Chau et al., 1976; Doran and Alexander, 1977; Reamer and Zoller, 1980; Ranjard et al., 2002; Bentley and Chasteen, 2002; Thomas et al., 2004).

In 1947, chemoautotrophic bacteria, probably of the *Thiobacillus* genus, were identified in cultures of acidic minesite drainage by Colmer and Hinkle (Colmer and Hinkle, 1947). This *Thiobacillus* genus of bacteria was first described in 1904 and reclassified in 2000 as *Acidithiobacillus*. Colmer and Hinkle's "preliminary" paper is said by some to be the "most quoted paper indicating the bacterial role in AMD formation" (Paine, 1987). In our experience, the most quoted paper is the 1970 paper by Singer and Stumm implicating *Thiobacillus* and *Ferrobacillus*, as discussed below.

This brings us to the wildly distorted ARD story of *Acidithiobacillus*. In particular, one species was sometimes considered the primary cause of pyrite oxidation and ARD. This species was often *A. ferrooxidans*, also known by its older synonyms of *Thiobacillus ferrooxidans*, *Ferrobacillus sulfooxidans*, and *Ferrobacillus ferrooxidans* (U.S. National Center for Biotechnology Information: [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

The large body of publications and studies on this bacterium spans more than a half century, and would require a separate book to review. Put simply, this bacterium could reportedly accelerate pyrite oxidation by one million times. Few ever asked questions like:

- "It might be able to do that, but like over-fertilizing farmland why would it do that?"
  - "This bacterium often represents a small portion of the biomass, so what are the other microorganisms doing?"
  - "If it accelerated the rate that much and the microbial population thus grows exponentially, how are the accumulating waste products removed to prevent toxicity?"
  - "What is the evidence that the presence of bacteria is the cause and not the effect of sulphide oxidation?"
  - "In this biotic world, could we ever expect to see a phenomenally lower abiotic oxidation rate?"
- Meditating on these questions may lead to biological enlightenment!

As mentioned above, a 1947 paper by Colmer and Hinkle and another in 1970 by Singer and Stumm are reportedly the most quoted papers attributing ARD primarily to bacteria, particularly *Acidithiobacillus*. It is informative here to return to those references and closely check their evidence, and also to watch closely for the changing importance of iron-oxidizing against sulphur-oxidizing bacteria.

Colmer and Hinkle in 1947 offered overwhelming and convincing proof that ferrous iron would not oxidize to ferric iron without bacteria. However, for some reason, they weakly

concluded,

“In this work, a bacterium, unidentified as yet, has been found in acid mine drainage which is involved in the oxidation of ferrous to ferric sulfate. A second bacterium similar, if not identical, in its morphological, cultural, and physiological characters to *Th. thiooxidans* been isolated repeatedly from the acid mine drainage of some bituminous coal mines. It is postulated that this latter or an unknown similar organism is involved in the oxidation of the sulfur and the sulfur compounds to sulfuric acid.”

This does not say that bacteria cause ARD, just they are “involved” based on preliminary work, yet this is reportedly a primary paper demonstrating the bacterial role. In 1951, a paper by Temple and Colmer would name these iron-oxidizing bacteria, *T. ferrooxidans* (Temple and Colmer, 1951).

Furthermore, the 1947 paper depends on papers by Waksman and Starkey published in the 1920's in the Journals of Bacteriology and of General Physiology (Waksman, 1922a and 1922b; Waksman and Starkey, 1923; Starkey, 1925a and 1925b). These papers focussed on sulphur-oxidizing species, like *Sulfomonas thiooxidans*, and other species, *Thiobacillus thiooxidans* and *T. thioparus*.

Later, in a three-page paper in *Science* published in 1970, Singer and Stumm explained that oxidation of ferrous to ferric iron, rather than the previous work on sulphur oxidation by bacteria, was the rate-controlling reaction in acid generation from pyrite or marcasite. They explained that a field study, with no details or references, at a site in West Virginia, USA, showed that the rate of ferrous-iron oxidation in acidic streams was much higher than laboratory rates. Several catalysts were considered:

“The catalytic effects of sulfate, iron(III), copper(I), manganese(II), aluminum(III), charcoal, iron pyrite, clay particles and their idealized counterparts, alumina and silica, and microorganisms were investigated and compared in synthetic mine waters in our laboratory. Of these, microorganisms (11) appeared to exhibit the greatest effect in accelerating the oxygenation of  $\text{Fe}^{2+}$ . Comparisons between the rates of oxidation of  $\text{Fe}^{2+}$  under sterile conditions after inoculation with un-treated and with sterilized natural mine showed that microbial mediation compared accelerates the reaction by a factor larger than  $10^6$ .”

