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Geomicrobiology: relative roles of bacteria and fungi as geomicrobial agents

HENRY L. EHRLICH

Introduction

The following definition of geomicrobiology will provide a proper context for the discussion in this essay. *Geomicrobiology* is a study of the role that microbes have played in the geologic past from the time of their first appearance on the planet Earth about 4 eons ago to the present, and the role they are playing today and are likely to play in the future in some of the processes that are of fundamental importance to geology. The discussion will be restricted to current geomicrobial activities because being able to observe them directly, we know most about them. Geomicrobial activities in the geologic past have been deduced from the detection in the geologic record of (1) microbial fossils that morphologically resemble present-day microorganisms of geologic significance and (2) relevant biomarkers. Past geomicrobial activities have also been inferred from present-day geomicrobial activities that occur under conditions similar to those presumed to have existed in the geologic past. Molecular phylogeny is providing information that supports inferences about ancient geomicrobial activity.

Geomicrobial agents

Phylogenetic distribution

Although geomicrobial agents that are presently recognized include members of the domains Bacteria (Eubacteria) and Archaea in the Prokaryota and members of Algae, Protozoa and Fungi in the Eukaryota, the following discussion will emphasize mainly geomicrobial activities of members of the Bacteria, Archaea and Fungi.

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Geomicrobial activities

Types of geomicrobial activities

Geomicrobial activities play a role in (1) mineral formation, (2) mineral degradation, (3) the cycling of organic and inorganic matter, (4) chemical and isotopic fractionation and (5) fossil-fuel genesis and degradation. Microbial mineral degradation includes phenomena such as weathering, bioleaching, and soil and sediment formation and transformation (diagenesis). Microbes contribute, to varying extents, to the genesis and degradation of fossil fuels, including methane, peat, coal and petroleum. Some geomicrobial activities can be commercially exploited in processes such as metal extraction from ores, biogas genesis, commercial tertiary petroleum recovery and environmental bioremediation.

Physiological processes involved in geomicrobial activity

The physiological basis for different forms of geomicrobial activity depends on the type of activity, the substance being transformed and the organism(s) involved. Some geomicrobial activity involves enzymatic oxidations or reductions of inorganic substances. Such reactions are promoted mostly by prokaryotic organisms and may contribute to mineral formation, mineral diagenesis and mineral degradation. Other geomicrobial activity involves enzymatic synthesis or degradation of naturally occurring organic carbon compounds in which both prokaryotes and eukaryotes participate extensively. Such organic transformations involve many other types of enzymatic reactions besides oxidations and/or reductions. In microbial physiology, microbial degradation of organic carbon to CO_2 is sometimes called *mineralization*, but in this book that term is strictly reserved for the process of mineral formation.

Some geomicrobial activity may involve non-enzymatic reactions in which inorganic or organic products of microbial metabolism serve as chemical reagents in reactions such as heavy metal precipitation, mineral weathering and dissolution, or mobilization of viscous petroleum hydrocarbons. Thus, heavy metals can be precipitated by H_2S formed by sulphate-reducing bacteria. Carbonates, silicate and aluminosilicate minerals and phosphate minerals may be weathered by microbially formed inorganic acids such as H_2SO_4, HNO_3, H_2CO_3, and organic acids such as acetic, oxalic, lactic, propionic, butyric and citric acids, or by microbially formed bases such as ammonia and amines. Metal constituents in some minerals may also be mobilized by microbially synthesized ligands, as for instance the mobilization of ferric iron by siderophores. Water-insoluble

components of petroleum may be emulsified and thereby mobilized through the action of microbially formed surface-active agents.

Some geomicrobial activity is attributable to physical effects exerted on the environment by growing microbes. Thus, growing microbes may transform an aerobic environment into an anaerobic one by consuming oxygen in their respiration faster than it can be replaced by contact with air. Conversely, oxygenically photosynthesizing microbes (cyanobacteria, algae) can transform a quasi-anaerobic environment into an aerobic one by generating O_2 faster than it is consumed by respiring organisms accompanying them. Microbes can raise or lower the pH of their environment, thereby rendering it more or less fit for other organisms present in the same environment. Microbes growing in rock fissures may contribute to the break-up of the rock by the pressure that their increasing biomass exerts on the rock fissures, causing the fissures to enlarge. Finally, some geomicrobial activity may be the result of a combination of several of the activities mentioned above.

