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Modeling and measuring biogeochemical reactions: system consistency, data needs, and rate formulations

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Abstract

This paper intends to lay-out a foundation of protocols for planning and analyzing biogeochemical experiments. It presents critical theoretical issues that must be considered for proper application of reaction-based biogeochemical models. The selection of chemical components is not unique and a decomposition of the reaction matrix should be used for formal selection. The decomposition reduces the set of ordinary differential equations governing the production–consumption of chemical species into three subsets of equations: mass action; kinetic-variable; and mass conservation. The consistency of mass conservation equations must be assessed with experimental data before kinetic modeling is initiated. Assumptions regarding equilibrium reactions should also be assessed. For a system with M chemical species involved in N reactions with N_I linearly-independent reactions and N_E linearly-independent equilibrium reactions, the minimum number of chemical species concentration vs. time curves that must be measured to evaluate the kinetic suite of reactions using a reaction-based model will be $(N_I - N_E)$. However, for a partial assessment of system consistency, at least one more species must be measured [i.e. $(N_I - N_E + 1)$]. For a complete assessment of system consistency, $(N_I - N_E + N_C)$ additional species would have to be measured, where N_C is the number of chemical components. Reaction rates for kinetic reactions that are linearly independent of other kinetic reactions can be determined based on only one profile of a kinetic-variable concentration vs. time for each kinetic reaction. Reaction rates for parallel kinetic reactions that are linearly dependent on each other cannot be uniquely segregated when they result in production of the same species, however, they must be included for simulation purposes. Kinetic reactions that are linearly dependent only on equilibrium reactions are redundant and do not have to be modeled. The bioreduction of ferric oxide is used as an example to functionally demonstrate these points, and shows that Henri–Michaelis–Benton–Monod kinetics should be applied with care to coupled abiotic and biotic systems.

Keywords: Geochemical modeling; Biochemical modeling; Kinetic modeling; System consistency; Data requirements; Bacterial iron reduction; Kinetic experiments

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1. Introduction

Innovative remediation technologies for subsurface contamination (e.g. geochemical transformation, biochemical degradation, electrokinetics, surfactant/cosolvent flushing, vapor extraction, natural attenuation) have become tenable alternatives to conventional pump-and-treat technologies. To properly evaluate, design, and optimize remediation technologies, appropriate reaction-based simulators are needed. Such simulators require rigorous treatment of biogeochemical reaction systems. Many different modeling approaches have been put forth to address a wide range of remediation problems, and the role of reactive biogeochemical modeling is growing.

Early work on geochemical modeling tended to emphasize the mathematical formulation of chemical processes. The earliest work focused on chemical equilibrium formulations of aqueous and surface complexation and precipitation/dissolution (Westall et al., 1976). This approach was extended to include ion exchange, oxidation–reduction and acid–base reactions (Yeh and Tripathi, 1989), and then reformulated to allow generic treatment of both equilibrium and kinetic reactions (e.g. Yeh et al., 1994, 1998). Modeling studies have highlighted different kinetic features of precipitation/dissolution reactions in earth systems and the implications of these to numerical simulation (Steefel and van Cappellen, 1990; van Cappellen and Berner, 1991; van Cappellen et al., 1993; Steefel and Lasaga, 1994; Steefel and Lichtner, 1994).

The developing field of bioremediation, combined with a greater awareness of the importance of subsurface bacteria in mediating key geochemical processes, has stimulated interest in the coupling of chemical and microbiologic models. Such coupled modeling has emphasized microbiologic and hydrologic aspects (Cheng, 1995; Rittmann and van Briesen, 1996), and microbiologic, geochemical and hydrologic aspects (Salvage and Yeh, 1998; Tebes-Stevens et al., 1998) of the problem.

The application of biogeochemical reaction models to both laboratory experiments and field-scale situations is increasing (Schafer and Therrien, 1995; Steefel and van Cappellen, 1998; Lu et al., 1999). In a recent special issue of the *Journal of Hydrology* (vol. 209, 1998), 15 papers dealt with various aspects of biogeochemical processes at laboratory (e.g. Szecsody et al., 1998) and field-scales (e.g. van Breukelen et al., 1998). The distinctions between mechanistic and empirical biogeochemical modeling were highlighted (Steefel and van Cappellen, 1998), and the dangers of model calibration were emphasized (Brusseau, 1998). While calibration with an empirical-based approach may guarantee a 'fit' of the data, it may limit the ability of the model to describe other systems because individual processes are not independently determined.

The basis of biogeochemical modeling is a set of ordinary differential equations describing time-variant changes in the concentration of chemical species in the system. This set of equations is generally constrained by a set of linear algebraic equations describing mass conservation of chemical components (Szecsody et al., 1994; Yeh et al., 1994; Salvage and Yeh, 1998). The choice of chemical components is commonly decided a priori. However, the selection of chemical components is not unique and a mathematical decomposition of the reaction matrix (e.g. Chilakapati et al., 1998) should be used for formal selection. If an initial selection of chemical components is not consistent with the reaction matrix, the decomposition procedure will automatically select a plausible set of components (Fang and Yeh, 2000). The Gauss-Jordian or QR decomposition, originally proposed to facilitate numerical integration of biogeochemical equations (Chen, 1994; Chilakapati, 1995), will be applied in this paper to assess system consistency and minimum data requirements.

System consistency is defined as when all mass conservation equations are satisfied. In some biogeochemical kinetic modeling studies, mass conservation equations are assumed to be satisfied and not experimentally validated. If the chemical species present in a system are not adequately identified, the use of mass conservation equations can generate erroneous evolutions of chemical species concentrations that can still be made to 'fit' experimental data with the calibration of reaction rates and parameters. Therefore, it is of ultimate importance to ensure system consistency with experimental data before biogeochemical kinetic modeling is initiated. The question then is, what is the minimum data required to ensure system consistency? These issues can be addressed by carefully evaluating the proposed biogeochemical reaction network.

The objectives of this paper are to: (1) present critical theoretical issues that must be considered for proper application of reaction-based biogeochemical models; and (2) functionally demonstrate the application of these concepts to the design, performance, and conceptual modeling of a relevant biogeochemical experiment. Theoretical considerations and definitions are first presented, and we review how a biogeochemical reaction network can be decomposed via the Gauss-Jordian elimination (Chilakapati, 1995; Steefel and MacQuarrie, 1996) or the QR decomposition (Chen, 1994) into three subsets of equations as shall be described below in Section 2.1. A procedure is then established to assess system consistency and data needs are discussed. A bacterial iron reduction experiment is used to demonstrate these theoretical considerations. An appropriate reaction network (conceptual modeling) is first defined for the example which is decomposed into the corresponding equation matrix. We show the importance of selecting a decomposition that yields

at least one mass conservation equation, and at least one kinetic-variable equation in which the concentration of the conserved and kinetic-variable, respectively, can be operationally measured. Finally, we compare reaction rate formulations for a biogeochemical reaction based on direct or indirect simulations, and formulate a non-elementary reaction rate for a geochemical reaction. An elementary reaction is one whose rate is described by the forward and backward rates with the order of the rate given by the stoichiometry of the reaction. A non-elementary reaction is one whose rate cannot be described by the elementary rate.

2. Theoretical considerations

A biogeochemical system is completely defined by specifying chemical species and biogeochemical reactions that produce them from chemical components. A set of M ordinary differential equations can be written for M species in a reactive system as

$$\left(\frac{\mathrm{d}C_i}{\mathrm{d}t}\right) = r_i|_{\mathrm{N}}, \quad i \in M \tag{1}$$

where C_i is the concentration of the i-th chemical species (mol l^{-1}), t is time, $r_i|_N$ is the production–consumption rate of the i-th species due to N biogeochemical reactions (mol l^{-1} per unit time), and M is the number of chemical species. The determination of $r_i|_N$ and associated parameters is a primary challenge in biogeochemical modeling. There are two general means of formulating $r_i|_N$: ad hoc and reaction-based formulations (Steefel and van Cappellen, 1998). Ad hoc formulations are most often applied to model biogeochemical experiments. In an ad hoc formulation, the production–degradation rate is an empirical function

$$r_i|_N = f_i(C_1, C_2, \ldots, C_M;\ p_1, p_2, \ldots) \tag{2}$$

where f_i is the empirical function for the i-th species and p_1, p_2, are rate parameters used to fit experimental data. The ad-hoc reaction parameters are not 'true' constants; thus, they are only applicable to the experimental conditions tested.

Ad hoc rate formulations [i.e. Eq. (2)] normally do not consider all the chemical reactions influencing a species. Instead, a rate formulation for the most significant (or obvious) reaction is proposed that may lump together the contributions of other reactions. In comparison, reaction-based approaches [see Eq. (3) below] attempt to formulate reaction rates for every significant reaction. When a reaction is not of the elementary type, its reaction rate must be empirically formulated or theoretically based on a reaction mechanism.

Biogeochemical parameters from reaction-based formulations have the potential to be applicable over a broader range of environmental conditions than ad-hoc parameters. In a reaction-based formulation, the rates of change of M chemical species are described by

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = \sum_{k=1}^{N} (\nu_{ik} - \mu_{ik}) R_k, \quad i \in M; \quad \text{or} \quad \mathbf{U}\frac{\mathrm{d}\mathbf{C}}{\mathrm{d}t} = \boldsymbol{\nu}\mathbf{R} \tag{3}$$

where N is the number of biogeochemical reactions, ν_{ik} is the reaction stoichiometry of the i-th species in the k-th reaction associated with the products, μ_{ik} is the reaction stoichiometry of the i-th species in the k-th reaction associated with the reactants, R_k is the rate of the k-th reaction (mol l^{-1} per unit time), $\mathbf{U}$ is a unit matrix, $\mathbf{C}$ is a vector with its components representing M species concentrations, $\boldsymbol{\nu}$ is the reaction stoichiometry matrix, and $\mathbf{R}$ is the reaction rate vector with N reaction rates as its components. Eq. (3) is a statement of mass balance for any species i in a reactive system that states that the rate of change of mass of any species is due to all reactions that produce or consume this species.