The “(11)” in this quotation is important, because it suggests the authors did not conduct much work on microorganisms, but were mostly referencing footnote (11) which said,

“Microbial catalysis of the oxygenation of  $\text{Fe}^{2+}$  by the autotrophs *Thiobacillus ferrooxidans* and *Ferrobacillus ferrooxidans* has often been demonstrated in laboratory systems [M. P. Silverman and H. L. Ehrlich, *Advan. Appl. Microbiol.* 6, 153 (1964)].”

That footnote refers to someone else's general paper on the microbial effects on the formation and degradation of minerals, within the optimal pH range of 3.0 to 3.6. Thus, Singer and Stumm did not specifically implicate *Acidithiobacillus* in their own work. Also, the exact basis for the iron-oxidation acceleration “by a factor larger than  $10^6$ ” is not clear, but as shown below no sulphide-mineral acceleration close to this has been reported by most researchers. Nevertheless, Singer and Stumm believed bacteria were the primary cause and concluded,

“The application of bactericides (16) should be a successful means of coping with the problem. Such application necessitates injection of the bactericidal agent into the system at a suitable location where the entire influent water can be treated. Strip (surface) mines and coal refuse piles are especially amenable to such treatment.”

Perhaps the credibility of a world-renown geochemist as a coauthor, and the paper's

publication in the prestigious journal of *Science*, account for its misconstrued larger-than-life reputation. In any case, we would estimate that hundreds of millions of dollars have been spent worldwide, based primarily on this paper, to study, predict, kill, and control *Acidithiobacillus*, with the ultimate objective of stopping ARD. Long-term success has not come from it. Yet the million-fold “Singer-Stumm model for pyrite oxidation in acidic solutions” is still in use (e.g., Caldeira et al., 2010; Sánchez España et al., 2005).

Field applications to minesite components of bactericides, particle coatings, toxic chemicals, bacteriophage, and other ideas have not stopped ARD (Doepker, 1988 and 1989; Erickson et al., 1985; Kleinmann and Erickson, 1981; Rastogi and Sobek, 1986a and 1986b; Zaburunov, 1987). Also, large-scale monocultures of bacteria like *Acidithiobacillus* have been cultivated for full-scale mineral processing using biohydrometallurgy to oxidize sulphides and recover economic metals (e.g., Brierley, 1982; Rawlings et al., 1999). However, these large bacterial cultures typically cannot be stabilized and maintained for long times, and thus have not met with widespread success. So bacterial contribution cannot be the primary cause of ARD, especially from a single species or genus. There must be more to this story.

Some have moderated the million-fold increase in iron oxidation by adding conditions, such as “Exponential growth occurs when there are no limiting conditions, and the rate of growth is limited only by the capacity of cellular synthesis for the specific microorganism” (Nordstrom, 2003). How often would such unlimited conditions be expected to occur in open environments like minesites? Never?

Furthermore, the rate of iron oxidation cannot be synonymous with the rate of sulphide-mineral oxidation in minesite drainage, because virtually all ferric iron would be “consumed” and converted back to ferrous iron. In reality, both ferrous and ferric iron are often detected in minesite drainage. This is consistent with the rapid ferrous-ferric redox couple accounting for measurable and stable Eh, but not with pyrite oxidation limited by the presence of ferric iron.

#### 4. CLOSER TO REALITY

During the *Acidithiobacillus* craze in the late 20<sup>th</sup> century, which seems to be rapidly dying out in the early 21<sup>st</sup> century, we talked with microbiologists who were frustrated. They knew that *Acidithiobacillus* and similar species comprised only a small percentage of the microbial biomass in minesite components. Also, the identified bacteria and archaea were mostly based on laboratory-based cultures. Many in-field bacteria could not be grown in laboratory-based cultures and thus could not be detected and identified (e.g., Goldscheider et al., 2006). They warned that the microbial contributions to sulphide oxidation and ARD (acid rock drainage) were thus not well understood, but these warnings were often ignored for decades. Even the microbial population of the human intestinal system is still not well known (Eckburg et al., 2005). Instead, a simple, one-dimensional, one-way, single-species cause-of-an-effect (ARD) was more popular. Now it is worthwhile to return to reality, recalling the previous discussions on predator-prey relationships and microbial ecosystems.

