Chapter 8
Microbiological Controls on Geochemical Kinetics 1: Fundamentals and Case Study on Microbial Fe(III) Oxide Reduction

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8.1 Introduction

The pervasive influence of microorganisms (abbreviated hereafter as “morgs”; see Table 8.1 for a list of abbreviations) on the geochemistry of low-temperature environments is well-recognized and has been the subject of voluminous experimental and observational research (Banfield and Nealson, 1997; Brezonik, 1994; Canfield et al., 2005; Chapelle, 2001; Ehrlich, 2002; Lovley, 2000b). Many of the foundational insights into the role of morgs as agents of geochemical reaction can be traced to basic discoveries in microbiology which took place in the 19th and early 20th centuries. Perhaps the most important contribution of all was Louis Pasteur’s definitive demonstration that decomposition of OM does not proceed in the absence of living morgs (Pasteur, 1860). Though not made in the context of geochemistry, his decisive defeat of the theory of spontaneous generation was a key step toward recognizing the role of microbial life as a direct agent of chemical transformation in natural, medical, and industrial settings. A long series of discoveries followed in which the participation of morgs in various aspects of elemental cycling and mineral transformation was revealed, many in the context of soil and aquatic microbiology (Clarke, 1985; Ehrlich, 2002; Gorham, 1991). These early discoveries, together with developments in the fields of general microbiology and biochemistry (e.g., as embodied in Kluyver (1957)’s synthesis of unity and diversity in microbial metabolism) laid the groundwork for our current understanding of microbial metabolism based on principles of biochemical energetics (thermodynamics) and enzymatic reaction kinetics.

Interest in the participation of morgs in geochemical and geological phenomena has accelerated greatly in the past several years (Banfield et al., 2005a; Nealson et al., 2001; Newman and Banfield, 2002). This burgeoning interest in geomicrobiology has been sparked in part by the rapid increase in our knowledge of the diversity and ubiquity of morgs in all kinds of natural systems, including extremely...
hot, cold, dry, alkaline, and acidic environments. This revolution is being facilitated by the application of molecular genetic tools, as well as ongoing advances in culturing techniques (Banfield et al., 2005b; Barns and Nierzwicki-Bauer, 1997; Keller and Zengler, 2004; Madsen, 2005; Oremland et al., 2005). In addition, advances in analytical methodologies such as high-resolution transmission electron microscopy and various spectroscopic methods for characterizing are offering new insights into the mechanisms of microbe-mineral interactions (see Geesey et al. (2002) for a review). Much of the work to date in this area has focused on qualitative descriptions of phenomena, in particular on the mineralogical and/or biochemical mechanisms leading to the production or destruction of minerals. With several notable exceptions (e.g., in the area of OM diagenesis in aquatic environments), generally less attention has been paid to development of quantitative descriptions (i.e., kinetic models) of microbial contributions to geochemical processes in natural systems. This distinction reflects the difference between the fields of Geomicrobiology and Microbial Biogeochemistry (Ehrlich, 2002): whereas Geomicrobiology examines the role that morgs have played in the past and continue to play in fundamental geological processes such as rock weathering, soil and sediment formation, genesis and degradation of minerals, and in the genesis and degradation of fossil fuels, Microbial Biogeochemistry deals primarily with the kinetics of microbially influenced chemical reactions, often in the context of material (mineral) cycles with emphasis on mass transfer and energy flow. The latter subject is the primary focus of this and the companion chapter (Chap. 8.4.5.5).
8 Microbiological Controls on Geochemical Kinetics

The overall goals of the two chapters are to (1) summarize the role of microbial processes in water-rock interactions (e.g., oxidation-reduction and mineral dissolution-precipitation reactions); (2) present and illustrate by example the essential kinetic models that are typically used to describe microbial processes in the context of biogeochemical processes; and (3) discuss two detailed case studies that illustrate how microbial processes control the kinetics of environmentally significant geochemical processes, namely the dissimilatory reduction of iron oxide minerals, and the biological oxidation of metal sulfide minerals. Both of the latter processes represent unique coupled microbial-geochemical reaction systems that embody the essential features of microbially catalyzed water-rock interactions. Finally, a brief summary and overview of future prospects for advances in understanding coupled microbial-geochemical kinetics is presented.

Before proceeding, a brief clarification of the term “microorganism”: with no exceptions, this term is used here to refer to prokaryotic organisms, which comprise two of the three domains of life, Bacteria and Archaea (Woese et al., 1990). Although microscopic eukaryotes are important in a wide variety of natural environments, we focus our attention here on prokaryotes because – as a result of their diverse metabolic potential (see next section) – they exert a much broader range of impacts on water-rock interactions than do eukaryotic organisms.

8.2 Overview of the Role of Mogs in Water-Rock Interactions

8.2.1 Mechanisms and Definitions

There are three basic mechanisms whereby mogs participate in water-rock interactions: (1) enzymatic reactions linked to energy generation or other biochemical processes that lead directly to production or consumption of dissolved and/or solid-phase compounds; (2) non-enzymatic reactions in the bulk phase which are promoted by enzymatic production or consumption of dissolved and/or solid-phase species; (3) non-enzymatic reactions which are promoted via coordination of reactants by reactive cell surface ligands (e.g., cell surface adsorption or surface nucleation/precipitation reactions). Figure 8.1 provides a simple illustration of these three mechanisms in the context of dissimilatory (microbial) iron oxide reduction.

An important distinction here is that between “biologically induced mineralization” and “biologically controlled mineralization” (also sometime referred to as “organic matrix-mediated mineralization) (Lowenstam, 1981). The latter refers to formation of a mineral via cellular activities that direct the nucleation, growth, and morphology of a mineral for a specific physiological or morphological purpose, usually via an enzymatically coordinated process that is under genetic control. Classic examples include production of calcium carbonate shells or silicate tests by eukaryotic organisms, and intracellular deposition of magnetite crystals by magnetotactic bacteria (Lowenstam and Weiner, 1989; Weiner...
Fig. 8.1 Illustration of three basic mechanisms of microbial participation in water-rock interactions (as defined in section 8.2.1), in the context of dissimilatory Fe(III) oxide reduction. **Left:** Reaction mechanisms: (1) transfer (e.g. by outer membrane cytochromes or other redox-active appendages) of electrons from the surface of a DIRM to the surface of an insoluble Fe(III) oxide (see Case Study #1); (2) biologically-induced formation of Fe(II)-bearing mineral phases such as siderite (FeCO$_3$), vivianite (Fe$_3$(PO$_4$)$_2$), or magnetite (Fe$_3$O$_4$) through reaction of biogenic Fe(II) with aqueous species (CO$_2^-$, PO$_4^{3-}$) or (in the case of magnetite) residual Fe(III) oxide surfaces; (3) surface precipitation of Fe(OH)$_2$ on DIRM cell surfaces, facilitated by accumulation of Fe$^{2+}$ ions on negatively-charged functional groups on the cell surface. **Right:** Photoillustrations: (a) physical contact between a DIRM (*Shewanella algae*) and a synthetic Fe(III) oxide (goethite, α-FeOOH) (M. Urrutia, unpublished), a critical step in the direct transformation of the oxide surface via enzymatically-catalyzed electron transfer; (b) stripping of Fe(III) oxide coatings on subsurface sediments via dissimilatory microbial reductive dissolution in hydrocarbon-contaminated sediments (Lovley, 2000); (c) conversion of synthetic hydrous ferric oxide (HFO) to siderite (FeCO$_3$) during dissimilatory reduction by *Shewanella putrefaciens* (Roden et al., 2002); (d) conversion of HFO to magnetite (Fe$_3$O$_4$) during dissimilatory reduction by *Desulfuromonas acetoxidans* (Roden and Lovley, 1993); (e) and (f) TEM image of Fe(OH)$_2$ surface precipitates on *Shewanella putrefaciens* cells formed during dissimilatory reduction of ferric citrate (Y. Gorby, unpublished) (see also color insert).
varies among different magnetotactic organisms, in all cases a given organism
produces crystals with very narrow size range within a specialized membrane-bound
prokaryotic organelle called a magnetosome (Bazylinski and Frankel, 2000). Recent
studies indicate that bacterial magnetosomes are invaginations of the cell membrane
flanked by cytoskeletal filaments composed of actin-like proteins analogous to those
present in eukaryotic cells (Komeili et al., 2006), and that these and other proteins
are likely to play a role in establishing the specific locus for production of the chains
of magnetite crystals (Scheffel et al., 2006). Magnetotactic bacteria produce intra-
cellular magnetite for a defined purpose, i.e., to align themselves vertically in the
Earth’s magnetic field in order to find and maintain a position of optimum dissolved
O$_2$ concentration within redox gradients in the water column or sediments of aquatic
systems (Bazylinski and Frankel, 2000).

In contrast to the magnetite produced by magnetotactic bacteria, the bulk
quantities of extracellular magnetite produced by DIR (see Fig. 8.1d) result from
the abiotic interaction of biogenic Fe(II) with ferrihydrite outside of the cell
(Lovley et al., 1987). The magnetite so produced is poorly crystalline and irregular
in shape, with a relatively broad size distribution, and with most particles being in
the superparamagnetic size range (<35 nm) (Moskowitz et al., 1989; Sparks et al.,
1990). Production of extracellular magnetite by DIR serves no known function for
DIRMs; in fact, it actually limits DIR activity, because the magnetite is not readily
reducible under the circumneutral pH conditions that are typical of DIR sys-
tems (Kostka and Nealson, 1995; Lovley and Phillips, 1986b; Lovley et al., 1987;
Zachara et al., 2002).

This chapter deals solely with examples of biologically induced mineralization,
and in particular on quantitative description of the physiological-biochemical
processes that are directly responsible for mineral-water reactions (mechanism 1
above). The term “microbial mineral transformation” (Ehrlich, 1999) is recom-
mended to refer collectively to mineral dissolution or precipitation reactions that
involve microbial activity. This label is preferable to one that includes “miner-
alization”, because for microbial physiologists and ecologists, as well as many
geochemists, mineralization means conversion of OM to its inorganic constituents
(Ehrlich, 1999).

### 8.2.2 Key Characteristics of Microorganisms

The significant quantitative role of morgs in water-rock interactions can be traced to
fundamental aspects of biological metabolism and to the tremendous metabolic di-
versity and unique physical and chemical structure of morgs. Like all other living or-
ganisms, morgs utilize enzymes (proteins) to catalyze reactions associated with en-
ergy generation and cellular biosynthesis (Madigan et al., 2000). Although enzymes
do not alter the overall thermodynamic favorability of a reaction, they lower the
activation energy required for a reaction to proceed (see Fig. 2.1), thereby permit-
ting reactions to occur under relatively low near-surface temperature and pressure
conditions. For example, DIRMs produce redox-active proteins and localize them
on the outer surface of the cell in order to transfer electrons from intracellular OC oxidation to Fe(III) oxide surfaces outside of the cell (see Fig. 8.11). In the absence of the whole suite of enzymes involved (intracellular and extracellular), there is no spontaneous reaction between Fe(III) oxides and OC. Similarly, Fe(II)- oxidizing morgs produce membrane-associated enzyme systems that catalyze the reaction between Fe(II) and O2 at low pH (see Fig. 9.3), a reaction which is extremely slow in the absence of enzymatic catalysis (see Fig. 9.1).

An extraordinarily wide range of reactions – far more diverse than those known in the eukaryotic world – are facilitated by prokaryotic morgs, many of which are directly relevant to the subject of water-rock interactions. Often such reactions occur solely through enzymatic catalysis at near-surface temperature and pressure, although several important reactions (e.g., oxidation of reduced Fe and S species) may also proceed through abiotic reaction pathways. Figure 8.2 provides a simple classification of microbial energy metabolism that can be used as a basis for understanding the diverse manner in which morgs may participate in water-rock interactions. Table 8.2, in turn, provides a representative listing, organized in the context of Fig. 8.2, of the role of morgs as catalysts of geochemical processes relevant to the subject of water-rock interactions. Nealson and Stahl (1997) provide a brief but thorough description of virtually all of the metabolic processes listed in Table 8.2, as well as the organisms responsible for catalyzing them.

Another key feature of morgs is their small size, which has several fundamental implications for their ability to participate intensively in biogeochemical processes (Nealson and Stahl, 1997). Prokaryotic cells with a diameter on the order of 1 µm have surface area-to-volume ratio 10–1000 times higher than typical eukaryotic cells. As a result, morgs generally possess a relatively high metabolic rate per unit mass compared to larger organisms, which in turn enhances the effective connection (chemically and energetically) between cells and their local environment. A related implication of the small size of morgs is their potential to exhibit rapid rates of growth and population turnover. In a review of the ecological implications of body size, Peters (1983) calculates that the time required for morgs
Table 8.2 Survey of the role of microorganisms as catalysts of water-rock interactions, organized in relation to the classification of microbial energy metabolism in Fig. 8.2

<table>
<thead>
<tr>
<th>Process type Chemotrophy</th>
<th>Process</th>
<th>Mechanisms of reaction</th>
<th>Representative organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemoorganotrophy</td>
<td>Oxidation of solid-phase organic matter</td>
<td>Enzymatic hydrolysis of polymeric OM and coupled fermentation-respiration of monomers</td>
<td>Wide range</td>
</tr>
<tr>
<td></td>
<td>Reductive dissolution of Fe(III) and Mn(IV) oxides (circumneutral pH) coupled to organic acid &amp; H₂ oxidation</td>
<td>Enzymatic (outer membrane redox-active cytochromes and/or pili) electron transfer to oxide surfaces</td>
<td>Geobacter, Geothrix, Shewanella, Geoglobus; some SRMs and MGMs; various thermophilic Archaea</td>
</tr>
<tr>
<td></td>
<td>Production of magnetite, siderite, vivianite, and rhodocrocite</td>
<td>Reaction of biogenic Fe(II) or Mn(II) with residual oxide surfaces, carbonate, or phosphate</td>
<td>Same as above</td>
</tr>
<tr>
<td></td>
<td>Production of U(IV), Tc(V), and other insoluble reduced metal/radionuclide phases</td>
<td>Enzymatic reduction of soluble oxidized metals and precipitation of insoluble reduced metals</td>
<td>Same as above</td>
</tr>
<tr>
<td></td>
<td>Production of pyrite and other iron and metal-sulfide minerals</td>
<td>Enzymatic reduction of sulfate, excretion of dissolved sulfide, and reaction of dissolved sulfide with Fe(II) and/or Fe(III)</td>
<td>δ-Proteobacteria and others</td>
</tr>
<tr>
<td>Chemolithotrophy Chemolithoautotrophy</td>
<td>Oxidation of metal-sulfide minerals and S⁰ (acidic pH) with O₂ or Fe³⁺</td>
<td>Enzymatic oxidation of Fe(II) and S⁰; electrochemical corrosion</td>
<td>Acidithiobacillus, Leptospirillum, Ferroplasma, Sulfolobus/Acidanus</td>
</tr>
<tr>
<td></td>
<td>Aerobic and nitrate-dependent oxidation of FeS and S⁰ (circumneutral pH)</td>
<td>Unknown</td>
<td>Beggiatoa, Thiothrix</td>
</tr>
<tr>
<td></td>
<td>Disproportion of S⁰, and S₂O⁵⁻</td>
<td>Unknown</td>
<td>Desulfoacidus</td>
</tr>
<tr>
<td></td>
<td>Oxidation of Fe(II) in FeS₂ and basalt glass</td>
<td>Unknown</td>
<td>α- and γ-Proteobacteria</td>
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</tbody>
</table>

(cont.)
<table>
<thead>
<tr>
<th>Process type</th>
<th>Chemotrophy</th>
<th>Process</th>
<th>Mechanisms of reaction</th>
<th>Representative organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixotrophy</td>
<td></td>
<td>Nitrate-dependent oxidation of aqueous Fe(II) &amp; solid-phase Fe(II)</td>
<td>Unknown</td>
<td>Geobacter, Ferroglobus, Thiobacillus denitrificans, various β-Proteobacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidative precipitation of Fe(III)/Mn(IV) oxides</td>
<td>Enzymatic Fe(II) (mechanism unknown) and Mn(II) (Cu-containing oxidases) oxidation</td>
<td>Bacillus, Leptothrix, Gallionella, ES1, TW2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aerobic and nitrate-dependent oxidation of FeS and S(^0) (circumneutral pH)</td>
<td>Unknown</td>
<td>Beggiatoa, Thiobacillus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrate-dependent oxidation of aqueous Fe(II) &amp; solid-phase Fe(II)</td>
<td>Unknown</td>
<td>Geobacter, Ferroglobus, Thiobacillus denitrificans, various β-Proteobacteria</td>
</tr>
<tr>
<td>Phototrophy</td>
<td></td>
<td>Production of calcium carbonate minerals</td>
<td>Photosynthesis-induced changes in alkalinity; coordination of ions on cell surfaces</td>
<td>Cyanobacteria and microeukaryotic algae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidative precipitation of Fe(III) oxides</td>
<td>Light-dependent enzymatic Fe(II) oxidation; mechanism unknown</td>
<td>Various anoxygenic photosynthetic β-Proteobacteria</td>
</tr>
</tbody>
</table>
to colonize an environment and reestablish an arbitrary biomass of 100mg m$^{-2}$ is on the order of a few days, whereas for larger organisms such as insects the time scale is on the order of a year or at least a seasonal period. These calculations explain conceptually how morgs can quickly exploit resources in environmental systems and therefore have immediate major impacts on materials and energy processing in changing natural environments. Finally, the small size of morgs permits them to associate with local physical and chemical microenvironments. In many instances, high rates of chemical flux (including mineral transformations) take place at reaction fronts that are characterized by micrometer (or even nanometer) to millimeter-scale microenvironments (Revsbech and Jorgensen, 1986). Such microenvironments are colonized by morgs specifically adapted to take advantage of sources of energy or other environmental conditions available within the microenvironments. Indeed, in most instances, the metabolic activities of morgs often dictate the physical location and specific chemical conditions of the microenvironment, and in this sense the microbial-geochemical interactions can be viewed as a holistic system that defines its own internal parameters relative to external boundary conditions (Revsbech and Jorgensen, 1986). Visscher et al. (1998) provide a comprehensive example of such a system involved in the formation of modern marine stromatolites.