An analytical solution of Eq. (3) is generally not possible, numerical integrations are typically applied. Numerical integration of Eq. (3) in its primitive form encounters two major difficulties. First, the N reaction rates range over several orders of magnitude. The time-step size used in numerical integration is dictated by the largest reaction rate among all reactions. If at least one of the reactions has an infinitely large rate (i.e. the reaction can reach equilibrium instantaneously), then the time-step size must be infinitely small, which makes integration impractical. Of course, the infinite rate of an equilibrium reaction is itself an abstraction or simplification. In reality, all of the rates are finite, although they may be quite large. The problem then becomes one of dealing with the stiffness of the reaction matrix, involving rate coefficients varying over many orders of magnitude. Second, for most practical problems the number of independent reactions (N_{I}) is less than the number of species (i.e. the rank of $\boldsymbol{\nu} = N_{\mathrm{I}} < M$). This implies that there are one or more chemical components whose masses must be conserved during the reactions, because the number of chemical components, $N_{\mathrm{C}} = (M - N_{\mathrm{I}})$. Under such circumstances, the integration of M simultaneous ordinary differential equations in Eq. (3) may not guarantee mass conservation of chemical components due to numerical errors. Thus, numerical integration of Eq. (3) is valid only when the following two conditions are

met: (1) all reactions are slow and their rates fall within a narrow range; and (2) the rank of $\boldsymbol{\nu}$ is equal to M. For most problems it is rare that these two conditions can be simultaneously met. Therefore, Eq. (3) must be manipulated to decouple fast from slow reactions, and to enforce component mass conservation. This can be done via the Gauss–Jordian elimination (Chilakapati, 1995; Steefel and MacQuarrie, 1996) or the QR decomposition of $\boldsymbol{\nu}$ (Chen, 1994). The original objective of decomposition was to facilitate numerical integrations. These decompositions are applied in this paper to enable the assessment of system consistency and minimum data requirements.

The application of Eq. (3) for biogeochemical calculations first requires the development of a reaction network (conceptual model) that includes the chemical components and species, and the reactions between them. This would be followed by experiment(s) to measure the concentration of selected chemical components/species as a function of time to parameterize the model. Because of analytical limitations and other experimental difficulties it is generally not possible to measure the concentrations of all species. Therefore, an explicit strategy must be developed to parameterize the model that takes into account the nature of the reactions in the network (i.e. equilibrium or kinetic) and their interdependence. Importantly, the mathematical equations used to describe the chemical reaction network should be decomposed to yield at least one mass conservation equation in which the conserved quantity can be operationally measured. By measuring all the species concentrations or operational quantities in one mass conservation equation, partial system consistency can be assessed. *Partial system consistency* means that at least one of the N_C mass conservation equations can be validated with direct experimental evidence.

2.1. System consistency

Eq. (3) can be decomposed based on the type of biogeochemical reactions. For now we will assume that among N reactions, there are N_E equilibrium (i.e. 'fast') reactions and N_K kinetic (i.e. 'slow') reactions ($N = N_E + N_K$). 'Fast' and 'slow' reaction speeds depend on 'time scales of interest' (Knapp, 1989 — from reviewer no. 1). For our purposes, a reaction is fast if it can instantaneously reach equilibrium (i.e. its rate is infinity). An infinite rate is mathematically represented by a mass action equation. For equilibrium reactions, we need only consider N_E reactions that are linearly independent since any equilibrium reaction that is linearly dependent on another equilibrium reaction is redundant. On the other hand, the rate of a kinetic reaction is finite, and parallel kinetic reactions (kinetic reactions that are linearly dependent on at least one other kinetic reaction) are allowed and must be included (if significant) in any kinetic formulation. Of course, whether a reaction is fast or slow, it depends on the scale of interest (Knapp, 1989). To analyze an experimental system, one must be aware of the scale of interest he or she is engaged in so he or she can correctly consider a reaction 'fast' or 'slow.'

If there are N_I linearly-independent reactions among N biogeochemical reactions, then the rank of the reaction stoichiometry matrix $\boldsymbol{\nu}$ is N_I. N_I must be less than or equal to M based on Eq. (3). Let us denote $N_C = M - N_I$, where N_C represents the number of chemical components, and $N_D = N - N_I$, where N_D represents the number of dependent reactions. Note that N_I linearly independent reactions are comprised of N_E equilibrium reactions (i.e. all N_E reactions are linearly independent) and a subset of N_K kinetic reactions. With these definitions, Eq. (3) is decomposed into the following equation via the Gaussian–Jordan elimination (Chilakapati, 1995; Steefel and MacQuarrie, 1996) or the QR decomposition (Chen, 1994)

$$\mathbf{B}\frac{\mathrm{d}\mathbf{C}}{\mathrm{d}t} = \begin{bmatrix} \mathbf{D} & \mathbf{K} \\ \mathbf{0_1} & \mathbf{0_2} \end{bmatrix}\mathbf{R} \tag{4}$$

where $\mathbf{B}$ is the reduced $\mathbf{U}$ matrix, $\mathbf{D}$ is the diagonal matrix representing a submatrix of the reduced $\boldsymbol{\nu}$ with size of $N_I \times N_I$ reflecting the effects of N_I linearly-independent reactions on the production–consumption rate of all kinetic-variables [a kinetic-variable is a combination of chemical species' concentrations (see the definition in Eq. (6)); the number of kinetic-variables is equal to $(N_I - N_E)$], $\mathbf{K}$ is a submatrix of the reduced $\boldsymbol{\nu}$ with size of $N_I \times N_D$ reflecting the effects of N_D-dependent kinetic reactions, $\mathbf{0}_1$ is a zero matrix representing a submatrix of the reduced $\boldsymbol{\nu}$ with size $N_C \times N_I$, and $\mathbf{0}_2$ is a zero matrix representing a submatrix of the reduced $\boldsymbol{\nu}$ with size $N_C \times N_D$.

The decomposition of Eq. (3) to Eq. (4) effectively reduces a set of M simultaneous ordinary differential equations into three subsets of equations: the first contains N_E non-linear algebraic equations representing mass action laws for the equilibrium reactions, the second contains $(N_I - N_E)$ simultaneous ordinary differential equations representing the rate of change of the kinetic-variables, and the third contains N_C linear algebraic equations representing mass conservation of the chemical components. These equation subsets are defined as

Mass action equations for equilibrium reactions

$$\frac{\mathrm{d}E_i}{\mathrm{d}t} = D_{kk}R_k + \sum_{j \in N_D} D_{ij}R_j;$$
$$i = 1,2,...,N_E;\ k \in N_E: \Rightarrow R_k = \infty \tag{5}$$

Governing equations for kinetic variables

$$\frac{dE_i}{dt} = D_{kk}R_k + \sum_{j \in N_D} D_{ij}R_j;$$
$$i = N_E + 1, N_E + 2, ..., N_I - N_E; \quad (6)$$

$$k \in N_I - N_E \quad \text{where} \quad E_i = \sum_{j=1}^{M} b_{ij}C_j$$

and *Mass conservation equations for N_C chemical components*

$$\frac{dT_i}{dt} = 0; \quad i = 1,2,...,N_C \quad \text{where}$$
$$T_i = \sum_{j=1}^{M} b_{ij}C_j \quad (7)$$

The decomposition of Eq. (3) to Eqs. (5)–(7) enables us to make the following inductions. First, from Eq. (5), any dependent, kinetic reaction that is linearly-dependent on only N_E equilibrium reactions is irrelevant to the system. Second, if all N reactions are linearly independent, then N_D in Eq. (6) is equal to zero. This implies that each kinetic reaction corresponds to a kinetic-variable. Third, from Eq. (7), the mass of each of the N_C chemical components must remain constant. Furthermore, the decomposition of Eq. (3) – Eqs. (5)–(7) is not unique. In the most general case, there will be M_d number of possible decompositions, where $M_d = M!/[(M - N_I)!N_I!]$. These inductions allow assessment of system consistency and data needs for biogeochemical kinetic experiments.

In the most rigorous case, all N_C mass conservation equations [Eq. (7)] must be validated with experimental data to demonstrate *complete system consistency*. If any of the N_C mass conservation equations are violated, then the system is not consistent. Inconsistency implies that either the number of species identified in the system is too many, or the number of linearly independent reactions is too few (recall $N_C = M - N_I$). In either case, the reaction network yields too many mass-conservation relationships that cannot be satisfied, and the reaction network would have to be revised. However, as noted above it is difficult, in practice, to measure all the species in all the mass conservation equations. Instead, one's strategy should be to establish at least partial system consistency by measuring all the species concentrations or operational quantities in one mass conservation equation. As will be shown in our example below, operationally defined chemical quantities (vs. discrete chemical species) can also be used to assess system consistency if the sum of the terms in the operational quantity appears directly in the mass conservation equation.

One major point of this paper is that system consistency must be validated with direct experimental evidence before reaction-based rates are formulated. When the system is assessed to be consistent, Eq. (5) can be used to determine if the assumptions of the equilibrium reactions are valid by checking if any of the N_E mass action equations are violated. If a mass action equation is not satisfied, then the corresponding reaction is not at equilibrium and should be treated as a kinetic reaction. When all assumed fast reactions are shown to be at equilibrium, then (finally) one can analyze the N_K kinetic reactions and formulate reaction-based rates.

2.2. Minimum data needs

An important issue is the minimum number of species that must be measured to adequately analyze the experimental data from a reaction-based standpoint. In the strictest sense where each measured quantity is a discrete independent chemical species concentration, our analysis indicates that $(N_I - N_E)$ chemical species must be measured if the number of chemical species is M, the number of equilibrium reactions is N_E, the constant for each equilibrium reaction is known, all N_C component equations are assessed to be consistent, and the number of linearly independent reactions is N_I. If $(N_I - N_E)$ species are measured then the kinetic suite of reactions can be evaluated. However, for a partial assessment of system consistency, at least one more species must be measured [i.e. $(N_I - N_E + 1)$]. For a complete assessment of system consistency, as many as $(N_I - N_E + N_C)$ species would have to be measured.

Given the measurement of the concentrations of $(N_I - N_E + 1)$ species, the concentrations of the remaining species can be obtained from $(N_C - 1)$ mass conservation equations and N_E mass action equations. However, in certain cases interdependent or operationally defined, 'lumped' chemical quantities can still satisfy our data needs. Lumped quantities will 'count' as one of the required species if the sum appears directly in either a kinetic-variable or mass conservation equation. Therefore, it may be erroneous to cite a firm number such as $(N_I - N_E + 1)$ as the minimum number of species that must be measured. Instead, experiments should be designed so that all the species or operational quantities can be measured in (1) at least one mass conservation equation, and (2) the most important kinetic-variable equation (i.e. central process under investigation).

