HISTORICAL OVERVIEW AND FUTURE DIRECTIONS OF THE MICROBIAL ROLE IN THE ACIDIC COAL MINE DRAINAGE SYSTEM[1]

by

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Abstract. Bacteria have been implicated and analyzed at every step in the production of acidic coal mine drainage (AMD). This review paper provides detailed information about microbial studies in mines, laboratory settings, waste piles, ground water, receiving streams, and downstream rivers and lakes. Research on AMD treatment, beneficial uses, and seasonal variability is also reviewed.

Additional Key Words: Thiobacillus, iron-oxidizing bacteria, sulfate-reducing bacteria

Introduction

The microbial role in the production of acidic coal mine drainage (AMD) is a multicomponent system. From breakdown of pyrite at the mineface, to the production of acids, flocculates, precipitates, and waste products in mine pools and surface mine lakes, to the ground water seeps moving through unknown pathways, to the discharge into nearby tributaries ("receiving streams"), to the discharge into downstream rivers and lakes, and finally to the discharge into treatment beds or facilities, bacteria have a role in producing deleterious products that enter the water. This complex waste stream is affected at every step by additional chemical, hydrological, and biological processes. To make this issue even more complex, once coal is mined, its overburden and other mine wastes, and some combustion products all form new environments and products with which bacteria can interact and produce acidic substances. Daily and seasonal climatic variations must also be factored in because they play an additional and quantifiable role in microbial reactions.

Part of the complexity of AMD is that it comprises a wide variety of products, each of which involves its own microbial community. Acidity generation has at least two components--hydronium ion and sulfuric acid production (Rose and Cravotta, in press). The major metals involved in the environmental impact of coal AMD are Fe, Mn, and Al. The interactions between acidity generation, alkalinity production, and metal release and uptake are often so intertwined and almost instantaneous that it may be impossible to separate them into individual reactions. For example, iron-oxidizing bacteria in the water generate energy from oxidizing iron and in so doing, they also release hydrogen ions.

Microbial studies in general typically begin with observation of a particular phenomenon, such as production of acids; the next step is often counting numbers of cells per milliliter of water or calculating rates of reactions. Characterization in terms of pH tolerance, physiology, and genetics often follows. The autotrophic bacteria, those that derive energy from inorganic substances, and the heterotrophic bacteria, those that require organic substances for energy, are often studied independently. Outright elimination of both types of bacteria using poisons comprised the initial studies of AMD; a recent trend has been toward using novel AMD bioremediation methods. Through time, ecologists eventually initiated studies to answer basic ecological questions; this line of inquiry led to ideas for shifting microbial populations to more benign forms. Beneficial use of particular microbial processes is an emerging field in AMD amelioration.

The earliest studies about bacteria in general were very useful to the next group of researchers that focused their attentions on AMD microbiology. Winogradsky (1887) suggested that bacteria could derive energy from oxidation of inorganic compounds, and Nathanson (1902) proved that microbial oxidation of sulfur compounds led to the fixation of carbon dioxide. Beijerink (1904) was first to isolate a pure culture of sulfur-oxidizing bacteria, and Omelyanskiy (1907) found that thiobacilli were involved with the oxidation of reduced sulfur compounds.

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Bacteria were initially implicated in AMD production by Powell and Parr (1919) who speculated that oxidation of the pyrite in coal may be catalyzed by bacteria. Other landmark studies in microbial acid production include the isolation of the acidophilic sulfide-oxidizing Thiobacillus thiooxidans by Waksman and Joffe (1921, 1922) and the discovery of the acidophilic iron-oxidizing T. ferrooxidans by Colmer and Hinkle (1947). Colmer and Hinkle also proved that acid production ceased with sterilization or the addition of antimicrobial agents such as mercuric chloride, phenol, toluene, and formaldehyde to coal mine water. Microbial numbers were first analyzed by Temple and Delchamps (1953) as the Pittsburgh coal bed was exposed in mining; they found the initial water from the bed to be neutral and neither of the two important Thiobacillus species to be present. After a few days, both species were present in high numbers and the water was acidic. Ashmeed (1955) later calculated that 4 tons of H_2SO_4 were produced by microbial action for each ton formed by chemical processes. The complexity of microbial physiological adaptations to low pH environments is one reason that so much acid is produced (Goodwin and Zeikus 1987).