A final key feature of prokaryotic cells is the ability of the cell surface to serve as an interface for sorption and concentration of metal cations in dilute environmental solutions. The long-standing attribution of this property to the presence of deprotonated carboxylate and phosphate groups on the bacterial outer membrane (Beveridge, 1989) was recently confirmed for both gram positive and gram negative organisms by infrared spectroscopy (Jiang et al., 2004). Thus there is an apparent universality of functional group chemistry on prokaryotic cell surfaces (i.e., carboxyl, phosphoryl, and hydroxyl sites, with pKa values of 4.8, 6.9, and 9.4, respectively; (Fein et al., 1997)), which validates the use of standard surface complexation models (see Chap. 4) for simulation of cation adsorption processes (Borrok and Fein, 2004; Fein et al., 2001; Yee and Fein, 2001). The key implication of this phenomenon for water-rock interactions is that once metal ions have interacted with electronegative sites on the outer membrane, they nucleate the formation of a wide range of fine-grained minerals (e.g., oxide/hydroxide, carbonate, sulfate/sulfide, silicate, and phosphate) by incorporating common anions from their surroundings (Schultze-Lam et al., 1996). This process in turn promotes further metal sorption and precipitation, and thus generally accelerates the kinetics of mineral precipitation in environments where prokaryotic cells are abundant. Two different modes of action can be distinguished in this context (Southam, 2000): passive microbially induced mineral formation refers to simple binding of cations and recruitment of solution anions, resulting in surface nucleation and mineral growth (essentially mechanism 3 above); active microbially induced mineral formation occurs via direct redox transformation of surface-associated metal ions (e.g., enzymatic electron transfer to or from a mineral surface), or by the production of metabolic end-products that form minerals on cell surfaces (a combination of mechanisms 1 and 3 above).
8.3 Kinetic Models in Microbial Geochemistry

8.3.1 Introduction

The literature on kinetic analysis and modeling of geochemical processes is vast and has been systematically reviewed several times over the past few decades (Berner, 1980; Boudreau, 1997; Brezonik, 1994; Lasaga and Kirkpatrick, 1981; Lasaga, 1998; Lerman, 1979; Schnoor, 1996; Stumm, 1990). The purpose of this section is to explain how standard kinetic rate laws can be applied to microbially catalyzed reactions. The subject is approached from a macroscopic point of view, in which rates of microbial metabolism are related to observed (or estimated) properties such as reactant concentration, microbial biomass, and the thermodynamic favorability (i.e., the $\Delta G$) of the reaction.

Virtually all biogeochemical reactions (as well as many abiotic geochemical reactions; see Chap. 2) are the net result of numerous elementary reactions. For example, the reduction of Fe(III) oxides coupled to oxidation of OM in sediments

$$\text{CH}_2\text{O} + 4\text{Fe(OH)}_3 + 7\text{H}^+ \rightarrow \text{HCO}_3^- + 4\text{Fe}^{2+} + 10\text{H}_2\text{O} \quad (8.1)$$

is the net result of a complex series of enzymatic reactions, carried out by multiple groups of morgs, during which polymeric organic carbon (OC) undergoes hydrolysis and fermentation to generate the organic acids (e.g., acetate) and $\text{H}_2$ that are the substrates for dissimilatory iron-reducing morgs (DIRMs) (Lovley, 1991). The metabolism of individual energy substrates such as acetate and $\text{H}_2$ coupled to DIR by DIRMs

$$\text{CH}_3\text{COO}^- + 8\text{Fe(OH)}_3 + 15\text{H}^+ \rightarrow 2\text{HCO}_3^- + 8\text{Fe}^{2+} + 20\text{H}_2\text{O} \quad (8.2)$$

$$\text{H}_2 + 2\text{Fe(OH)}_3 + 4\text{H}^+ \rightarrow 2\text{Fe}^{2+} + 6\text{H}_2\text{O} \quad (8.3)$$

are also complex processes that involve several different enzyme systems both inside and outside of the cell membrane (Gorby et al., 2006; Lovley et al., 2004; Methe, 2003; Reguera et al., 2005). Even in cases where the rate-limiting step in a microbial reaction can be traced to a specific enzymatic process (e.g., uptake of an organic substrate or a soluble electron acceptor across the cell membrane, or an intracellular enzymatic reaction), that biochemical process is never the result of a single elementary reaction, but rather a multistep process involving one or more enzymes. Thus, for our purposes kinetic expressions for rates of microbial reaction are by definition macroscopic descriptions of overall reactions. Fortunately, in many cases, it is possible to use elementary rate expressions to describe the dependence of an overall multistep reaction on the concentration of one or a few reactions that control the rate of the overall reaction.
8.3.2 Zero-Order Kinetics

A zero-order reaction is one whose rate is (apparently) not dependent on the concentration of the reactant of interest (see Chap. 1). Although such a reaction would appear to violate the principle of mass action, the violation is only an apparent one. Zero-order kinetics are common in homogeneous chemical reactions in which the concentration of a catalyst controls the rate of reaction, and in reactions at surfaces where a physical constraint such as surface area limits the reaction rate (Brezonik, 1994). A zero-order rate law may also describe biogeochemical reactions in which the reactant concentration(s) are above saturation, or to describe reaction rates in experiments where the period of observation is sufficiently short so as to avoid curvature in a plot of reactant concentration versus time.

A classic example of the latter situation comes from Martens and Berner’s (1974) experiments on microbial sulfate reduction (SR) in marine sediments. Rates of microbial SR in marine sediments are independent of sulfate concentration down to sulfate concentrations of ca. 3 mM (Boudreau and Westrich, 1984; Roden and Tuttle, 1993), tenfold lower than the concentration of sulfate in seawater (28 mM). As a result, Martens and Berner (1974) observed a linear decrease in sulfate concentration during their salt marsh sediment incubation experiment (Fig. 8.3a), in which the rate of SR was limited by the abundance and reactivity of OM rather than sulfate concentration down to sulfate concentrations of \( \leq \) ca. 1 mM.

Another good example of a zero-order rate process in a geomicrobiological reaction system comes from studies of the kinetics of microbial (enzymatic) reduction of synthetic Fe(III) oxides with differing SAs (Roden, 2003a, 2006). Although the long-term kinetics of DIR can be well-described as a first-order process dependent on the abundance of microbially reducible surface sites (discussed in detail in Case Study #1), rates of reduction are generally constant during the first few days of incubation, such that initial, zero-order rates of reaction can be extracted from linear plots of Fe(II) versus time (e.g., Fig. 8.3b).

8.3.3 First-Order Kinetics

Several major classes of geomicrobiological processes (e.g., oxidation of dissolved and solid-phase OM, oxidation and reduction of insoluble mineral phases) can be well-described by first-order kinetics, in which the reaction rate is directly proportional to the concentration of the reactant (see Chap. 1). The first-order dependence of bulk DIR and FeS\(_2\) oxidation rates on the abundance of mineral SA is examined in detail in Case Studies 1 and 2, respectively. We focus here on oxidation of sediment OC as premier example of first-order reaction kinetics in microbial geochemistry. The development provides a segue into descriptions of the use of mixed
Fig. 8.3 Two examples of zero-order rate processes: (A) Consumption of sulfate during laboratory incubation of organic-rich saltmarsh sediments (data from Martens and Berner (1974), used with permission); (B) Production of Fe(II) during reduction of different synthetic Fe(III) oxides by S. putrefaciens (data not shown in Roden (2003)). Solid lines show the results of linear least-squares regression analysis of the concentration vs. time data. The zero-order rate of sulfate consumption in panel A is 0.91 mM d\(^{-1}\); the zero-order rates of Fe(II) production in panel B range from ca. 0.09 for crystalline hematite (squares) to 0.9 mmol L\(^{-1}\) d\(^{-1}\) for poorly crystalline hydrous ferric oxide (asterisks). The dashed line in panel A illustrates the influence of sulfate limitation on sulfate consumption at very low (< 1 mM) sulfate concentration.

first-order/Monod kinetics to describe consumption of soluble EAs during OM oxidation (see Sect. 8.3.4).

Berner (1964) proposed the use of a first-order rate model to depict decay of POC in order to explain the observed depth distribution of sulfate in marine sediment porewater (see Berner (1980) for review). This early work led to a generation of kinetic models in which first-order decay of POC is the primary driving force for a wide range of sediment diagenetic processes (Berner, 1977; Boudreau, 1996; Dhakar and Burdige, 1996; Klump and Martens, 1989; Soetaert et al., 1996; VanCappellen et al., 1993; VanCappellen and Wang, 1996). Although multiple fractions of POC, with differing intrinsic reactivities (see Boudreau (1991) for review),
must typically be invoked to properly describe the overall kinetics of degradation, introduction of the basic first-order rate model for POC metabolism was crucial in that it provided an adequate tool for quantifying the impact of OM degradation on sediment geochemistry (VanCappellen and Gaillard, 1996). Such impacts include a wide variety of mineral dissolution and precipitation reactions (Berner, 1981) that strongly influence both present day biogeochemical dynamics as well as long-term geochemical cycles on earth, e.g., the role of FeS$_2$ burial in planetary redox balance (Berner, 1982, 1989).

The basic assumption underlying application of the first-order rate model to POC metabolism is that hydrolysis of solid-phase OM by attached morgs is the rate-limiting step in the overall degradation process. Thus, POC oxidation can be viewed as a surface-controlled process analogous to microbial mineral transformations such as Fe(III) oxide reduction and FeS$_2$ oxidation. Westrich and Berner (1984) provided two pieces of direct experimental evidence in support of this model. First, loss of POC mass during aerobic oxidation of coastal phytoplankton could be well-described by a so-called “two-G” rate model (where “G” is the generic symbol used to represent POC), in which $G_1$ represents the most labile fraction of POC, and $G_2$ represents a less-labile (but still metabolizable on a time scale of years) fraction (Fig. 8.4a). Second, Westrich and Berner (1984) demonstrated that addition of increasing amounts of either fresh or partially degraded phytoplankton POC to organic-poor coastal marine sediment lead to a linear increase in the rate of OM oxidation coupled to microbial SR (Fig. 8.4b). The latter experiments were performed using short-term incubation experiments (after a 10-day preincubation period following POC addition) with $^{35}$S-labeled sulfate, which (after analyzing data on the accumulation of $^{35}$S-labeled sulfide using a zero-order rate model) yielded instantaneous rates of OM metabolism for each level of POC addition. These results provided conclusive support for the first-order rate model for POC decay first proposed by Berner 20 years earlier (Berner, 1964). Subsequent studies of marine sediment OM metabolism have also supported this approach (e.g., (Burdige, 1991; Klump and Martens, 1987; Roden and Tuttle, 1996)), as well as studies in freshwater sediments in which Fe(III) oxide reduction and methanogenesis (MG) were the predominant pathways for OM oxidation (Roden and Wetzel, 2002).

### 8.3.4 Hyperbolic Kinetics: Enzyme Activity and Microbial Growth/Metabolism

The basic rate expressions that describe the kinetics of enzyme activity and microbial metabolism are referred to as “Michaelis-Menton” and “Monod” kinetics, respectively, in honor of the scientists who first introduced them (Michaelis and Menten, 1913; Monod, 1942, 1949). The term “hyperbolic kinetics” is often used in reference to these rate laws, since a plot of rate against reactant concentration takes the form of a rectangular hyperbola through the origin. Table 8.3 provides derivations of the hyperbolic rate law applied to enzyme and microbial metabolic activity.
Hyperbolic rate expressions find broad use in microbiology, engineering, and environmental science. Because of the importance of these rate laws, their history is briefly reviewed prior to examining examples of their applications in geomicrobiology.

The Michaelis-Menton (1913) equation was derived to describe the activity of the invertase enzyme (which splits sucrose into glucose and fructose) in terms of the law of mass action, based on the formation of an enzyme-substrate complex and its subsequent conversion to reaction products. In contrast, the Monod (1942, 1949) equation was originally used as a strictly empirical tool to describe the influence of
Table 8.3A  Derivation of classical Michaelis-Menton enzyme kinetics

Overall reaction:

$$S \xrightarrow{E} P$$

(1)

where:

- S = substrate
- P = product
- E = enzyme (catalyst)

Reaction mechanism:

$$E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} P + E$$

(2)

where:

- ES = enzyme-substrate complex

Rate of P accumulation:

$$\frac{d[P]}{dt} = k_2[ES]$$

(3)

Assume d[ES]/dt = 0 and solve for [E]:

$$[E] = \frac{k_{-1} + k_2}{k_1[S]} [ES]$$

(4)

Assuming total amount of E ($E_T$) is constant:

$$[E]_T = [E] + [ES]$$

(5)

Substitute for [E] in (4) and solve for [ES]:

$$[ES] = \frac{[S][E]_T}{(k_{-1} + k_2)/k_1 + [S]}$$

(6)

$$\frac{d[P]}{dt} = V_{\text{max}} \frac{[S]}{K_m + [S]}$$

(7)

where:

$$V_{\text{max}} = k_2[E]_T$$

(9)

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

(10)
Table 8.3B Derivation of general Monod rate law for microbial metabolism

Overall reaction:

\[ S \xrightarrow{B} P \]  

(1)

where:

- \( S \) = substrate
- \( P \) = product
- \( B \) = cell biomass (catalyst)

Reaction mechanism:

\[ B + S \xrightarrow{k_1} BS \xrightarrow{k_2} P + B \]  

(2)

where:

- \( BS \) = cell-substrate complex

Rate of \( P \) accumulation:

\[ \frac{d[P]}{dt} = k_2[BS] \]  

(3)

Assume \( \frac{d[BS]}{dt} = 0 \) and solve for \( [B] \):

\[ [B] = \frac{k_{-1} + k_2}{k_1[S]}[BS] \]  

(4)

Assuming total biomass of cells \( (B_T) \) is constant:

\[ [B]_T = [B] + [BS] \]  

(5)

Substitute for \( [B] \) in (4) and solve for \( [BS] \):

\[ [BS] = \frac{[S][B]_T}{(k_{-1} + k_2)/k_1 + [S]} \]  

(6)

Substitute for \( [BS] \) in (3)

\[ \frac{d[P]}{dt} = V_{\text{max}} \frac{[S]}{K_s + [S]} \]  

(7)

where:

\[ V_{\text{max}} = k_2[B]_T \]  

(9)

\[ K_s = \frac{k_{-1} + k_2}{k_1} \]  

(10)
limiting substrate concentration on the specific growth rate (e.g., in units of d\(^{-1}\)) of morgs during exponential growth (Koch, 1998). Subsequent work by Monod and colleagues (Rickenberg et al., 1956) adapted the basic framework of the Michaelis-Menton equation to describe the kinetics of lactose uptake across the bacterial cell membrane in terms of a substrate-enzyme (permease) complex. Both of these applications of the Monod equation can be recovered from the general derivation given in Table 8.3B. To account for substrate uptake across the cell membrane, S can be assumed to represent substrate on the outside of the cell, and P substrate on the inside of the cell. To account for microbial growth kinetics, B is substituted for P such that the reaction mechanism leads to production of an additional unit of cell biomass as a function of substrate concentration. This leads to the following rate expression:

\[
\frac{d[B]}{dt} = k_2 \frac{[S]}{K_s + [S]} \tag{8.4}
\]

Dividing both sides by the total biomass at time t, \([B]_T\), yields

\[
\frac{d[B]/dt}{[B]_T} = k_2 \frac{[S]}{K_s + [S]} \tag{8.5}
\]

in which the left hand term represents the instantaneous rate of biomass production relative to the current total biomass (i.e., the specific growth rate), and in which \(k_2\) can be identified as the maximum specific growth rate at very high substrate concentration. The specific and maximum specific growth rates are typically referred to by the symbols \(\mu\) and \(\mu_{\text{max}}\), which leads to the following classical expression for Monod growth kinetics (Koch, 1998):

\[
\mu = \mu_{\text{max}} \frac{[S]}{K_s + [S]} \tag{8.6}
\]

The Michaelis-Menton and Monod equations can be generalized to the following rate expression for use in describing biogeochemical process rates

\[
R = R_{\text{max}} \frac{[S]}{K_m + [S]} \tag{8.7}
\]

where \(R\) is the rate of substrate metabolism (e.g., in units of mol L\(^{-1}\) d\(^{-1}\)), \(R_{\text{max}}\) is the maximum rate of metabolism (at saturating substrate concentration), \([S]\) is the substrate concentration (e.g. in units of mol L\(^{-1}\)) and \(K_m\) is the half-saturating substrate concentration at which \(R = 0.5R_{\text{max}}\). Formal association of Eq. (8.7) with the Michaelis-Menton or Monod equation would require the assumption that the biogeochemical process of interest is limited by the activity of a specific enzyme or substrate uptake system with fixed bulk abundance in the reaction medium. In practice such an assumption is unnecessary, since a wide variety of more complicated enzyme-catalyzed reactions (i.e., processes that involve multiple enzymatic steps) obey the general form of Eq. (8.7). Thus, Eq. (8.7) provides a robust expression
for representing the overall rate of substrate consumption in a complex microbial process, even when detailed information on the reaction pathway(s) involved is not available (VanCappellen and Gaillard, 1996). In situations where morg biomass is included in the overall rate law (i.e., in biomass-dependent models), Eq. (8.7) becomes

$$R = \frac{R_{\text{max}}[S]}{K_m + [S][B]}$$

(8.8)

where $[B]$ represents biomass (e.g., in units of g L$^{-1}$), and the parameter $R_{\text{max}}$ now has units of mol g biomass$^{-1}$ d$^{-1}$. The corresponding rate expression for biomass growth is

$$\frac{d[B]}{dt} = \frac{Y_{\text{R}} R_{\text{max}}[S]}{K_m + [S][B]} - k_d[B]$$

(8.9)

where $Y$ is the growth yield in units of g biomass produced per mol substrate metabolized, and $k_d$ is a first-order endogenous cell decay constant (Rittmann and McCarty, 2001), e.g., in units of d$^{-1}$.

Hyperbolic rate laws analogous to Eqs. (8.7)–(8.9) have been used to describe a wide variety of biogeochemical processes in natural systems, most notably the consumption of natural and contaminant DOC, soluble inorganic electron donors such as Fe$^{2+}$ and HS$^-$, and soluble EAs such as O$_2$, NO$_3^-$, and SO$_4^{2-}$. Figure 8.5 shows an example of the kinetics of microbial SR in oligohaline (salinity < 2 ppt, SO$_4^{2-}$ < 2 mM) and mesohaline (salinity 10–20 ppt, SO$_4^{2-}$ 7–15 mM) estuarine sediments. The results illustrate an important principle in microbial geochemistry, namely that environmental conditions can lead to adaptation of the kinetic properties of the resident microbial populations to local conditions. In this case, long-term differences in sulfate availability between low-salinity and high-salinity estuarine environments has led to the development of SRM populations with different $K_s$ values for sulfate uptake.

Multiplicative hyperbolic equations have been adopted to describe rate limitations imposed by more than one limiting substrate. A good example is the so-called “dual Monod” equation (Bader, 1982):

$$R = \frac{R_{\text{max}}[S_1]}{K_{m_{s1}} + [S_1]} \frac{[S_2]}{K_{m_{s2}} + [S_2]}$$

(8.10)

where the rate of reaction is limited by two different substrates ($S_1$ and $S_2$). This formulation is commonly used to describe rates of natural or contaminant DOC oxidation coupled to the reduction of a specific EA (e.g., Lovley (1986), Molz (1986), Bae and Rittmann (1995)), where $S_1$ and $S_2$ would correspond to DOC and EA, respectively (see further discussion below). In addition, Eq. (8.10) provides a mechanistic basis for use of a second-order rate law to model secondary redox reactions between EAs and reduced end-products of primary redox reactions associated with organic matter decay (VanCappellen and Wang, 1996) (some of which are known only to proceed via biological catalysis, e.g., oxidation of Mn(II) and CH$_4$ by O$_2$), since Eq. (8.10) reduces to a second-order equation when $S_1 \ll K_{m_{s1}}$ and $S_2 \ll K_{m_{s2}}$. 