Conditions that determine whether a geomicrobial attack of a mineral is enzymatic or non-enzymatic

Direct enzymatic attack Direct enzymatic attack of a mineral is either oxidative or reductive and can occur if three conditions are met. The first condition is the presence of one or more oxidizable or reducible mineral constituents. The second condition is that the cells involved in the oxidation or reduction of an appropriate mineral constituent attach to the mineral surface. The third condition is that the enzyme capable of catalysing the oxidation or reduction of a mineral constituent resides at the cell surface. Besides being in contact with the mineral surface, this enzyme must also be in contact with other enzymes and electron carriers residing below the cell surface. In a mineral oxidation by a Gram-negative bacterium, enzymes and electron carriers below the cell surface in the periplasm and plasma membrane convey electrons removed from an oxidizable mineral constituent by the oxidase at the cell surface (outer membrane) to a terminal electron acceptor, which is oxygen in an aerobic process (Fig. 1.1a). In a mineral reduction by a Gram-negative bacterium, the enzymes and electron carriers below the cell surface in the plasma membrane and the periplasm convey electrons from an electron donor within the cell to the reductase at the cell surface (outer membrane) in contact with the mineral, an appropriate constituent of which will be reduced in serving as terminal electron acceptor (Fig. 1.1b). Only prokaryotic

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microbes have the capacity for direct enzymatic attack of minerals because only they include representatives with oxidases or reductases at their cell surface capable of interacting with an oxidizable or reducible mineral. So far, such enzymes with a cell-surface location have only been identified in Gram-negative bacteria, i.e. in the aerobe *Acidithiobacillus ferrooxidans* (Yarzábal *et al.*, 2002), and in anaerobically growing *Shewanella oneidensis* MR-1, a facultative organism (Myers & Myers, 1992), and in the strict anaerobe *Geobacter sulfurreducens* (Lovley, 2000). Such enzymes probably also exist in Gram-negative marine isolates strains BIII 32, BIII 41 and BIII 88 (Ehrlich, 1980, 1993a, b). Circumstantial evidence suggests that Gram-positive *Bacillus* 29 and *Bacillus* GJ33 are capable of MnO_2 reduction by a direct mechanism similar to that proposed for marine strain BIII 88 when it reduces MnO_2 aerobically (Ghiorse & Ehrlich, 1976; Ehrlich, 1993a, b; 2002a, p. 451). Although *Sulfolobus* spp. and *Acidianus brierleyi*, which
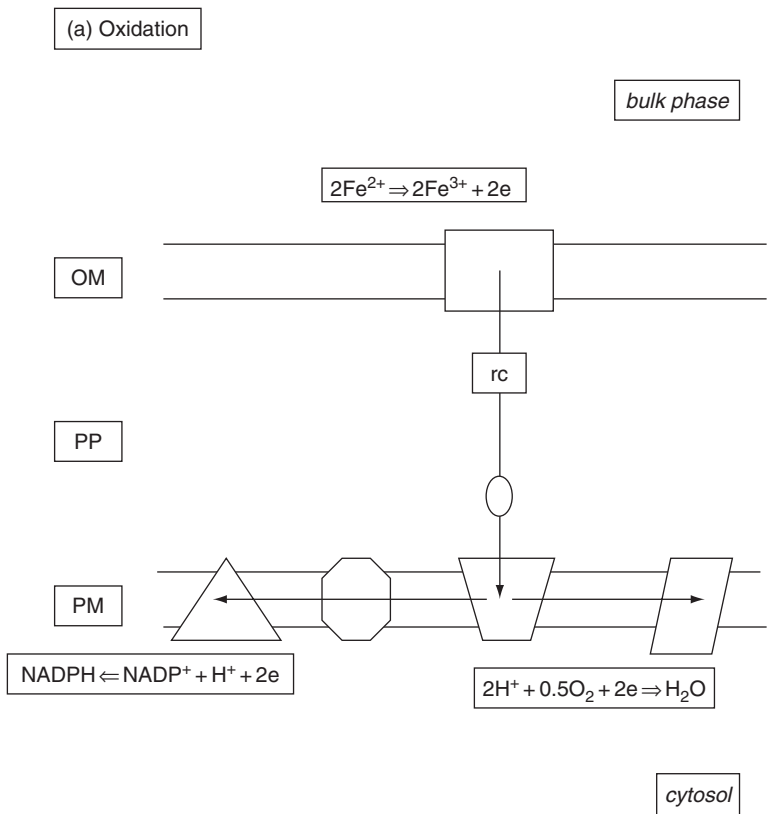
(a) Oxidation

