Biodegradation **10:** 315–330, 1999.

Modeling speciation effects on biodegradation in mixed metal/chelate systems

Jeanne M. VanBriesen & Bruce E. Rittmann
Department of Civil Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3109, USA

Accepted 16 July 1999

Key words: biogeochemistry, biodegradation modeling, coupled processes, citrate, citric acid

Abstract

A new model, CCBATCH, comprehensively couples microbially catalyzed reactions to aqueous geochemistry. The effect of aqueous speciation on biodegradation reactions and the effect of biological reactions on the concentration of chemical species (e.g. H_2CO_3, NH_4^+, O_2) are explicitly included in CCBATCH, allowing systematic investigation of kinetically controlled biological reactions. Bulk-phase chemical speciation reactions including acid/base and complexation are modeled as thermodynamically controlled, while biological reactions are modeled as kinetically controlled. A dual-Monod kinetic formulation for biological degradation reactions is coupled with stoichiometry for the degradation reaction to predict the rate of change of all biological and chemical species affected by the biological reactions. The capability of CCBATCH to capture pH and speciation effects on biological reactions is demonstrated by a series of modeling examples for the citrate/Fe(III) system. pH controls the concentration of potentially biologically available forms of citrate. When the percentage of the degradable substrate is low due to complexation or acid/base speciation, degradation rates may be slow despite high concentrations of substrate Complexation reactions that sequester substrate in non-degradable forms may prevent degradation or stop degradation reactions prior to complete substrate utilization. The capability of CCBATCH to couple aqueous speciation changes to biodegradation reaction kinetics and stoichiometry allows prediction of these key behaviors in mixed metal/chelate systems.

Introduction

Predicting the ultimate fate of mixed chemical contaminants requires an understanding of the chemical and biological processes affecting contaminants and naturally occurring organic and inorganic constituents. While fundamental chemical and biological reactions may, in some cases, be well characterized, the interactions among these reactions for complex systems are largely undefined. In particular. the interactions among bacteria, biologically degradable organic species, and biologically affected inorganic species may significantly affect contaminant fate in the environment.

Laboratory-scale experimentation is often used to identify impacts of biological reactions on contaminants, however, the complexity of the interactions can produce experimental results that are difficult to interpret. On the other hand, these interactions can be assessed and quantified through modeling efforts in a rapid, cost-effective manner. The study of chemical and biological reactions of mixtures of contaminants is, therefore, an area of research well suited to mathematical modeling. In this paper, we present CCBATCH a model designed to deal with chemical and biological interactions in aqueous batch systems. (The acronym CCBATCH stands for *C*o-*C*ontaminants in a *BATCH* reactor.) First, we describe the development and coupling of models for aqueous speciation and biological degradation. Then, a modeling example highlights the key functionality of CCBATCH by predicting complex, but expected behavior in a mixed metal/chelate system. Finally, model verification and limitations are discussed.

CCBATCH methodology foundation

The biogeochemistry that influences the fate of mixed contaminants can be grouped into two reaction classes The first type of reactions are those that take place in the bulk aqueous phase (homogeneous reactions) and include acid/base and complexation reactions. These reactions normally reach thermodynamic equilibrium rapidly, although slow complexation reactions have been reported (Pohlmeier and Knoche 1996; Xue et al. 1995; Nowak et al. 1997). In CCBATCH, bulk-phase reactions are modeled as thermodynamically controlled. The second type includes slower reactions, involving kinetically controlled biological reactions or abiotic interphase reactions. Biological reactions are catalyzed by bacteria either suspended in the aqueous phase or attached to surfaces. These biological reactions are oxidations and reductions, but they frequently consume or produce acids or bases present in solution (Rittmann and VanBriesen 1996). Abiotic surface reactions (Baer 1990) that are slow to reach equilibrium include sorption, precipitation and dissolution, abiotic oxidation and reduction, and radioactive decay, and may also affect the solution pH. In CCBATCH, biological and abiotic surface reactions are modeled as kinetically controlled.

In this paper, we present specifics for how CCBATCH couples bulk-phase equilibrium reactions and kinetic biological reactions. CCBATCH is designed so that subprograms for all kinds of kinetic reactions can be added easily. Contaminant transport also can be considered by coupling the batch routines to a transport code (Tebes-Steves et al. 1998). The rest of this section describes the mathematical solution methods for equilibrium and kinetic submodels and the coupling of the submodels.

Components and equilibrium reactions

Equilibrium-based modeling in CCBATCH follows the well established pattern of multicomponent thermodynamic equilibrium modeling (Morel and Morgan 1972; Lichtner 1985; Yeh and Tripathi 1989; Engesgaard and Kipp 1992; Lichtner 1996) utilized in several commonly used software packages for chemical speciation modeling (e.g. MINEQL, Westall et al. 1976; MINTEQA2, Allison et al. 1991; PHREEQE, Parkhurst et al. 1980; and The Geochemist's Workbench, Bethke, 1992). Chemical components are selected so that they can be combined to form all possible species, but no component is formed by combination of the other components (Morel and Hering 1993).