Other early papers were very important in leading future research in the directions of downstream effects, treatment of acidified water, and pre-processing of coal. Lackey (1938) investigated 62 acidified streams lacking fish in West Virginia and discovered that algae and protozoans were abundant along with bacteria. Leathen et al. (1956) suggested that T. ferrooxidans plays a role in purifying water because they accelerated the oxidation and precipitation of iron close to the source of production. Experiments on desulfurization of coal by Zerubina et al. (1959) discovered that microbial processing was too slow to use for coal purification at an industrial scale.

The effects of AMD-producing microbial processes ripple from the mine and then downstream through the food chain and preservation in the sedimentary record. This paper therefore looks at AMD production as part of a multicomponent system and explores past and current research at each step along the flow stream. Although coal mine microbiology will be the major emphasis, important findings from metal mine studies are included where processes are universal.

Mine Microbiology

Following the quantification of acidophilic bacteria by Temple and Delchamps (1953), Ashmeed (1955) analyzed bacteria in two underground coal mines in Scotland that had high sulfur in the range of 6 to 7 percent. One mine was worked for around 50 years; the mine pool had a pH of 2.8 and Fe 550 mg/L. The second mine operated 7 years, and had low Fe (0.8 m/L), and a mine pool pH of 7.2. In the last two years of data collection, this second mine showed a rise in dissolved Fe and a decrease in the number of bases. Ashmeed showed that T. thiooxidans and T. ferrooxidans were always present in the mine pools.

Other mine research compared differences in microbial populations in mines to receiving streams (Dugan and Randles 1968; Dugan 1970); acid, sulfate, and ferric iron were formed in the mines and gob piles. Acid bacteria were also analyzed in a sealed instrumented drift mine in Vinton County, Ohio; Smith and Shumate (1968) added a nitrogen atmosphere and found acid production from bacteria decreased approximately 50% after oxygen dropped below 10 percent. At the Decker coal mine in Montana, microbial activity was limited to pyrite microzones in the coal (Olson et al. 1979); carbonate minerals in the coal quickly neutralized any in situ acid production. Furthermore, sulfate reducing bacteria were present in the mine waters (Olson et al. 1979). Microbial succession includes T. ferrooxidans, Leptospirillum ferrooxidans and a Metallogenium-type as pH drops during pyrite oxidation (Walsh and Mitchell 1972).

The microbiology of strip-mine lakes in old surface mines has been another focus. Water chemistry of surface mine lakes can eventually change to more alkaline conditions where growth of anaerobic bacteria can be encouraged (King et al. 1974). pH rises with the onset of stratification and concomitant increase in sulfate reduction (Gyure et al. 1987, 1990); heterotrophic bacteria were most abundant at the sediment/water interface. Sulfate reduction was found to be widespread in surface mine lakes (Wicks et al. 1991); lakes in Ohio, Kentucky, Kansas, Oklahoma, and Texas shared chemical characteristics. Not surprisingly, ordinary trophic level interactions occur between bacteria and protozoans in mine lakes (Johnson 1995b).

Laboratory Microbiology

A great deal of research on the microbial role in AMD production has been performed in the laboratory. Following the landmark research on iron oxidation by T. ferrooxidans (Temple and Colmer 1951; Leathen et al. 1953) determined that T. ferrooxidans was essential to production of AMD. They inoculated pyrite and marcasite with cultures of iron-oxidizing bacteria and measured the increase in acidity, sulfuric acid, and soluble sulfates; pH dropped from 3.6 to 1.2. Based on that information, Silverman and Lundgren (1959) were able to design what is now considered to be the classical growth medium for acidophilic iron-oxidizing bacteria.

Isolation, identification, and genetics. Among the many bacteria isolated from coal, a mycoplasma, Thermoplasma acidophilum, was discovered growing in a coal refuse pile (Darland et al. 1970). Fluorescent antibody techniques show that the microbial population in AMD is a consortium of a small number of thiobacilli and a much larger number of other bacteria that remain to be isolated and characterized in the future (Apel et al. 1976). All together, 37 acidophilic heterotrophs were isolated in AMD waters (Wichlacz and Unz 1981); these were unable to grow at pH values greater than 6 (Wichlacz et al. 1986. The presence of many genetic differences in the 37 sulfur- and iron-oxidizing bacteria including Thiobacillus, Acidophilium, and "Leptospirillum" illustrates that the ability to oxidize sulfur and iron are widespread (Lane et al. 1992). Seven chemical variations have been found in the lipopolysaccharide fraction of T. ferrooxidans (Southam and Beveridge 1993); this means there are at least 7 different chemospecies of this taxon.