bulk phase

$$2Fe^{2+} \Rightarrow 2Fe^{3+} + 2e$$

OM

rc

PP

PM

$$NADPH \Leftarrow NADP^+ + H^+ + 2e$$

$$2H^+ + 0.5O_2 + 2e \Rightarrow H_2O$$

cytosol

Fig. 1.1. (cont.)

(b) Reduction

bulk phase

$MnO_2 + 2H^+ + 2e \Rightarrow Mn^{2+} + H_2O$

OM

PP
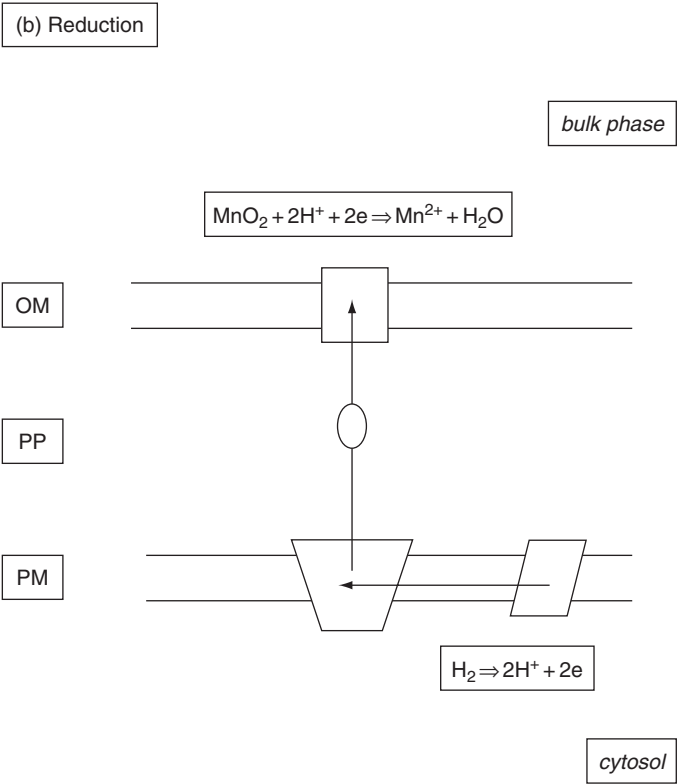
PM

$H_2 \Rightarrow 2H^+ + 2e$

cytosol

Fig. 1.1. Schematic representation of electron flow in oxidation of an inorganic electron donor (a) and reduction of a terminal inorganic electron acceptor (b) at the cell surface of respective Gram-negative bacteria. The inorganic electron donors or acceptors do not penetrate the outer membrane in these bacteria. The diagram in (a) summarizes electron flow in Fe^{2+} oxidation by *Acidithiobacillus ferrooxidans* (Ehrlich *et al*., 1991; Yarzábal *et al*., 2002). The diagram in (b) summarizes electron flow in reduction of MnO_2 by the anaerobe *Geobacter sulfurreducens* (Lovley, 2000). The general reduction scheme for *G. sulfurreducens* also applies to anaerobically grown *Shewanella oneidensis* MR-1 when reducing MnO_2 (Myers & Myers, 1992). OM, outer membrane; PP, periplasm; PM, plasma membrane. The arrows indicate direction of electron flow. In (a), the rectangle (OM), oval (PP) and trapezoid (PM) represent different c-type cytochromes, the parallelogram (PM) represents cytochrome a_1, the octagon (PM) represents a bc1 complex and the triangle represents NADPH dehydrogenase complex. The framed rc in the periplasm represents rusticyanin. In (b), the parallelogram (PM) represents a NADH dehydrogenase complex, and the trapezoid (PM), oval (PP) and rectangle (OM) represent different cytochromes of the c-type.

belong to the domain Archaea, are known to attack various sulphide minerals such as pyrite (FeS_2), chalcopyrite ($FeCuS_2$), arsenopyrite (FeAsS) and nickel sulphide (NiS) (see, for instance, summary by Ehrlich, 2002a, p. 632), the mechanism of attack employed by them is not known.