As an example of an organic/metal mixture we consider citric acid ($H_3C_6H_5O_7$) in the presence of the complexing metal iron(III). This system was selected because the aqueous chemistry is well understood and includes competition of acidic hydrogen and iron for the citrate, competition of hydroxide and citrate for the iron, and the production of acidic hydrogen during the course of biodegradation of the citrate. Thus, the general behavior of this system under various conditions can be predicted and the ability of CCBATCH to capture speciation changes and the potential impact of these changes on the biological system can be evaluated. The components are the citrate anion ($C_6H_5O_7^{3-}$ or Cit^{3-}, the iron cation (Fe^{3+}), acidic hydrogen (H^+), and water (H_2O). This system includes acid/base and complexation reactions for the organic acid and the cationic metal. In order to fully characterize the chemistry in this system under biodegradation conditions, the additional acid/base species of H_2CO_3 and NH_4^+ must be considered, as biodegradation reactions have the potential to utilize and generate inorganic carbon and nitrogen. Further, in order to consider the kinetic reactions of citrate degradation by micro-organisms, as well as cell growth and decay, the system must include the cells (by convention, $C_5H_7O_2N$) and their electron acceptor (O_2 in this case). No equilibrium acid/base or complexation reactions occur for these components in this system.

Table 1 presents the component set and the stoichiometric coefficients for the aqueous complexes that can form along with the log of the overall formation constant ($^c\beta$) for the ionic strength selected for the example (0.1 M). While ionic strength can vary during biodegradation due to changes in the chemical composition of the solution and CCBATCH includes an activity correction routine that can adjust the β values at each timestep, for simplicity in the example presented we assume a swamping buffer concentration fixes the ionic strength at 0.1 M.

For each component, a mass balance to determine the total concentration of the component in the system is required. Substitution of mass action equations for the reactions described in Table 1 into the mass balances produces equations expressed only in terms of the components. These mass balances are a set of coupled non-linear algebraic equations that must be solved simultaneously. They are further coupled to an appropriate mass balance on the acidic hydrogen component, discussed below, and a Newton-Raphson

Table 1. Components and complexes with corresponding stoichiometric coefficients and log β and log ${}^{c}\beta$ values for the citrate/Fe(III) example

Complexes	H_2O	H^+	Cit^{3-}	Fe^{3+}	H_2CO_3	NH_4^+	Cells ($C_5H_7O_2N$)	O_2	Log β	Log ${}^{c}\beta$
H_3Cit	0	3	1	0	0	0	0	0	14.29	13.08
H_2Cit	0	2	1	0	0	0	0	0	11.16	10.06
$HCit^{2-}$	0	1	1	0	0	0	0	0	6.40	5.74
$FeOH^{2+}$	1	−1	0	1	0	0	0	0	−2.2	−2.64
$Fe(OH)_2^+$	2	−2	0	1	0	0	0	0	−5.7	−6.36
$Fe(OH)_3^0$	3	−3	0	1	0	0	0	0	−12.8	−13.32
$Fe(OH)_4^-$	4	−4	0	1	0	0	0	0	−21.06	−22.04
$Fe_2(OH)_2^{4+}$	2	−2	0	2	0	0	0	0	−2.90	−3.0
$FeCit^0$	0	0	1	1	0	0	0	0	13.50	11.52
$FeOHCit^-$	1	−1	1	1	0	0	0	0	10.1	9.81
$Fe(OH)_2Cit^{2-}$	2	−2	1	1	0	0	0	0	28.3	25.11
OH^-	1	−1	0	0	0	0	0	0	14.0	13.78
HCO_3^-	0	−1	0	0	1	0	0	0	−6.35	−6.13
CO_3^{2-}	0	−2	0	0	1	0	0	0	−16.68	−16.02
NH_3	0	−1	0	0	0	1	0	0	−9.4	−9.28

Constants from Stumm and Morgan (1996) and Hamm et al. (1954).

iterative solution method is used (Press et al. 1992) for the component array. The mass action equations are then solved for the reactions described in Table 1 for the complex concentrations.

Acid hydrogen, the proton condition and the initial conditions

Typically, variable-pH modeling is accomplished by adding H^+, or acidic hydrogen, as another component in the general matrix of components. Its mass balance is coupled to all other mass balances for components without special consideration (Lichtner 1985). This is a mathematically sound method for computation and works well under many conditions for strictly equilibrium-controlled models. However, in coupled equilibrium-kinetic models that involve reactions that consume or produce acidic hydrogen, this key mass balance is better understood as a formal proton condition, which is a modification of the standard mass balance on acidic hydrogen utilized by most multicomponent equilibrium solvers. First, we present the general utility and formulation methodology for the proton condition. Second, we discuss utilizing the proton condition in conjunction with an initial speciation to set the starting conditions for the system. Finally, we show how we utilize the proton condition to integrate the equilibrium solver with the kinetically controlled biodegradation reactions.

In CCBATCH, the mass balance on the acidic hydrogen is defined by the proton condition (Stumm and Morgan 1996), which is a special mass balance on acid and base equivalents produced and is formulated relative to defined reference species. All species that contain more protons than the reference species are placed on the left side of the equation, and all species that contain fewer protons than the reference species are placed on the right side of the equation. The difference in protons from the reference species multiplied by the molar concentration of the species defines the concentration term in the proton condition. In this way, the number of acid equivalents (left side) created by all acid/base reactions is matched by the number of base equivalents (right side) created by all acid/base reactions, regardless of any changes that take place in the speciation of components. The proton condition thus quantifies the fundamental principle of acid/base chemistry: For every acid equivalent formed, a conjugate base equivalent must be formed.