Experiments with growth on pyrite. Experimental growth of bacteria on pyrite was first explored by Lorenz and Tarpley (1963). T. ferrooxidans was found to attach onto specific pyrite sites (Bennet and Tributsch 1978). Chemosynthetic autotrophs were found to use mineral oxidation in general for their source of chemical energy (Ehrlich 1990), and they use a variety of methods to dissolve pyrite for this energy (Ehrlich 1990). Leaching patterns by bacteria can actually be identified (Rodriguez-Leiva and Tributsch (1988) and intergranular porosity formed by bioleaching can be quantified (Mustin et al. 1992). Selective attachment of T. ferrooxidans onto fresh pyrite is 90% complete within 5 minutes (Bagdigian and Myerson 1986), whereas Sulfolobus acidocaldarius performed the same activity within 2 minutes (Chen and Skidmore 1987, 1988). Non-hydrophobic attachment may be an important attachment mechanism (Takaeuchi and Suzuki 1997). The interface between bacteria and pyrite has been envisioned as a nanoenvironment (Nordstrom and Southam 1997).

Physiology and growth characteristics. Elucidation of physiology and growth of Thiobacillus thiooxidans, a focus of Suzuki (1958, 1974), is important because the species is ubiquitous (Brown et al. 1990). It also changes its leaching activities depending on the growth conditions (Suzuki et al. 1990). The ferrous- oxidizing enzyme has been purified from T. ferrooxidans (Fukumori et al. 1988), and its concentrations of metabolites has been analyzed (Liu et al. 1988). Large populations of T. ferrooxidans have been found even in neutral pH waters (Southam and Beveridge 1992). A single strain of T. ferrooxidans can acidify mine tailings because of the ability to grow into large populations (Southam and Beveridge 1993). The lower pH limit of thiobacilli growth has been determined to be pH 2 (Sokolova and Karavaika 1962). The transport of elementary sulfur is one mechanism by which thiobacilli oxidize reduced sulfur species (Karavaiko and Pivovarova 1977). Once Unz (1965) showed that Ferrobacillus ferrooxidans could behave nutritionally like T. ferrooxidans after an induction period for sulfur utilization, Ingledew (1982) was able to lump Ferrobacillus with Thiobacillus. Useful review articles include physiology and growth of T. ferrooxidans by Ingledew (1982), dissimilatory sulfate reduction by Tuttle et al. (1969a), and the entire acidophilic microbial cycle interacting with iron by Pronk and Johnson (1992).

Utilization of different sulfur species. The breakdown of pyrite results in a variety of sulfur-bearing compounds, each of which can be utilized by different bacteria or at different places along the AMD pathway. The sulfate reducing bacteria can use a variety of sulfur species as electron acceptors (Postgate 1979). Thiobacilli can be cultivated on thiosulfate (Karavaiko 1962; Vishniac and Santer 1957). Sulfate is assimilated into cellular material of one strain of T. ferrooxidans but not another (Tuovinen 1977). Leptospirillum ferrooxidans produces three different sulfur-bearing compounds and sulfur-bearing acids are needed for oxidative pyrite degradation (Shippers et al. 1996).

Ecological considerations. The influence of AMD on populations of bacteria were traced in a non-acidified stream; control of acidity and sulfate were both important (McCoy and Dugan 1968). Successional changes in an artificial coal spoil were monitored by Harrison (1978) who noted that shifts were driven by acidification and that other organisms began to bloom as the pH rose. pH-controlled succession was not necessary because T. ferrooxidans can theoretically acidify the environment without assistance (Kleinmann and Crerar 1979). Baker and Mills (1982) demonstrated that T. ferrooxidans could survive long exposures to circumneutral water, and that transport in non-acidic water could provide a suitable inoculum for acidogenic oxidation of freshly exposed sulfide minerals. A combination of a fluorescence technique with enumeration showed that succession on coal involved T. ferrooxidans, which was no longer detectable after 16 days, followed by a large number of other microorganisms (Muyzer et al. 1987).