2.3. Reaction rate formulations

A general, biogeochemical reaction can be written as

$$\sum_{i=1}^{M} \mu_{ik} G_i \Leftrightarrow \sum_{i=1}^{M} \upsilon_{ik} G_i, \quad k \in N \tag{8}$$

where G_i is the chemical formula of the i-th species involved in k reactions. The key in modeling biogeochemical experiments using a reaction-based approach is the formulation of reaction rates for all N reactions specified by Eq. (8). For an equilibrium reaction, the reaction rate is infinity resulting in the law of mass action as

$$R_k = \infty \Rightarrow K_k^e = \frac{\left(\prod_{i=1}^{M} (A_i)^{\nu_{ik}} \right)}{\left(\prod_{i=1}^{M} (A_i)^{\mu_{ik}} \right)}, \quad k \in N_E \tag{9}$$

where K_k^e is the equilibrium constant of the k-th reaction and A_i is the activity of the i-th species. The activity of a species is related to its concentration via an activity coefficient. Any reaction that is linearly dependent on N_{E} reactions is redundant because its mass action equation can be obtained from Eq. (9). The equilibrium constants may be determined separately from the parameterization of kinetic reactions. The determination of such constants is not our main concern here.

For an elementary kinetic reaction, the rate equation based on collision theory (Smith, 1981; Atkins, 1986) may be represented

$$R_k = \left(K_k^f \prod_{i=1}^{M} (A_i)^{\mu_{ik}} - K_k^b \prod_{i=1}^{M} (A_i)^{\nu_{ik}} \right), \quad k \in N_K \tag{10}$$

where R_k is the reaction rate, K_k^f is the forward rate constant, and K_k^b is the backward rate constant of the k-th kinetic reaction. For a kinetic system, the forward and backward rate constants cannot be determined individually by measurement of all concentration vs. time profiles because the N_K equations in Eq. (10) are coupled. Our analysis indicates that the concentration vs. time profiles must be measured for $(N_{\mathrm{I}} - N_{\mathrm{E}})$ chemical species (i.e. the number of linearly independent kinetic reactions) to parameterize the kinetic suite of the reaction network. Additional concentration vs. time profiles can be used to check system consistency and assumptions regarding equilibrium reactions.

When a kinetic reaction is not elementary, its rate may be formulated based on empirical or mechanistic approaches. In an empirical approach, an nth-order rate equation is postulated and reaction parameters including rate constants are determined from the experimental analysis of concentration vs. time profiles. In a mechanistic-based approach, rate equations are derived from proposed reaction pathways. A mechanism consists of a sequence of elementary steps that describes how the final products are formed from the initial reactants and determines the overall reaction. An elementary step is a reaction that can be described by the forward and backward rates with the order of the rate given by the stoichiometry of the reaction. An advantage of a mechanistic-based approach is that the reaction parameters may be applicable to environmental conditions other than the experiments, but in reality the determination of the mechanism is difficult.

Mechanistic-based approaches may involve the direct simulation of the proposed mechanism or the indirect simulation of the overall rate process. In the direct simulation, all elementary reactions in the mechanism are included. The advantage is that either mass action equations for equilibrium reactions or only one type of rate law for kinetic reactions needs to be considered, while the disadvantage is that all intermediate species have to be included. In the indirect simulation, an overall rate equation for the proposed mechanism must be derived. The advantage of the indirect simulation is that all intermediate species can be eliminated, while the disadvantage is that one needs to derive a reaction rate for every reaction.

3. Demonstrative example

For our example we will consider the bioreduction of ferric oxide (Fe_2O_3) by dissimilatory iron reducing bacteria (DIRB). Experimental variables can include the DIRB culture, the iron oxide and its characteristics, the electron donor, the carbon source, and constituents of the growth media including buffer and presence/absence of nutrients. Depending on the experimental conditions an extremely complex system composed of multiple species (e.g. $M > 40$) and reactions (e.g. $N > 30$) may have to be evaluated. For this paper we will consider only the most simple experimental conditions and reaction network without the loss of generality in our analysis. We will also discuss how experiments should be designed to collect sufficient data to model with a reaction-based approach.

Under anaerobic conditions where ferric iron is the predominant electron acceptor, DIRB reduce both crystalline (Roden and Zachara, 1996) and non-crystalline (Lovley and Phillips, 1986) ferric oxides producing Fe(II). The secondary reactions of ferrous iron may include aqueous complexation (if chelators present) (Roden et al., 1999; Zachara et al., 1999), surface complexation to the residual ferric oxide, precipitation

of ferrous minerals (e.g. $FeCO_3$) (Fredrickson et al., 1998), biosorption to DIRB cells (Urrutia et al., 1998), and re-oxidation. Additionally, chemical species may participate in acid–base reactions (e.g. buffers) and mineral conversions. Thus, the overall process of biologically-mediated iron reduction is complex, and many issues are still poorly understood.

The goals of our experiment would be to collect enough kinetic data to (1) elucidate the reaction mechanism of DIRB-mediated Fe(II) production coupled to H_2 oxidation, and (2) determine all parameters associated with the corresponding rate equations. To accomplish this goal we would use 'model' systems in which characterized materials and controlled conditions are used. For example, by using chemically unreactive electrolytes, buffer and media, excluding a carbon source, and conducting the experiments under non-growth conditions, the system could be simplified to the smallest chemical species and reaction network. Non-growth conditions would avoid complications resulting from cell division. This approach will significantly reduce the minimum number of species concentrations that must be measured, and make modeling a more tractable activity. While this simple model system is an abstraction of the environment, complexity could be added for further hypothesis testing.

3.1. Reaction network

We will consider an experiment containing crystalline hematite (α-Fe_2O_3), H_2 provided as the sole electron donor, no carbon source, and a DIRB pure culture. A non-growth supporting buffered media [e.g. 1,4-piperazinediethanesulfonic acid (HPIPES)] with no phosphate, trace metals, vitamins or other nutrients would be used to maintain pH and prevent growth. Under these conditions the DIRB-mediated bioreduction of ferric oxide may occur as

$$Fe_2O_3 + H_2(aq) + 4H^+ \rightleftharpoons 2Fe^{2+} + 3H_2O \text{ slow} \quad \text{(R1)}$$

Because DIRB-mediated bioreduction is generally slow (e.g. Zachara et al., 1998), Reaction (R1) is considered a kinetic reaction.

A significant challenge in modeling the bioreduction of ferric oxides is that while bacteria require direct contact to the oxide surface (Arnold et al., 1988; Lovley and Phillips, 1988), the reductive dissolution of the oxide will alter the available surface area, and sites for bacterial attachment and Fe^{2+} sorption. Thus, a distinction between the bulk and surface phases of the ferric oxide must be made. The formation of surface sites (for the 001 face of hematite) can be represented by the following hydration reaction

$$Fe_2O_3 + 3H_2O \rightleftharpoons 2[(OH)_2 = FeOH] \text{ (slow or fast)} \quad \text{(R2)}$$

While surface ferric atoms are coordinated to three surface hydroxyls (Fig. 1), a more common convention is to simply use =FeOH to designate surface hydroxyl sites. Reaction (R2) can be treated as a kinetic or equilibrium reaction depending on how fast the hydration reaction occurs. For now this will be considered a kinetic reaction. Ferric oxide surface ionization reactions are commonly described with a diprotic acid representation known as the 2-pKa model (Dzombak and Morel, 1990):

$$= FeOH_2^+ \rightleftharpoons = FeOH + H^+ \text{(fast)} \quad \text{(R3)}$$

$$= FeOH \rightleftharpoons FeO^- + H^+ \text{(fast)} \quad \text{(R4)}$$

Reactions (R3) and (R4) will be considered equilibrium reactions.

The adsorption–desorption of Fe^{2+} to the ferric oxide surface species may occur as

$$= FeOH_2^+ + Fe^{2+} \rightleftharpoons = FeOFe^+ + 2H^+ \text{ (slow)} \quad \text{(R5)}$$

$$= FeOH + Fe^{2+} \rightleftharpoons = FeOFe^+ + H^+ \text{ (slow)} \quad \text{(R6)}$$

$$= FeO^- + Fe^{2+} \rightleftharpoons = FeOFe^+ \text{ (slow)} \quad \text{(R7)}$$

Reactions (R5) to (R7) represent the surface complexation of Fe^{2+} to the various ferric oxide surface species. If all three Reactions (R5) to (R7) were equilibrium reactions, then any one of these three reactions [e.g. (R5)] along with Reactions (R3) and (R4) could form a set of three linearly independent equilibrium reactions and the remaining two reactions [(R6) and (R7) in this case] would be redundant [in fact, if we had presumed all five reactions, i.e., Reactions R3 through R7, were equilibrium reactions right away, then any three of the five reactions could have been taken as the basis and the remaining two reactions could have been considered redundant]. If any two of Reactions (R5) to (R7) were equilibrium reactions [e.g. (R5) and (R6)], then one of these reactions [e.g. (R5)] along with Reactions (R3) and (R4) would form three linearly independent equilibrium reactions [in fact, if we had presumed that Reactions R3, R4, R5 and R6 were equilibrium reactions in the first place, then any three of these four reactions could have been considered linearly independent equilibrium reactions]. The other equilibrium reaction [(R6) in this case] would be redundant and the lone kinetic reaction [(R7) in this case] would be irrelevant to the system [in fact, any of the four equilibrium reactions can be considered redundant]. If just one of

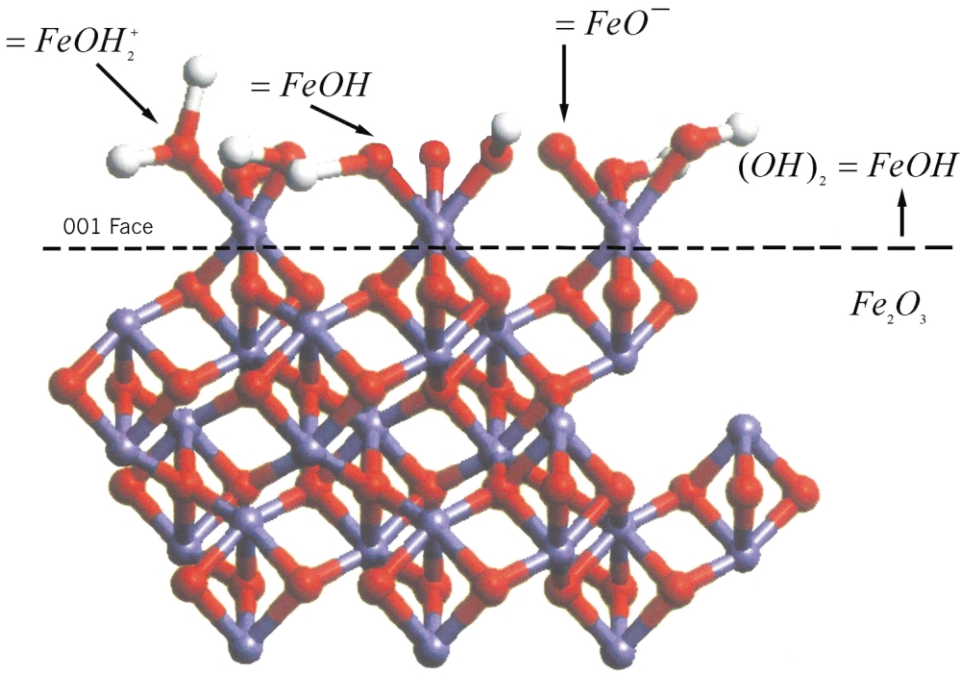

Fig. 1. Molecular model of the 001 face of hematite to differentiate between ferric surface species (=$FeOH_2^+$, =FeOH, and =FeO^-), and the bulk ferric oxide species (Fe_2O_3). The model was created with the Visualizer module of Cerius2 (Molecular Simulations Inc.).