Within the structure of a generic multicomponent equilibrium solver, however, the formality of a reference species is lost. The mass balance on H^+ is created with reference to the components regardless of their acid/base 'status'. While mathematically sound, this methodology results in a numerical value for the total concentration of acidic hydrogen in the system that may have no inherent meaning. If all the components are chosen in their least protonated form, the total concentration of acidic hydrogen represents the base-neutralizing capacity of the solution; likewise, if the most protonated forms are selected for the reference level, the acid-neutralizing capacity is represented (Morel and Morgan 1972). However, often the components are chosen for reasons other than being systematic with regard to their acid/base status. Then, the value of the total concentration of acidic hydrogen is without direct meaning.

The effect of this absence of meaning in the arbitrary proton mass balance is seen most clearly when the coupled biological reactions utilize acidic hydrogen. As biodegradation proceeds and acidic hydrogen is utilized, the value of the total concentration of acidic hydrogen decreases, however, as the starting value is arbitrary, the total concentration term can become negative during the modeling simulation.

In CCBATCH, the user selects a system of components in the traditional manner, and acidic hydrogen (H^+) is a component. In order to ensure that the acidic hydrogen balance is a true proton condition, the components are taken as the reference species for acid/base reactions. In our example, Fe^{3+} and Cit^{3-} are the reference species for iron and citrate, respectively. This formal constraint allows CCBATCH to track a 'pool' of available acidic hydrogen to be utilized in biological reactions. The proton condition created relative to this fixed reference species can be utilized with the known pH of the starting solution to determine the 'true' available acidic hydrogen in the system.

As with all multicomponent equilibrium models, the selection of components is independent of the actual species predominating at the system starting time. In order to predict accurately changes in speciation, the initial speciation mist be determined, and the starting total aqueous hydrogen concentration must be reset to account for the difference between the selected reference level and the dominant species of each component at the initial conditions. This is accomplished in CCBATCH by linking an initial speciation routine to a resetting routine based on the formulation of the proton condition for the system. Since the actual starting conditions differ from reference levels, the proton condition is adjusted during the pre-iterative equilibration using a method called *equivalent additions*. The equivalent addition methodology is based on the fact that the difference between the starting condition and the reference level is known. All the changes to the proton condition are in the same direction, and the effect of rewriting the condition will be to change the 'sum' of acidic hydrogen. Instead of rewriting the proton condition for each new starting condition, the change to this 'sum' is computed, and an equivalent addition made to alter the proton condition.

As an example, the proton condition can be formulated for the citric acid system described above. The selected reference species for this system are taken as identical to the components – the deprotonated citrate ion (Cit^{3-}); uncomplexed, cationic iron (Fe^{3+}); fully protonated carbonic acid (H_2CO_3) and ammonium (NH_4^+), water (H_2O), molecular oxygen, and cells ($C_5H_7O_2N$). The proton condition is written as described above:

$$3[H_3Cit] + 2[H_2Cit^-] + [HCit^{2-}] + [H^+] = [FeOHCit^-] + 2[Fe(OH)_2Cit^{2+}] + [FeOH^{2+}] + 2[Fe(OH)_2^+] + 3[Fe(OH)_3^0] + 2[Fe_2(OH)_2^{4+}] + [OH^-] + 2[CO_3^{2-}] + [HCO_3^-] + [NH_3] + ACID \qquad (1)$$

We rearrange Eq. (1) to be consistent with the format of the other mass balances for the component set:

$$ACID = 3[H_3Cit] + 2[H_2Cit^-] + [HCit^{2-}] + [H^+] - [FeOHCit^-] - 2[Fe(OH)_2Cit^{2-}] - [FeOH^{2+}] - 2[Fe(OH)_2^+] - 3[Fe(OH)_3^0] - 2[Fe_2(OH)_2^{4+}] - [OH^-] - 2[CO_3^{2-}] - [HCO_3^-] - [NH_3] \qquad (2)$$

The term ACID replaces the total aqueous concentration of acidic hydrogen compared to the reference levels. The ACID term can be used to represent increases in acid or base equivalents in the system due to the dominant aqueous species at the starting conditions being different from the reference species. When the starting species are more acidic than the reference level, ACID is positive, when the added species are more basic than the reference level, ACID is negative. Because ACID can be used to adjust for kinetic reactions that produce or consume acidic equivalents, it is a key link between the equilibrium solver and the kinetic solver.

For the Fe/Citric acid system, at starting pH values below the first pK_a of citric acid (3.08), carbonic acid (6.35), and ammonium (9.24), the fully protonated forms of citric acid, carbonic acid and ammonium are the initial species, and the iron ion remains uncomplexed with hydroxide; thus, ACID is positive. At starting pH values above the third pK_a of citric acid (5.74), the pK_fs of the iron hydroxides, and the pK_a values of ammonium and the carbonates, the deprotonated citrate ion, the complexed iron hydroxide species, NH_3, and free carbonate (CO_3^{2-}) dominate the system; hence, ACID is negative. In between these two extreme cases, due to the complexity of the system involving multiple competing equilibrium reactions, the value and sense of ACID requires a complete speciation computation. The first iteration of CCBATCH must begin with a set of solution-phase concentrations that conform to mass balance limitations (Steefel and MacQuarrie 1996), and are in thermodynamic equilibrium prior to the iterative stepping between the equilibrium and kinetic routines. Thus, CCBATCH uses a separate pre-iterative solution to the equilibrium problem. Using the total concentrations of components defined by the user and an initial pH, CCBATCH computes the equivalent addition necessary to define a proton condition for the system: i.e. ACID is computed. CCBATCH then solves the equilibrium problem for the fixed initial pH and determines the concentrations of all components and complexes.