Kinetic rate reactions. Microbial oxidation of ferrous to ferric iron controls the rate of pyrite oxidation (Singer and Stumm 1970). Bacteria have multiple roles in the oxidation kinetics (Lau et al. 1970). Analyzing the kinetics of reactions that require microbial intervention, Baker and Wilshire (1970) found that the concentration of acidity, ferrous and total iron, and sulfate in aerobic beds is not affected by flow rate. Oxidation rates by different iron- and sulfur-oxidizing bacteria varied primarily by the rate of utilization of the sulfur moiety in thiosulfate (Bounds and Colmer 1972). In systems lacking bacteria, the oxidation rate of ferrous to ferric iron is too slow to affect the oxidation of pyrite (Jaynes et al. 1984a). Acid production by bacteria has been found to limit pyrite oxidation (Prein 1993). Isotopic differences in sulfate values have been used to help assess microbial rate reactions (Taylor et al. 1984). Rate of microbial ferrous oxidation depends on successional state (Mitchell 1972), species (Lizama and Suzuki 1989b, 1991), and strain (Olson 1991).

Analysis of metals, metal binding, and types of microbially-mediated minerals. The relationship between acidophilic bacteria and metals has many research directions. At the most basic level, Beveridge and Murray (1980) provided the first demonstration that metals were bound to the bacterial wall. Geesey et al. (1988) showed the sites of metal binding on bacterial extracellular polymers. Beveridge et al. (1983) also provided the first simulation to show that bacterial metal complexation may have a profound effect on sediment chemistry. Stanton and Goldhaber (1991) followed microbiological formation of iron monosulfides via bacterial sulfate reduction to discern the effect on early sediment diagenesis.

Specific minerals and metals have been analyzed. Ferrihydrite to be the iron oxide phase that precipitates iron in an acidified stream (Ferris et al. 1989). Other metals containing Ni (Ferris et al. 1988), Mn (Robbins et al. 1997b), and Al (Robbins et al. 1997a) have been reported. Magnetite formation has been studied as a method of binding AMD-derived iron (Svanks and Shumate 1973).

Leaching studies. In leach tests of cores from Montana coal beds, unexpected acid layers were found bearing both sulfur- and iron-oxidizing bacteria (Kimble and Temple 1980). Multileaching studies on the same coals learned that acidity increased with successive leaching (Means et al. 1987). Dissolution of metals from coal under aerobic conditions is due to direct microbial oxidation, whereas under anaerobic conditions, it is due to direct microbial reduction (Francis et al. 1987); leachates of low sulfur coal contained autotrophic iron-oxidizers and organosulfur-utilizing heterotrophs. Coals stored at different temperatures and different oxygen tensions were leached (Tuttle et al. 1990); chemoautotrophic bacteria controlled the leaching of dissolved organic carbon by production of acid. Leptospirilli are as common as thiobacilli in leach experiments of a pyrite ore (Sand et al. 1992). Protozoan predators of bacteria were found to increase with leaching (Johnson and Rang 1993).

Coal Waste Piles Microbiology

Bacteria perform many roles in the tailings environment (Gould et al. 1994), some of which can be exploited for treatment (Kalin et al. 1991). Bacterial reduction of sulfates occurs below spoil banks (Schopel 1985), whereas biological reduction of Fe and Mn and other metals that co-precipitated with the oxides occurs below coal-combustion byproduct piles (Francis 1985). Biological production of acid is intense in gob piles (Dugan and Randles 1968). Thiobacilli cells counted in mine tailings were $>10^8$/gm (Beveridge and Southam 1992), and numerous heterotrophs have been found in refuse piles (Belly and Brock 1974). Percolating water has been found to remove the soluble reaction products of bacteria (Jaynes et al. 1984b).

The data from the western coal fields, where more alkalinity-production is available, point out major differences from the above studies. Spoil samples from a strip mine in Wyoming contained relatively low numbers of acidophilic iron- and sulfur-oxidizing bacteria where pH values are near neutral or alkaline even where pyrite is present in coal (Olson et al. 1980).

Inhibition of microbial activity in waste piles is an important research topic (Watzlaf 1986, 1988). Leaching of acid from coal refuse can be inhibited with the application of sodium lauryl sulfate and benzoic acid (Dugan 1987a, b).