Reactions (R5) to (R7) were equilibrium reactions [e.g. (R5)], then the other two kinetic reactions, being linearly dependent on the three equilibrium Reactions (R3) to (R5), would be irrelevant to the system (see Eqs. (12.1), (12.2) and (13.3*) in Section 3.2). Finally, if all three Reactions (R5) to (R7) are slow, they constitute parallel kinetic reactions that all contribute to the production of $=FeOFe^+$ and they must all be considered. For the time being, all three reactions are considered kinetic reactions.

Although adsorption–desorption of metals to bacterial surfaces can be modeled as a series of surface complexation reactions to different functional groups such as carboxylate, phosphate and phenolate (Fein et al., 1997), we will treat it as a single surface reaction without loss of generality in terms of modeling

$$\mathrm{DIRB} + \mathrm{Fe}^{2+} \rightleftharpoons \mathrm{DIRB\text{--}FE}^{2+}\,(\mathrm{slow}) \qquad \text{(R8)}$$

Reaction (R8) represents the biosorption of ferrous iron to metal binding sites on the DIRB cell surfaces. The buffering of HPIPES would occur as

$$\mathrm{HPIPES} \rightleftharpoons \mathrm{PIPES}^- + \mathrm{H}^+\,(\mathrm{fast}) \qquad \text{(R9)}$$

where HPIPES and $PIPES^-$ are the protonated and deprotonated forms of the buffer, respectively. Finally, the dissolution of $H_2(g)$ from the experimental reactor headspace into solution would occur as

$$\mathrm{H_2(aq)} \rightleftharpoons \mathrm{H_2(g)(fast)} \qquad \text{(R10)}$$

For this example, reaction (R10) will be considered an equilibrium reaction.

3.2. Equation matrix decompositions

The reaction network described by Reactions (R1) though (R10) includes 10 reactions ($N = 10$) and 14 species (Fe_2O_3, $=FeOH_2^+$, =FeOH, $=FeO^-$, $H_2(aq)$, $H_2(g)$, H^+, H_2O, Fe^{2+}, $=FeOFe^+$, DIRB, DIRB--Fe^{2+}, HPIPES, $PIPES^-$). However, the activity of H_2O will be assumed equal to 1.0 and its concentration approximately equal to 55 moles l^{-1}. Thus, there are 13 species ($M = 13$) and 13 equations are needed to calculate their concentrations. According to Eq. (3), these 13 equations can be written as

$$\frac{\mathrm{d}[=\mathrm{FeOH}]}{\mathrm{d}t} = 2R_2 + R_3 - R_4 - R_6, \qquad \text{(11.1)}$$

$$\frac{\mathrm{d}[=\mathrm{FeOH_2^+}]}{\mathrm{d}t} = -R_3 - R_5, \qquad \text{(11.2)}$$

$$\frac{d[=FeO^-]}{dt} = R_4 - R_7, \tag{11.3}$$

$$\frac{d[DIRB]}{dt} = -R_8, \tag{11.4}$$

$$\frac{d[DIRB\text{--}Fe^{2+}]}{dt} = R_8 \tag{11.5}$$

$$\frac{d[HPIPES^+]}{dt} = -R_9 \tag{11.6}$$

$$\frac{d[PIPES]}{dt} = R_9 \tag{11.7}$$

$$\frac{d[Fe_2O_3]}{dt} = -R_1 - R_2, \tag{11.8}$$

$$\frac{d[Fe^{2+}]}{dt} = 2R_1 - R_5 - R_6 - R_7 - R_8, \tag{11.9}$$

$$\frac{d[=FeOFe^+]}{dt} = R_5 + R_6 + R_7, \tag{11.10}$$

$$\frac{d[H_2(aq)]}{dt} = -R_1 - R_{10}, \tag{11.11}$$

$$\frac{d[H^+]}{dt} = -4R_1 + R_3 + R_4 + 2R_5 + R_6 + R_9 \tag{11.13}$$

The straightforward formulation of the above 13 equations for the evolution of 13 chemical species as widely done in the current literature on reaction-based kinetic modeling suffers several difficulties. First, in Eq. (11.1) both R_3 and R_4 are infinite, so how does one define infinity minus infinity? Second, R_1 or R_2 does not appear alone in any of the above 13 equations, therefore, one cannot determine the reaction rate R_1 or R_2 by simply plotting the concentration of one species vs. time. Third, mass conservation of chemical components is not explicitly stated, therefore, the assessment of even partial system consistency cannot be verified. To overcome these difficulties, a diagonalization decomposition of the reaction matrix (Chilakapati et al., 1998) should be performed so that each equation would not contain more than one linearly independent reaction and mass conservation of chemical components is explicitly stated.

A matrix analysis of this 10 reaction–13 species system would yield 8 linearly independent reactions ($N_I = 8$). Therefore, there must be five chemical components ($N_C = M - N_I = 13 - 8$) described by five mass conservation equations. Since we have assumed that Reactions (R3), (R4), (R9) and (R10) are equilibrium reactions, then $N_E = 4$. These four equilibrium reactions will result in four mass action equations. Since all equilibrium reactions are linearly independent, four kinetic reactions ($N_I - N_E = 8 - 4$) must also be linearly independent.

Using this assumption regarding N_E, Eq.11 can be decomposed to the form of Eqs. (5)–(7) in many ways. The first decomposition shown below was chosen because (1) all the species or operational quantities in at least one mass conservation equation can be measured [Eq. (14.2)], and (2) all the species or operational quantities in at least one kinetic-variable equation can be measured [Eq. (13.1)]. For this experiment, evaluation of reaction rate R_1 [represented by Eq. (13.1)] is our objective as it represents the overall reduction rate. These considerations are important because the first will allow assessment of partial system consistency, and the second will allow reaction rate formulation based on direct experimental evidence. The equation decomposition is as follows

N_E mass action equations

$$\frac{d[=FeO^-]}{dt} = R_4 - R_7:$$

$$R_4 = \infty \Rightarrow [=FeOH] = \frac{1}{K_4^e}[=FeO^-][H^+] \tag{12.1}$$

$$\frac{d([=FeOH_2^+] + [=FeOFe^+])}{dt} = -R_3 + R_6 + R_7:$$

$$R_3 = \infty \Rightarrow [=FeOH_2^+] = \frac{1}{K_3^e}[=FeOH][H^+] = \frac{1}{K_3^e}\frac{1}{K_4^e}[=FeO^-][H^+]^2 \tag{12.2}$$

$$\frac{d[HPIPES]}{dt} = -R_9:\ R_9 = \infty \Rightarrow [HPIPES] = \frac{1}{K_9^e}[PIPES^-][H^+] \tag{12.3}$$

$$\frac{d[H_2(g)]}{dt} = R_{10}:\ R_{10} = \infty \Rightarrow [H_2(g)] = K_{10}^e[H_2(aq)] \tag{12.4}$$

$(N_I - N_E)$ kinetic variable equations

$$\frac{1}{2}\frac{d([Fe^{2+}] + [=FeOFe^+] + [DIRB\text{--}Fe^{2+}])}{dt} = R_1 \tag{13.1}$$

$$\frac{1}{2}\frac{\mathrm{d}([{=}\mathrm{FeOH}_2^+]+[{=}\mathrm{FeOH}]+[{=}\mathrm{FeO}^-]+[{=}\mathrm{FeOFe}^+])}{\mathrm{d}t}=R_2 \tag{13.2}$$

$$\frac{\mathrm{d}[{=}\mathrm{FeOFe}^+]}{\mathrm{d}t}=R_5+R_6+R_7 \tag{13.3}$$

$$\frac{\mathrm{d}[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}]}{\mathrm{d}t}=R_8 \tag{13.4}$$

N_C mass conservation equations

$$\begin{aligned}\mathrm{TOT}_{\mathrm{Fe_2O_3}} &= [\mathrm{Fe_2O_3}]+\tfrac{1}{2}[{=}\mathrm{FeOH}_2^+]+\tfrac{1}{2}[{=}\mathrm{FeOH}]+\tfrac{1}{2}[{=}\mathrm{FeO}^-]\\ &\quad+[{=}\mathrm{FeOFe}^+]+\tfrac{1}{2}[\mathrm{Fe}^{2+}]\\ &\quad+\tfrac{1}{2}[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}]\end{aligned} \tag{14.1}$$

$$\begin{aligned}\mathrm{TOT}_{\mathrm{H_2}} &= [\mathrm{H_2(aq)}]+[\mathrm{H_2(g)}]+\tfrac{1}{2}[\mathrm{Fe}^{2+}]\\ &\quad+\tfrac{1}{2}[{=}\mathrm{FeOFe}^+]+\tfrac{1}{2}[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}]\end{aligned} \tag{14.2}$$

$$\begin{aligned}\mathrm{TOT}_{\mathrm{H^+}} &= [\mathrm{H}^+]+[{=}\mathrm{FeOH}_2^+]-[{=}\mathrm{FeO}^-]\\ &\quad+[{=}\mathrm{FeOFe}^+]+[\mathrm{HPIPES}]+2[\mathrm{Fe}^+]\\ &\quad+2[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}]\end{aligned} \tag{14.3}$$

$$\mathrm{TOT}_{\mathrm{DIRB}}=[\mathrm{DIRB}]+[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}] \tag{14.4}$$

$$\mathrm{TOT}_{\mathrm{PIPES}}=[\mathrm{PIPES}]+[\mathrm{HPIPES}] \tag{14.5}$$

where the symbol TOT means the total analytical concentration and the subscript associated with TOT denotes the corresponding component.