An important variant on the dual Monod approach to model rates of POC decay with different EAs is the following mixed first-order/hyperbolic rate scheme:

$$R = k_1[POC] \frac{[EA]}{K_m + [EA]}$$  \hspace{1cm} (8.11)

where $k_1$ is a first-order decay constant for POC and $[POC]$ is the bulk reactive POC concentration. Boudreau (1992) provides a mechanistic derivation of Eq. (8.11) based on the formation of a transient complex (similar to the enzyme-substrate complex in the Michaelis-Menton framework) between the EA and a reactive POC surface site.
Some sediment biogeochemical modeling studies have adopted a modified version of Eq. (8.11) to model EA limitation and competition among different TEAPs in redox stratified environments such as aquatic sediments and groundwater aquifers (Boudreau and Westrich, 1984; Gaillard and Rabouille, 1992; Hunter et al., 1998; VanCappellen and Wang, 1995, 1996; Wang and VanCappellen, 1996). In this “modified Monod” approach (which is identical in form to the empirical model devised by Blackman (1905)), the hyperbolic rate term is simplified by assuming that the rate of reaction is independent of the EA concentration above a certain limiting concentration, and directly proportional to it below that concentration:

\[
R = R_{\text{max}} \quad \text{where} \quad [\text{EA}] \geq [\text{EA}]_{\text{lim}} (8.12)
\]

\[
R = R_{\text{max}} \frac{[\text{EA}]}{[\text{EA}]_{\text{lim}}} \quad \text{where} \quad [\text{EA}] < [\text{EA}]_{\text{lim}} (8.13)
\]

This approach offers a convenient way to model the temporal-spatial sequencing of different TEAPs (VanCappellen and Wang, 1995, 1996): when the concentration of a more energetically favorable EA exceeds the limiting concentration for that EA, the rate of EA consumption is independent of EA concentration, and all other less energetically favorable TEAPs are suppressed. When the concentration of the EA falls below the limiting concentration, the rate of EA consumption is proportional to EA concentration, and less favorable TEAPs become active. This scheme allows for a smooth transition in space and/or time from one predominant TEAP to another. It should be noted that the limiting concentration for a given EA is a semi-empirical parameter that accounts collectively for the influence of kinetic limitations on EA consumption, inhibition of enzyme systems by higher redox potential electron acceptors, and the influence of competition for energy substrates (discussed further below) on the spatial/temporal segregation of different TEAPs (VanCappellen and Wang, 1996). Table 8.4 provides a summary of this computational scheme applied to the typical sequence of TEAPs found in aquatic sediments and groundwater aquifers, and Fig. 8.6a,b shows an application of this approach to modeling the temporal sequence of TEAPs in a slurry experiment with anoxic freshwater wetland sediment.

An attractive aspect of the modified Monod approach for modeling competing microbial respiratory reactions is that it alleviates the need for terms to depict inhibition of TEAPs by more energetically favorable EAs. Although a wide variety of equations are available to describe inhibition of metabolic pathways (cf. Humphrey (1972), Rawn (1983)), the “noncompetitive” inhibition approach is most commonly used to depict inhibition of a given TEAP by a more favorable EA (VanCappellen et al., 1993; Widdowson et al., 1988). For example, inhibition by NO$_3^-$ of Fe(III)
Table 8.4 Computational scheme for the “modified Monod” rate law applied to temporal-spatial sequencing of major TEAPs in sediments

If \([O_2] > [O_2]_{\text{lim}}\) Then
\[R_{O2} = R_{\text{max}}\]
\[R_{NO3} = R_{\text{Mn(IV)}} = R_{\text{Fe(III)}} = R_{SO4} = R_{CH4} = 0\]

Otherwise
\[R_{O2} = R_{\text{max}} \times \frac{[O_2]}{[O_2]_{\text{lim}}}\]
If \([NO_3^-] > [NO_3^-]_{\text{lim}}\) Then
\[R_{NO3} = (R_{\text{max}} - R_{O2})\]
\[R_{\text{Mn(IV)}} = R_{\text{Fe(III)}} = R_{SO4} = R_{CH4} = 0\]

Otherwise
\[R_{NO3} = (R_{\text{max}} - R_{O2}) \times \frac{[NO_3^-]}{[NO_3^-]_{\text{lim}}}\]
If \([Mn(IV)] > [Mn(IV)]_{\text{lim}}\) Then
\[R_{\text{Mn(IV)}} = (R_{\text{max}} - R_{O2} - R_{NO3})\]
\[R_{\text{Fe(III)}} = R_{SO4} = R_{CH4} = 0\]

Otherwise
\[R_{\text{Mn(IV)}} = (R_{\text{max}} - R_{O2} - R_{NO3}) \times \frac{[Mn(IV)]}{[Mn(IV)]_{\text{lim}}}\]
If \([Fe(III)] > [Fe(III)]_{\text{lim}}\) Then
\[R_{\text{Fe(III)}} = (R_{\text{max}} - R_{O2} - R_{NO3} - R_{\text{Mn(IV)}})\]
\[R_{SO4} = R_{CH4} = 0\]

Otherwise
\[R_{\text{Fe(III)}} = (R_{\text{max}} - R_{O2} - R_{NO3} - R_{\text{Mn(IV)}}) \times \frac{[Fe(III)]}{[Fe(III)]_{\text{lim}}}\]
If \([SO_4^{2-}] > [SO_4^{2-}]_{\text{lim}}\) Then
\[R_{SO4} = (R_{\text{max}} - R_{O2} - R_{NO3} - R_{\text{Mn(IV)}} - R_{\text{Fe(III)}})\]
\[R_{CH4} = 0\]

Otherwise
\[R_{SO4} = (R_{\text{max}} - R_{O2} - R_{NO3} - R_{\text{Mn(IV)}} - R_{\text{Fe(III)}} - R_{SO4})\]
\[R_{CH4} = (R_{\text{max}} - R_{O2} - R_{NO3} - R_{\text{Mn(IV)}} - R_{\text{Fe(III)}} - R_{SO4})\]


\(R_{\text{max}}\) represents the maximum collective rate of all TEAPs, e.g. as limited by the first-order rate of POC hydrolysis (see section 8.3.3). \(R_{O2}, R_{NO3}, R_{\text{Mn(IV)}}, R_{\text{Fe(III)}}, R_{SO4},\) and \(R_{CH4}\) refer to rates of OC oxidation coupled to \(O_2\) reduction (aerobic respiration), \(NO_3^-\) reduction (e.g. to \(N_2\) or \(NH_4^+\)), \(Mn(IV)\) oxide reduction, \(Fe(III)\) oxide reduction, \(SO_4^{2-}\) reduction, and methanogenesis, respectively. The subscript “lim” refers to the limiting concentration for a given EA.

Reduction coupled to first-order decay of sediment POC is expressed as follows:

\[
R_{\text{Fe(III)}} = k_1 [\text{POC}] \frac{[\text{Fe(III)}]}{K_{\text{m,Fe(III)}} + [\text{Fe(III)}]} \frac{K_{I,NO3}}{K_{I,NO3} + [NO_3^-]} \tag{8.14}
\]

where \(K_{\text{m,Fe(III)}}\) is the standard half-saturating concentration of Fe(III) as an electron acceptor for OC oxidation, and \(K_{I,NO3}\) is the noncompetitive inhibition constant for \(NO_3^-\). Note here that the inhibition constant corresponds to the concentration of the inhibitor at which the rate of reaction is one-half of what it would be in the absence of the inhibitor; thus, when \([NO_3^-] = K_{I,NO3}\), the rate of Fe(III) reduction \((R_{\text{Fe(III)}})\) is equal to one-half of it would be in the total absence of \(NO_3^-\). When
more than one inhibitor is present, the combined effect of all inhibitors is described by the product of multiple terms analogous to the last term in Eq. (8.14). To illustrate this approach, the sediment slurry data in Fig. 8.6a were simulated using a standard Monod scheme based on Eq. (8.11) (summarized in Table 8.5), with inhibition functions for NO$_3^-$, Fe(III), and SO$_4^{2-}$. As a first approximation, the inhibition constants for NO$_3^-$, Fe(III), and SO$_4^{2-}$ were set equal to the half-saturation constants for these EAs (Van Cappellen and Gaillard, 1996). The simulation including the inhibition functions produced results similar to those from the modified Monod model (Fig. 8.6c,d).

Use of the modified Monod approach, or the standard Monod equation together with noncompetitive inhibition functions, provides a convenient way to model the effects of competition between different microbial respiratory groups without having to include microbial biomass in the reaction network. However, there are established approaches to model microbial competition and associated populations dynamics, and their use is an often overlooked aspect of biogeochemical
Table 8.5 Computational scheme for the standard Monod rate law with noncompetitive inhibition applied to temporal-spatial sequencing of major terminal electron acceptor processes (TEAPs) coupled to first-order POC decay in sediments

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Law</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{RO}_2 )</td>
<td>( r = \frac{k_1 [\text{POC}]}{K_{m,02} + [\text{O}_2]} )</td>
</tr>
<tr>
<td>( \text{RNO}_3 )</td>
<td>( r = \frac{k_1 [\text{POC}]}{K_{m,NO}_3 + [\text{NO}<em>3^-]} \frac{K</em>{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{O}_2]} )</td>
</tr>
<tr>
<td>( \text{RMn}^{(IV)} )</td>
<td>( r = \frac{k_1 [\text{POC}]}{K_{m,Mn}^{(IV)} + [\text{Mn}^{(IV)}]} \frac{K_{L,Mn}^{(IV)}}{K_{L,Mn}^{(IV)} + [\text{Mn}^{(IV)}]} \frac{K_{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{NO}<em>3^-]} \frac{K</em>{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{O}_2]} )</td>
</tr>
<tr>
<td>( \text{RFe}^{(III)} )</td>
<td>( r = \frac{k_1 [\text{POC}]}{K_{m,Fe}^{(III)} + [\text{Fe}^{(III)}]} \frac{K_{L,Fe}^{(III)}}{K_{L,Fe}^{(III)} + [\text{Fe}^{(III)}]} \frac{K_{L,Mn}^{(IV)}}{K_{L,Mn}^{(IV)} + [\text{Mn}^{(IV)}]} \frac{K_{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{NO}<em>3^-]} \frac{K</em>{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{O}_2]} )</td>
</tr>
<tr>
<td>( \text{RSO}_4 )</td>
<td>( r = \frac{k_1 [\text{POC}]}{K_{m,SO}^{(IV)} + [\text{SO}<em>4^{2-}]} \frac{K</em>{L,Fe}^{(III)}}{K_{L,Fe}^{(III)} + [\text{Fe}^{(III)}]} \frac{K_{L,Mn}^{(IV)}}{K_{L,Mn}^{(IV)} + [\text{Mn}^{(IV)}]} \frac{K_{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{NO}<em>3^-]} \frac{K</em>{L,NO}<em>3}{K</em>{L,NO}_3 + [\text{O}_2]} )</td>
</tr>
</tbody>
</table>

Adapted from Van Cappellen and Gaillard (1996). The \( K_m \) terms refer to half-saturating EA concentrations in the generalized hyperbolic rate expression (see Eq. 8.7). The \( K_I \) values refer to noncompetitive inhibition constants that depict inhibition of a given TEAP by one or more favorable EAs.

reaction modeling (Rittmann and VanBriesen, 1996). From a mechanistic standpoint, it makes sense that a kinetic model that includes microbial reactions should include the morgs themselves together with the factors that determine their abundance and metabolic rates (Rittmann and VanBriesen, 1996); otherwise rates of energy metabolism and associated geochemical processes (e.g., mineral dissolution and precipitation) are functionally disconnected from the temporal/spatial evolution of the microbial communities responsible for those processes. Since the next wave of microbial reaction models is likely to include multiple groups of interacting populations with different physiological properties (Rittmann and McCarty (2001); see Watson et al. (2003), Wirtz (2003), and Maurer and Rittmann (2004) for recent examples), we examine the basic framework for depicting competition and population dynamics in some detail below, using sediment TEAPs as an example. In this case, the kinetic expressions depict utilization of dissolved monomeric substrates such as acetate or \( \text{H}_2 \), which are in fact the major substrates for TEAPs in anaerobic sediments (Christensen, 1984; Lovley and Klug, 1982; Lovley and Phillips, 1989; Sorensen et al., 1981). This kinetic analysis sets the stage for subsequent consideration of thermodynamic effects on microbial metabolism. \textbf{Moreover, it demonstrates the important general principle that the physiological properties}
of different groups of morgs can directly control the relative rates of competing geochemical processes.

### 8.3.5 Microbial Population Dynamics and Competition

To illustrate the microbial population dynamics approach, the sediment slurry data from Fig. 8.6 was simulated using the model described in Table 8.6, in which POC was assumed to undergo first-order hydrolytic/fermentative decay, liberating acetate as an energy source for the metabolism and growth of anaerobic respiratory morgs. This structure is based on Lovley and Klug’s (1986) model of the competition between SR and MG in freshwater lake sediments. Numerous studies have shown that acetate is the main carbon and energy substrate for respiration in anaerobic sediments (Christensen, 1984; Lovley and Klug, 1982; Lovley and Phillips, 1989; Sorensen et al., 1981), which justifies the use of acetate as the sole ED for the microbial population-based simulation of TEAPs in sediments. Parameter values were chosen from data available in the literature and research in the author’s laboratory. Yield coefficients for the different morg groups were estimated using the energy balance approach described in Rittmann and McCarty (2001), which provides a theoretically sound and practical alternative in situations where detailed information on growth yield for different types of physiological reactions are not available. The approach involves comparison of the amount of energy required to convert a carbon source to cellular carbon (assuming pyruvate is the central carbon intermediate) with the amount of energy liberated from energy-generating reactions, taking into account the efficiency of cellular energy transfer. The free energies for acetate oxidation coupled to DN, DIR, SR, and MG were estimated (see ΔG values listed in Table 8.6A) using reactant and product ΔGf values from Stumm and Morgan (1990), assuming dissolved acetate, NO3 −, SO4 2−, HCO3 −, Fe(II), HS −, and CH4 concentrations of 100 µM, 1 mM, 2 mM, 5 mM, 1 mM, 20 µM, and 1 µM, respectively. All these values are reasonable for the conditions present in the slurry experiment. Pyruvate was assumed to be the main cellular carbon intermediate, and NH4 + was assumed to be available in excess for incorporation into cell biomass. The estimated yield coefficients for SRMs and MGMs (see Parameter Values in Table 8.6D) agreed within ca. 30% of the values for these microbial groups used in the model of Lovley and Klug (1986).

The only parameters adjusted to fit the slurry incubation data were the initial population densities of the DNMs, DIRMs, SRMs, and MGMs. Using initial biomass values equivalent to 0.02–1.0 g L−1, the model reproduced the observed biogeochemical data (Fig. 8.7a) as well as (to a first approximation) the results of the biomass-independent modified and standard Monod models shown in Fig. 8.6. In other words, the microbial population dynamics approach accounted for the temporal sequence of TEAPs without the need for semi-empirical “limiting concentration” or “inhibition constant” parameters. Although accurate depiction of the myriad of microbial competitive interactions in natural systems (e.g., in
Table 8.6 Description of microbial population-based model used to depict competition between TEAPs for acetate (CH$_3$COO$^-$) in the sediment slurry experiment (see Fig. 8.7)

A. Stoichiometric Equations

(1) \[ \text{CH}_3\text{COO}^- + 1.6\text{NO}_3^- + 0.6\text{H}^+ \rightarrow 2\text{HCO}_3^- + 0.8\text{N}_2 + 0.8\text{H}_2\text{O} \quad \Delta G = -798 \text{ kJ/mol CH}_3\text{COO}^- \]

(2) \[ \text{CH}_3\text{COO}^- + 8\text{Fe(OH)}_3 + 15\text{H}^+ \rightarrow 2\text{HCO}_3^- + 8\text{Fe}^{2+} + 20\text{H}_2\text{O} \quad \Delta G = -171 \text{ kJ/mol CH}_3\text{COO}^- \]

(3) \[ \text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{HS}^- \quad \Delta G = -62.4 \text{ kJ/mol CH}_3\text{COO}^- \]

(4) \[ \text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{CH}_4 \quad \Delta G = -39.1 \text{ kJ/mol CH}_3\text{COO}^- \]

B. Metabolic Rate Expressions

(1) \[ R_{\text{POC}} = k_1[\text{POC}] \]

(2) \[ R_{\text{CH}_3\text{COO},\text{DNM}} = R_{\text{max,CH}_3\text{COO},\text{DNM}} \frac{[\text{CH}_3\text{COO}^-]}{K_{m,\text{CH}_3\text{COO},\text{DNM}} + [\text{CH}_3\text{COO}^-]} \frac{[\text{NO}_3^-]}{K_{m,\text{NO}_3} + [\text{NO}_3^-]} \]

(4) \[ R_{\text{CH}_3\text{COO},\text{DIRM}} = R_{\text{max,CH}_3\text{COO},\text{DIRM}} \frac{[\text{CH}_3\text{COO}^-]}{K_{m,\text{CH}_3\text{COO},\text{DIRM}} + [\text{CH}_3\text{COO}^-]} \frac{[\text{Fe(III)}]}{K_{m,\text{Fe(III)}} + [\text{Fe(III)}]} \]

(5) \[ R_{\text{CH}_3\text{COO},\text{SRM}} = R_{\text{max,CH}_3\text{COO},\text{SRM}} \frac{[\text{CH}_3\text{COO}^-]}{K_{m,\text{CH}_3\text{COO},\text{SRM}} + [\text{CH}_3\text{COO}^-]} \frac{[\text{SO}_4^{2-}]}{K_{m,\text{SO}_4} + [\text{SO}_4^{2-}]} \]

(6) \[ R_{\text{CH}_3\text{COO},\text{MGM}} = R_{\text{max,CH}_3\text{COO},\text{MGM}} \frac{[\text{CH}_3\text{COO}^-]}{K_{m,\text{CH}_3\text{COO},\text{MGM}} + [\text{CH}_3\text{COO}^-]} \]

(cont.)
Table 8.6 (Continued)