Despite the endearing popularity of phenomenal acceleration of sulphide-mineral oxidation by bacteria, aspects of Singer and Stumm (1970) were actually discredited shortly after its

publication, with others reporting accelerations by factors of only ten to fifty (Morth et al., 1972; Ohio State University Research Foundation, 1971a and 1971b). For that matter, studies in the 1950's, more than fifteen years before Singer and Stumm (1970), also showed that bacterial acceleration of pyrite oxidation and iron oxidation was only by factors of two to five, and thus not the sole cause of acidic drainage (Leathen et al., 1953a and 1953b).

More recently, studies under laboratory and field conditions with biotic and abiotic samples showed similar oxidation rates, some over a large range of pH from 1 to 8 (Nicholson, 1994; Van Stempvoort and Krouse, 1994; Kwong et al., 1995). These contradicted the widely believed million-fold effect of *Acidithiobacillus* at acidic pH. A compilation of ferrous-iron rates also failed to show reliable pH dependence (Figure 2). All this might have simple explanations, like the micro-environments around sulphide minerals are often acidic whatever the macro-scale pH, or this might have complex explanations not yet identified.

More recent techniques for microbiological characterization in minesite drainages include DNA-, protein-, lipid-, and enzyme-based methods. Based on these, we read statements like, “microbial communities in these environments are much more diverse than originally thought”, “contrary to previous interpretations”, “most microorganisms are uncultivated, and their roles in natural systems are unclear”, “[t]he majority of the dominant organisms detected were newly discovered”, and “functionally diverse and much more complex than has yet been revealed” (e.g., Figure 3). We wonder about the naive people who had those “original thoughts”. Also, at some locations, *Acidithiobacillus* is not even detected (Druschel et al., 2004).

Bacteria and archaea are capable of exchanging and recombining DNA. This accounts for their impressive adaptability to various environmental conditions, and renders the delineations of species and genus more ambiguous (Lo et al., 2007). Even a basic recognition of microbial processes shows that simple human explanations of microbial effects are not close to reality (Figure 4). Furthermore, newly documented microbes have been discovered in minesite drainage, including one of the smallest known cellular life forms (Baker et al., 2006; Edwards et al., 2000).

Despite the past studies of iron oxidation by *Acidithiobacillus*, in-field surveys have shown that *Leptospirillum* and *Ferroplasma* can be the dominant iron oxidizers in ARD, sometimes representing at least 50% of the biomass (Bond et al., 2000; see also Figure 3). Other reported bacteria, archaea, and microbes in minesite drainage include:  $\alpha$ -*Proteobacteria*,  $\beta$ -*Proteobacteria*,  $\gamma$ -*Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Nitrospira* (Xiao et al., 2008), *Acidiphilium*, *Flavobacterium*, and *Bacillus* (Leduc et al., 2002), and fungi (Fang et al., 2007; Leduc et al., 2002). In such field studies, *Acidithiobacillus* was typically minor, at less than a few percent of the biomass, or not mentioned.

Isotopes of elements like oxygen and sulphur are sometimes used to distinguish biotic and abiotic reactions, because biological reactions can change the fractionation ratios. However, oxygen and sulphur isotopes within sulphate, derived from anaerobic ferric-iron pyrite oxidation, cannot clearly distinguish biotic from abiotic oxidation (Balci et al., 2007).