In some cases, mixed contaminant systems have significant buffering, which effectively fixes the pH and acidic hydrogen concentration. In this special case, the acidic hydrogen component is constant and is determined from the set pH: $[H^+] = \{H^+\}/\gamma_{H+}$, where γ_{H+} is the activity coefficient for the proton. Once the initial pH has been used to set the ACID term, CCBATCH can be operated in the fixed-pH mode for these systems.

In most situations, however, the potential for biodegradation (or other) reactions to change the system pH is significant. Many degradation reactions produce or consume acidic hydrogen and, therefore, change the pH of the system. For example, the aerobic degradation of citrate and the microbially catalyzed reduction of ferric iron to ferrous iron are acid/base reactions that consume and produce acidic hydrogen, respectively (Francis et al. 1992; Lovley and Phillips 1988). Furthermore, biodegradation consumes substrates with acid/base character (like citrate) or produces other species with acid/base character (like carbonates). The changes in total concentration of these components also causes respeciation and pH change. Since bacteria can be quite sensitive to shifts in pH, rates of degradation and bacterial growth may depend on the prevailing pH.

CCBATCH incorporates the effects of kinetic reactions in three ways: (1) adjusting the ACID term for consumption or production of acidic hydrogen; (2) changing the total component concentration of produced or consumed components; and (3) respeciating to determine the new equilibrium position and pH. Details of the coupling of the equilibrium and kinetic routines are discussed below.

Biodegradation modeling

Micro-organisms utilize nutrients (C, N, P, O and H), electrons, and energy to build new cells. They acquire these necessary materials from environmental sources and are sinks for them. Micro-organisms also produce a range of products, making them sources of these product materials. The rate at which cells grow and thereby serve as sinks or sources for materials in their environment is controlled by inherent characteristics of the cells and by system-dependent factors, such as the amount of available substrates and the total number of cells present in the system.

In CCBATCH, we use dual-limitation Monod kinetics (Bae and Rittmann 1996) to describe the rate of utilization of the primary electron-donor substrate:

$$r_{\text{util}} = -q_m X_a \frac{S}{S + K_S} \frac{A}{A + K_A} \qquad (3)$$

in which X_a is the concentration of metabolically active organisms in the system (M_xL^{-3}), S is the concentration of the electron-donor substrate (M_sL^{-3}), K_s is the half-maximum-rate concentration for the electron-donor substrate (M_sL^{-3}), A is the concentration of the electron-acceptor substrate (M_aL^{-3}), K_A is the half-maximum concentration of the electron-acceptor substrate (M_aL^{-3}), q_m is the maximum specific rate of substrate utilization for the bacterial species ($M_sM_x^{-1}T^{-1}$), and r_{util} is the rate of utilization of the rate-limiting substrate ($M_sL^{-3}T^{-1}$). The rate of substrate utilization is directly related to the rate of biomass synthesis, or cell growth, through the true yield coefficient (Y), expressed as the amount of cell mass produced per unit of substrate consumed ($M_xM_s^{-1}$). Multiplying r_{util} in Eq. (3) by $-Y$ gives $r_{\text{synthesis}}$.

The dual Monod formulation reduces to the familiar single Monod (1946) formulation when one of the substrates is in high (and, therefore, non-limiting) concentration. It also reduces to a first-order dependency on a particular substrate when that limiting substrate(s) has a very low concentration (below K_s for this substrate), and to zero order with respect to that substrate when its concentration is high. Thus, the dual Monod model has wide applicability in modeling biodegradation reactions.

In addition to utilizing environmental resources to build new cells, cells also have maintenance requirements (Pirt 1965; Van Uden 1967; Cherry and Thompson 1997; Horn and Hempel 1997). In CCBATCH, we consider the maintenance requirements of cells to be met by self-oxidation, or utilization of biomass as an electron-donor substrate. In this formulation, a decay rate is computed as proportional to the concentration of biomass: $r_{\text{decay}} = bX_a$ in which r_{decay} is the rate of cell decay, b is the endogenous decay constant (T^{-1}), and X_a is the concentration of organisms in the system (M_xL^{-3}).

Substrate utilization and cell decay consume or produce other chemical species (e.g. oxygen, acidic hydrogen, ammonium, or carbonate species), in addition to affecting cell yields. In CCBATCH, the stoichiometry between substrate utilization or cell decay and production or consumption of other chemical species of interest is based on balanced chemical equations for the biological reactions. The coefficients for all species in the degradation reaction are computed following an expanded McCarty (1969, 1971, 1972a, b, 1975) method that is discussed in detail elsewhere (VanBriesen and Rittmann, 1999). In brief this method is based on developing an oxidation half-reaction for the electron donor substrate and combining it with reduction half-reactions for the electron acceptor and cell synthesis. Combining the half reactions divides all the electrons released in the oxidation of the electron donor between the electron acceptor (for generating energy) and the environmental sources of nutrients necessary for cell synthesis (for reducing nutrient C, N and O, to the oxidation state necessary for incorporation into cell mass).