C. Conservation Equations

\[\begin{align*}
(1) \quad \frac{d[POC]}{dt} &= -k_1[POC] \\
(2) \quad \frac{d[CH_3COO^-]}{dt} &= -0.5k_1[POC] - R_{CH3COO,DNM} - R_{CH3COO,DIRM} - R_{CH3COO,SRM} - R_{CH3COO,MGM} \\
(3) \quad \frac{d[NO_3^-]}{dt} &= -R_{CH3COO,DNM} \alpha_{NO3/CH3COO} \\
(4) \quad \frac{d[Fe(III)]}{dt} &= -R_{CH3COO,DIRM} \alpha_{Fe(III)/CH3COO} \\
(5) \quad \frac{d[Fe(II)]}{dt} &= R_{CH3COO,DIRM} \alpha_{Fe(III)/CH3COO} \\
(6) \quad \frac{d[SO_4^{2-}]}{dt} &= -R_{CH3COO,SRM} \alpha_{SO4/CH3COO} \\
(7) \quad \frac{d[CH_4]}{dt} &= -R_{CH3COO,MGM} \alpha_{CH4/CH3COO} \\
(8) \quad \frac{d[DNM]}{dt} &= Y_{DNR}R_{CH3COO,DNM} - k_d[DNM] \\
(9) \quad \frac{d[DIRM]}{dt} &= Y_{DIRM}R_{CH3COO,DIRM} - k_d[DIRM] \\
(10) \quad \frac{d[SRM]}{dt} &= Y_{SRM}R_{CH3COO,SRM} - k_d[SRM] \\
(11) \quad \frac{d[MGM]}{dt} &= Y_{MGM}R_{CH3COO,MGM} - k_d[MGM]
\end{align*}\]
## D. Parameter Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{POC}]_0$</td>
<td>20.8</td>
<td>mmol L$^{-1}$</td>
<td>Experimental data</td>
</tr>
<tr>
<td>$[\text{NO}_3^-]_0$</td>
<td>0.97</td>
<td>mM</td>
<td>Experimental data</td>
</tr>
<tr>
<td>$[\text{Fe(III)}]_0$</td>
<td>20.8</td>
<td>mmol L$^{-1}$</td>
<td>Experimental data</td>
</tr>
<tr>
<td>$[\text{SO}_4^{2-}]_0$</td>
<td>2.0</td>
<td>mM</td>
<td>Experimental data</td>
</tr>
<tr>
<td>$[\text{CH}_4]_0$</td>
<td>0.0</td>
<td>mmol L$^{-1}$</td>
<td>Experimental data</td>
</tr>
<tr>
<td>$[\text{DNM}]_0$</td>
<td>0.1</td>
<td>g L$^{-1}$</td>
<td>Trial and error to achieve fit to slurry data</td>
</tr>
<tr>
<td>$[\text{DIRM}]_0$</td>
<td>0.1</td>
<td>g L$^{-1}$</td>
<td>Trial and error to achieve fit to slurry data</td>
</tr>
<tr>
<td>$[\text{SRM}]_0$</td>
<td>0.1</td>
<td>g L$^{-1}$</td>
<td>Trial and error to achieve fit to slurry data</td>
</tr>
<tr>
<td>$[\text{MGM}]_0$</td>
<td>0.02</td>
<td>g L$^{-1}$</td>
<td>Trial and error to achieve fit to slurry data</td>
</tr>
<tr>
<td>$k_1$</td>
<td>0.04</td>
<td>d$^{-1}$</td>
<td>Roden &amp; Wetzel (2002)</td>
</tr>
<tr>
<td>$k_d$</td>
<td>0.01</td>
<td>d$^{-1}$</td>
<td>Lovley &amp; Klug (1986)</td>
</tr>
<tr>
<td>$R_{\text{max,CH}_3\text{COO, DNM}}$</td>
<td>188</td>
<td>mmol CH$_3$COO$^-$ g$^{-1}$ d$^{-1}$</td>
<td>Rittmann &amp; McCarty (2001) (see section 3.3)</td>
</tr>
<tr>
<td>$R_{\text{max,CH}_3\text{COO, DIRM}}$</td>
<td>121</td>
<td>mmol CH$_3$COO$^-$ g$^{-1}$ d$^{-1}$</td>
<td>Roden, unpublished data for <em>G. metallireducens</em></td>
</tr>
<tr>
<td>$R_{\text{max,CH}_3\text{COO, SRM}}$</td>
<td>9.9</td>
<td>mmol CH$_3$COO$^-$ g$^{-1}$ d$^{-1}$</td>
<td>Lovley &amp; Klug (1986)</td>
</tr>
<tr>
<td>$R_{\text{max,CH}_3\text{COO, MGM}}$</td>
<td>15.7</td>
<td>mmol CH$_3$COO$^-$ g$^{-1}$ d$^{-1}$</td>
<td>Lovley &amp; Klug (1986)</td>
</tr>
<tr>
<td>$K_m,\text{CH}_3\text{COO, DNM}$</td>
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<td>mM</td>
<td>Arbitrarily set equal to $K_{m,\text{CH}_3\text{COO, DIRM}}$</td>
</tr>
<tr>
<td>$K_m,\text{CH}_3\text{COO, DIRM}$</td>
<td>0.001</td>
<td>mM</td>
<td>Roden &amp; Wetzel (2003)</td>
</tr>
<tr>
<td>$K_m,\text{CH}_3\text{COO, SRM}$</td>
<td>0.003</td>
<td>mM</td>
<td>Lovley &amp; Klug (1986)</td>
</tr>
<tr>
<td>$K_m,\text{CH}_3\text{COO, MGM}$</td>
<td>0.05</td>
<td>mM</td>
<td>Lovley &amp; Klug (1986)</td>
</tr>
<tr>
<td>$Y_{\text{DNM}}$</td>
<td>0.0203</td>
<td>g cells/mmol CH$_3$COO$^-$</td>
<td>Energetics approach of Rittmann &amp; McCarty (2001) (see section 3.3)</td>
</tr>
<tr>
<td>$Y_{\text{DIRM}}$</td>
<td>0.0123</td>
<td>g cells/mmol CH$_3$COO$^-$</td>
<td>Energetics approach of Rittmann &amp; McCarty (2001) (see section 3.3)</td>
</tr>
<tr>
<td>$Y_{\text{SRM}}$</td>
<td>0.00484</td>
<td>g cells/mmol CH$_3$COO$^-$</td>
<td>Energetics approach of Rittmann &amp; McCarty (2001) (see section 3.3)</td>
</tr>
<tr>
<td>$Y_{\text{MGM}}$</td>
<td>0.00286</td>
<td>g cells/mmol CH$_3$COO$^-$</td>
<td>Energetics approach of Rittmann &amp; McCarty (2001) (see section 3.3)</td>
</tr>
<tr>
<td>$K_m,\text{NO}_3$</td>
<td>0.01</td>
<td>mM</td>
<td>Simulation in Fig. 8.6B</td>
</tr>
<tr>
<td>$K_m,\text{Fe(III)}$</td>
<td>2.0</td>
<td>mmol surface sites L$^{-1}$</td>
<td>Simulation in Fig. 8.6B</td>
</tr>
<tr>
<td>$K_m,\text{SO}_4$</td>
<td>0.068</td>
<td>mM</td>
<td>Lovley &amp; Klug (1986)</td>
</tr>
<tr>
<td>$\alpha_{\text{NO}_3^-/\text{CH}_3\text{COO}}$</td>
<td>1.6</td>
<td>mmol NO$_3^-$ /mmol CH$_3$COO$^-$</td>
<td>Stoichiometric equation (1)</td>
</tr>
<tr>
<td>$\alpha_{\text{Fe(III)/CH}_3\text{COO}}$</td>
<td>8.0</td>
<td>mmol Fe(III)/mmol CH$_3$COO$^-$</td>
<td>Stoichiometric equation (2)</td>
</tr>
<tr>
<td>$\alpha_{\text{SO}_4^{2-}/\text{CH}_3\text{COO}}$</td>
<td>1.0</td>
<td>mmol SO$_4^{2-}$ /mmol CH$_3$COO$^-$</td>
<td>Stoichiometric equation (3)</td>
</tr>
<tr>
<td>$\alpha_{\text{CH}_4/\text{CH}_3\text{COO}}$</td>
<td>1.0</td>
<td>mmol CH$_4$/mmol CH$_3$COO$^-$</td>
<td>Stoichiometric equation (4)</td>
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</table>
Fig. 8.7 Microbial population-based simulation of TEAPs in the same slurry of anoxic freshwater wetland sediment depicted in Fig. 8.6. See section 8.3.5 and Table 8.6 for model description. Solid and dashed lines show results of simulations with and without, respectively, a thermodynamic control term based on the free energy of acetate oxidation for each TEAP (see section 8.3.6). Symbols in panel C show results of simulation without thermodynamic control term; dashed lines show simulation with thermodynamic control term.
situations where the same group of morgs can utilize more than one EA) will likely require the development of more complicated models that include inhibition effects (such as the repression of denitrification by O$_2$ (Tiedje, 1988), the inhibition of DIR by NO$_3^-$ (Sorensen, 1982; Weber et al., 2006), and the inhibition of acetoclastic MG by Fe(III) (Bond and Lovley, 2002; vanBodegom et al., 2004)) the results of this simple simulation provide important insight into how microbial competition, as dictated by morg physiological parameters, can govern reaction dynamics in a biogeochemical system.

Predicted acetate concentrations displayed a step-wise increase (Fig. 8.7b) as the more favorable EAs were depleted (Fig. 8.7a) and different TEAP reactions became dominant (Fig. 8.7c). This pattern can be explained on the basis of the physiological properties of the different acetate-utilizing microbial groups. A key parameter governing the outcome of competitive interactions among different microbial populations is the ratio of the maximum rate of substrate uptake to the half-saturation constant for uptake ($R_{\text{max}, \text{CH}_3\text{COO}}/K_{\text{m,CH}_3\text{COO}}$ in the present context). This ratio is often referred to as the “clearance rate”, since it has units of inverse time. Populations with higher clearance rates dominate substrate uptake under limiting substrate conditions (as in the case of the sediment slurry experiment), because they are able to maintain substrate concentrations at values much lower than the $K_{\text{m}}$ values for less competitive populations. Clearance rates for the DNM, DIRM, SRM, and MGM groups in our model were 750,000, 121,000, 3300, and 314, respectively. The upshot of this wide range of clearance rates is that the mean acetate concentration during the initial nitrate-reducing phase of the simulation, ca. 0.015 µM, was 100–1000 times lower than the $K_{\text{m,CH}_3\text{COO}}$ values for the DIRM, SRM, and MGM populations (Fig. 8.7b). Likewise, the mean acetate concentration during the Fe(III)-reducing phase of the simulation (ca. 0.06 µM) was 10–100 times lower than the $K_{\text{m,CH}_3\text{COO}}$ for the SRMs and MGMs; and the mean acetate concentration during the sulfate-reducing phase (ca. 1.3 µM) was 10 times lower than the $K_{\text{m,CH}_3\text{COO}}$ for the MGMs.

The importance of considering microbial population dynamics in kinetic analyses of geomicrobiological processes becomes even more apparent in situations where the reaction system approaches steady-state over long time periods. Although this did not occur in the slurry experiment discussed above, there are many examples of steady-state or near steady-state biogeochemical reaction systems in nature, notably aquatic sediments undergoing steady-state chemical diagenesis (VanCappellen and Gaillard, 1996), and ancient, deeply buried groundwater aquifers which display stable redox zonation over very long time scales (Chapelle and Lovley, 1992). Long-term microbial redox zonation translates into long-term patterns of geochemical flux that can have a profound impact on the mobility of trace and contaminant compounds in natural systems (see Davis et al. (2004) for an interesting discussion of this topic). Hence it is worthwhile to examine how microbial population dynamics can affect the steady-state configuration of a biogeochemical reaction system, once again using the sediment TEAP system as a means of illustration.

The basic mass balance equation for microbial biomass in the sediment TEAP system (Eqs. (8) to (11) in Table 8.6C) can be manipulated to show that, at steady-state (i.e., when the rates of change in biomass and other substrate
concentration are equal to zero), the dissolved acetate concentration is solely a function of the physiological parameters $R_{\text{max}}$, $K_m$, $Y_i$, and $k_d$ (Lovley, 1988); see also Lovley and Chapelle (1998):

$$[\text{CH}_3\text{COO}^-]_{ss} = \frac{K_{m,\text{CH}_3\text{COO},i} \times C}{1 - C} \quad (8.15)$$

where

$$C = \frac{k_{d,i} (K_{m,\text{EA},i} + [\text{EA}_i])}{R_{\text{max,CH}_3\text{COO},i} \times Y_i \times [\text{EA}_i]}, \quad (8.16)$$

the subscript $i$ denotes a given TEAP pathway. It is easy to show that the following condition must be met in order for the rate of change of biomass (BM) to be greater than zero at any given point in time:

$$Y_i R_{\text{CH}_3\text{COO},i} = k_{d,i}[\text{BM}_i] \quad (8.17)$$

The key insight here is that if the physiological properties of one group of morgs (as modified by the abundance of their EA) allows them to maintain the concentration of an energy substrate (e.g., acetate) at a value where the left hand side of Eq. (8.17) is less than the value of the right hand side for a second group of organisms (i.e., where $Y_2 R_{\text{CH}_3\text{COO},2} < k_{d,2}[\text{BM}_2]$, where the subscript 2 indicates the second group of organisms), then the population of the second group of organisms will undergo decline, whereas the biomass of first group of organisms will increase. The net effect of this interaction is that the physiologically more competitive groups will always dominate over less competitive groups – until, that is, their supply of EA acceptor becomes limiting, at which time the next most efficient group of organisms will become dominant. In situations where sufficient abundance of a given EA is maintained (e.g., through mass transport in sedimentary environments), all other less competitive groups will be completely excluded over long time scales.

To illustrate this concept, the microbial population-based TEAP model was modified to simulate situations in which either nitrate reduction, Fe(III) reduction, SR, or MG became the dominant TEAP at steady-state. This was easily achieved by keeping the concentrations of all EAs (as well as the labile POC) fixed over time, and running the model for a long enough period of time (e.g., 10 years) to achieve steady-state. With the nitrate concentration fixed at a constant value of 1 mM, the system became completely dominated by DNMs; with nitrate set to zero and Fe(III) fixed at 20 mmol L$^{-1}$, the system became dominated by DIRMs; with nitrate and Fe(III) set to zero and sulfate fixed at 2 mM, the system became dominated by SRMs; and finally with nitrate, Fe(III) and sulfate all set to zero the system was dominated by MGMs. The predicted acetate concentrations for the different TEAP conditions evolved to steady-state values that were very similar to the critical concentration at which $dB/dt \approx 0$, i.e., the $[\text{CH}_3\text{COO}^-]_{ss}$ concentrations dictated by Eqs. (8.15) and (8.16) for a given TEAP. The long-term presence of more thermodynamically efficient EAs led to steady-state acetate concentrations lower than the critical values for acetate metabolism by organisms using less favorable EAs, which
in turn led to extinction of these less competitive populations. This result illustrates the general principle that biological populations tend to draw the concentration of a limiting substrate down to the lowest possible level (as defined by their physiological capacities and other environmental tolerances) so as to maximize their ability to compete with other groups of organisms (Blackman, 1905).

### 8.3.6 Kinetic Versus Thermodynamic Control of Microbial Reaction Rates

An important consideration in interpreting the kinetics of microbially driven geochemical reactions is the relative influence of kinetic versus thermodynamic effects on the rate and extent of reaction. A key feature of the rate laws discussed above is that they represent irreversible reactions that ignore the potential impact of thermodynamic driving force on reaction rate. Many metabolic reactions proceed at conditions close to thermodynamic equilibrium (Crabtree and Nicholson, 1988). This phenomenon has important implications for understanding the rates and spatial-temporal distribution of geomicrobiological processes such as oxidation of natural and contaminant organics via different TEAP pathways (Curtis, 2003). The basic issue is whether irreversible kinetic models based on morg physiological properties can universally explain spatial-temporal patterns of microbial metabolism, or whether models that consider thermodynamic driving force are required.

In a lucid review of this question, Banwart and Thornton (2003) refer to the irreversible kinetic/physiological approach (i.e., the one employed above to simulate the results of the sediment slurry experiment) as the “competitive exclusion” (CE) approach, whereas approaches that include thermodynamic considerations are referred to as the “partial equilibrium” (PE) approach. The term PE was coined by Postma and Jakobsen (1996), based on the idea that the overall irreversible reaction between OM and EAs could be subdivided into two basic steps: (1) a slow, overall rate limiting hydrolysis/fermentation step (e.g., one that could be depicted as a zero or a first-order reaction), and (2) a fast, electron-accepting step that approaches thermodynamic equilibrium. In the PE approach, the concentration of an energy substrate (e.g., acetate or H$_2$) is used together with other geochemical information to calculate the available free energy ($\Delta G$) for one or more TEAPs. If the $\Delta G$ for a given TEAP is more positive than the minimum amount of energy required for conservation of energy via ATP biosynthesis (on the order of $-20$kJ/rxn; Schink (1997)), then this TEAP cannot proceed under ambient conditions. TEAPs for which the $\Delta G$ is sufficiently negative can potentially coexist, subject to constraints posed by competitive effects as described in the previous section. A major motivation for development of the PE approach was the apparent coexistence (in space and time) of TEAPs in both pristine (Jakobsen and Postma, 1999) and organic-contaminated (Jakobsen et al., 1998; Vroblesky et al., 1997) aquifer environments, which would not occur if competitive exclusion effects were strictly enforced.
The sediment slurry TEAP simulations discussed above can be used to gain insight into potential thermodynamic versus kinetic controls on microbial metabolism under idealized conditions. The acetate concentrations predicted in the steady-state microbial population-based simulations were used to estimate $\Delta G$ values for the four different TEAP reactions, using the same reactant and product concentrations employed to compute $\Delta G$ values for the purpose of estimating yield coefficients (see Table 8.6A). The results suggest that acetate scavenging by DNMs and DIRMs could in principle have led to conditions under which MG would not have been thermodynamically feasible, assuming a minimum energy requirement of $-20kJ/rxn$. In all other cases, however, the computed $\Delta G$ values for competing TEAP reactions were more negative $-20kJ/rxn$, even though competitive exclusion took place. This result suggests a simple explanation for the failure of the CE model to explain the distribution of TEAPs in certain shallow aquifer environments, namely that resident microbial populations may not have been able to achieve long-term steady-state conditions in the face of spatial-temporal heterogeneity in environmental conditions, most importantly the abundance of EDs and EAs.

Although the foregoing analysis suggests that competitive exclusion provides a robust explanation for segregation of microbial redox processes (at least under steady-state conditions), there are some situations in which consideration of thermodynamic driving force must be invoked to explain reaction dynamics. A clear example of this can be identified in the oxidation of H$_2$ via different groups of respiratory morgs. Laboratory experiments have shown that anaerobic morgs have a threshold level for H$_2$ scavenging that is related to the redox potential of the EA used (Cord-Ruwisch et al., 1988; Lovley, 1985). The threshold concentration represents the lowest dissolved H$_2$ concentration that can support H$_2$-driven respiration by a given group of organisms; as H$_2$ concentrations approach the threshold value, rates of H$_2$ uptake approach zero. As a result of this phenomenon, dissolved H$_2$ concentrations in sediments dominated by different TEAPs (Lovley and Goodwin, 1988) may reflect thermodynamic rather than competitive control of substrate metabolism (Ho and Cord-Ruwisch, 1996; Hoehler et al., 1998). Another well-known example of this phenomenon comes from anaerobic digestion of domestic and industrial organic waste, in which accumulation of end-products such as acetate and H$_2$ can limit the overall rate of OM digestion (Ho and Cord-Ruwisch, 1996). An analogous example in the context of microbial geochemistry can be identified in the influence of Fe(II) on enzymatic reductive dissolution of Fe(III) oxides. Although (as discussed in detail in Case Study #1) thermodynamic driving force does not appear to strongly influence initial rates of electron transfer from DIRM to Fe(III) oxide surfaces, it can clearly play an important role in governing the long-term extent of oxide reduction. Moreover, from the practical point of view of kinetic modeling, a thermodynamic “control switch” of some sort is required to simulate the observed cessation of oxide reduction activity after only partial reduction in closed reaction systems. To set the stage for this analysis, the basic framework for including thermodynamic control of microbial metabolic activity is presented below.