Before we describe a second decomposition for alternatively assessing system consistency, we note that if Reaction (R5) is fast (i.e. $R_5=\infty$), then Eq. (13.3) is replaced by the mass action equation for Reaction (R5) as

$$\frac{\mathrm{d}[{=}\mathrm{FeOFe}^+]}{\mathrm{d}t}=R_5+R_6+R_7:$$

$$\begin{aligned}R_5=\infty \Rightarrow [{=}\mathrm{FeOFe}^+] &= K_5^e\frac{[{=}\mathrm{FeOH}_2^+][\mathrm{Fe}^{2+}]}{[\mathrm{H}^+]^2}\\ &= \frac{K_5^e}{K_3^eK_4^e}[{=}\mathrm{FeO}^-]\end{aligned} \tag{13.3*}$$

As a result, the reaction rates R_6 and R_7 would be absent from the governing equations [Eqs. (12.1), (12.2) and (13.3*)]. Hence Reactions ($R6$) and ($R7$) would be irrelevant to the system as stated earlier. In fact, any kinetic reaction that is linearly dependent on only equilibrium reactions is irrelevant to the system because the replacement of kinetic-variable equations with mass action equations will make the reaction rate of this kinetic reaction vanish from the governing equations. Since for our example, we assume that Reactions ($R5$)–($R7$) are kinetic, they form parallel reactions that contribute to the production of $={\rm FeOFe}^+$. The problem is that parallel reactions cannot be uniquely segregated when all contribute to the production of the same species. Therefore, one may wish to design experiments such that only one reaction is contributing to the production of $={\rm FeOFe}^+$ in order to segregate these three reactions for their parameterization. After they are parameterized (via separate experiments), they must be included for simulations when all three reactions are comparably dominant. However, for this example, evaluation of Reactions ($R5$)–($R7$) is not an experimental objective.

The decomposition shown in Eq. (12.1) to Eq. (14.5) is not unique, and others may provide additional insights and testing power to evaluate system consistency. For example, the second decomposition shown below was chosen because kinetic-variable Eq. (13.1) and Eq. (13.1*) result from the same reaction, and all the species in Eq. (13.1*) can be directly measured. Different decompositions that yield kinetic-variable equations from the same reactions must be equivalent to one another. In other words, $\{1/2([\mathrm{Fe}^{2+}]+[{=}\mathrm{FeOFe}^+]+[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}])\}$ and $\{-([\mathrm{H_2(aq)}]+[\mathrm{H_2(g)}])\}$ are equivalent kinetic variables because both result from Reaction ($R1$). The direct comparison of kinetic variable vs. time profiles [i.e. Eq. (13.1) vs. Eq. (13.1*)] from different decompositions of Eq.11 will provide an alternative assessment of system consistency. A second assessment of system consistency would not be provided because both assessments are based on the same experimental measurements. The second decomposition of Eq.11 would result in an identical set of governing equations to that from the first decomposition except for the following four equations

$$-\frac{\mathrm{d}([\mathrm{H_2(aq)}]+[\mathrm{H_2(g)}])}{\mathrm{d}t}=R_1 \tag{13.1*}$$

$$\begin{aligned}\mathrm{TOT}_{\mathrm{Fe^{2+}}} &= [\mathrm{Fe}^{2+}]+[{=}\mathrm{FeOFe}^+]\\ &\quad+[\mathrm{DIRB}\text{--}\mathrm{Fe}^{2+}]+2[\mathrm{H_2(aq)}]\\ &\quad+2[\mathrm{H_2(g)}]\end{aligned} \tag{14.1*}$$

$$\begin{aligned}\mathrm{TOT}_{\mathrm{Fe_2O_3}} &= [\mathrm{Fe_2O_3}]+\tfrac{1}{2}[{=}\mathrm{FeOH}_2^+]+\tfrac{1}{2}[{=}\mathrm{FeOH}^+]\\ &\quad+\tfrac{1}{2}[{=}\mathrm{FeO}^-]+\tfrac{1}{2}[{=}\mathrm{FeOFe}^+]\\ &\quad-[\mathrm{H_2(aq)}]-[\mathrm{H_2(g)}]\end{aligned} \tag{14.2*}$$

$$\mathrm{TOT}_{\mathrm{H}^+} = [\mathrm{H}^+] + [\mathrm{HPIPES}] + [=\mathrm{FeOH}_2^+] - [=\mathrm{FeO}^-] - [=\mathrm{FeOFe}^+] - 4[\mathrm{H}_2(\mathrm{aq})] - 4[\mathrm{H}_2(\mathrm{g})] \quad (14.3^*)$$

where the (Eq. numbers)* correspond to those from the first decomposition. All the species or operational quantities in mass conservation Eq. (14.1*) can be measured. However, Eqs. (14.1*) and (14.2) are equivalent and no additional assessment of system consistency is provided by Eq. (14.1*). The mass conservation equations from the first diagonalization [Eq. (14.1) to Eq. (14.5)] should be familiar to geochemists, while some of the mass conservation equations from the second diagonalization [e.g. Eq. (14.1*) to Eq. (14.3*)] may not be as intuitive. From a modeling perspective, however, either set of mass conservation equations is valid. How, then, does one obtain a decomposition among many, which contains intuitively obvious or recognizable quantities? This is a problem of specific computer applications. It is beyond the scope of this paper, and the problem will be addressed in another manuscript in which a set of rules is used as a guideline (Fang and Yeh, 2000). The rule is obtained based on the following observations in the column reduction: (1) when a row is chosen as pivoting, its corresponding species is a product species; (2) the species corresponding to the row that has not been chosen as pivoting is a component species so that one can exert control of components based on his or her understanding of the problem; (3) the species involved most frequently in reactions is preferably chosen as a component; (4) all columns representing equilibrium reactions in the reaction matrix should be reduced first and a subset of linearly independent equilibrium reactions is used as the basis; and (5) a linearly dependent reaction will appear only in the rows that contain linearly independent reactions (each row has one) that this reaction depends on after the completion of decomposition.

3.3. Experimental design considerations — minimum data needs

The goal of a kinetic experiment should be to measure a suitable analyte suite to unambiguously define reaction progress and to test the scientific hypothesis being evaluated. If more species than the minimum number can be measured, additional assessments of system consistency and equilibrium assumptions may be made. Also, if the number of species identified or the reactions hypothesized are incomplete, the original estimate of the minimum number of species may be too low. If one cannot measure at least the minimum number of species then no reaction-based information can be obtained from the experiment.

Our conceptual model [Reactions (R1)–(R10)] is one of the simplest reaction networks for dissimilatory iron oxide reduction, yet additional factors must be considered to maintain this simplicity. Although we have selected a buffered media (i.e. HPIPES) we will assume HPIPES and PIPES^- are chemically unreactive with ferric oxide and DIRB surfaces. If a chemically unreactive electrolyte were not used, then sorption reactions of the background ions would have to be included in the reaction network if their sorption affected the mass distribution/conservation of any of the chemical components. However, conditional constants could be used specific to the electrolyte that 'lump' these interactions. If CO_2 were not excluded from our experiment (and a C-source), carbonate species and FeCO_3(s) would have to be included, along with all reactions these species participate in. If phosphate were present, phosphate species and $\mathrm{Fe}_2\mathrm{PO}_3 \cdot 8\mathrm{H}_2\mathrm{O}$(s) would have to be included. The addition of a new chemical component will involve several more reactions and always increase the minimum number of species concentrations that must be measured. The allowable complexity of the system must be constrained by our ability to measure the minimum number of species.

For as many equilibrium reactions as possible, preliminary experiments would be conducted to measure the corresponding equilibrium constants. For example, acid–base titrations with the ferric oxide could be performed to provide data for the estimation of K_3^e and K_4^e [for Reactions (R3) and (R4), respectively]. Acid–base titrations could also be performed with HPIPES solutions to directly measure K_9^e, although the literature would be relied upon for relevant constants that are not system specific.

To evaluate the proposed kinetic reactions and establish partial system consistency, 5 $(= N_\mathrm{I} - N_\mathrm{E} + 1)$ species or operational quantities must be measured. One suitable approach would be to use a single large-volume well-mixed reactor (at controlled temperature) equipped with a pH-stat to maintain pH (e.g. pH 6.8). Because pH is truly a master variable in this system, maintaining it as a constant will simplify subsequent data interpretation. The bioreduction of hematite [Reaction (R1)] consumes $[\mathrm{H}^+]$ and increases pH, while the sorption of biogenic Fe^{2+} [e.g. Reactions (R5)–(R7)] produces $[\mathrm{H}^+]$ and decreases pH. The experimental variable would be $\Delta[\mathrm{H}^+]/\Delta[\mathrm{OH}^-]$ added vs. time, which is equivalent to measuring $[\mathrm{H}^+]$. At discrete time intervals the headspace gas would be analyzed for H_2(g), and suspension samples would be removed for analyses. The frequency of sampling will be controlled by a variety of factors but the majority of data should be collected during the initial 'fast' stage of the experiment. For non-growth conditions, short-term experiments (e.g. days) would minimize any effects of cell death and lysis.

$[H_2(aq)]$ would be calculated by Eq. (12.4) based on the measurement of $[H_2(g)]$ and the known Henry's constant (i.e. K^e_{10}) for H_2. Suspension samples would be used to measure $[Fe^{2+}]$, acid extractable ferrous iron ($HCl_{Fe^{2+}} = [Fe^{2+}] + [= FeOFe^+] + [DIRB\text{--}Fe^+]$), residual ferric iron ($HCl_{Fe^{III}} = 2\ [Fe_2O_3] + [= FeOFe^+] + [= FeOH_2^+] + [= FeOH] + [= FeO^-]$), and the surface area (S_A) of the residual ferric iron. Dissolved $[Fe^{2+}]$ would be measured by filtering the sample (e.g. 0.22 μm) and analyzing by Ferrozine. $HCl_{Fe^{2+}}$ would be measured by adding HCl to the suspension to yield a 0.5 N HCl concentration. The acidified suspension would be mixed overnight, filtered and analyzed by Ferrozine (Zachara et al., 1999). The residual ferric iron remaining after the 0.5 N HCl extraction would be split to measure $HCl_{Fe^{III}}$ and the remaining S_A. $HCl_{Fe^{III}}$ would be measured by complete reductive dissolution [e.g. dithionite-citrate procedure (Rueda et al., 1992)] or by strong acid dissolution (e.g. 6 N HCl) with subsequent analysis of Fe(II) or Fe(III). The S_A of the residual ferric iron would be measured by 5-point BET N_2 adsorption (after 0.5 N HCl extraction).