The relative proportioning of electrons is dependent on the energy yield of the donor-acceptor redox couple and the energy requirements of the cell synthesis reaction. In some cases, this partitioning is established from empirical data on the cells' true yield. In that case, the fraction of donor electrons going to biomass synthesis (f_s^o) is proportional to the true yield. A units conversion expressing Y in electron equivalent biomass synthesized per electron equivalent of donor consumed gives f_s^o. The fraction of the electrons going to the acceptor, f_e^o, is obtained by difference ($f_e^o = 1 - f_s^o$) for reactions that do not involve intermediate formation. In cases for which reliable empirical yield data are not available, f_s^o and f_e^o can be predicted utilizing thermodynamic properties of all involved species (McCarty 1971, 1975). Continuing with the citrate example, based on the thermodynamic approach of McCarty (1971, 1975), the fraction of electrons going to synthesis and to oxygen are, respectively, $f_s^o = 0.718$ and $f_e^o = 0.282$. That partitioning gives the overall stoichiometry of the reaction to oxidize citrate with oxygen and grow cells:

$$C_6H_5O_7^{3-} + 0.64\ NH_4^+ + 1.27\ O_2 + 0.071\ H_2O + 2.34\ H^+ \rightarrow 2.77\ H_2CO_3 + 0.64\ C_5H_7O_2N \tag{4}$$

Reaction (4) gives the molar ratios of all chemical components affected during the aerobic degradation of citric acid and the synthesis of new biomass. Note that all reactants and products are components in the equilibrium speciation model. Reaction (4) appears to show that the anionic, uncomplexed form of citrate is being degraded. This is not the case. In reality, the reaction accounts for the rate of loss of the total component of citrate, because Cit^{3-} is the reference species for the citrate component. Thus, reaction (4) gives the change in all components (e.g. O_2, cells, acidic hydrogen) in proportion to the loss of the total citrate and in reference to Cit^{3-}. The use of the components in the stoichiometry reactions is key to linking these kinetically controlled biodegradation reactions to the equilibrium-controlled reactions.

One or more chemical forms of the substrate (H_3Cit, H_2Cit^-, $HCit^{2-}$, Cit^{3-}, $FeCit^o$, $FeOHCit^-$, $Fe(OH)_2Cit^{2-}$) may be biologically available to the micro-organisms, and biodegradation kinetics can depend strongly on the chemical form of the substrate (Lauff et al. 1990; Palumbo et al. 1994; Nortemann 1992; Joshi-Tope and Francis 1995; Bolton et al. 1996). In these systems, a range of bacterial species appear to have rapid degradation kinetics for one or two chemical forms, but slow or zero kinetics for other forms. CCBATCH allows the user to specify degradable and non-degradable forms of each electron-donor substrate component. It is the concentration of the biologically available, degradable substrate form (whether a component, complex, or sum of many different complexes) that controls the rate of degradation. Therefore, although the concentrations of all chemical forms are computed by the speciation routine, only the concentrations of the degradable forms (as specified by the user) are included in S, the substrate available for degradation that controls the rate in the Monod Eq. (3). However, it is the total concentration of the substrate (C_T for the component) that is changed as a result of the degradation. If reliable information for the kinetics and degradable form are not available, the full substrate concentration can be considered degradable or CCBATCH can be used along with experimentation to explore hypotheses relating to the biologically-available substrate form (VanBriesen et al. 1999c; Banaszak et al. 1996, 1997a, 1999).

The stoichiometry of the decay or endogeneous respiration reactions can be based on cell mineralization or a proportioning of carbon between mineralization products and soluble microbial by-products (SMP). While different decay formulations can be used (DeSilva and Rittmann, 1999; VanBriesen et al. 1999a), for simplicity in this example, we assume that the cells are mineralized. With oxygen as the electron acceptor, then, the decay reaction is:

$$C_5H_7O_2N + 3\,H_2O + H^+ + 5\,O_2 \rightarrow 5\,H_2CO_3 + NH_4^+ \quad (5)$$

Once the full degradation and cell decay reactions are known, the substrate utilization rate is combined with stoichiometric values from these reactions to compute the rates of consumption or production for all components affected. In our example, for the components other than citrate, rates are computed based on the utilization rate of the primary electron donor substrate (in moles citrate/L-day) computed following Eq. (3) and the stoichiometries of reactions (4) and (5).

Linking equilibrium and kinetics

Once CCBATCH has determined the rates of change of all components due to the kinetic biodegradation reactions, these rates are used, along with a timestep, to adjust the total concentrations of the components. The adjusted total concentrations are used in the next call of the speciation routine to predict the concentrations of the components and complexes at the new equilibrium state. The sequential solution of the equilibrium and biodegradation submodels continues until a user-determined stop criterion is reached. Examples of stop criteria are: a substrate is exhausted, all cells are eliminated by decay, or the maximum number of timesteps set by the user is reached.

This method represents a sequential non-iterative approach (Steefel and MacQuarrie 1996; Tebes-Steves et al. 1998). The time step must be chosen small enough to avoid numerical error or instability (Valocchi and Malmstead 1992). In this work, which involves modeling batch reactions, the simple forward, non-iterative approach has proved suitable. However, CCBATCH can be integrated into complex subsurface flow models, in which case more sophisticated iterative-sequential methods based on complete coupling of the mass balances are necessary to reduce the computational burden (Tebes-Steves et al. 1998).

Modeling example

An example demonstrates the utility of CCBATCH for multicomponent systems involving biological degradation. The components and relevant reactions for the equilibrium system have been presented in previous sections. Kinetic parameters for the growth of *Pseudomonas fluorescens*, a species known to degrade citrate, are available in the literature (Joshi-Tope and Francis 1995) or through estimation (McCarty 1969) and presented in Table 2. For this example, we select a starting concentration of approximately 10^4 CFU/ml (1.0×10^{-8} M cells) and a starting citrate concentration of 10^{-4} M. We assume that adequate oxygen exists in the system and its transfer into aqueous solution is not rate limiting. The goal of the modeling example is to show the ability of CCBATCH to predict expected trends in a mixed metal/chelate system along the course of a simulated experiment.