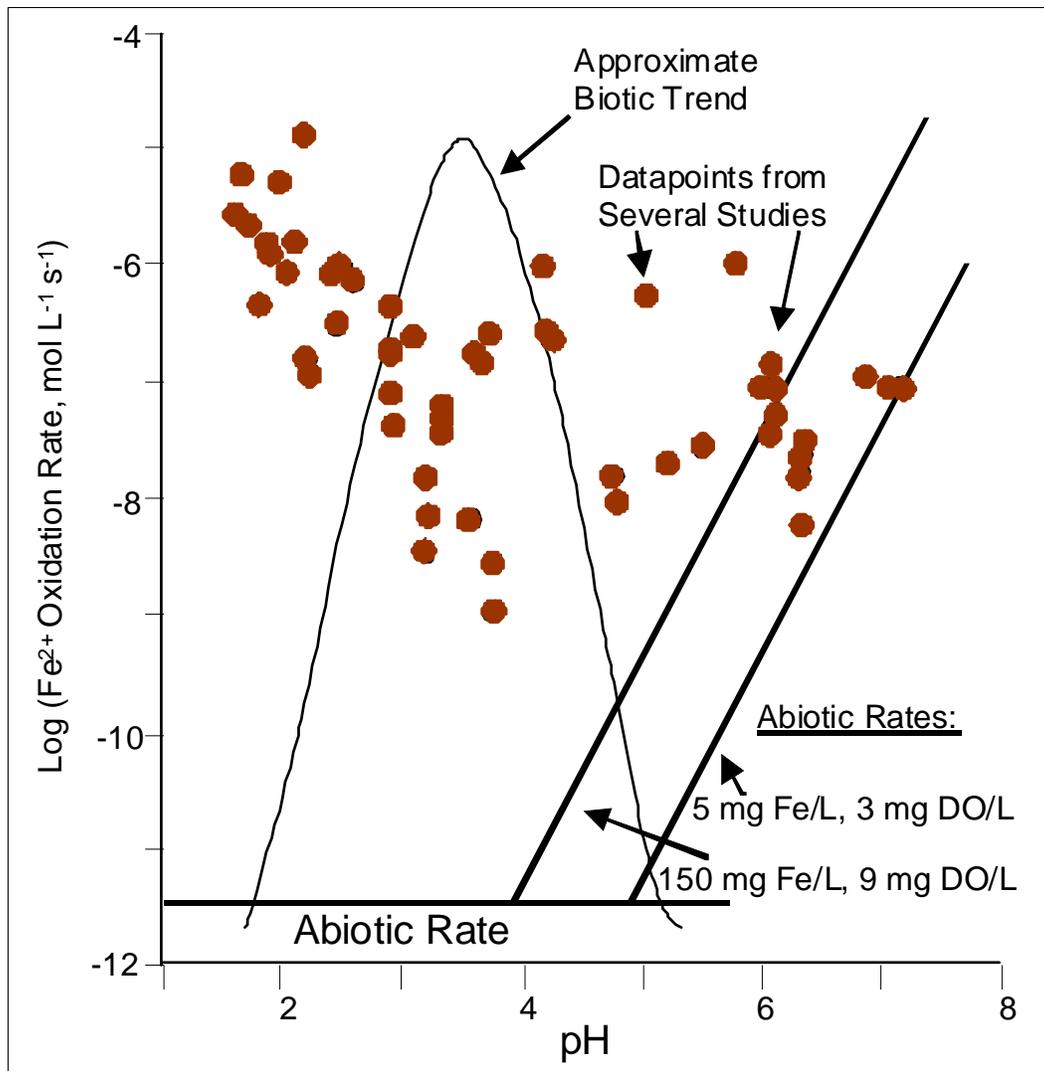
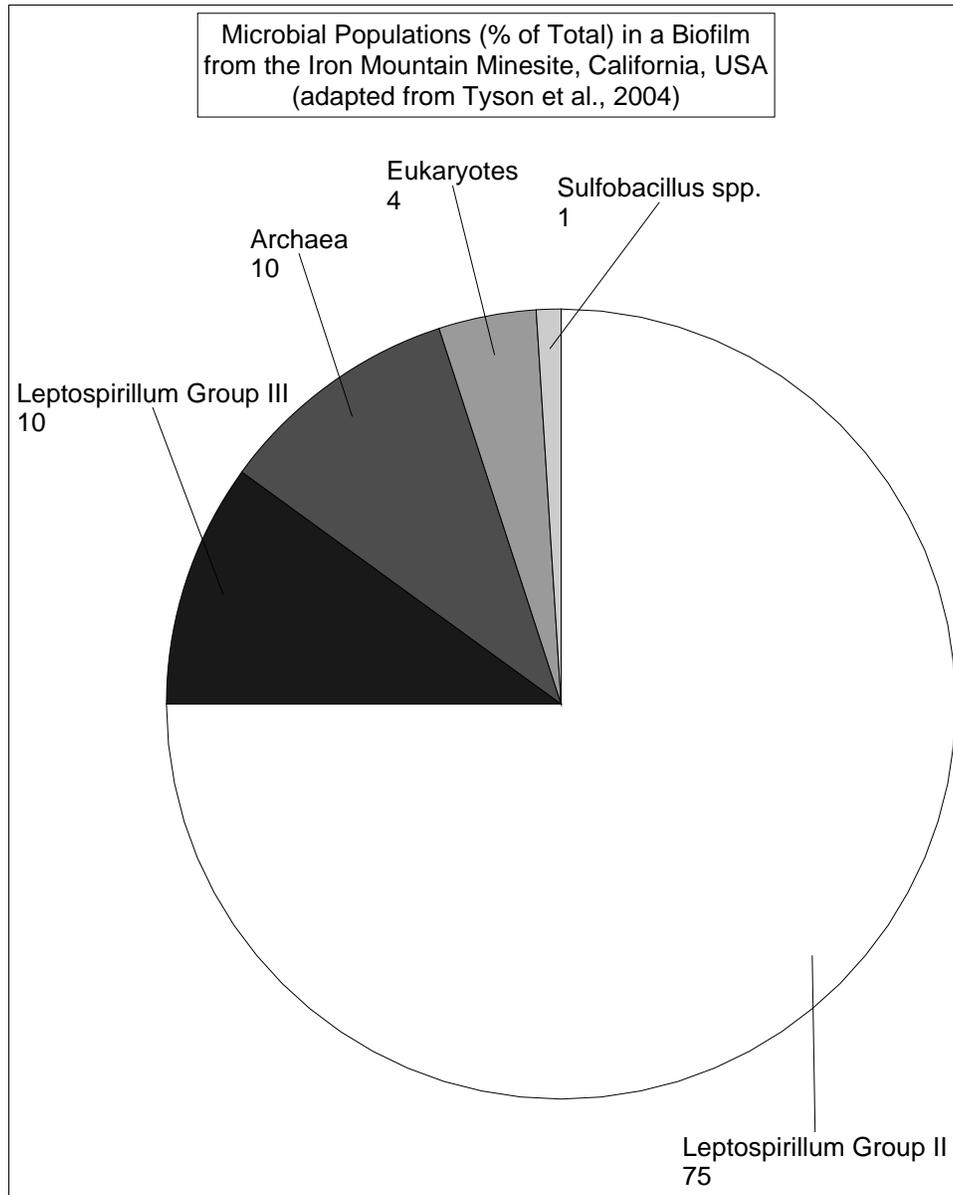