Non-enzymatic attack In non-enzymatic attack of minerals by microbes, reactive products of microbial metabolism come into play. The microbial enzymes responsible for metabolic product formation are located below the cell envelope, in the cytoplasm of prokaryotes (Bacteria and Archaea) and in cell organelles and/or the cytoplasm of eukaryotes (e.g. fungi, algae, lichens). In these instances of microbial attack, physical contact of the microbial cells with the surface of a mineral being attacked is not essential. The reactive metabolic products are formed intracellularly and are then excreted into the bulk phase where they are able to interact chemically, i.e. non-enzymatically, with a susceptible mineral. Depending on the type of metabolic product and mineral, the interaction with the mineral may result in mineral dissolution or mineral diagenesis by oxidation or reduction or acid or base attack. Mineral dissolution or diagenesis may also be the result of complexation by a microbial metabolic product with that capacity. In some instances mineral attack may involve a combination of some of these reactions.

Enzymatically catalysed inorganic geomicrobial transformations

Oxidations

Aerobic oxidation of dissolved inorganic substances resulting in end-product immobilization and mineral formation Aerobic bacterial oxidation of dissolved Fe^{2+} to a Fe(III) oxide or oxyhydroxide and of Mn^{2+} to Mn(IV) oxide are examples of end-product immobilization by mineral formation (Ehrlich, 1999).

Aerobic mineral oxidation resulting in mineral degradation and product mobilization Aerobic bacterial oxidation of elemental sulphur (S^o), of various mineral sulphides such as pyrite (FeS_2), chalcopyrite ($CuFeS_2$), arsenopyrite (FeAsS), sphalerite (ZnS), cobalt sulphide (CoS) and nickel sulphide (NiS) to corresponding metal sulphates, and of uraninite (UO_2) to UO_2^{2+} are examples in which oxidizable minerals undergo dissolution of one or more of their constituents, which are thus mobilized (see Ehrlich, 2002a).

Anaerobic oxidation of dissolved Fe(II) to Fe(III) oxide Ferrous iron has been shown to be anaerobically oxidized to Fe(III) by some bacteria using

nitrate as terminal electron acceptor (Straub *et al.*, 1996). Ferrous iron has also been shown to be anaerobically oxidized to Fe(III) in anoxygenic bacterial photosynthesis in which the oxidation of Fe(II) is the source of reducing power for assimilation of CO_2, i.e. carbon fixation (Widdel *et al.*, 1993).

Aerobic and anaerobic oxidation of arsenite to arsenate Aerobic oxidation of arsenite to arsenate by bacteria has been known for a long time (see Ehrlich, 2002a, pp. 305–8). Recently, anaerobic oxidation of arsenite by a chemoautotrophic bacterium, strain MLHE-1, using nitrate as terminal electron acceptor was discovered in the monimoliminion of meromictic Mono Lake, CA, USA by Oremland *et al.* (2002a). This organism together with heterotrophic, anaerobically arsenate-respiring organisms (see later, p. 9) from the sediment in Mono Lake make possible a complete arsenic cycle that is promoted entirely by bacteria in the anaerobic region of the lake (Oremland *et al.*, 2004).

Reductions

Bacterial reduction in air of MnO_2 to Mn^{2+} and of CrO_4^{2-} to Cr^{3+} Several examples of enzymatic reduction of MnO_2 to Mn^{2+} in air have been reported (see Ehrlich, 2002a, pp. 449–55). Glucose and acetate have been shown to be effective electron donors in Mn(IV) reduction. In at least one instance of Mn(IV) reduction, some energy appeared to be conserved in the process (Ehrlich, 1993a, b).