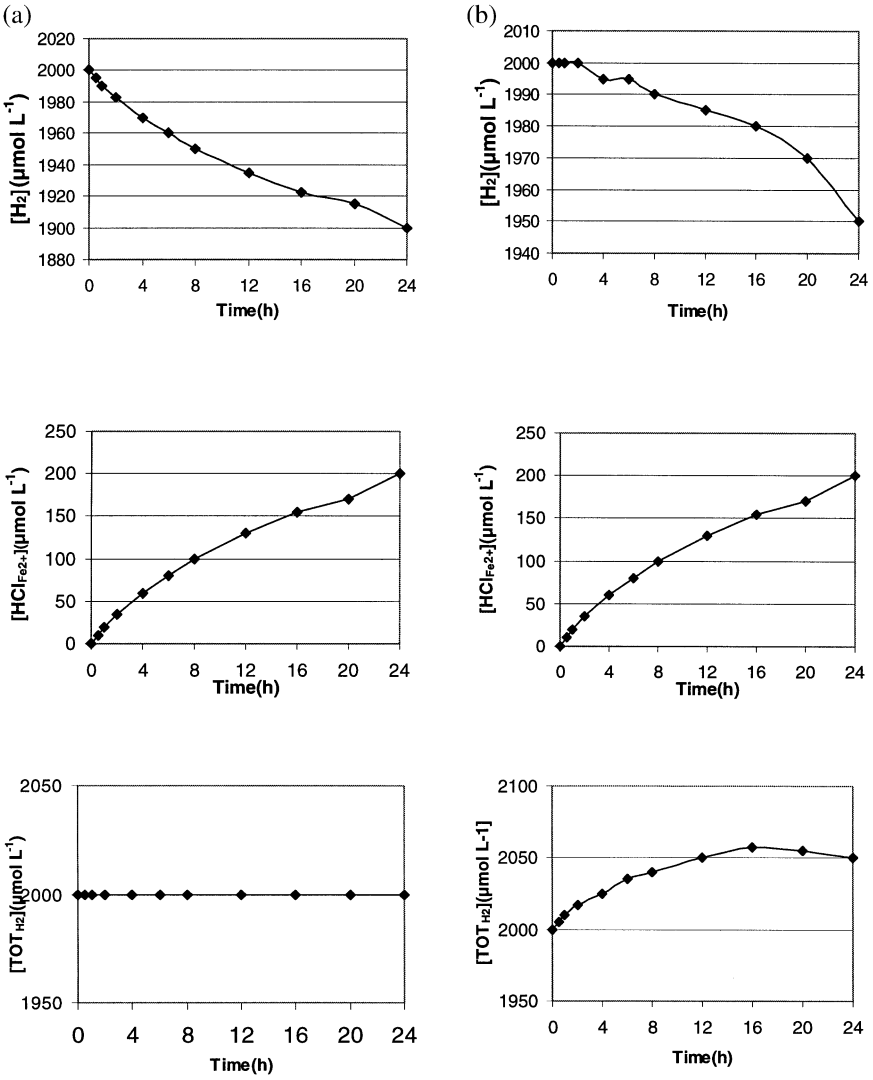

Fig. 2. Hypothetical data for a DIRB-mediated ferric oxide bioreduction kinetic experiment. The two top panels in column (A) show the consumption of the electron donor ($[H_2(aq)]$ calculated based on $[H_2(g)]$ measured in reactor headspace and known Henry's constant (i.e. K^e_{10}) for H_2), and the corresponding production of the reduced form of the electron acceptor ($HCl_{Fe^{2+}}$ measured by acid extraction of reactor suspension). The bottom panel in column (A) shows the calculated values of the chemical component concentration (TOT_{H_2}, Eq. (14.2)) based on these two measurements. Thus, the experimental data in column (A) would demonstrate partial system consistency. Conversely, the experimental data in column (B) would demonstrate an inconsistent system, and a revised reaction network would need to be proposed and tested.

Five of these measurements ($\Delta[H^+]/\Delta[OH^-]$, $[H_2(g)]$, $[Fe^{2+}]$, $HCl_{Fe^{2+}}$, and $HCl_{Fe^{III}}$) will be needed to evaluate kinetic Reactions (R1) and (R2), and assess partial system consistency. The measurement of S_A will be required to formulate a reaction rate for Reaction (R2).

3.4. Assessment of system consistency

System consistency is determined by evaluating whether the mass conservation equations [Eq.14] are satisfied. In other words, a plot of the total concentration of a component vs. time must remain constant. For example, to assess if Eq. (14.2) is satisfied we plot the concentrations of $([H_2(aq)] + [H_2(g)] + 1/2\ [Fe^{2+}] + 1/2\ [= FeOFe^+] + 1/2\ [DIRB\text{--}Fe^{2+}])$ vs. time and determine if the profile remains constant. This assessment of partial system consistency is possible because we would measure $[H_2(g)]$ and $HCl_{Fe^{2+}}$. If the profile remains constant, partial system consistency has been assessed (e.g. Fig. 2A). As discussed above, another means to assess system consistency is to compare kinetic-variable vs. time profiles from different decompositions of Eq.11 that result from the same reactions. For the two decompositions shown, these yield the equivalent kinetic-variable Eqs. (13.1) and (13.1*). If we plot $1/2([Fe^{2+}] + [= FeOFe^+] + [DIRB\text{--}Fe^{2+}])$ vs. time and $(-[H_2(aq)] - [H_2(g)])$ vs. time for Eqs. (13.1) and (13.1*), respectively, the slopes of these two profiles (Fig. 3) must be the same if the system is consistent. This alternative assessment of partial system consistency is also possible because we would measure $[H_2(g)]$ and $HCl_{Fe^{2+}}$. If the system is shown to be inconsistent for any case (e.g. Figs. 2b and 3b), a revised reaction network must be formulated and tested.

The reactions that are assumed to reach equilibrium should also be tested with respect to these assumptions. As noted in Section 3.3, the best approach would be to measure all equilibrium constants in preliminary experiments or rely upon published values based on thermodynamic data. After one is satisfied with the system consistency and assumptions regarding equilibrium, the experimentally measured kinetic data can be used to study kinetic reactions and to optimally obtain reaction constants of proposed or derived rate laws using a numerical biogeochemical model (e.g. Yeh et al., 1994; Salvage and Yeh, 1998).

For this example, system consistency can be assessed with only two species or operational quantities, yet we stated that five must be measured to use a reaction-based model. What value and use are the remaining three measurements ($\Delta[H^+]/\Delta[OH^-]$, $[Fe^{2+}]$, and $HCl_{Fe^{III}}$) that we proposed? As stated earlier, $(N_I - N_E)$ species concentrations or operational quantities must be measured to evaluate and simulate the kinetic suite

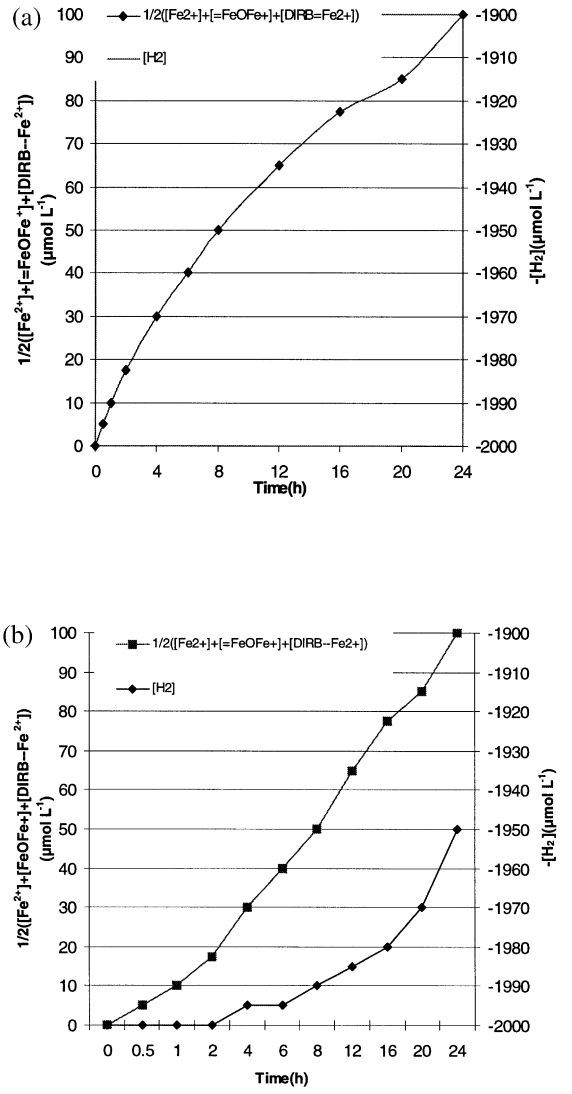

Fig. 3. Hypothetical data for a DIRB-mediated ferric oxide bioreduction kinetic experiment. System consistency can also be assessed by the comparison of kinetic-variable vs. time profiles from different decompositions of Eq. (3) that result from the same reactions, as these quantities must be equivalent. For this example, the kinetic-variable Eq. (13.1) from the first decomposition (values correspond to primary y-axis in both panels) is compared to the kinetic-variable Eq. (13.1*) from the second decomposition (values correspond to secondary y-axis in both panels). Experimental data in panel (A) would demonstrate partial system consistency, while data in panel (B) would demonstrate an inconsistent system.

of reactions in the reaction network. A reaction-based model would use these $(N_I - N_E)$ measurements along with mass action and mass conservation equations to calculate the production of all species in the reaction network. In other words, these measurements are required to calculate the remaining species concentra-

tions not measured in the system. Having obtained the concentrations of all species, we can finally use the kinetic-variable equations to iteratively formulate reaction rates and optimize reaction constants and parameters associated with the rate equations.