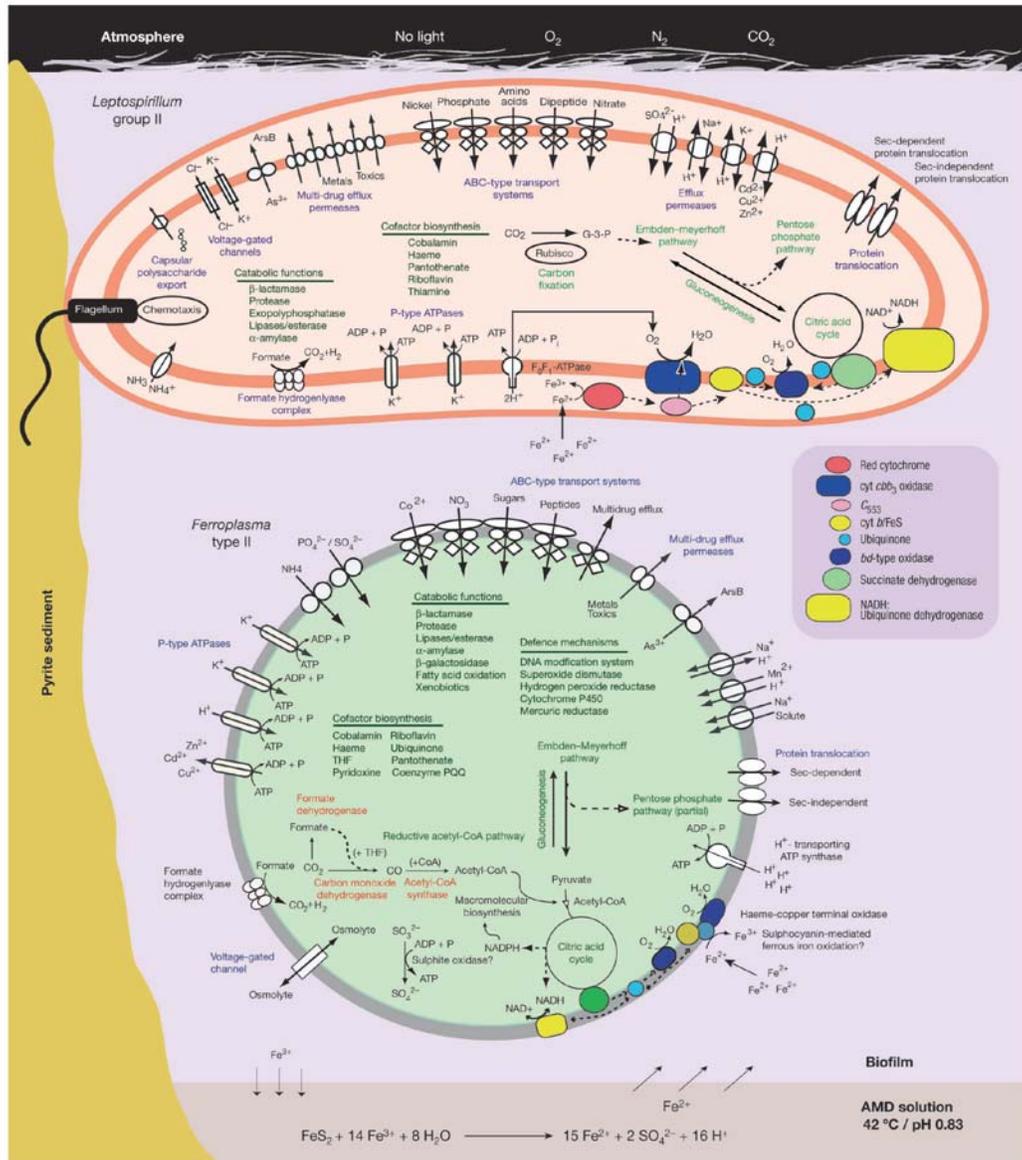