The basis of all kinetic rate versus thermodynamic driving force considerations is the idea that the reaction system is a reversible one (in contrast to the irreversible
rate laws discussed previously). This basic feature is common to all derivations of thermodynamic control terms, including ones based on transition state theory that are common in chemistry and earth science (Laidler, 1987; Lasaga, 1998), and ones based on the laws of mass action used in enzyme kinetics (Haldane, 1930; Roels, 1983). Jin and Bethke (2002) recently provided a mechanistic derivation to explain coupled kinetic and thermodynamic controls on electron transport during microbial respiration, as well as several applications of the resulting new rate law to microbial metabolism in geochemical systems (Jin and Bethke, 2003, 2005). The authors produced the following compact expression:

$$R = R_{\text{max}} F_D F_A F_T$$  \hspace{1cm} (8.18)

where $F_D$ and $F_A$ are hyperbolic rate terms that account for the influence (kinetic) of electron donor and electron acceptor concentration on respiration rate, and $F_T$ is a thermodynamic driving force term which takes the form

$$F_T = 1 - \exp \left( \frac{f}{\chi RT} \right)$$  \hspace{1cm} (8.19)

in which $f$ is the thermodynamic driving force, $R$ is the ideal gas constant, $T$ is temperature in degrees Kelvin, and $\chi$ is the average stoichiometric number, which corresponds to the number of times a rate-determining step occurs during an overall reaction. For respiratory reactions involving proton translocation across the cell membrane, the value of $\chi$ can be constrained by the number of protons translocated per rate-limiting electron transfer step. However, the general form of Eq. (8.19) can be applied to other processes in which there is no mechanistic basis for assigning a value to $\chi$. In this case, and for our purposes below, $\chi$ can be arbitrarily assigned a value of 1.0. The usual way to express thermodynamic driving force is the $\Delta G$ value. In the case of biologically catalyzed reactions that involve energy conservation, a modification is required to take into account the minimum amount of free energy required to synthesize ATP. In some situations, the amount of energy required to transport a substrate across a biological membrane can also strongly influence the minimum free energy required for a microbial reaction to proceed (Jackson and McInerney, 2002). Liu et al. (2001a) assigned the term $\Delta G_{\text{min}}$ to this minimum energy requirement, and expressed $f$ as

$$f = (\Delta G - \Delta G_{\text{min}})$$  \hspace{1cm} (8.20)

so that $F_T$ becomes (assuming $\chi = 1$)

$$F_T = 1 - \exp \left( \frac{\Delta G - \Delta G_{\text{min}}}{RT} \right)$$  \hspace{1cm} (8.21)

A value of ca. −20kJ mol$^{-1}$ is typically assumed to be the smallest quantum of metabolically convertible energy for prokaryotic cells, which corresponds to the energy required to synthesize one-third of a mol of ATP during transport of one ion (e.g., a proton) across the cytoplasmic membrane (Schink, 1997).
To illustrate the use of Eq. (8.21), the microbial population-based simulation of the sediment slurry experiment was re-run including a $F_T$ term (along with the usual $F_D$ and $F_A$ Monod terms for acetate and the different external EAs, respectively) applied to each of the TEAP reactions with a common $\Delta G_{\text{min}}$ value of $-20\text{kJ/mol}$ acetate. The $\Delta G$ values for each TEAP were computed dynamically during the simulation using the predicted concentrations of reactants and products. The simulated reactant/product concentrations and metabolic rates (Fig. 8.7, dashed lines) were not much different from those produced by the model lacking the thermodynamic control term. This result reinforces the general conclusion that microbial competition is likely to play the dominant role in governing the distribution of TEAPs and associated geochemical processes in sedimentary environments.

8.4 Case Study #1 – Microbial Fe(III) Oxide Reduction

8.4.1 Introduction

Dissimilatory microbial reduction of Fe(III) oxides has broad range of influences on the aqueous/solid-phase geochemistry and behavior of natural and contaminant compounds in modern circumneutral, nonsulfidogenic subsurface sedimentary environments. In addition, there is compelling evidence for the role of DIRMs in the formation of mineral phases (e.g., magnetite and siderite) on the ancient Earth (Walker, 1984, 1987), as well as for their potential role in the early evolution of microbial respiration (Lovley, 2004; Vargas et al., 1998). It is therefore not surprising that Fe(III) oxide reduction and its associated geochemical effects has emerged as a major focal point for sediment biogeochemical research. There is ongoing interest in the factors controlling the rate and long-term extent (i.e., the kinetics) of enzymatic DIR. The rate and extent of DIR are governed by complex surface-chemical and physiological interactions which are still only partially understood. In this case study information on factors that govern the kinetics of DIR is presented and analyzed. Studies with natural wetland sediments as well as pure culture experiments with synthetic and natural Fe(III) oxides are discussed.

8.4.2 Mechanisms of Enzymatic Fe(III) Oxide Reduction

Enzymatic DIR involves transfer of electrons from organic substrates or $H_2$ to insoluble Fe(III) oxides through the enzymatic activity of DIRMs under anoxic conditions in soil and sedimentary environments. Although certain morgs can transfer small quantities of reducing equivalents to Fe(III) during fermentative growth, the vast majority of DIR activity in nature is linked to oxidation of organic carbon or $H_2$ coupled to respiration with Fe(III) (see Lovley (1987, 1991) for review). Certain
organics (reducing acids and thiols) can abiotically reduce Fe(III) oxides (Stone and Morgan, 1987), however abiotic reduction by such compounds is likely to be only a minor process compared to DIRM respiration (Lovley and Phillips, 1991). Moreover, a wide variety of organic carbon compounds can be readily oxidized in the presence of DIRM, but do not react spontaneously with Fe(III) oxides under abiotic conditions (Arnold et al., 1988; Lovley and Phillips, 1991; Munch and Ottow, 1980, 1983; Ottow, 1968, 1971; Ottow and Glathe, 1971). Likewise, the mere presence of a low system redox potential (e.g., as a result of microbial consumption of oxidants such as oxygen and nitrate, or production of fermentation intermediates) does not lead to spontaneous Fe(III) oxide reduction (Lovley and Phillips, 1991; Munch and Ottow, 1983). Thus classical models that treat Fe(III) oxide reduction as a freely reversible process dependent on system redox potential (Hem, 1972; Ponnamperuma, 1972; Starkey and Halvorson, 1927; Zehnder and Stumm, 1988), while providing a convenient framework for conceptualizing Fe geochemistry, do not accurately represent reality (Lovley, 1991). In fact, low redox potentials are the result of DIR rather than the cause of Fe(III) oxide reduction, since the redox potential of non-sulfidogenic environments is typically controlled by the Fe(II)-Fe(III) redox couple (Stumm and Morgan, 1996). Reaction with hydrogen sulfide produced by dissimilatory SRMs is the only significant abiotic pathway for abiotic Fe(III) oxide reduction in low-temperature sedimentary environments. This is a quantitatively significant process in shallow coastal marine sediments, and there is strong competition between enzymatic and abiotic Fe(III) reduction in such environments (Canfield and DesMarais, 1993; Canfield et al., 1993; Kostka et al., 1999, 2002; Thamdrup et al., 1994, 2000). In contrast, DIR is the dominant mechanism for Fe(III) reduction in low-S freshwater systems (Thamdrup, 2000).

There are three basic mechanisms whereby enzymatic DIR can take place (Nevin and Lovley, 2002), which are summarized in Fig. 8.8a: (1) direct electron transfer from the DIRM cell surface to the Fe(III) oxide surface; (2) dissolution of Fe(III) oxides by organic chelators followed by reduction of chelated Fe(III) at the surface of or within the DIRM; and (3) indirect electron transfer to the oxide surface via electron shuttling compounds (e.g., quinone-bearing humic substances), which are reduced at the surface of or within the DIRM cell and subsequently react with the oxide surface. The discussion and analysis below focuses implicitly on mechanism 1. While there are undoubtedly situations in nature where mechanisms 2 and 3 are operative (e.g., organic-rich sediments, and biofilm environments where chelators and/or electron shuttling compounds produced by DIRMs may accumulate and thereby promote oxide reduction (Hernandez and Newman, 2001; Hernandez et al., 2004; Lies et al., 2005; Nevin and Lovley, 2002), metal chelators and electron shuttling compounds are likely to be relatively scarce in most natural environments. As a result, direct electron transfer is likely to be the default means by which DIRMs “earn a living” in nature. The energetic cost of producing endogenous chelators or electron-shuttling compounds (as opposed to exogenous compounds already present in the environment) is undoubtedly very high, resulting in a relatively low growth yield per unit energy substrate metabolized for organisms that utilize such compounds to reduce Fe(III) oxides. Organisms that require such compounds
A. Mechanisms of enzymatic Fe(III) oxide reduction

1. Direct reduction
   \[ \text{Fe(III)(s) + DIRM} \rightarrow \text{Fe(II)(aq), Fe(II)(s)} \]

2. Chelator-promoted reduction
   \[ \text{Fe(III)(s) + Chelator} \rightarrow \text{Fe(III)-Chelator(aq)} \]
   \[ \text{Fe(III)-Chelator(aq) + DIRM} \rightarrow \text{Fe(II)-Chelator(aq)} \]

3. Electron shuttling-promoted reduction
   \[ \text{e- shuttle}_{\text{a} \text{(aq)}} + \text{DIRM} \rightarrow \text{e- shuttle}_{\text{a} \text{(aq)}} \]
   \[ \text{e- shuttle}_{\text{a} \text{(aq)}} + \text{Fe(III)(s)} \rightarrow \text{e- shuttle}_{\text{a} \text{(aq)}} + \text{Fe(II)(aq)}, \text{Fe(II)(s)} \]

B. Existing model for direct reduction (Lovley et al., 2004)
C. New model for direct reduction (Reguera et al., 2005; Gorby et al., 2006)

\[ \text{Fe(III)(s)} \]
\[ \text{Outer membrane cytochromes} \]
\[ \text{Periplasmic electron carriers} \]
\[ \text{Inner membrane electron carriers} \]
\[ \text{NADH dehydrogenase} \]

\[ \text{Fe(III)(s)} \]
\[ \text{“Geopilins”} \]
\[ \text{Outer membrane cytochromes} \]
\[ \text{Periplasmic electron carriers} \]
\[ \text{Inner membrane electron carriers} \]
\[ \text{NADH dehydrogenase} \]

**Fig. 8.8** Mechanisms and biochemical models of enzymatic DIR. See section 8.4.2 for details

would therefore not be expected to compete effectively with DIRMs that transfer electrons directly to Fe(III) oxide surfaces in situations where exogenous chelators or electron shuttles are scarce (Lovley et al., 2004).

Although the exact molecular mechanism(s) for direct electron transfer from DIRMs to Fe(III) oxides surfaces is not known, there has been rapid progress in the last few years in our understanding of how this process can take place. A system of inner membrane-, periplasmic-, and outer membrane-associated cytochromes and other electron carriers is known to participate in the transfer of electrons from the cytoplasm (e.g., from NADH) to the cell surface (DiChristina et al., 2005; Lovley, 2000a, 2002; Lovley et al., 2004; Richardson, 2000; Ruebush et al., 2006a, 2006b). Until recently, c-type cytochromes embedded in the outer face of the outer membrane were thought to be solely responsible for electron transfer to Fe(III) oxides in direct association with the DIRM cell surface (see Fig. 8.8b). However, two very recent studies have demonstrated that pili attached to the outer membrane of DIRMs such as *Geobacter sulfurreducens* (Reguera et al., 2005) and *Shewanella oneidensis* (Gorby et al., 2006), which were previously thought to be involved
in attachment to Fe(III) oxide surfaces (Childers et al., 2002), are electrically conductive. Pili are extracellular appendages that are present in a wide variety of morgs. It is possible that pili produced by DIRMs (referred to by Reguera et al. (2005) as “geopilins”) serve as microbial “nanowires” (ca. 5 nm in diameter and up to 20 μm in length) that form the final electrical connection between the cell and the surface of the Fe(III) oxides (Fig. 8.8c). The pili are anchored in the periplasm and outer membrane of the DIRM cell, and may thus be able to accept electrons from the various electron carriers (e.g., c-type cytochromes) that are known (from biochemical and genetic studies) to be involved in Fe(III) oxide reduction, thereby completing the circuit between these electron carriers and the Fe(III) oxide. Such appendages extend the capacity for electron transfer well beyond the outer surface of the DIRM cell, which is likely to be important in natural soils and sediments where Fe(III) oxides typically exist as coatings on various mineral and organic phases. Current knowledge thus provides a plausible mechanism whereby direct electron transfer from DIRMs to Fe(III) oxides may take place.

8.4.3 Fe(III) Oxide Mineralogy and Microbial Reducibility

Prior to discussing the kinetics of enzymatic DIR, it is necessary to briefly consider the physical and thermodynamic properties of different Fe(III) oxides in soils and sediment, as these have a fundamental influence on the susceptibility of Fe(III) oxides to enzymatic reduction, and on the nature of the Fe(II)-bearing end-products of oxide reduction. Fe(III) oxides occur in nature as a spectrum of phases ranging from completely (or near completely) amorphous minerals such as ferrihydrite, to minerals such as goethite and hematite with well-defined crystal structures (Cornell and Schwertmann, 1996). For a particular Fe(III) oxide phase, a range of crystallinity can exist which is correlated with the particle size, SA, and solubility of the mineral. With regard to microbial Fe(III) oxide reduction it is possible to draw a fundamental distinction between amorphous (or poorly crystalline) and crystalline Fe(III) oxides. The pioneering studies of Lovley and colleagues (Lovley and Phillips, 1986a, 1987) showed that amorphous Fe(III) oxides, operationally defined by their solubility in 0.25 M NH₂OH-HCl/0.25 M HCl (Chao and Zhou, 1983) or dilute HCl (0.5 or 1.0 M) (Roden and Lovley, 1993; Wallmann et al., 1993), in both riverine and hydrocarbon-contaminated aquifer sediments were subject to essentially complete microbial reduction to a combination of dissolved and solid-phase Fe(II) compounds. In contrast, both synthetic and natural crystalline Fe(III) oxides, which are poorly dissolved in NH₂OH-HCl or dilute HCl, were found to undergo much lower degrees of reduction, and to be preserved in highly reducing (methanogenic) sediments (Lovley and Phillips, 1987; Phillips et al., 1993). Subsequent studies of microbial Fe(III) oxide reduction in a variety of surface and subsurface sediments have verified the potential for near complete reduction of amorphous Fe(III) oxides, and for the preservation of substantial quantities of crystalline Fe(III) oxides (Albrechtsen et al., 1995; Amirbahman et al.,
1998; Cozzarelli et al., 1999, 2000; Roden and Wetzel, 1996; Roden and Edmonds, 1997; Tuccillo et al., 1999; Wallmann et al., 1993).

Although phase transformations induced during enzymatic reduction may in some situations limit the long-term reducibility of amorphous Fe(III) oxides (see Hansel et al. (2003) for analysis and review), the results of studies to date lead collectively to the view that, because of their relatively high degree of reducibility, amorphous Fe(III) oxides are the dominant forms of solid-phase Fe(III) subject to microbial reduction in sedimentary environments (Lovley, 1991, 1993, 2000a). While this is very likely to be the case when substantial quantities of amorphous Fe(III) oxides are present, in sediments where crystalline Fe(III) oxides are more abundant than amorphous phases, crystalline Fe(III) bioreduction may contribute substantially to Fe(II) generation and attendant effects on aqueous/solid-phase geochemical conditions.

8.4.4 Kinetics of Amorphous Fe(III) Oxide Reduction in Sediments

Analysis of the kinetics of microbial Fe(III) reduction in sediments presents a unique problem relative to other TEAPs (e.g., oxygen, nitrate, and sulfate reduction), because the process involves the interaction of bacterial cell surfaces with particulate oxide phases which are not transported into the cell (Ghiorse, 1988; Lovley, 1987). For the sake of simplicity, the TEAP models developed in Sect. 8.3 treated Fe(III) oxide reduction kinetics using a Monod-style rate term (dependent on bulk Fe(III) oxide surface site concentration) analogous to that used for soluble TEAPs. However, there are two important reasons why such a rate formulation is not appropriate for describing Fe(III) oxide reduction kinetics: (1) the Monod equation was designed to depict saturation of intracellular reaction rates (or substrate uptake) with respect to a soluble substrate; and (2) empirical evidence suggests a robust first-order linear relationship between reduction rate and Fe(III) oxide concentration.

8.4.4.1 Empirical Evidence for First-Order Rate Law

Roden and Wetzel (2002) reported on the kinetics of amorphous Fe(III) oxide (abbreviated hereafter as AIO) reduction in sediments from a freshwater wetland in northcentral Alabama, USA. Concentrations of AIO decreased exponentially over time to a nonzero asymptote during anaerobic incubation of oxidized sediment slurries (Fig. 8.9a) and homogenized surface sediments (Fig. 8.9b). Similar patterns of AIO depletion were observed during anaerobic incubation of slurries containing different proportions of oxidized and reduced sediment and homogenized sediments from various depth intervals in the upper 3 cm of wetland sediment cores. The Fe(III) versus time data from these experiments were normalized to the initial AIO concentration (Fe(III)$_0$) in order to permit nonlinear regression analysis of pooled
Fig. 8.9 Panels A and B: Fe(III) reduction during anaerobic incubation of (A) oxidized wetland sediment slurries and (B) wetland surface sediments. Symbol legends indicate initial Fe(III) oxide concentrations in µmol cm⁻³. Solid lines are nonlinear least-squares regression fits of pooled data to Eq. (8.22). Open symbols show ΣCO₂+CH₄ production during anaerobic incubation; the lines are a linear least-squares regression fits. Panels C and D: Initial Fe(III) reduction rate vs. initial Fe(III) oxide concentration in slurries (C) and surface sediments (D) containing different initial amounts of AIO. Initial rates were computed from the first derivative of the nonlinear regression fits to Eq. (8.22), evaluated at t = 0. The different symbols show averages of triplicate bottles for two separate experiments. Error bars were computed from the standard error of the k_red and (Fe(III)₀ − Fe(III)ₙ₉) regression parameters via error propagation (Bevington and Robinson, 1992). The line is a linear least-squares regression fit; the error term for the slope is the standard error of the regression parameter. All data are from Roden and Wetzel (2002), used with permission.

data from experiments with different starting Fe(III) concentrations. The data were fit by nonlinear least-squares regression analysis to the following equation:

\[
\frac{\text{Fe(III)}(t)}{\text{Fe(III)}_0} = \left(\frac{\text{Fe(III)}_0 - \text{Fe(III)}_{\text{nr}}}{\text{Fe(III)}_0}\right) \exp\left(-k_{\text{red}}t\right) + \frac{\text{Fe(III)}_{\text{nr}}}{\text{Fe(III)}_0}
\]  

(8.22)

where \(\text{Fe(III)}(t)\) is the AIO concentration at time t, \(\text{Fe(III)}_0\) is the initial AIO concentration, \(\text{Fe(III)}_{\text{nr}}\) is the nonreactive AIO concentration, and \(k_{\text{red}}\) is a first-order rate constant. The nonreactive (i.e., nonmicrobially reducible) Fe(III) oxide probably represents moderately crystalline Fe(III) oxides (e.g., nanocrystalline goethite; Vanderzee et al. (2003)) which were subject to dissolution by 0.5 M HCl but not readily susceptible to microbial reduction on the time scale of our experiments. Equation (8.22) is an integrated form of the first-order rate expression

\[
\frac{\text{dFe(III)}(t)}{\text{dt}} = -k_{\text{red}} \left(\text{Fe(III)}(t) - \text{Fe(III)}_{\text{nr}}\right) = -k_{\text{red}} \text{Fe(III)}_{\text{react}}
\]  

(8.23)
which is analogous to the equation used by Westrich and Berner (1984) for kinetic analysis of POC decomposition. The close adherence of the AIO reduction time course data to Eq. (8.22) provides explicit evidence that rates of microbial AIO reduction in the wetland sediment are first-order with respect to AIO concentration.