Monitoring and exploiting microbial activity in the waste environment has important economic potential. Guthrie et al. (1981) performed a very important experiment on microbial communities and waste products in a coal ash settling basin; they monitored annual changes showing there were seasonal peaks in microbial populations and these affected the downstream environments. Guthrie et al. (1978a and b) compared genera growing in pH 6.5 bottom ash to those in pH 4.6 fly ash; Pseudomonas, Flavobacterium, Chromobacterium, Bacillus, and Brevibacterium dominated at the lower pH value. Metal-rich sludges from AMD can be treated with bacterially-generated H_2S

to improve settling and sludge density characteristics (Hustwit et al. 1996).

Ground Water Microbiology

Ground water has been found to be affected by AMD. Sulfur-oxidizing bacteria in aquifers underlying lignite are limited by the amount of soluble carbon in the water (Houghton et al. 1985). Microorganisms can influence the chemical evolution of ground water underlying coal mine overburden by altering oxygen concentration, Eh, and pH (Wallis and Ladd 1985). They can also impact ground water quality by shifting electron acceptors and energy sources (Chapelle 1993).

Stream Microbiology

Acid tolerant populations in receiving streams have been found to revert to normal microflora once acid production from the mines ceased (Dugan and Randles 1968). The presence and amount of naturally occurring calcium carbonate may affect the acid-producing bacteria in receiving streams (Caruccio (1968). The diversity of microbial populations in AMD-receiving streams was found to be quite high (Johnson 1991b), whereas the heterotrophic bacteria which attach to surfaces were stressed in the stream receiving AMD from abandoned pyrite mines (Mills and Mallory 1987).

Many papers have been written on the co-occurrence of acid-producing bacteria with other organisms in receiving streams. Yeast, fungi, algae, protozoans, and plants have been reported by Lackey (1938), Temple and Koehler (1954), Cooke (1966), Hargreaves et al. (1975), Rao (1989), and McGinness and Johnson (1992). Heterotrophic bacteria are particularly abundant in the presence of algae (Dugan et al. 1970 a, b).

Microbiology of Downstream Rivers, Lakes, and Reservoirs

Bacteria and their relationship to other members of the aquatic food chain have been analyzed downstream from AMD sources. Actinomycetes, fungi, green algae, diatoms have been reported downstream from AMD by Joseph (1953). Microbial numbers, types, and other organisms have been compared in rivers receiving AMD to those free of AMD (Weaver and Nash 1968); indigenous iron-oxidizing bacteria were found in AMD water. Bacteria cement iron onto grain surfaces as iron oxyhydroxide minerals downstream from AMD (Bigham et al. 1990).

Microbial studies in lakes downstream from AMD-producing coal mines have not been reported, but there is one large lake downstream from pyrite mines that has received detailed analysis; the sulfate budget in Lake Anna in Louisa County, Virginia, has been analyzed in detail (Wassel and Mills 1983), Herlihy and Mills (1985, 1986), Herlihy et al. (1987). Mills (1985) hypothesized that manipulation of the microbial community could play a major role in recovery of downstream waters. Sedimentation was tracked after a storm and sulfate reduction had removed the signal of acid flushing within two weeks in Lake Anna (Bell et al. 1990).

AMD Treatment Microbiology

The literature on microbial treatment of AMD is voluminous. In this review article, I will discuss the wide variety of laboratory, pilot plant, and field treatments, but only highlight some of the many studies.

Biocides. Following the landmark study of Colmer and Hinkle (1947), biocide research first branched out into use of a variety of other toxic compounds including chromates (Bufton 1958). A wide variety of processes were found to kill bacteria (Hugo 1967). Compounds that are non-toxic to fish and humans have been isolated (Kleinmann 1979; Kleinmann et al. 1981; Kleinmann and Erickson 1983) including surfactants (essentially soaps) such as mixtures of sodium lauryl sulfate and sodium benzoate (Olem et al. 1983, Dugan and Appel 1983) and other unnamed anionic surfactants (Rastogi 1996) and anti-bacterial agents (Shearer et al. 1968). Formic, hexanoic and other acids were effective microbial inhibitors (Dugan 1987a, b). Controlled release of biocides (Sobek et al. 1990) has turned out to be a very important aspect in cessation of AMD. Furthermore, a useful workshop manual was reprinted and includes some of the most important papers on use of biocides (Kleinmann and Rastogi 1996). Thiodiazoles have been used to block microbial oxidation of sulfur-bearing compounds (Gould et al. 1996). A word of caution in biocide research was injected by Brickett et al. (1996) who demonstrated that sulfide leaching experiments using improper inhibitors can overestimate the importance of bacterial involvement.