3.5. Reaction rate formulations

Let us consider the rate formulation of Reaction (R1). If an ad hoc approach is employed to formulate the reaction rate R_1, one may simply assume an empirical rate equation based on the $[H_2(aq)]$ vs. time profile, e.g. with a first-order rate such as

$$\frac{d[H_2(aq)]}{dt} = -R_1 = -k[H_2(aq)] \tag{15}$$

As shall be seen, this first order rate constant k is not a true constant, but in fact contains the effect of the concentration of the electron acceptor, Fe_2O_3.

An alternate approach is to postulate a reaction mechanism as a basis for simulation. Emulating Henri–Michaelis–Benton–Monod's description of biodegradation involving both electron donor/carbon sources (in the event of a microbial growth reaction) and an electron acceptor, we may hypothesize the following mechanism (Segel, 1975)

$$\begin{array}{ccccc} E + H_2(aq) & \overset{K_D}{\rightleftharpoons} & EH_2 \\ + & & + \\ Fe_2O_3 & & Fe_2O_3 \\ K_A \updownarrow & & \updownarrow \alpha K_A \\ EFe_2O_3 + H_2(aq) & \overset{\alpha K_D}{\rightleftharpoons} & EH_2Fe_2O_3 + 4H^+ \\ & & \downarrow k_p \\ & & E + 2Fe^{2+} + 3H_2O \end{array}$$

where E is an enzyme; EH_2 is the enzyme–electron donor complex; EFe_2O_3 is the enzyme–electron acceptor complex; $EH_2Fe_2O_3$ is the enzyme–electron donor–electron acceptor complex; K_D and K_A are the half saturation constants for the electron donor and electron acceptor, respectively; α is a characteristic parameter of the enzymatic site ($0 \le \alpha \le 1$); and k_p is the rate constant. The half saturation constants are the inverse of their corresponding stability constants. For simplicity, we have assumed that the enzyme E contains both hydrogen oxidation and iron reduction capabilities (i.e. dual hydrogenase-iron reductase functions). These complexes are intermediate species leading to the final products, Fe^{2+} and H_2O. In one mechanism, the electrons donated by hydrogen (represented by reaction in top line) are accepted by Fe_2O_3 (represented by reaction at top of right column) to form the complex $EH_2Fe_2O_3$. Since, the order of donation and acceptance may be random, there is another way to state this donation–acceptance reaction. The electrons accepted by Fe_2O_3 (represented by reaction in left column) are from the donation of hydrogen to form the complex $EH_2Fe_2O_3$ (represented by reaction in bottom line). Finally, the complex $EH_2Fe_2O_3$ is transformed by the DIRB to the final products (represented by reaction at bottom of right column). The above mechanism can be written as the following five reactions

$$E + H_2(aq) \rightleftharpoons EH_2(\text{equilibrium reaction: } K_D) \tag{r1}$$

$$E + Fe_2O_3 \rightleftharpoons EFe_2O_3(\text{equilibrium reaction: } \alpha K_A) \tag{r2}$$

$$EH_2 + Fe_2O_3 \rightleftharpoons EH_2FE_2O_3 \times(\text{equilibrium reaction: } \alpha K) \tag{r3}$$

$$EH_2Fe_2O_3 + 4H^+ \rightarrow E + 2Fe^{2+} + 6H_2O \times(\text{irreversiable kinetic reactions: } k_p) \tag{r4}$$

$$EFe_2O_3 + H_2(aq) \rightleftharpoons EH_2Fe_2O_3 \times(\text{equilibrium reaction: } \alpha K_D) \tag{5}$$

3.6. Indirect simulation of hydrogen consumption

To employ an indirect simulation, an overall reaction rate must be formulated for Reaction (R1). The overall reaction rate may be formulated by eliminating [E], $[EH_2]$, $[EFe_2O_3]$, and $[EH_2Fe_2O_3]$ from Reactions (r1) to (r3), utilizing a first-order representation of Reaction (r4), and defining the total enzyme concentration with Eq. (16)

$$TOT_E = [E] + [EH_2] + [EFe_2O_3] + [EH_2Fe_2O_3] \tag{16}$$

With an appropriate algebraic manipulation, one can obtain the reaction rate as

$$\frac{R_1}{\mathrm{TOT_E}} = \frac{k_p[\mathrm{H_2(aq)}][\mathrm{Fe_2O_3}]}{\alpha K_D K_A + \alpha K_A[\mathrm{H_2(aq)}] + \alpha K_D[\mathrm{Fe_2O_3}] + [\mathrm{H_2(aq)}][\mathrm{Fe_2O_3}]} \quad (17)$$

where R_1 is the overall reaction rate for Reaction (R1) and $\mathrm{TOT_E}$ is the total enzymatic site concentration (note: $k_p \cdot \mathrm{TOT_E} \cdot Y$ is commonly referred to as the maximum reaction rate, μ_{max}, with Y being the specific yield). When $\alpha = 1$, Eq. (17) becomes the hyperbolic rate law of dual Monod kinetics as

$$R_1 = \frac{\mu_{max}}{Y}\left(\frac{[\mathrm{H_2(aq)}]}{K_D + [\mathrm{H_2(aq)}]}\right)\left(\frac{[\mathrm{Fe_2O_3}]}{K_A + [\mathrm{Fe_2O_3}]}\right) \quad (17a)$$

In deriving the overall reaction rate we have not referred to Reaction (r5) because (r5) is equal to [(r3) − (r2) + (r1)]. Since Reaction (r5) is linearly dependent on three equilibrium reactions, it is redundant and is excluded in the foregoing analysis, and in the direct simulation analysis addressed below. Comparing Eq. (15) and Eq. (17) we see that the empirical rate constant k in Eq. (15) is equivalent to

$$k = \frac{\mathrm{TOT_E} k_p[\mathrm{Fe_2O_3}]}{\alpha K_D K_A + \alpha K_A[\mathrm{H_2(aq)}] + \alpha K_D[\mathrm{Fe_2O_3}] + [\mathrm{H_2(aq)}][\mathrm{Fe_2O_3}]} \quad (18)$$

Thus, k includes the effects of $[\mathrm{Fe_2O_3}]$, $[\mathrm{H_2}]$, and $\mathrm{TOT_E}$; hence, it is not a 'true' rate constant, even if k_p, α, K_A, and K_D are intrinsic.

After deriving the overall reaction rates for Reactions (R1) and (R2) (discussed below), we can simulate the production of all 13 species using 13 equations: 4 mass action equations [Eqs. (12.1)–(12.4)]; four kinetic-variable equations [Eqs. (13.1)–(13.4)] with four elementary rate laws for Reactions (R5)–(R8), and the overall rate equations Eq. (17) and Eq. (22) for Reactions (R1) and (R2), respectively; and five mass conservation equations [Eqs. (14.1)–(14.5)]. The concentration vs. time profile of the kinetic-variable $1/2([\mathrm{Fe^{2+}}] + [=\mathrm{FeOFe^+}] + [\mathrm{DIRB\text{--}Fe^{2+}}])$ could serve the purpose for the determination of the four reaction parameters, k_p, K_A, K_D, and α, given the enzymatic site $\mathrm{TOT_E}$. It should be noted that in the indirect simulation, the simulations of the intermediate species E, $\mathrm{EH_2}$, $\mathrm{EFe_2O_3}$, and $\mathrm{EH_2Fe_2O_3}$ are not necessary. The feasibility of this approach depends on our ability to derive the overall reaction rate and measure $\mathrm{TOT_E}$, and its validity depends on (1) whether the simplifying assumptions that Reactions (r1) to (r3) are equilibrium reactions and Reaction (r4) is an irreversible kinetic reaction are valid, and (2) whether Reactions (r1) to (r4) can be decoupled approximately from Reactions (R2) to (R10) in the analysis. In deriving the overall reaction rate we have implicitly assumed that Reactions (r1) to (r4) are decoupled from Reactions (R2) to (R10). If these assumptions are not true then an indirect simulation (i.e. overall rate approach) should not be pursued.

3.7. Direct simulation of hydrogen consumption

For the direct simulation of the proposed reaction mechanism, we simply replace Reaction (R1) by Reactions (r1)–(r4). In this approach, we have a total of 13 reactions [$N = 13$; Reactions (R2)–(R10) and Reactions (r1)–(r4)] and 17 species [the replacement of Reaction (R1) by four reactions (r1)–(r4) has added four intermediate species E, $\mathrm{EH_2}$, $\mathrm{EFe_2O_3}$, and $\mathrm{EH_2Fe_2O_3}$; thus, now $M = 17$]. A matrix analysis of this 13 reaction−17 species system would yield 11 linearly independent reactions. If we assume Reactions (r1)–(r3), (R3), (R4), (R9) and (R10) are equilibrium reactions, then $N_E = 7$. Based on this assumption for N_E, there must be four linearly independent kinetic reactions ($N_I - N_E = 11 - 7$) resulting in four kinetic-variable equations, and six chemical components ($N_C = M - N_I = 17 - 11$) described by six mass conservation equations. Compared to the indirect simulation, three more reactions and four more species have to be considered within the direct simulation. However, because both N_I and N_E increased by the same number (i.e. from 8 to 11, and 4 to 7, for N_I and N_E, respectively) the minimum number of chemical species vs. time that must be measured ($N_I - N_E$) has not changed.

The solution for the direct simulation can be decomposed in many ways, with one shown below.

N_E mass action equations

$$\frac{\mathrm{d}(\frac{1}{2}[\mathrm{Fe^{2+}}] + \frac{1}{2}[=\mathrm{FeOFe^+}] + \frac{1}{2}[\mathrm{DIRB\text{--}Fe^{2+}}] + [\mathrm{EH_2}] + [\mathrm{EH_2Fe_2O_3}])}{(\mathrm{d}t)} = r_1: \quad (19.1)$$

$$r_1 = \infty \Rightarrow [\mathrm{E}][\mathrm{H_2(aq)}] = K_D[\mathrm{EH_2}]$$

$$\frac{\mathrm{d}[\mathrm{EFe_2O_3}]}{\mathrm{d}t} = r_2: \quad r_2 = \infty \Rightarrow [\mathrm{E}][\mathrm{Fe_2O_3}] = K_A[\mathrm{EFe_2O_3}] \quad (19.2)$$

$$\frac{\mathrm{d}(\frac{1}{2}[\mathrm{DIRB\text{--}Fe^{2+}}] + \frac{1}{2} = [\mathrm{FeOFe^+}] + \frac{1}{2}[\mathrm{Fe^{2+}}] + [\mathrm{EH_2Fe_2O_3}])}{\mathrm{d}t} = -r_3: \quad (19.3)$$

$r_3 = \infty \Rightarrow [E][H_2(aq)][Fe_2O_3] = \alpha K_A K_D [EH_2Fe_2O_3]$

and Eqs. (12.1)–(12.4).