We begin by considering a simplified system without the presence of the complexing metal, Fe^{3+}. We start with a base case trial at a fixed pH of 7 and with all forms of citrate considered to be equally degradable; in other words, for Eq. (3), $S=C_{Tcitrate}$. Figure 1 shows the concentration changes for the citrate, cells, and ammonia-nitrogen (panel a). Also shown are the H_2CO_3 released and oxygen and acidic hydrogen utilization for the same case (panel b). H_2CO_3 and O_2 are assumed to re-equilibrate with the atmosphere for this example, and pH is fixed; therefore, these figures show the amount of H_2CO_3 formed and O_2 and H^+ utilized in the biodegradation reaction rather than the dissolved concentrations of these components, which remain constant. Panel c shows how the substrate utilization and net cell growth rates change with time for this trial. This figure indicates that biodegradation of the citrate is complete in 10.8 hours. All the species affected by the biodegradation reaction follow the same pattern: a slow change at first, followed by a rapid change, and then a slow down again as the substrate is exhausted and cell decay dominates. This pattern is typical of batch systems (Monod 1946). The early slow phase occurs as the cells grow from an initially small inoculum to a concentration able to degrade the

Table 2. Kinetic biodegradation modeling parameters for the citrate/Fe(III) example

Parameter	Value	Source
$q_{\max,\text{citrate}}$ maximum rate of substrate utilization	1.369 mole citrate/mole cell-hour	Estimated following McCarty (1969)
Y, true yield	0.64 mole cells/mole citrate	Stoichiometry
$K_{s,\text{citrate}}$, Monod half maximum rate constant for citrate	3.0×10^{-6} mole/L	(Joshi-Tope and Francis 1995)
K_a, Monod half maximum rate constant for electron acceptor substrate (O_2)	6.25×10^{-6} mole oxygen/L	(Kinzelbach et al. 1991)
b, endogenous decay constant	0.05 day^{-1}	(Odencrantz 1992)

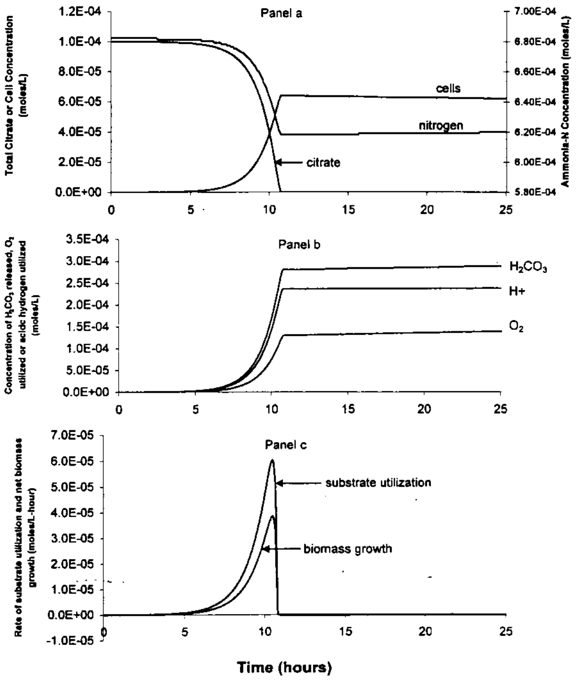

Figure 1. Model output for total concentration of citrate, cells, and ammonia-nitrogen (panel a); concentration of H_2CO_3 produced, and O_2 and H^+ utilized (panel b); and substrate utilization and biomass growth rates (panel c) for the base case at fixed pH 7 and with all citrate considered degradable.

substrate rapidly. When the substrate is driven to a low concentration, metabolism slows until the substrate is eliminated. In the long term, the cells slowly decay, utilizing oxygen and producing H_2CO_3 in the endogeneous decay reaction. Thus, CCBATCH captures the expected behavior of this simple biological system.

Next, we want to demonstrate CCBATCH's utility for considering the impact of speciation on biodegradation. We consider the case of either $HCit^{2-}$ or Cit^{3-} as the actively degraded species in a solution of citrate at a fixed pH of 7.0; again no Fe is considered for this trial. At pH 7, 5% of the citrate is found as $HCit^{2-}$, and 95% as Cit^{3-}. As Figure 2 panel a indicates, citrate loss in solution is more rapid when Cit^{3-} is degradable (complete in 10.8 hours) and slower when $HCit^{2-}$ is degradable (complete in 20 hours). Thus, full degradation of citrate takes longer when the degradable form is substantially less than the total citrate concentration. Once all the citrate is degraded, the total cell growth; ammonia, O_2, and acidic hydrogen utilized; and H_2CO_3 released are comparable for the two cases (data not shown). Figure 2 (panel b) shows the substrate utilization rate for the two situations. When Cit^{3-} is the degradable form, we would expect its higher initial concentration to lead to a faster rate of substrate utilization. The resulting higher concentration of cells would then increase the rate even further. The CCBATCH simulation captures this behavior, shown as a sharp rate peak for Cit^{3-} as the degradable form. When $HCit^{2-}$ is degradable, we would expect the initial lag phase that has a slow rate of substrate utilization to be extended. Fewer cells are grown initially, and the substrate concentration is much less than the total citrate. Again the CCBATCH simulation predicts this behavior with the utilization rate showing a more gradual rise and never reaching the rate predicted when the more abundant Cit^{3-} is degradable. Despite the slower rates when $HCit^{2-}$ is degradable, CCBATCH predicts all the citrate will be degraded, indicating that full mineralization is possible even when the degradable form makes up only 5% of the total substrate initially.