Bioreactors. Bioreactor experiments have been used to learn about microbial interactions with Fe, Mn, and sulfate. A bioreactor experiment tested the efficiency rates of bacterial versus chemical oxidization of reduced iron (Bigham et al. 1984); removal efficiencies of 90% were found in the bioreactors having bacteria; chemical (abiotic) oxidation was insignificant in the three monthlong experiment. Using a bioreactor study, Gordon and Chuang (1990) unexpectedly discovered that Mn precipitating bacteria were light sensitive. Bioreactor experiments have also been used to measure rates of sulfate reduction (Herrera et al. 1993); rates as high as 2,765 mg/L/d were possible. Different substrates have been tested to increase bacterial sulfate reduction rates including straw (Bechard et al. 1993, 1994; Christensen et al. (1996), polylactic acid (Edenborn et al. 1996b), and polymers (Edenborn et al. 1996a).

Burial and sealing. Burial and sealing of coal tailings was found to be one of the most economical method for preventing growth of acidophilic iron oxidizing bacteria (Walsh and Mitchell 1975).

Electrical methods. Experiments have shown that electrobiochemical fuel cell treatment could neutralize AMD following microbial oxidation (Sisler et al. 1977).

In line treatment. Multiple in line treatment has been shown to stop the wide variety of bacteria that participate in AMD (Walsh and Mitchell 1972). A flow stream that included physical, chemical, and biological processes provides the most inexpensive treatment (Ackman and Kleinmann, 1985).

Limestone drains. Removing manganese from AMD water has proved to be a major problem. Recent work on microbial populations in buried limestone drains that are slightly oxygenated have been shown to efficiently remove Mn (Vail and Riley 1993, 1997).

Microbial mats. Use of microbial mats to precipitate AMD metals is another relatively new technique. Mixtures of cyanobacteria and algae are able to remove Mn (Wildeman et al. 1993). Key organisms have been isolated (Phillips et al. 1995), rates and mechanisms of metal uptake have been provided (Bender et al. 1995), and spatial variability has been analyzed (Phillips et al. 1996; Phillips and Bender, in press).

Sulfate reduction. Once it was understood that sulfate reduction raises pH (Tuttle et al. 1969b), and that high quality but low cost sources of organic carbon were needed by these bacteria (Ogg 1972), the field blossomed with field experiments to treat AMD (McIntire and Edenborn 1990; Dvorak et al. 1992; Vile and Wieder 1993; Fortin et al. 1994; Hammack et al. 1996). Understanding microbial processes such as sulfate reduction has led to successful reclamation efforts in Canada (Visser 1985) and West Virginia (Harris and Birch 1990).

Waste water. A detailed review of waste water treatment of coal mine waste by bacteria appeared in the Journal of the Water Pollution Control Federation (Anonymous 1965). Experiments at the Hollywood, Penna., demonstration site have led to technological advances using rotating discs to oxidize mine waste water and thereby reduce growth by iron-oxidizing bacteria (Lovell (1973; Olem 1975; Olem and Unz 1980).

Wetlands. Publication of studies on AMD treatment by microbial populations in wetlands began with analyses of iron and manganese precipitating bacteria in bogs (Burris 1984). Microbially-mediated iron oxidation was predicted by Henrot et al. (1989) to play a key role in iron retention and then measured by (Henrot and Wider 1990). Manganese fixation in wetlands has been found to require an oxidizable substrate (Gordon and Burr 1988). Incubation experiments with iron and manganese showed that mineral crystallization slows down dissolution reactions (Tarutis and Unz 1994).

Detailed overviews of the many microbial experiments that have been conducted in wetlands are available (Kleinmann 1985; Kleinmann et al. 1992). Size scaling has been analyzed for constructed wetlands that use microbial and chemical reactions to raise pH and drop metals (Hedin et al. 1989). Sulfide generation in anaerobic wetlands has been found to remove a wide variety of metals (Machemer et al. 1993. The ability to calculate sulfate reduction rates in a constructed wetland (Reynolds et al. 1997) means that rates can also be estimated for metal retention. Numbers of bacteria are different in the aerobic versus the anaerobic parts of wetlands (Wildeman et al. 1994) where metals are precipitating as sulfides.