Further support for a first-order relationship between AIO reduction rate and concentration comes from experiments which demonstrated that initial rates of AIO reduction (computed from the first derivative of nonlinear regression fits of Fe(III) versus time data to Eq. (8.22), evaluated $t = 0$) were directly (linearly) correlated with starting AIO concentrations in sediment slurries (Fig. 8.9c) and surface sediments (Fig. 8.9d). Studies in marine sediments have also demonstrated direct correlations between Fe(II) production rate and AIO abundance (Hines et al., 1997; Thamdrup, 2000).

8.4.4.2 Theoretical Basis for First-Order Rate Model

None of the Fe(III) reduction rate versus concentration data discussed above provided obvious evidence of saturation behavior at high AIO abundance. Although rates of Fe(III) reduction at the highest AIO concentrations were often not statistically different from one another, the general trend of the rate versus concentration data was clearly linear rather than hyperbolic. The observed first-order kinetics of AIO reduction can be rationalized in terms of the principles of chemical (abiotic) mineral transformation discussed in Chap. 5. Chemical dissolution of metal oxide and silicate minerals is commonly described by the following generalized rate law (Hering and Stumm, 1990; Stumm and Sulzberger, 1992):

$$R_{\text{surf}}(t) = -k \times C_{\text{surf}}(t)$$ (8.24)

where $R_{\text{surf}}(t)$ is the SA-normalized dissolution rate (e.g., in $\mu$mol m$^{-2}$ d$^{-1}$) at time $t$, $k$ is a rate constant (d$^{-1}$), and $C_{\text{surf}}(t)$ is the concentration of surface species ($\mu$mol m$^{-2}$) involved in the dissolution reaction present at time $t$. Here surface species refers to an oxide surface site coordinated with $H^+$, $OH^-$, or organic ligands which polarize, weaken, and ultimately break the metal-oxygen bonds in the lattice of the oxide surface (Stumm and Sulzberger, 1992). This formulation assumes that reactions at the Fe(III) oxide surface are surface-controlled, i.e., that reactions at the surface are slow in comparison with other reaction steps, such as association of the ligand with the mineral surface to produce a surface species (Stumm and Morgan, 1996). A well-known example of abiotic mineral dissolution is the reductive dissolution of crystalline hematite ($\alpha$-Fe$_2$O$_3$) by ascorbic acid (Sulzberger et al., 1989; Suter et al., 1991). The $R_{\text{surf}}$ for this reaction is dependent on the concentration of ascorbate adsorbed to the oxide surface, which in turn is related to the concentration of ascorbic acid in solution according a Langmuir adsorption isotherm (Hering and Stumm, 1990).

A conceptual analogy can be drawn between abiotic Fe(III) oxide reductive dissolution by surface bound chemical reductants and enzymatic AIO reduction by DIRM. Because Fe(III) oxides are not taken up into the cell, microbial Fe(III)
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Oxide reduction is a surface-controlled reaction between DIRM cells and particulate Fe(III) oxides. As such, SA-specific rates of Fe(III) oxide reduction must be controlled by the abundance of cellular Fe(III) reduction moieties in contact with oxide surface sites. In other words, the abundance of active cellular reduction moieties will determine the rate constant for SA-specific enzymatic Fe(III) oxide reduction. The magnitude of this rate constant will be a complex function of the rate at which electron donors for Fe(III) oxide reduction are liberated through particulate OM hydrolysis and fermentation, the abundance of DIRM cells, and the relative susceptibility of oxide surface sites to reduction. The abundance of DIRM, in turn, will be dynamically controlled by the rate of electron donor supply, the abundance of reducible Fe(III) oxide surface sites, and the growth and death rates of the DIRM cells.

Given some specified rate constant for electron transfer to particulate Fe(III) oxide surfaces, the relationship between SA-specific and bulk volumetric mineral transformation rate can be defined as follows:

\[ R_{\text{bulk}}(t) = R_{\text{surf}}(t) \times \psi_s(t) \times \text{SA}(t) = -k \times C_{\text{surf}}(t) \times \psi_s(t) \times \text{SA}(t) \]  

where \( R_{\text{bulk}}(t) \) is the bulk volumetric reaction rate (\( \mu \text{mol cm}^{-3} \text{d}^{-1} \)) at time \( t \), \( \psi_s(t) \) is the concentration of solids per unit volume (\( g \text{ cm}^{-3} \)) at time \( t \), and \( \text{SA}(t) \) is the specific SA of the mineral (\( m^2 g^{-1} \)) at time \( t \). The bulk molar concentration of mineral is defined by

\[ C_{\text{bulk}}(t) = \psi_s(t)/MW \]  

where \( MW \) is the molecular weight of the mineral (\( g \mu \text{mol}^{-1} \)). Substituting this expression into Eq. (8.25) yields the following expression for volumetric mineral reaction rate:

\[ R_{\text{bulk}}(t) = dC_{\text{bulk}}(t)/dt = -k' \times C_{\text{surf}}(t) \times \text{SA}(t) \times MW \times C_{\text{bulk}}(t) \]  

The term \( kC_{\text{surf}}(t) \times \text{SA}(t) \times MW \) in Eq. (8.27) can be identified as an effective rate constant, \( k'(t) \):

\[ k'(t) = kC_{\text{surf}}(t) \times \text{SA}(t) \times MW \]  

If we assume that \( C_{\text{surf}}(t) \) and \( \text{SA}(t) \) remain constant over time, i.e., that reactive surface sites are efficiently regenerated and that mineral morphology and surface site density remain constant during reaction, Eq. (8.27) reduces to a simple first-order rate expression:

\[ R_{\text{bulk}} = dC_{\text{bulk}}/dt = -k'C_{\text{bulk}} \]  

which when integrated yields an exponential decay equation which is identical in form to the first-order rate law for microbial Fe(III) oxide reduction depicted by Eq. (8.22):

\[ C_{\text{bulk}}(t) = C_{0,\text{bulk}} \exp(-k't) \]
Equations (8.25)–(8.30) show that bulk-phase concentrations of an oxide mineral can decrease exponentially over time as a result of enzymatic (or abiotic) reduction, while surface-controlled reactions at the mineral-water interface are in steady-state with respect to SA-specific reaction rate. Because rates of electron donor supply through organic carbon mineralization, as indicated by $\Sigma CO_2 + CH_4$ production, were essentially constant during Fe(III) oxide reduction in our experiments (Fig. 8.9a,b, open symbols), effective rate constants for SA-specific enzymatic reduction were apparently approximately constant over time. Hence, a direct analogy can be drawn between the exponential decline in bulk AIO concentration observed in the microbial reduction experiments and the evolution of bulk-phase mineral concentration predicted by Eq. (8.30).

8.4.4.3 Influence of Labile OM Abundance on Fe(III) Reduction Kinetics

Thamdrup (2000) emphasized that the abundance and decomposition rate of labile OM is expected to exert a major influence on the kinetics of AIO reduction in sediments. Roden and Wetzel (2002) explicitly evaluated the relationship between labile OC and Fe(III) oxide reduction kinetics by adding different amounts of heat-killed Baker’s yeast to AIO rich wetland sediment slurries, and monitoring rates of OC mineralization and Fe(III) consumption over time (see Fig. 8.10). The results demonstrated a direct correlation between the Fe(III) reduction rate constant, as well as the initial Fe(III) reduction rate, and the abundance (Fig. 8.10c) and mineralization rate (Fig. 8.10d) of labile OC, i.e., rates of AIO reduction were directly controlled by bulk OC abundance and decomposition rate. These findings are analogous to the demonstration by Westrich and Berner (1984) of a linear correlation between SR rate and reactive OC concentration in marine sediments (Fig. 8.4b).

It is possible to interpret the results of the OM addition experiment in relation to the SA-controlled framework for microbial Fe(III) oxide reduction discussed above. The correlation between $k_{\text{red}}$ and OC mineralization can be attributed to a progressive increase in the SA-specific rate constant for AIO reduction ($k$ in Eq. (8.24)) with increasing OC mineralization rate. These SA-specific rate constants translate into increased effective rate constants for bulk Fe(III) depletion according to Eq. (8.28), which are equivalent to the $k_{\text{red}}$ parameters obtained from curve-fits of the Fe(III) time course data.

8.4.4.4 Relationship Between DIRM Abundance and Fe(III) Reduction Kinetics

The assumption of a time-invariant SA-specific Fe(III) oxide reduction rate constant ($k$ value) ignores the potential influence of changes in DIRM population density on rates of enzymatic electron transfer. This is tantamount to assuming that DIRM populations respond instantaneously to changes in the rate of electron donor supply, i.e., as dictated by rates of OC hydrolysis and fermentation. This assumption
Fig. 8.10 Panels A and B: Fe(III) reduction (A) and organic carbon mineralization (B) during anaerobic incubation of oxidized wetland sediment slurries amended with different amounts (in % dry weight) of labile OM. Solid lines in panel A are nonlinear least-squares regression fits of pooled data to Eq. (8.22). Solid lines in panel B show nonlinear least-squares regression fits of the data to an equation of the form $C(t) = C_{\text{max}}[1 - \exp(-at)]$, where $C(t)$ represents the amount of $\Sigma CO_2+CH_4$ produced at time $t$, $C_{\text{max}}$ represents the maximum (asymptotic) amount of $\Sigma CO_2+CH_4$ produced during the incubation period, and $a$ represents a first-order rate constant. Panels C and D: First-order Fe(III) reduction rate constants ($k_{\text{red}}$) and initial $\Sigma CO_2+CH_4$ production rates for oxidized wetland sediment slurries amended with different amounts of labile OM, and Fe(III) reduction rate constants and initial Fe(III) reduction rates versus initial $\Sigma CO_2+CH_4$ production. All data are from Roden and Wetzel (2002), used with permission.

is probably defensible for modeling long-term Fe(III) oxide reduction kinetics in sediments (Roden and Wetzel, 2002). However, as discussed in Sect. 8.3.5, proper mechanistic models of competitive interactions among different TEAPs should include the biomass of different functional respiratory populations. This points out the need for a framework for depicting biomass-dependent Fe(III) oxide reduction kinetics.

DIRM pure culture experiments with synthetic Fe(III) oxides have shown that rates of Fe(III) oxide reduction are a hyperbolic function of DIRM cell density (Roden and Zachara, 1996; Roden, 2003a, 2006) (see following section). This relationship is analogous to that between mineral dissolution and ligand concentration in abiotic systems (Hering and Stumm, 1990), which makes sense given that redox-active components on DIRM cell surfaces can be viewed as the “ligands” responsible for enzymatic Fe(III) oxide reduction. Controlled experiments with Fe(III) oxide-rich wetland sediment clearly demonstrate this analogy (Fig. 8.11a,b). The results can be used to assemble a kinetic expression for biomass- and SA-dependent Fe(III) oxide reduction in the wetland sediment. To facilitate this, the biological reduction data in Fig. 8.11b were re-expressed in terms of a SA-specific rate
Fig. 8.11 Rates of abiotic (A) and biotic (B) wetland sediment AIO reduction as a function of ligand (ascorbate or DIRM) concentration (E. Roden, unpublished). The abiotic reduction experiments were conducted with a suspension of ca. 1 mmol Fe(III) L$^{-1}$ in 10 mM ascorbate at pH 2. The biotic reduction experiments were conducted with a suspension of ca. 5 mmol Fe(III) L$^{-1}$ in 30 mM NaHCO$_3$ at pH 6.8, inoculated with different quantities of washed acetate/Fe(III) citrate-grown *G. metallireducens* cells. An average cell mass of $4 \times 10^{-10}$ mg cell$^{-1}$ (E. Roden, unpublished) was used to convert cell numbers to dry weight cell density. The solid lines show nonlinear least-squares regression fits of the data to the equation for a rectangular hyperbola (see Eq. (8.7)). SA-normalized rates and cell densities shown in panel C were computed using an assumed AIO SA of 600 m$^2$ g$^{-1}$ (see section 8.4.4.4 for details)
constant versus SA-normalized DIRM cell density (Fig. 8.11c). This relationship leads to the following expression for the biomass-dependent reduction rate constant $k_{\text{DIRM,surf}}$

$$k_{\text{DIRM,surf}} = k_{\text{max,DIRM,surf}} \frac{[\text{DIRM}]_{\text{surf}}}{K_{\text{DIRM,surf}} + [\text{DIRM}]_{\text{surf}}}$$  \hspace{1cm} (8.31)

where $k_{\text{max,DIRM,surf}}$ and $k_{\text{DIRM,surf}}$ have units of $\mu$mol m$^{-2}$ d$^{-1}$, and $[\text{DIRM}]_{\text{surf}}$ and $K_{\text{DIRM,surf}}$ have units of mg cells m$^{-2}$. The bulk rate of Fe(III) oxide reduction is then computed from the expression

$$R_{\text{Fe(III),bulk}}(t) = k_{\text{DIRM,surf}} \times \psi_{s}(t) \times \text{SA}(t)$$  \hspace{1cm} (8.32)

where $\psi_{s}(t)$ and SA(t) are defined in Eq. (8.25).

A standard (and time-invariant) SA value of 600 m$^{2}$ g$^{-1}$ for AIO (Dzombak and Morel, 1990) was used to compute the values for $k_{\text{max,DIRM,surf}}$ and $K_{\text{DIRM,surf}}$ shown in Fig. 8.11c from the bulk reaction rate and cell density data in Fig. 8.11b. Equations (8.31) and (8.32), together with an expression such as

$$\frac{d[\text{DIRM}]}{dt} = YR_{\text{Fe(III),bulk}} - k_{d}[\text{DIRM}]$$  \hspace{1cm} (8.33)

for DIRM growth (where Y is the yield coefficient and $k_{d}$ the cell decay coefficient), provide a means for computing rates Fe(III) oxide in a manner consistent with the known relationship between DIRM cell density and oxide reduction kinetics. Figure 8.12 illustrates the application of this approach to simulation of the Fe(III) reduction and CH$_{4}$ production data from the OM addition experiment shown in Fig. 8.10. DIRMs and MGMs were assumed to compete for acetate as the primary energy source for respiration as in previous simulations (Fig. 8.7). The rate of acetate production from POM hydrolysis and fermentation was computed from
curve-fits to the $\Sigma$CO$_2$ + CH$_4$ production data shown in Fig. 8.10b. The rate law for Fe(III) oxide reduction (Eq. (8.32)) was modified to account for dependence on acetate concentration using a standard Monod function and the same $K_m$ value (1 µM) used in previous simulations. The rate equations for MGM metabolism and growth were the same as in previous simulations. Assuming an initial DIRM and MGM cell density of 0.02 mg cm$^{-3}$, the model generally reproduced the timing and magnitude of the switch between Fe(III) reduction and methanogenesis in the sediment slurries (Fig. 8.12). Inclusion of a thermodynamic control term (Eq. (8.21)) had little influence on the predicted pattern of TEAPs. Although other rate formulations (e.g., biomass-independent Modified Monod and mixed first-order/Monod models, and a standard biomass-dependent Monod model) were also able to reproduce the experimental results with comparable accuracy, the biomass- and SA-dependent rate law summarized in Eq. (8.32) is mechanistically the most realistic way to describe the reaction system.

A final word regarding actual determination of DIRM cell densities in natural sediments: Roden and Wetzel (2002) employed a most probable number (MPN) enumeration procedure (with synthetic growth medium) to estimate changes in DIRM population size in response to addition of different amounts of labile OM. The results indicated that although DIRM abundance increased ca. 10-fold in OM-amended sediments compared to unamended slurries, there was no correlation between DIRM abundance and OM addition above 0.25% OM. This is perhaps not surprising in light of the relatively small changes in DIRM biomass ($< 2.5$-fold) predicted in the biomass-dependent model simulations. It is well known that MPN procedures employing synthetic growth media typically underestimate the abundance of anaerobic respiratory morgs such as SRMs by one or more orders of magnitude in both marine (Gibson et al., 1987; Jorgensen, 1978; Ramsing et al., 1996; Vester and Invorsen, 1998) and freshwater (Bak and Pfenning, 1991) environments, which complicates quantitative analysis of DIRM abundance under changing biogeochemical conditions. Thus, it is not possible to accurately verify the changes in DIRM abundance predicted in the biomass-dependent model simulations. Refinement of MPN procedures for enumeration of DIRM (e.g., through use of natural media (Vester and Invorsen, 1998) and/or application of molecular genetic techniques for nonculture-based estimation of DIRM abundance (e.g., MPN-PCR (Anderson et al., 1998; Rooney-Varga et al., 1999; Snoeyenbos-West et al., 2000), or real-time PCR (Stults et al., 2001)) will be required definitively to link variations in DIRM biomass to the kinetics of Fe(III) oxide reduction in sediments.

### 8.4.5 Pure Culture Studies of Fe(III) Oxide Reduction Kinetics

Although AIO is the most bioavailable and in many cases the dominant form of Fe(III) oxide reduced in sedimentary environments (Lovley, 2000a), in many situations crystalline Fe(III) oxides (CIOs) may be much more abundant than AIO (Roden and Urrutia, 2002). Because of their relatively high abundance, enzymatic
reduction of ClOs may contribute significantly to the long-term potential for Fe(II) generation and associated impacts on the aqueous/solid-phase geochemistry of subsurface environments, even if only a minor fraction of the crystalline Fe(III) oxide content is subject to microbial reduction. In addition, slow reduction of crystalline Fe(III) could play a major role in altering Fe geochemistry over geological time scales in nonsulfidogenic subsurface environments (Lovley et al., 1990; Lovley, 1992).

The studies discussed in this section explored how the surface chemical (e.g., specific SA) and thermodynamic properties (e.g., oxide reduction potential) of various Fe(III) oxides influence the rate and extent of microbial versus chemical Fe(III) oxide reduction. The results lead to a general quantitative model of enzymatic Fe(III) oxide reduction at circumneutral pH that can be compared and contrasted with models of abiotic mineral dissolution and enzymatic reduction of soluble metals and other aqueous EAs.

8.4.5.1 Initial Rates of Enzymatic Versus Abiotic Reduction

Initial rates of enzymatic Fe(III) oxide reduction were examined for a wide range of synthetic oxide phases using *S. putrefaciens* and *G. sulfurreducens* as model DIRMs (Roden, 2003a, 2006). Transmission electron microscope images of four representative oxide phases in the presence of a related DIRM (*Shewanella algae*) are shown in Fig. 8.13. The results showed that initial bulk rates of microbial Fe(III) oxide reduction were directly controlled by oxide SA (Fig. 8.14a). However, initial SA-normalized reduction rates (Fig. 8.14b) were independent of oxide SA, as well as oxide reduction potential (Eh, as inferred from long-term abiotic reduction experiments; see Fig. 8.14 legend). These results contrast with SA-normalized rates of abiotic Fe(III) oxide reduction by ascorbate and AH2DS, which were significantly correlated with oxide SA and Eh (Fig. 8.14c).

More than 65% of total dilute (0.5 M) HCl-soluble Fe(II) produced in the biological reduction experiments accumulated as Fe$^{2+}_{(aq)}$. Hence, enzymatic Fe(III) oxide reduction was mainly a reductive dissolution process in these experiments, e.g., as opposed to the Fe(II) mineralization processes that typically dominate AIO reduction systems (Zachara et al., 2002). If Fe(II) detachment from the oxide surface during enzymatic reduction was affected by the thermodynamic properties of the oxide and thus controlled the bulk reduction rate, we would have expected to see a significant correlation between initial surface-area normalized reductive dissolution rate and oxide Eh – as was clearly the case for reductive dissolution by ascorbate and AH2DS. Since this was not observed, we conclude that the mechanism and/or the rate-limiting step during enzymatic Fe(III) oxide reduction is fundamentally different than that for abiotic reductive dissolution. The simplest explanation is that the rate of electron transfer, rather than Fe(II) detachment, is the rate limiting step during enzymatic reduction, and that rates of enzymatic electron transfer are not strongly affected by oxide thermodynamic properties. This phenomenon
provides a clear example of a fundamental difference in the kinetic controls on an enzymatic versus an abiotic mineral-water reaction.