Since pH affects the concentration of different forms of citrate, we would expect biodegradation to depend significantly on pH if only certain citrate forms are available to the micro-organisms. CCBATCH should capture these predictable pH effects on the biological reactions. To evaluate this, we consider the case of $HCit^{2-}$ as the degradable form and with degradation taking place at a fixed pH of 6, 7 or 8. $HCit^{2-}$ makes up 37, 5 and less than 1% of the total

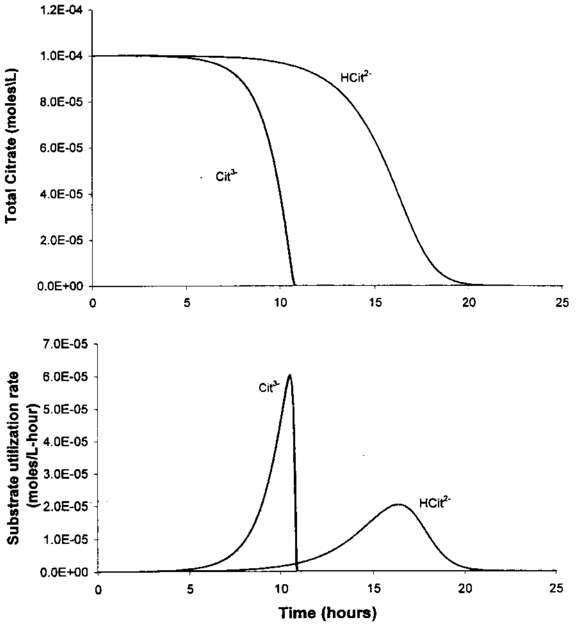

Figure 2. Loss of citrate (panel a) and substrate utilization rate (panel b) for the case with fixed pH 7 with $HCit^{2-}$ or Cit^{3-} as degradable substrate form.

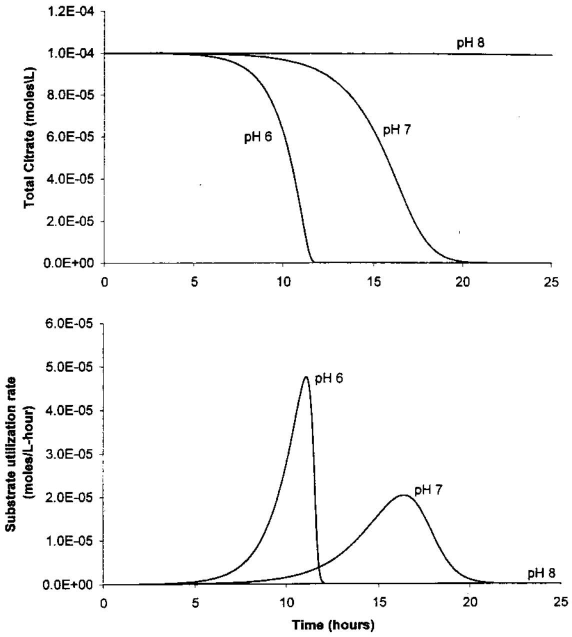

Figure 3. Loss of citrate (panel a) and substrate utilization rate (panel b) for the case where $HCit^{2-}$ is the degradable form at fixed pH 6, 7 and 8.

citrate at pH 6, 7 and 8, respectively. Figure 3 shows that the degradation is most rapid for pH 6 (complete at 12 hours) and slower for pH 7 (complete at 20 hours). However, for pH 8, degradation is only 7% complete by 50 hours. Likewise, Figure 3 (panel b) shows rapid utilization rates for pH 6 and 7, but no appreciable rate of substrate utilization or cell growth for pH 8. Whether, or not, the complete utilization of the substrate will eventually take place at pH 8 is largely controlled by the rate of endogenous decay. If the substrate utilization rate is high enough to sustain the cell population and the percentage of $HCit^{2-}$ remains low but constant, the cells will utilize the citrate eventually. If, however, the growth rate due to citrate utilization is less than the endogenous decay rate, and no other electron donor is available to the cells, the cell population will slowly decline, and undegraded citrate will persist in the system. For the conditions of this trial, the substrate utilization rate exceeds the cell decay rate, and eventual citrate mineralization is predicted.