Beneficial Use of AMD Microbes

Recovery of metals and desulfurization experiments are both important current directions in microbial AMD research. Metals are being recovered from metal-mine wastewater (Brierley et al. (1989) and coal-mine wastewater (Hammack et al. 1993, 1994) using both aerobes and anaerobes. Desulfurization of coal has been successful using acidophilic bacteria concentrated from AMD (Johnson 1991a).

Seasonal Variability and Microbes

A few studies have analyzed the effect of diurnal and seasonal changes on microbial processing of acidic components from coal and its wastes. In Wyoming, where the summer is dry, bacterial numbers in spoils piles were low; in the wetter spring months, numbers were still relatively low (Olson et al. 1980). Seasonal differences were analyzed in a stream draining a surface mine and compared to another stream where no mining had occurred in the watershed (Weaver and Nash 1968); T. ferrooxidans was present all year round in the mine-affected stream but only in the summer in the non-mine-affected streams.

Three important studies looked at changes downstream from metal and coal mines but did not perform the microbial analyses that might have elucidated the microbial role in the effects. Ward and Walton-Day (1995) analyzed seasonal variations resulting in decrease in metals downstream. Wieder (1994) studied day-night changes in iron chemistry in treatment wetlands and found that during the day, most of the iron was ferric and that during the night, most of the iron was ferrous, similar to the findings of McKnight and Bencala (1988) downstream from a metal mine in Colorado.

Microbiology Review Articles

Through the years, there have been some very useful comprehensive overviews of the microbial role in AMD production. The three most important books were written or edited by Kuznetsov et al. (1962), Mitchell (1972), and Ehrlich (1990). Lorenz and Stephen (1967) provided a valuable service by reviewing world wide literature on all aspects of AMD. Dugan (1975) followed the microbial AMD system from acidic production, through reactions in spoils; he reviewed the data on biocides and bacterial sulfate reduction. Renton (1985) and Evangelou (1995) looked at AMD microbiology from the point of view of mineralogists. Powell (1988) discussed all aspects of AMD production and showed that AMD preceded mining in the United States. Wildeman and Updegraff (in press) review bioremediation methods and show how metal removal in wastes is essentially a microbially mediated process.

Future Microbial Studies

A group of microbiologists (see acknowledgements) consulted for this paper provided the following ideas on future directions in AMD microbiology. Five directions that require study emerged from this exercise:

1) In the mine. Research is needed on the differences between bacteria in anoxic, suboxic, and oxygenated mine pools. Paul Petzrick (Maryland DNR, oral commun. 1997) is particularly interested in learning if different mine pools have endemic microflora. H.M. Edenborn and L.A. Brickett (written commun. 1997) are currently examining microbial diversity within the mine located at the DOE Federal Energy Technology Center in Pittsburgh, PA.

2) At seeps. Mines have numerous seep sites that become active when mine pools fill. Seep microbiology is an unstudied environment. There should be distinct differences between populations around oxygenated or anoxic seeps.

3) In the affected ground water and hyporheic zones. More studies need to be conducted to learn about microbial interactions of AMD with ground water and the hyporheic zone between the ground water and surface water.

4) Synergisms between microbial acid production and the metal-stripping capabilities of other aquatic organisms. Microbial mats are obviously complex communities that have individnals that compete, cooperate, or are neutral in their relationships to one another. Krishnaswamy (1996) has begun a study looking into such synergisims.

5) Reactions in different climates. Climate must play a major role in the types and numbers of bacteria involved in AMD production, and yet this subject is barely touched upon in the present worldwide literature.

Discussion and Conclusions

The microbial role in AMD production and treatment has been studied for almost 100 years. Species, numbers, interactions, processes, treatment, beneficial use, and daily and annual variations have been analyzed in the mine, in the waste piles, in the receiving streams, in the downstream direction, in the ground water, and in the laboratory. Many of the references are in obscure publications; this is attributable to several factors. Microbial research in this subject is generally applied to specific problems, leading to reports written specifically for project officers. Conferences on this subject have been numerous through time and researchers working on the subject have been willing to get the information into immediate print as conference papers rather than waiting for the long review process that results in peer reviewed journals. These are only some of the reasons that the voluminous literature is generally unknown to chemists and reclamationists who toil in the laboratory and in the field trying to eliminate acidic coal mine drainage.

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