Ongoing studies with well-characterized DIRMs such as Shewanella and Geobacter have provided evidence that low redox potential, outer membrane-associated $c$-type cytochromes and/or electrically conductive pili are involved in electron transfer from DIRMs to Fe(III) oxides (see Sect. 8.4.2). In addition, a atomic force microscopy study by Lower et al. (2001) demonstrated apparent molecular “recognition” of Fe(III) oxide surface sites by a putative ca. 150 kDa outer membrane protein of the dissimilatory DIRM Shewanella oneidensis (formerly S. putrefaciens strain MR-1), a close relative of the organism used in this study. Together these findings suggest the possibility that the similarity of SA normalized electron transfer rates across a broad range of oxide minerals results from the fact that DIRMs “recognize” different Fe(III) oxide surfaces more-or-less equally, independent of the underlying crystal structure, such that initial rates of electron transfer subsequent to recognition are not strongly dependent on crystal structure. This suggestion is consistent with an argument presented by Fischer (Fischer, 1988) to account for the relatively minor influence of oxide solubility on rates of synthetic
Fig. 8.14 Initial (2–3 day incubation) rates of bacterial (A,B) and abiotic (C) reduction of synthetic Fe(III) oxides as a function of oxide surface area and standard reduction potential (E$_{0h}$). E$_{0h}$ values for the different oxides were estimated from the final (after reduction ceased) dissolved Fe(II) concentration and pH values achieved in the AH$_2$DS reduction experiments, together with the E$_{0h}$ of the AQDS/AH$_2$DS couple (+0.23V; (Clark, 1960)). Different symbols correspond to different Fe(III) oxide phases as indicated in the legend. Filled and thick-lined symbols refer to biological reduction by *S. putrefaciens* or abiotic reduction by ascorbate; open and thin-lined symbols refer to biological reduction by *G. sulfurreducens* or abiotic reduction by AH$_2$DS. Starting Fe(III) concentrations in the biotic and abiotic reduction experiments were 10 mmol L$^{-1}$ and 5 mmol L$^{-1}$, respectively. *S. putrefaciens* and ascorbate reduction data are reproduced with permission from Roden (2003). *G. sulfurreducens* data are reproduced with permission from Roden (2006).
Fe(III) oxide reduction by Corynebacteria at pH 7. Fischer reasoned that if the redox potential of the microbial cells (i.e., their outer membrane redox-active components) is sufficiently negative for reduction of well-crystallized oxide phases like hematite or goethite, each contact (or “recognition”) of a redox-active DIRM cell surface component with an oxide particle will trigger reduction of a Fe(III) surface site. Therefore the amount of Fe(II) produced during each contact event will not increase markedly with increasing oxide solubility. This conclusion is supported by the results of a literature survey of enzymatic versus abiotic (ascorbate-catalyzed) SA-specific rates of synthetic Fe(III) oxide reduction (Roden, 2003a). The survey revealed that initial ascorbate catalyzed reduction rates vary by more than three orders of magnitude, with rates for highly ordered minerals such as goethite and hematite being far lower than those for ferrihydrite and lepidocrocite. In contrast, initial microbial reduction rates are much more uniform, with rates for 2-line ferrihydrite actually being the lowest due to the effect of particle aggregation during dehydration. No statistically significant difference was detected between SA normalized initial enzymatic rates of AIO, goethite, hematite, and lepidocrocite reduction.

8.4.5.2 Influence of DIRM Cell Density and Association with Oxide Particles on Reduction Kinetics

As described above for wetland sediment AIO reduction (Fig. 8.11b,c), SA-normalized rates of enzymatic oxide reduction are directly influenced by the density of DIRM cells, and by inference the abundance of their redox active surface components. This phenomenon can be traced to the requirement for contact between DIRM and Fe(III) oxide surfaces in order for electron transfer to take place in the absence of soluble chelators or electron-shuttling compounds (see Sect. 8.4.2). It is relevant to note here that Fe(III) oxide particles are generally much smaller than the DIRM cells. Hence, “contact” between DIRMs and oxide surfaces corresponds to binding of Fe(III) oxides by DIRM cells (discussed in detail in Glasauer et al. (2001)). Figure 8.15 summarizes the results of DIRM cell loading experiments conducted with S. putrefaciens or G. sulfurreducens and a variety of synthetic Fe(III) oxide phases with excess H₂ as an energy source. Although the data show considerable scatter, they suggest a maximum SA-specific reduction rate on the order of 6 µmol m⁻² d⁻¹, and a half-saturating SA-normalized cell density of ca. 1 mg cells m⁻² (Fig. 8.15a). Initial rates of oxide reduction correlated directly with the abundance of DIRM cells associated with Fe(III) oxide surfaces (Fig. 8.15b), which in turn could be related to free (non-oxide-associated) cell density according to a Langmuir isotherm (Fig. 8.15c). Previous work by Das and Caccavo (2000, 2001), as well as a recent study by Bonneville et al. (2006), revealed qualitatively similar results for the association of Fe(III) oxides with DIRM cell surfaces, and the relationship between oxide-adhered DIRM cell density and Fe(III) reduction rate. The association of DIRMs with Fe(III) oxides is probably driven by hydrophobic rather than electrostatic forces, since both DIRM cells and Fe(III) oxides bear a net negative charge at pH (Caccavo et al., 1997; Das and Caccavo, 2000, 2001). Cell surface...
Fig. 8.15 Initial SA-normalized rates of enzymatic Fe(III) oxide reduction as a function of total (A) or attached (B) DIRM cell density, and relationship between oxide-associated and free cell density (C). *S. putrefaciens* and *G. sulfurreducens* data in (A) are reproduced with permission from data not shown in Roden (2003) and Roden (2006), respectively. Data in (B) and (C) are unpublished data of E. Roden. The concentration of surface sites was calculated based on oxide loading (10 mmol L\(^{-1}\) ≈ 0.89 g L\(^{-1}\)), the measured mineral SA areas, and an assumed mineral surface site density of 3.84 × 10\(^{-6}\) mol sites m\(^{-2}\) (Davis and Kent, 1990). The abundance of attached cells was determined by direct cell counting as described in Caccavo et al. (1997). The non-linear regression fit in panel A is defined by Eq. 1 in Table 8.7; best-fit values for \(k_{\text{max,DIRM, surf}}\) and \(K_{\text{DIRM,surf}}\) are 6.98 μmol m\(^{-2}\) d\(^{-1}\) and 1.18 mg cells m\(^{-2}\), respectively. Solid line in panel B shows results of a linear least-squares regression. Solid line in panel C shows nonlinear least-squares regression fit of the data to a Langmuir isotherm.
proteins and/or flagella are likely to be involved in the binding of Fe(III) oxides by DIRM cells (Caccavo, 1999; Caccavo and Das, 2002). Current evidence suggests that the latter components are required for the irreversible adhesion of Fe(III) oxides to DIRM cells (Caccavo and Das, 2002) that has been observed in several studies (Bonneville et al., 2006; Caccavo, 1999; Caccavo et al., 1996; Glasauer et al., 2001). However, irreversible adhesion is not required for enzymatic reduction to take place (Caccavo et al., 1997; Caccavo and Das, 2002; Grantham et al., 1997), and detachment and transport of DIRM cells clearly takes place in hydrologically open reaction systems (Rodent and Urrutia, 1999; Roden et al., 2000). The physiochemical mechanisms and controls on DIRM-Fe(III) oxide association are still not well-understood, and represent an important area of future research (Caccavo and Das, 2002).

Although the above relationships are logically consistent, uncertainties exist with respect to the quantitative relationship between DIRM abundance, DIRM-Fe(III) oxide association, and oxide reduction rate. An example of such uncertainty is evidenced by Fe(III) oxide loading experiments conducted with *G. sulfurreducens* and *S. putrefaciens*. Initial reduction rates were determined in the presence of 5–200 mmol L\(^{-1}\) of synthetic goethite (ca. 55 m\(^2\) g\(^{-1}\)) and 1 \(\times\) 10\(^8\) cells mL\(^{-1}\) of washed DIRM cells under non-growth conditions (Fig. 8.16). A linear correlation was observed between Fe(III) oxide loading and initial reduction rate, analogous to the first-order kinetics documented in wetland surface sediment (Fig. 8.9c,d), and similar to the results of several previous pure cultures studies of synthetic Fe(III) oxide reduction kinetics (Liu et al., 2001a; Roden and Zachara, 1996; Roden and Urrutia, 1999). In contrast, rates predicted using the relationship between SA-normalized reduction rate and DIRM cell density shown in Fig. 8.15a (and defined by Eqs. (8.31–8.32)) were invariant and considerably lower than observed values above 50 mmol Fe(III) L\(^{-1}\); i.e., the saturation behavior predicted by the rate equation was clearly not observed (Fig. 8.16). The cell-oxide binding isotherm shown in Fig. 8.15c predicts that all the DIRM cells present in these experiments should have been associated with Fe(III) oxides. Thus, it appears that the attached DIRM cells were somehow able (at least initially) to transfer electrons to Fe(III) oxides at SA-normalized rates considerably higher than those predicted by the data shown in Fig. 8.15a. Although the basis for this phenomenon is unclear, it is possible that aggregation of cells and oxides at high particle loading (visibly obvious in these experiments) facilitated contact between cell surface redox-active components and oxide surface sites – an effect that is obviously not taken into account in the rate law depicted in Eqs. (8.31–8.32).

### 8.4.5.3 Controls on Long-Term Reduction Kinetics

A common pattern of Fe(II) accumulation was observed during long-term (3-week incubation) reduction of the various synthetic Fe(III) oxides by *G. sulfurreducens* with H\(_2\) as the ED (Fig. 8.17a). After an initial period of rapid Fe(II) accumulation, rates of reduction decreased and Fe(II) levels approached an asymptote.
Similar patterns have been observed in many previous microbial reduction experiments with synthetic and natural Fe(III) oxide phases (see Roden and Urrutia (2002) for review). Solution pH was correlated with Fe$^{2+}$($aq$) accumulation during synthetic Fe(III) oxide reduction (Fig. 8.17b), as expected from the stoichiometry of Fe(III) oxide reduction coupled to H$_2$ oxidation:

$$\text{H}_2 + 2\text{FeOOH} + 4\text{H}^+ \rightarrow 2\text{Fe}^{2+} + 4\text{H}_2\text{O}$$

Measured pH values and Fe$^{2+}$($aq$) concentrations were used, together with the estimated $E_h^0$ values for the different Fe(III) oxides, to compute the free energy of Fe(III) oxide reduction ($\Delta G$ values) for the different synthetic phases during the course of the long-term reduction experiments (Fig. 8.17c). The results indicate that the cessation of oxide reduction activity cannot be attributed to free energy constraints posed by dissolved Fe(II) (Fe$^{2+}$($aq$)) accumulation and pH increase, because reduction rates approached zero at $\Delta G$ values substantially lower than the theoretical minimum of ca. $\sim$20 kJ mol$^{-1}$ required for energy conservation during biological energy metabolism (Schink, 1997). Similar conclusions were reached in a recent study of synthetic Fe(III) oxide reduction coupled to acetate oxidation by *G. metallireducens* (Dominik and Kaupenjohann, 2004), a result supported by an experiment examining reduction of synthetic goethite (55 m$^2$ g$^{-1}$) by *G. sulfurreducens* with acetate as the electron donor (asterisks in Fig. 8.17c; estimated $\Delta G$ data were converted to kJ/2e$^-$ to allow direct comparison with H$_2$ oxidation metabolism). The solid line in Fig. 8.17c corresponds to a thermodynamic control function which accounts for the influence of thermodynamic driving force on reaction rate, modified to account for the minimum free requirement for biological energy conservation as described in Liu et al. (2001a) (see Eq. (8.21) in Sect. 8.3.6). The experimental data clearly do not correspond to this function, in contrast to results obtained in studies of abiotic ligand-promoted dissolution of $\delta$-Al$_2$O$_3$ and goethite (Kraemer and Hering, 1997).

Both the total amount of Fe(II) generated and the amount of Fe(II) associated with oxide surfaces (referred to as “sorbed Fe(II)” at the end of the reduction experiments correlated directly with the measured (BET) SA of the mineral phases (Fig. 8.18). These results suggest that accumulation of Fe(II) on oxide surfaces was
Fig. 8.17 Panels A and B: Total (0.5M HCl extraction) Fe(II) production (A) and relationship between pH and aqueous Fe(II) accumulation (B) during reduction of synthetic Fe(III) oxides by *G. sulfurreducens* with H₂ as the electron donor (data from Roden (2006)). Symbols as in Fig. 8.14, except thick asterisks, which show results of an experiment with acetate as the electron donor. Lines in panel A show nonlinear regression fits of the data to an equation of the form $C(t) = C_{\text{max}}[1 - \exp(-at)]$, where $C(t)$ represents the amount of Fe(II) produced at time $t$, $C_{\text{max}}$ represents the maximum (asymptotic) amount of Fe(II) produced during the incubation period, and $a$ represents a first-order rate constant. Line in panel B shows result of a linear least-squares regression analysis. Panel C: Rate of Fe(III) reduction as a function of the estimated free energy of reaction during the Fe(III) reduction experiments. Fe(III) reduction rates were computed from derivatives of the nonlinear curve-fits shown in panel A. The solid line shows the relationship between reaction rate and energetics predicted by Eq. (8.21)
Fig. 8.18 Correlation between oxide mineral SA and (A) the final concentration of total HCl-extractable Fe(II) or (B) surface-associated (“sorbed”) Fe(II) during long-term *G. sulfurreducens* experiments. Symbols as in Fig. 8.1.4. Data for the HFO reduction system are omitted, since the mineral was transformed to goethite during partial reductive dissolution (Hansel et al., 2004). Lines show linear least-squares regression fits of the data; data for lepidocrocite (diamond) were omitted from the regression analysis shown in panel A.

Responsible for cessation of oxide reduction activity, with higher surface area CIoS supporting production of greater amounts of Fe(II) by virtue of their higher Fe(II) binding capacity per unit mass. This conclusion is consistent with many previous studies of CIO reduction at circumneutral pH, and has been attributed to kinetic and/or thermodynamic impacts of surface-associated Fe(II) on enzymatic electron transfer, including impacts of Fe(II) sorption/surface precipitation on FeRB cell surfaces (Roden and Urrutia, 2002). Recent Mossbauer studies (Williams and Scherer, 2004) as well as experiments on the behavior of Fe isotopes during DIR (Crosby et al., 2005; Icopini et al., 2004) verify previous speculations (Coughlin and Stone, 1995; Roden and Urrutia, 2002) that electron (and Fe atom) exchange takes place between Fe(II) and CIO surfaces. These studies indicate that Fe(II) atoms accumulate within the surface of the CIO crystal lattice during DIR, the net effect of which is to render the bulk oxide progressively more resistant to enzymatic reduction.
8.4.5.4 General Conceptual Model and Rate Law for Microbial Fe(III) Oxide Reduction

The experimental findings summarized above point to a conceptual model of microbial Fe(III) oxide reduction kinetics in which oxide SA exerts primary control on both the initial rate and the long-term extent of reduction. The explicit SA control of the initial rate and long-term extent of oxide reduction leads to a general rate law for oxide reduction as a function of electron acceptor and DIRM abundance that differs fundamentally from that for reduction of chelated Fe(III) and other soluble metal species. Table 8.7 provides a summary of rate laws that are appropriate for use in modeling solid-phase as compared to dissolved metal (and other soluble electron acceptor) reduction. Equation (1) in Table 8.7 states that the bulk Fe(III) oxide reduction rate is a linear function of “free”, microbially reducible oxide surface abundance (discussed further below) at fixed DIRM cell density. DIRM cell density in turn defines the Fe(III) reduction rate constant (e.g., in units of $\mu$mol m$^{-2}$ d$^{-1}$) according to a hyperbolic function (Fig. 8.15a). The observed relationships between DIRM cell density and oxide reduction rate are analogous to the well-recognized dependence of abiotic reductive dissolution rate on total and surface-associated ligand concentration (Hering and Stumm, 1990), and are the opposite of those for reduction of soluble metals and other electron acceptors. As illustrated for Fe(III)-citrate in Fig. 8.19, rates of soluble EA reduction are a hyperbolic function of EA concentration, and a linear function of DIRM cell density (Eq. (8.3) in Table 8.7). Similar patterns hold for reduction of various soluble metals such as U(VI), Co(III), Cr(VI), and Tc(VI) (Liu et al., 2001b, 2002; Roden, 2005; Roden and Scheibe, 2005; Truex et al., 1997).

A key issue in the quantitative interpretation and modeling of CIO reduction is how to conceptualize controls on the long-term extent of oxide reduction. Several studies have shown that it is possible to model the long-term extent of CIO reduction by assuming that oxide surface sites occupied by surface-bound (sorbed and/or surface-precipitated) Fe(II) are no longer available for microbial reduction (Burgos et al., 2002, 2003; Hacherl et al., 2003; Liu et al., 2001a; Roden and Urrutia, 1999). In this approach, mass-action relationships between surface-associated and Fe$_{2+}^{(aq)}$, together with bulk oxide SA loading, control the availability of microbially reducible Fe(III) oxide SA. This strategy and its limitations is illustrated below using data on synthetic Fe(III) oxide reduction by *G. sulfurreducens*.