A number of examples of enzymatic reduction of CrO_4^{2-} to Cr(III) in air have also been reported (see Ehrlich 2002a, pp. 531–4). Glucose and citrate were found to be effective electron donors. Involvement of the electron transport system in the plasma membrane of *Pseudomonas fluorescens* LB300 suggested that some energy may be conserved in this process.

Anaerobic bacterial reduction of MnO_2 to Mn^{2+} A number of different bacteria have been shown to reduce MnO_2 to Mn^{2+} only anaerobically (see Lovley, 2000; Ehrlich, 2002a). Some of these, like *S. oneidensis*, are facultative, whereas others, like *G. metallireducens*, are obligately anaerobic. Depending on the specific organism, effective electron donors include H_2, acetate, lactate, glucose and others. Regardless of the electron donor, all these reductions of MnO_2 are a form of anaerobic respiration from which energy required by the organism to function is conserved.

Anaerobic bacterial reduction of Fe(III) oxide to Fe_3O_4 or Fe^{2+} A variety of different bacteria have been shown to use Fe(III) as a terminal electron

acceptor in anaerobic respiration (see Lovley, 2000; Ehrlich, 2002a). In some cases (e.g. *S. oneidensis* and *S. putrefaciens*) these bacteria are facultative, whereas in many other instances they are strict anaerobes (e.g. *Geobacter* spp., *Geospirillum barnesii; Geothrix fermentans, Geovibrio ferrireducens; Pyrobaculum islandicum, Desulfobulbus propionicus; Desulfovibrio desulfuricans; Desulfuromonas acetoxidans; Desulfuromusa* spp.; *Ferribacterium limneticum*). Depending on the organism, H_2, acetate and a variety of other organic compounds, including aromatic compounds, can serve as electron donors. Reduction of Fe(III) is a very important respiratory process in anaerobic carbon decomposition in soil and sediment environments.

Anaerobic bacterial reduction of SO_4^{2-} to HS^- In anaerobic marine environments, especially in estuaries and coastal nearshore regions, bacterial sulphate reduction is a very important respiratory process for degradation of organic carbon because of the ready availability of sulphate in seawater. The importance of sulphate reduction in anaerobic carbon decay was not recognized until a seminal observation by Widdel and Pfennig (1977). They found that their newly discovered *Desulfotomaculum acetoxidans* was able to oxidize acetate to CO_2 and H_2O with sulphate as terminal electron acceptor. Before that time it was thought that the sulphate reducers known up to then (*Desulfovibrio* spp., *Desulfotomaculum nigrificans*, and *Desulfomonas pigra*) were only able to degrade a limited number of carbon compounds, in particular lactate, pyruvate, fumarate, malate and ethanol, to acetate but not to CO_2 and H_2O. The discovery of *Desulfotomaculum acetoxidans* led to the isolation of many other sulphate reducers that collectively are able to degrade a wide range of organic compounds. In addition, some were isolated that could reduce sulphate autotrophically using H_2 or formate as electron donor. Until the early 1980s all known sulphate reducers were eubacterial, but since then some archaeal sulphate reducers, e.g. *Archeoglobus fulgidus* (Stetter *et al.*, 1987) and *A. profundus* (Burggraf *et al.*, 1990), have been identified as well.

Anaerobic reduction of SeO_4^{2-} and SeO_3^{2-} to Se^o and methylated selenides Selenate and selenite reduction by microorganisms has been noted for some time. Organisms with this capacity include bacteria and fungi (Trudinger *et al.*, 1979; Stolz & Oremland, 1999). In bacteria the reductions are mostly a form of anaerobic respiration and result in selenium immobilization with the formation of water-insoluble Se^o. In fungi the reductions involve the formation of methylated selenium,

e.g. $(CH_3)_2Se$ and $(CH_3)_2Se_2$. The formation of the methylated selenium compounds is a form of selenium detoxification because these compounds are volatile and can thus be vented from soil, sediment and water into the atmosphere. In trace amounts, selenium meets a nutritional requirement of diverse organisms and is assimilated by them.