Figure 2. Compilation of ferrous-iron oxidation rates (adapted from Kirby and Brady, 1998).



**Figure 3. Microbial populations in a biofilm within acidic minesite drainage at Iron Mountain, California, USA (adapted from Tyson et al., 2004).**



**Figure 4** Cell metabolic cartoons constructed from the annotation of 2,180 ORFs identified in the *Leptospirillum* group II genome (63% with putative assigned function) and 1,931 ORFs in the *Ferroplasma* type II genome (58% with assigned function). The cell cartoons are shown within a biofilm that is attached to the surface of an acid mine

drainage stream (viewed in cross-section). Tight coupling between ferrous iron oxidation, pyrite dissolution and acid generation is indicated. Rubisco, ribulose 1,5-bisphosphate carboxylase-oxygenase. THF, tetrahydrofolate.

**Figure 4. Schematic diagram of complex microbial functions and interactions with minesite-drainage chemistry (from Tyson et al., 2004; reproduced here with permission).**

## 5. CONCLUSION

For all the above reasons, studies of microbial populations in minesite-drainage chemistry are still rudimentary and sometimes misinterpreted, but this is not a barrier to environmental assessments and predictions of minesite-drainage chemistry. This is not because microbial contributions are unimportant. Instead, we conclude that (1) microbes are always present in our environment, (2) microbial effects on full-scale aqueous chemistry cannot be reliably isolated and quantified at this time, and (3) microbes cannot be eliminated from minesite components. In other words, microbial activity can be considered an ever-present and ubiquitous factor in minesite-drainage chemistry. As a result, microbial effects are already typically included in empirical testwork (Morin and Hutt, 1997, 2001, and 2007), and are actually difficult to exclude. Where explicitly considered, microbial effects can lead to equilibrium chemistry even in relatively fast-flowing subsurface systems (Turner and Fein, 2007).

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