The partitioning of biogenic Fe(II) between the aqueous phase and oxide/DIRM surfaces during the reduction experiments (Fig. 8.20) was generally consistent with previous studies of Fe(II) sorption to synthetic goethite at similar pH and ionic strength (Liu et al., 2001a; Urrutia et al., 1998), with maximum sorption densities of 2–4 $\mu$mol Fe(II) m$^{-2}$. Nonlinear-fitting of the data to a Langmuir isotherm (Eq. (8.2) in Table 8.7) yielded a best-fit maximum sorption density value of 3.67 $\mu$mol m$^{-2}$, which is close to the “universal” mineral surface site density of 3.84 $\mu$mol m$^{-2}$ recommended by Davis and Kent (1990) for use in surface complexation modeling. Although DIRM cells possess a mass-normalized Fe(II) sorption capacity comparable to that of 55 m$^2$ g$^{-1}$ synthetic goethite (Liu et al., 2001b; Urrutia et al.,
A. Solid-Phase

\[ R_{\text{Fe(III)}} = k_{\text{max,DIRM,surf}} \frac{[\text{DIRM}_{\text{surf}}]}{K_{\text{DIRM,surf}}} [\text{Fe(III)}_{\text{fsa}}] \]

where:

- \( R_{\text{Fe(III)}} \) = bulk volumetric rate of Fe(III) oxide reduction (e.g. mmol/L/d)
- \( k_{\text{max,DIRM,surf}} \) = Maximum SA-normalized reduction rate constant (e.g. \( \mu \text{mol/m}^2 \text{ free oxide surface/d} \)) at very high SA-normalized DIRM density (see Fig. 8.15A)
- \([\text{DIRM}_{\text{surf}}] \) = SA-normalized DIRM cell density (e.g. mg cells/m² free oxide SA) (see Fig. 8.15A)
- \( K_{\text{DIRM,surf}} \) = Half-saturation constant for relationship between DIRM cell density and oxide reduction rate constant (e.g. mg cells/m² free oxide SA) (see Fig. 8.15A)
- \([\text{Fe(III)}_{\text{fsa}}] \) = bulk volumetric abundance of “free” (i.e. microbially-reducible) Fe(III) oxide surface (e.g. m²/L)

In 1998), the mass of cells (ca. 40 mg L\(^{-1}\)) was much lower than that of Fe(III) oxides (890 mg L\(^{-1}\)) in these experiments, such that the cells contributed only negligibly to Fe(II) sorption capacity. The sorption isotherm shown in Fig. 8.20 was used in conjunction with the relationship between SA-normalized reduction rate and DIRM cell density (Eq. (8.1) in Table 8.7) to simulate the time course data shown in Fig. 8.17a. The bulk concentration of microbially reducible Fe(III) oxide surface (e.g., in m² L\(^{-1}\)) was computed dynamically during as the simulation (using

B. Aqueous

\[ R_{\text{Fe(III)}} = \alpha_{\text{DIRM}} [\text{DIRM}]_{\text{bulk}} \frac{[\text{Fe(III)}_{\text{aq}}]}{K_{\text{Fe(III)}} + [\text{Fe(III)}_{\text{aq}}]} \]

where:

- \( R_{\text{Fe(III)}} \) = bulk volumetric rate of Fe(III) oxide reduction (e.g. mmol/L/d)
- \( \alpha_{\text{DIRM}} \) = proportionality constant for relationship between bulk DIRM cell density and soluble Fe(III) reduction rate in the presence of excess soluble Fe(III) (e.g. mmol/cell/d) (see Fig. 8.19B)
- \([\text{DIRM}]_{\text{bulk}} \) = bulk DIRM cell density (e.g. cells/L)
- \([\text{Fe(III)}_{\text{aq}}] \) = Concentration of aqueous Fe(III) (e.g. mmol/L)
- \( K_{\text{Fe(III)}} \) = Half-saturation constant for relationship between Fe(III)\(_{\text{aq}}\) concentration and reduction rate (e.g. mmol/L) (see Fig. 8.19A)
Fig. 8.19 Kinetics of Fe(III)-citrate reduction by *G. sulfurreducens* (data from Roden, (2006), used with permission). Solid lines in panels A and B show results of nonlinear and linear least-squares regression fits to the following expressions, respectively: 

$$R_{Fe}^{(III)} = \frac{[Fe(III)_{aq}]}{(K_{Fe}^{(III)} + [Fe(III)_{aq}])}; \quad \text{and} \quad R_{Fe}^{(III)} = \alpha_{DIRM} [DIRM]_{bulk} \, (\text{see Table 7B})$$.

Best-fit values for $K_{Fe}^{(III)}$ and $\alpha_{DIRM}$ are 0.25 mmol L$^{-1}$ and $5.9 \times 10^{-11}$ mmol cell$^{-1}$ d$^{-1}$, respectively.

The results illustrate a fundamental problem with this approach, which is that cessation of oxide reduction does not take place because some quantity of free surface sites remains available until all the oxide is consumed (data not shown). It is possible to account for the cessation of oxide reduction in an “ad hoc” manner by fixing the oxide SA at a pre-chosen value and subtracting the Fe(II) surface coverage computed at each time step until no surface is left available (Roden and Urrutia, 1999). However, this approach suffers from obvious theoretical limitations in that the mechanistic link between available SA and the sorption
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\[ [\text{Fe(II)}_{\text{sal}}]_{\text{max}} = 3.67 \times 10^{-6} \text{ mol m}^{-2} \]

\[ K_{\text{ads}} = 1.38 \times 10^{-4} \]

Fig. 8.20 Aqueous/solid-phase partitioning of Fe(II) during long-term synthetic Fe(II) oxide reduction experiments with *G. sulfurreducens* (data from experiments shown in Figs. 8.17 and 8.18; symbols as in Fig. 8.15). Asterisks show data for sorption of Fe(II) to synthetic goethite (55 m² g⁻¹) from Urrutia et al. (1998). Solid line shows nonlinear regression fit of the data to a Langmuir isotherm (Eq. 2 in Table 8.7). Dashed line indicates the “universal” mineral surface site density of 3.84 µmol m⁻² recommended by Davis and Kent (1990) for use in surface complexation modeling.

Some investigators have assumed that precipitation of siderite on oxide surface leads to blockage of available SA and thereby contributes to the cessation of oxide reduction (Hacherl et al., 2003; Liu et al., 2001a). While the latter approach may be defensible in situations where dissolved inorganic carbon (DIC) is abundant, it does not apply to the reactions systems considered here, which contained very little DIC and therefore were not likely to involve siderite precipitation.

Liu et al. (Liu et al., 2001a) introduced the concept of a mixed kinetic/thermodynamic approach for simulation of long-term Fe(III) oxide reduction kinetics, which is equivalent to combining Eq. (8.21) with Eqs. (8.31–8.32). In this approach the value of the thermodynamic control term \( F_T \) is computed based on the measured (or estimated) concentrations of reactants and products, and an assumed \( \Delta G_f \) value for the oxide phase undergoing reduction. For the purposes of simulating the oxide reduction data in Fig. 8.21, the oxide \( \Delta G_r \) can be treated as an adjustable, empirical parameter (referred to hereafter as \( \Delta G_f^* \)) whose value defines the point at which \( F_T \) (and thus the rate of oxide reduction) approaches zero. Using this approach together with the expressions for enzymatic oxide reduction kinetics and Fe(II) sorption (Eqs. (8.1) and (8.2) in Table 8.7), it was possible to reproduce the observed reduction of various synthetic Fe(III) oxides by *G. sulfurreducens* (Fig. 8.21). It is important to note that the \( \Delta G_f^* \) values listed in Fig. 8.25 are not the same as the \( \Delta G_f \) values that correspond (vis-à-vis the Nernst equation) to the \( E_h^0 \) values for the different oxide phases indicated in Fig. 8.18. Although it is not appropriate to assign a formal meaning to the \( \Delta G_f^* \) values, they could be interpreted as values that account (albeit indirectly) for the effect of sorbed Fe(II) accumulation on the thermodynamic favorability of enzymatic electron transfer. The fact that it was possible to simulate reduction of all four goethite phases (i.e., those formed...
Fig. 8.21  Simulation of synthetic Fe(III) oxide reduction coupled to H₂ oxidation by *G. sulfurreducens* (data from Fig. 8.17) using a combination of Eqs. (8.21) and (8.31–8.32) together with the Fe(II) sorption isotherm shown in Fig. 8.20 to compute (dynamically during the simulation) the abundance of free, microbially-reducible oxide surface as described in Table 8.7A. The free energy of formation for the oxide phase undergoing reduction was treated as an adjustable parameter ($\Delta G_f^*$) in order to obtain a reasonable fit (by eye) to the observed data. Filled symbols and solid lines refer to total Fe(II); open symbols and dashed lines refer to aqueous Fe(II). Dotted line shows the value of the thermodynamic control function ($F_T$ in Eq. (8.21))
by $\text{Fe}^{3+}$ precipitation at either 7, 20, 30, or 90$^\circ$C with the same $\Delta G_f^*$ value of $-487 \text{kJ mol}^{-1}$ suggests that these oxides respond (relative to enzymatic reduction) in a common way to the accumulation of surface-associated Fe(II).

In order to evaluate the robustness of the above approach, data from the goethite loading experiment with *G. sulfurreducens* described in Fig. 8.16 (and associated text) were simulated using a $\Delta G_f^*$ value of $-487 \text{kJ mol}^{-1}$. The results illustrate an important limitation of the kinetic framework, which reflects an impact of the geochemical environment on DIRM cell viability that is not included in the framework. Although the data for the lowest levels of goethite loading (5 and 10 mmol Fe(III) L$^{-1}$) were reproduced reasonably well, the simulations over-predicted the extent of reduction 2–3 fold at higher levels of loading (Fig. 8.22). These results can be tentatively attributed to the inhibitory influence that Fe(II) sorption onto DIRM cell surfaces has on enzymatic reduction activity, particularly under non-growth conditions (Roden and Urrutia, 2002; Urrutia et al., 1998). Increased production of Fe(II) at higher levels of goethite loading probably led to coating of DIRM surfaces with sorbed and/or surface-precipitated Fe(II) (see Fig. 8.1e,f), thereby inhibiting Fe(III) reduction. If this explanation is correct, then it follows that the $\Delta G_f^*$ values listed in Fig. 8.21 account (in a lumped manner) for the influence of Fe(II) binding by both oxide and DIRM surfaces on long-term oxide reduction. Further studies of the relative influence of these two phenomena will be required to formulate and parameterize a more accurate kinetic model for controls on the long-term extent of ClO reduction.

### 8.4.5.5 Implications and Application to Natural Soils and Sediments

Virtually all experimental work to date on microbial synthetic Fe(III) oxide reduction indicates that oxide mineral heterogeneity in natural soils and sediments is likely to affect initial reduction kinetics (e.g., during the early stages of anaerobic metabolism following the onset of anoxic conditions) mainly via an influence on reactive surface site density. This assertion is supported by experiments with a mixed culture of wetland sediment morgs and a range of synthetic Fe(III) oxides, which showed that DIRMs could outcompete MGMs for acetate with equal effectiveness when the different oxides are present at comparable SA loadings – despite major differences in computed $\Delta G$ values for acetate oxidation coupled to DIR (Roden, 2003b). An immediate implication of these findings is that the SA of different oxides phases present in a natural soil or sediment will exert primary control on initial rates of enzymatic reduction, e.g., in situations where DIR contributes to the oxidation of natural or contaminant organic compounds.

During the later stages of DIR in permanently reduced sediments, accumulation of aqueous and (more importantly) surface-bound Fe(II) is expected to exert a dominant control on apparent Fe(III) oxide reactivity toward enzymatic reduction, particularly in situations where removal of Fe(II) end-products is slow compared to the kinetics of reduction (Roden and Urrutia, 1999, 2002). As discussed above, there is a general relationship between oxide SA and long-term extent of oxide
Fig. 8.22 Simulation of the results of the synthetic goethite loading experiment with *G. sulfurreducens* described in Fig. 8.16 (and associated text) using the same expressions and parameter values used in Fig. 8.21

Reduction in closed reaction systems (see Fig. 8.18), which results from the function of oxide surfaces as a repository for sorbed and/or surface-precipitated biogenic Fe(II). Although the existence of this relationship implies a connection between extent of microbial reduction and oxide thermodynamic properties, evidence suggests that this connection is not directly related to thermodynamic properties such as $E_h^0$ or $\Delta G_f$, but rather results mainly from the correlation between these properties and oxide SA. Consequently, inferences regarding the ability of DIR to compete with other TEAPs in soils and sediments should be based on estimates of bulk reactive (i.e., microbially accessible) surface site density – rather than the thermodynamic properties of the oxide(s) identified as the dominant phase(s) in a particular soil or sediment.

In order to formally evaluate changes in the apparent reactivity of Fe(III) oxides during long-term enzymatic versus abiotic reduction, data from long-term experiments on reduction of three different natural CIOs by *S. putrefaciens* versus ascorbate (Roden, 2004) were interpreted in relation to a standard generalized rate law for mineral dissolution (Larsen and Postma, 2001; Postma, 1993):

$$\frac{J(t)}{m_0} = k'(\frac{m}{m_0})^\gamma$$  \hspace{1cm} (8.34)

where $J(t)$ is the rate of dissolution and/or reduction at time $t$, $m_0$ is the initial mass (or molar concentration) of oxide, $k'$ is a rate constant, $m/m_0$ is the unreduced (or undissolved) mineral fraction, and $\gamma$ is a parameter that accounts for changes in reactivity over time that may arise as a result of (i) heterogeneity in the reactivity
of the initial population of minerals undergoing dissolution, and/or (ii) changes in reactivity of the dissolving mineral phase over time. Equation (8.34) corresponds to a power rate law, which reduces to a first-order rate law when $\gamma \approx 1$.

The Fe(III) oxide pool in each of the materials was dominated ($\geq 97\%$) by crystalline phases, as indicated by the ratio of hydroxylamine HCl- to total citrate/dithionite-extractable Fe. The natural Fe(III) oxide assemblages demonstrated significant changes in reactivity during long-term abiotic reductive dissolution (Fig. 8.23a), as indicated by $\gamma$ values in excess of 1 for curve-fits of the data to the generalized rate law (Larsen and Postma, 2001; Postma, 1993). Much larger changes in reactivity were estimated for the microbial reduction experiments (Fig. 8.23b, solid symbols). However, when the analysis was restricted to the long-term “microbially reducible” fraction of the Fe(III) oxide content of the natural solids (Fig. 8.23b, open symbols), the data could be well-approximated with $\gamma$ values of ca. 1, i.e., by a first-order rate law. Note here that the “microbially reducible” fraction refers simply to that fraction of total Fe(III) that was subject to reduction, directly analogous to Fe(III)$_{\text{reac}}$ in Eq. (8.23).

Kinetic and thermodynamic considerations indicated that neither the abundance of electron donor (lactate) nor the accumulation of aqueous end-products of oxide reduction (Fe(II), acetate, dissolved inorganic carbon) are likely to have posed significant limitations on the long-term kinetics of oxide reduction. Rather, accumulation of biogenic Fe(II) on residual oxide surfaces appeared to play a dominant role in governing the long-term kinetics of natural CIO, as described for synthetic Fe(III) oxide reduction above. Exploratory numerical simulations of SADIR (based on a constrained model for synthetic goethite reduction) indicate that this phenomenon can account for the pseudo-first order kinetics of reduction of the operationally defined “microbially reducible” fraction of the sediment Fe(III) oxide pool (see Roden (2004) for more a more detailed explanation).

To illustrate how the above-developed reaction framework for the kinetics of DIR can be applied to a natural soil or sediment, data on the reduction of shallow subsurface Atlantic coastal plain sediment (designated as material AP in Fig. 8.23) by $S$. putrefaciens (under growth conditions with lactate as an energy source) was simulated using an approach that incorporates Fe(II) partitioning onto mineral surfaces, analogous to that used to model synthetic Fe(III) oxide reduction shown in Figs. 8.21 and 8.22. Two important additions to the approach were to (i) include DIRM growth using a yield coefficient (ca. 1 mg of cells per mmol Fe(III) reduced) estimated using the energy balance approach of Rittmann and McCarty (2001); and (ii) include an auxiliary solid-phase capable of sorbing biogenic Fe(II). Inclusion of such a phase is required because the total amount of solid-associated Fe(II) produced during Fe(III) reduction, when normalized to the residual (after reduction ceased) Fe(III) content of the sediment, was at least 10-fold higher (ca. 0.4 mol Fe(II) mol Fe(III)$^{-1}$) than that observed for synthetic Fe(III) oxide reduction (ca. 0.003–0.04 mol Fe(II) mol Fe(III)$^{-1}$); in other words, the Fe(III) oxides present in the sediment cannot account for the majority of the solid-associated Fe(II) that accumulated during enzymatic reduction. In addition, previous experimental studies have shown that the presence of mineral phases (e.g., aluminum oxide and layered
Fig. 8.23 Kinetic analysis of ascorbate (A) and microbial (S. putrefaciens) (B) reduction of three different Fe(III) oxide-bearing subsurface materials. All data are from anaerobic batch reactor experiments reported in Roden (2004), used with permission. Squares, triangles, and circles represent the HC, CP, and AP materials described in detail in Roden (2004). Ascorbate reduction experiments were conducted with shaken (well-mixed) particle suspensions containing 1 mmol Fe(III) L$^{-1}$ in 10 mM ascorbic acid, pH 2. Microbial reduction experiments were conducted with static (unmixed) suspensions containing 12 mmol Fe(III) L$^{-1}$ in Pipes (10 mM, pH 6.8) buffer with excess lactate (10 mM) as the electron donor. Open symbols in panel B refer to reduction of the “microbially-available” fraction of the oxide pool (equivalent to Fe(III)$_{\text{react}}$ in Eq. (8.23)). The terms $m_0$ and $m_t$ refer to the mass (concentration) of Fe(III) oxide present at the start of the experiment and at time $t$, respectively. Solid lines show nonlinear regression fits of the data to the integrated form of the generalized mineral dissolution rate law (Eq. (8.34)). $\gamma$ values refer to the results of the nonlinear curve fits.

Silicates capable of binding Fe(II) can dramatically extend the degree of CIO reduction by serving as a sink for biogenic Fe(II) that would otherwise down-regulate Fe(III) oxide reduction by the mechanisms discussed above (Urrutia et al., 1999). Thus, inclusion of the auxiliary solid-phase is important for accurate simulation of both aqueous/solid-phase Fe(II) partitioning as well as Fe(III) oxide reduction kinetics.

In order to minimize the number of adjustable parameters in the sediment Fe(III) oxide reduction model, the following values were chosen a priori based on available information: (1) the mass of the auxiliary Fe(II) sorbing phase was set equal to the total mass of solids per unit volume in the reaction system (142 g L$^{-1}$), to which the
mass of Fe(III) oxide contributed less than 1% (ca. 9 mg/g); (2) the SA of the auxiliary phase was set equal to the measured (BET) SA of the sediment (5.6 m² g⁻¹); (3) the surface site density of the auxiliary phase was set equal to the universal site density of 3.84 μmol m⁻² recommended by Davis and Kent (1990); (4) the Fe(III) oxide pool in the sediment was assumed to be dominated by goethite, in accordance with previous detailed characterization of similar materials (Penn et al., 2001); (5) the initial bulk Fe(III) oxide abundance in the reaction system (ca. 12 mmol Fe(III) L⁻¹) was computed from the total citrate/dithionite-extractable Fe(III) content of the sediment (ca. 100 μmol g⁻¹) and the total solid mass loading; (6) the ΔG_f° value (see Sect. 8.4.5.4) for the sediment Fe(III) oxide pool was set equal to −485 kJ mol⁻¹, which is the mean of the ΔG_f° values estimated for the synthetic goethite reduction systems depicted in Fig. 8.21; (7) the maximum SA-normalized sorption capacity and binding constant for Fe(II) sorption by Fe(III) oxides were set equal to the best-fit values for the synthetic phases shown in Fig. 8.20; and (8) the kmaxbiomass_surf and KDIRM_surf values for enzymatic Fe(III) reduction (see Table 8.7) were set equal to the best-fit values for the synthetic oxide phases shown in Fig. 8.15.

With the above parameter values fixed, only two additional parameters needed to be defined to run the model: (1) the average SA for the sediment Fe(III) oxide pool; and (2) the binding constant (log K_ads value) for Fe(II) sorption by the auxiliary phase. Systematic testing showed that only a limited combination of values for these two parameters could reproduce the observed total and Fe²⁺(aq) versus time data (SA values of 100–150 m² g⁻¹ and log K_ads values of −3.5 to −3.7), all of which are reasonable for soil/sediment mineral phases. The results of a simulation using an SA value of 120 and a log K_ads value of −3.5 are shown in Fig. 8.24. The results of
this exercise, together with the analysis of wetland sediment Fe(III) oxide reduction kinetics, suggest that it is possible to use mechanistic, reaction-based models (which incorporate a wide array of microbial and geochemical phenomena) to describe the kinetics of DIR in natural sediments.

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