Anaerobic reduction of $AsO_4{}^{3-}$ to $AsO_2{}^-$ and methylated arsenides Although the ability of bacteria and fungi to reduce arsenate has been known for many decades (see Stolz & Oremland, 1999; Ehrlich, 2002a), clear recognition that bacterial reduction of arsenate to arsenite is mostly a form of anaerobic respiration has come only recently. Glucose, lactate and acetate have been found to be effective electron donors in this process (Oremland *et al.*, 2002b). *Chrysiogenes arsenatis* was the first organism found to be able to use acetate as reductant (Macy *et al.*, 1996). At least one of the organisms that can respire with arsenate as terminal electron acceptor is an archeon, *Pyrobaculum arsenaticum* (Huber *et al.*, 2000). Fungi as well as bacteria can form methylated arsenides from arsenite, bacteria forming $(CH_3)_2As$ and fungi forming $(CH_3)_3As$ in a process that is a form of arsenic detoxification.

Anaerobic reduction of CO_2 to CH_4 Carbon dioxide can serve as terminal electron acceptor in anaerobic hydrogen respiration. The product of this reduction is methane when certain members of the domain Archaea, the methanogens, are involved:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{1.1}$$

The product is acetate when certain members of the domain Bacteria, homoacetogens, are involved:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \tag{1.2}$$

These transformations are an important phase of the anaerobic part of the carbon cycle.

Non-enzymatic inorganic transformations of geomicrobial significance

Mineral formation

Metal sulphides Sulphide produced in bacterial sulphate respiration can precipitate heavy metal ions from solution when the sulphide concentration is in excess of that demanded by the solubility product of the

corresponding metal sulphide. Although most microbial metal sulphide precipitation is attributable to the bacterial reduction of sulphate, at least one instance of CuS precipitation by fungal reduction of S^o has been observed in the laboratory (Ehrlich & Fox, 1967).

Carbonates Biogenic carbonate can precipitate calcium ions as calcite or aragonite, ferrous ions as siderite and manganous ions as rhodochrosite when the carbonate ion concentration is in excess of that demanded by the solubility product of the respective carbonate precipitates and if some other factors do not interfere with the reaction. The carbonate ions may be formed from CO_2 generated in microbial respiration or fermentation of organic carbon (see below, p. 14), or it may be the result of photosynthesis in an aqueous environment in which the assimilation of dissolved CO_2 causes an increase in the concentration of carbonate ion due to the removal of CO_2 from HCO_3^--containing solution ($2HCO_3^- \leftrightarrow CO_3^{2-} + CO_2\uparrow + H_2O$). Although the formation of the carbonate anions in these instances depends on enzymatic processes, the subsequent carbonate precipitations are not enzymatically catalysed. Ehrlich (2002a, pp. 184–211) reviewed this topic in more detail.

Phosphates Microbially mobilized phosphate resulting from the enzymatic breakdown of phosphate esters may precipitate as calcium phosphate (e.g. apatite) when encountering critical environmental concentrations of Ca^{2+}. Such precipitation may occur in the bulk phase (see Ehrlich, 2002a, pp. 275–7) or at a cell surface (Macaskie *et al.*, 1987, 1992).

Mineral weathering

Carbonate, silicate and phosphate minerals may be subject to weathering by corrosive products of bacterial and fungal metabolism. Corrosive metabolic products produced by bacteria and/or fungi include the mineral acids H_2CO_3, H_2SO_4 and HNO_3, and organic acids such as formic, acetic, oxalic, propionic, pyruvic, lactic, succinic, butyric, gluconic, 2-ketogluconic, citric and others. An organic acid may corrode a mineral by promoting acid dissolution or by withdrawing cationic constituents like Ca^{2+} from the mineral surface through complexation, thereby promoting eventual collapse of the crystal lattice. Thus, the calcium of limestone may be mobilized as a result of dissolution of its $CaCO_3$ by H_2CO_3 formed from respiratory CO_2 produced by bacteria and/or fungi growing on the limestone surface. Similarly, the calcium of limestone may be mobilized by HNO_3 formed in the oxidation of NH_4^+ by nitrifying