($N_I - N_E$) *kinetic variable equations*

$$\frac{1}{2}\frac{d([Fe^{2+}] + [=FeOFe^{+}] + [DIRB\text{--}Fe^{2+}])}{dt} = r_4 \tag{20.1}$$

and Eqs. (13.2), (13.3) and (13.4).

N_C *Mass Conservation Equations*

$$TOT_{Fe_2O_3} = [Fe_2O_3] + \tfrac{1}{2}[=FeOH_2^{+}] + \tfrac{1}{2}[FeOH] + \tfrac{1}{2}[=FeO^{-}] + [=FeOFe^{+}] \tag{21.1}$$

$$+ \tfrac{1}{2}[Fe^{2+}] + \tfrac{1}{2}[DIRB\text{--}Fe^{2+}] + [EFe_2O_3] + [EH_2Fe_2O_3]$$

$$TOT_{H_2} = [H_2(aq)] + [H_2(g)] + \tfrac{1}{2}[Fe^{2+}] + \tfrac{1}{2}[=FeOFe^{+}] + \tfrac{1}{2}[DIRB\text{--}Fe^{2+}] + [EH_2] + [EH_2Fe_2O_3] \tag{21.2}$$

and Eqs. (14.3)–(14.5) and (16). The *italicized* terms in Eqs. (21.1) and (21.2) are additional terms compared to the corresponding Eqs. (14.1) and (14.2), respectively. These additional terms were absent in the indirect simulation because the contribution of the reactions in this pathway to the mass conversation of other chemical components was not considered in deriving its overall-rate equation; i.e. the proposed pathway was assumed to be decoupled from the other chemical reactions.

The indirect simulation is identical to the direct simulation when (1) $[EFe_2O_3]$ and $[EH_2]$ are negligible in their contribution to the mass conservation of Fe_2O_3, and (2) $[EH_2]$ and $[EH_2Fe_2O_3]$ are negligible in their contribution to TOT_{H^+}. Only when these two conditions are met can the effects of the proposed pathway of DIRB-mediated bioreduction of ferric oxide on the mass conservation of chemical components be ignored in the analysis, as normally done in modeling a system involving both abiotic and biotic reactions (Tebes-Stevens et al., 1998; Salvage and Yeh, 1998). Therefore, Henri–Michaelis–Benton–Monod kinetics should be applied with care to coupled abiotic and biotic systems, and the nature of intermediate species should be considered. Unless one can be sure that a proposed pathway for a biotic reaction can be decoupled from all other abiotic reactions in the analysis, the indirect approach of using an overall rate law such as the Henri–Michaelis–Benton–Monod kinetics and ignoring intermediate species is invalid. Under such circumstances, the direct simulation approach of the proposed pathway along with all other abiotic reactions must be employed; i.e. the contribution of intermediate species to the mass conservation of chemical components must be included.

3.8. Simulation of hydration reaction

The rate of the hydration reaction (R2) must be formulated because we do not expect it to be approximated by an elementary rate law. The bioreduction of hematite [Reaction (R1)] has already been assumed to be a kinetic reaction. Since bioreduction will 'uncover' additional ferric oxide surface sites, the subsequent hydration reaction [Reaction (R2)] will also be assumed to be a kinetic reaction. As Fe_2O_3 is chemically reduced to Fe^{2+}, the hematite particles are presumably physically reduced in size. As the size of the residual hematite particles is changed, the unit surface area of the particles is also changed. Thus, the total surface sites are not constant but are a function of $[Fe_2O_3]$, residual surface area, and reduction extent. Based on this rationale, the reaction rate equation R_2 can be written as

$$R_2 = k_2 S_A \frac{N_S}{N_A}[Fe_2O_3] \tag{22}$$

where k_2 is a first-order rate constant ($time^{-1}$), S_A is the specific surface area of the residual hematite particles ($m^2 \cdot g^{-1}$), N_s is the number of sites per unit surface area (mol sites $\cdot m^{-2}$), N_A is Avogadro's number (mol sites $\cdot mol^{-1}$), and $[Fe_2O_3]$ is the bulk ferric oxide concentration ($mol \cdot l^{-1}$). For this example, we will assume that N_s remains constant. The rate constant k_2, therefore, can only be determined if both S_A and $[Fe_2O_3]$ vs. time are known. As discussed above, S_A would be measured directly, however, an independent or operational measurement of $[Fe_2O_3]$ cannot be made from the proposed measurements (recall definitions for $HCl_{Fe^{2+}}$ and $HCl_{Fe^{III}}$ in Section 3.3). Instead, the simulated values of $[Fe_2O_3]$ vs. time must be used for the evaluation of R_2.

If Reaction (R2) were an equilibrium reaction, then Eq. (13.2) would be replaced by the mass action equation for Reaction (R2) as

$$\frac{1}{2}\frac{d([=FeOH_2^{+}] + [=FeOH] + [=FeO^{-}] + [=FeOFe^{+}])}{dt} = R_2:$$

$$R_2 = \infty \rightarrow TOT_{=FeOH} = S_A \frac{N_s}{N_A}[Fe_2O_3] = [=FeOH_2^{+}] + [=FeOH] + [=FeO^{-}] + [=FeOFe^{+}] \tag{13.2*}$$

Note that whether Reaction (R2) is a kinetic or equilibrium reaction, the total surface sites are not constant. That is, the evolution of reactive surface sites depends on the extent of the bioreduction of Fe_2O_3.

4. Conclusions

The objectives of this paper were to: (1) present critical theoretical issues that must be considered for proper application of reaction-based biogeochemical models; and (2) functionally demonstrate the application of these concepts to the design, performance, and conceptual modeling of a relevant biogeochemical experiment. Our first major point was that the selection of chemical components is not unique and a mathematical decomposition of the reaction matrix (e.g. Chilakpati, 1995) should be used for formal selection. This decomposition procedure effectively reduces a set of M simultaneous ordinary differential equations governing the production–consumption of M chemical species into three subsets: N_E non-linear mass action equations; $(N_I - N_E)$ simultaneous ordinary differential kinetic-variable equations, and; N_C linear algebraic mass conservation equations.

Our second major point was that the consistency of mass conservation equations must be assessed with experimental data before kinetic modeling is initiated. If the reaction matrix (conceptual model) is not adequately defined, then assumptions of mass conservation can generate erroneous evolutions of chemical species. If even one mass conservation equation can be validated with experimental data, partial system consistency can be assessed, and greater confidence in the reaction matrix and kinetic modeling will be obtained. Another means to assess system consistency is to compare kinetic-variable vs. time profiles from different decompositions of Eq. (3) that result from the same reactions, as these quantities must be equivalent to each other. If a system is shown to be inconsistent, a revised reaction network must be formulated and tested. Similarly, assumptions regarding equilibrium reactions should also be validated, although this could be done in separate, preliminary experiments. If a mass action equation is not satisfied then the corresponding reaction must be treated as a kinetic reaction.

Our third major point was that a minimum number of chemical species or operational quantities must be measured to use a reaction-based model. A reaction matrix must be proposed that identifies all significant chemical species (M) and reactions (N). Analysis of this reaction matrix is used to determine the number of linearly-independent reactions (N_I). Based on the assumption of the number of linearly independent equilibrium reactions (N_E), the minimum number of chemical species concentration vs. time curves that must be measured to evaluate the kinetic suite of reactions using a reaction-based model will be $(N_I - N_E)$. However, for a partial assessment of system consistency, at least one more species must be measured [i.e. $(N_I - N_E + 1)$]. For a complete assessment of system consistency, $(N_I - N_E + N_C)$ additional species would have to be measured, where N_C is the number of chemical components.

The bioreduction of ferric oxide by DIRB was used as an example to functionally demonstrate these points. Through this example we also showed that reaction rates for kinetic reactions that are linearly independent of other kinetic reactions can be determined based on only one profile of kinetic-variable concentration vs. time for each reaction. Reaction rates for kinetic reactions that are linearly dependent on each other can not be segregated when they result in production of the same species; thus, experiments should be designed to avoid parallel kinetic reactions for parameterization purposes; however, they must be included for simulation purposes. Kinetic reactions that are linearly dependent only on equilibrium reactions are redundant and do not have to be included or modeled. Our final major point was that Henri–Michaelis–Benton–Monod kinetics should be applied with care to coupled abiotic and biotic systems, and the nature of the intermediate species should be considered.

A_i: activity of the i-th species
B: matrics of reduced **U**
b_{ij}: ij-th entry (i-th row, j-th column) or the matrix **B**
C_i: concentration of the i-th chemical species
C: species concentration vector
DIRB: dissimilatory iron reducing bacteria
D: diagonal matrix representing a submatrix of reduced $\boldsymbol{\nu}$ with size $N_I \times N_I$
D_{kk}: k-th diagnonal entry of the matrix **D**
E_i: concentration of the i-th kinetic variable
f_i: empirical function for production-degradation of the i-th species
G_i: chemical formula of i-th species
K_k^b: backward rate constant of k-th kinetic reaction
K_k^f: equilibrium constant of k-th reaction
K_k^f: forward rate constant of k-th reaction
K: submatrix of reduced $\boldsymbol{\nu}$ with size $N_I \times N_D$
M: number of chemical species in reaction network
M_d: number of possible decompositions of the reaction matrix
N: total number of biogeochemical reactions in reaction network
N_C: number of chemical components

N_D: number of dependent reactions
N_E: number of equilibrium reactions
N_I: number of linearly independent reactions
N_K: number of kinetic reactions
$\mathbf{0}_1$: diagonal matrix representing a submatrix of reduced $\boldsymbol{\nu}$ with size $N_C \times N_I$
$\mathbf{0}_2$: zero matrix representing a submatrix of reduced $\boldsymbol{\nu}$ with size $N_C \times N_D$
p_1, p_2: empirical rate parameters
$r_{i|N}$: production–consumption rate of the i-th species due to N biogeochemical reactions
R_k: rate of the k-th reaction
$\mathbf{R}$: reaction rate vector
t: time
T_i: total analytical concentration of i-th component
$\mathbf{U}$: unit matrix vector
μ_{ik}: reaction stoichiometry of the i-th species in the k-th reaction associated with the reaction reactants
ν_{ik}: reaction stoichiometry of the i-th species in the k-th reaction associated with the reaction products
$\boldsymbol{\nu}$: reaction stoichiometry matrix

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