The profound effect of speciation on the biological degradation of a substrate like citrate has been observed in mixed metal/citrate systems when pH is not controlled (Francis et al. 1992; Joshi-Tope and Francis 1995). Once again, to evaluate the predictive ability of CCBATCH in these systems, we consider a simulation where $HCit^{2-}$ is the degradable form of citrate. We begin with the pH at 6 or 7 and allow the model to predict the pH variation in response to the utilization of acidic hydrogen during the degradation reaction. Figure 4 shows the total concentration of citrate predicted during the variable-pH trials. The results for fixed pH 6, 7 and 8 are shown for comparison. For both variable-pH cases, complete citrate degradation is predicted, but it is delayed when compared to the fixed-pH case. When degradation begins at pH 6 and pH is not controlled, CCBATCH predicts complete degradation by 18 hours, which compares with 11 hours at fixed pH 6. Likewise when degradation begins at pH 7, it is complete by 27 hours when pH is allowed to vary, which compares with 20 hours at fixed pH 7. Figure 5 shows the substrate utilization rates and the predicted speciation for the systems starting at pH 6 and 7. The system behavior predicted by CCBATCH in Figure 5 again agrees with our intuitive understanding of the system. Initially, the $HCit^{2-}$ form is a much lower percentage of the total citrate for pH 7 (3%) compared with pH 6 (37%), and we would expect a slower substrate utilization rate for pH 7 runs compared to pH 6 runs. Both the variable-pH runs predict a more gradual rate increase than their fixed-pH counterparts, and the pH effect on citrate speciation

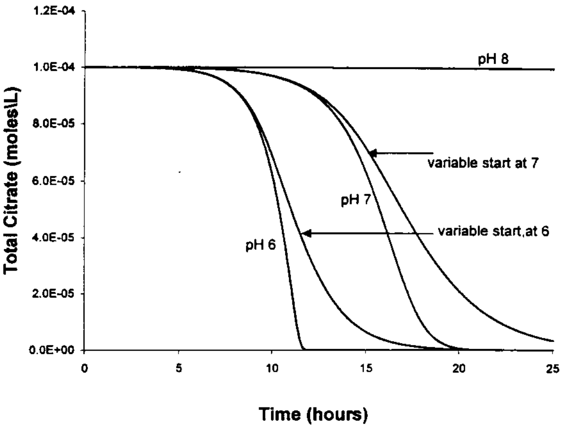

Figure 4. Total citrate concentration for the case where $HCit^{2-}$ is the degradable form and pH is allowed to vary. Results starting at pH 6 and 7 are shown along with results for the cases were pH is fixed at 6, 7 and 8.

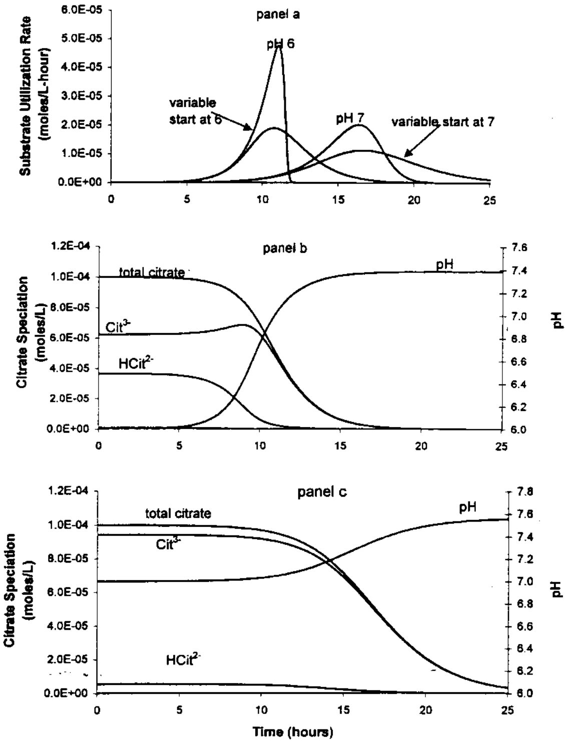

Figure 5. Substrate utilization rate (panel a) for case where $HCit^{2-}$ is the degradable form and pH is allowed to vary. Results starting at pH 6 and 7 are shown along with results for the cases where pH is fixed at 6 and 7. Panels b and c show speciation for pH starting at 6 and 7, respectively.

(bottom panels) is the cause. As the pH rises due to utilization of acidic hydrogen in the degradation of citrate, the percentage of $HCit^{2-}$ decreases in favor of the Cit^{3-} form. This is seen most clearly in Figure 5 (panel b), where the Cit^{3-} concentration shows a brief rise in concentration as the pH increases past 6.8, despite the fact that overall citrate is declining throughout the trial. In panels b and c, the $HCit^{2-}$ concentration declines faster than the decline in total citrate. Thus, we see that CCBATCH accurately captures trends we would expect in biodegradation of organic compounds with significant pH dependence.

The citrate degradation example becomes more complex when we consider the addition of iron to the system. As mentioned, the selection of iron for the example was made because of its well understood aqueous chemistry with citrate. Iron forms three complexes with citrate: FeCit, $FeOHCit^{-}$, and $Fe(OH)_2Cit^{2-}$. An equimolar Fe/citrate solution at 10^{-4} M and at pH 7 contains 92% of the citrate as $FeOHCit^{-}$, 7% as $Fe(OH)_2Cit^{2-}$, and less than 1% as the sum of all acid species. Therefore, in this system, if either $HCit^{2-}$ or Cit^{3-} is the biologically available form, degradation of citrate should be very slow. In fact, CCBATCH predicts negligible biodegradation rates for this case (results not shown).

Instead, if we consider the case in which the iron concentration is half the citrate concentration ($C_{T,Fe(III)} = 5 \times 10^{-5}$, and $C_{T,citrate} = 10^{-4}$), the initial equilibrium condition at pH 7 predicts 27% $FeOHCit^{-}$, 22% $Fe(OH)_2Cit^{2-}$, 3% $HCit^{2-}$, and 47% Cit^{3-}. In this case, we would expect to observe degradation with either $HCit^{2-}$ or Cit^{3-} as the degradable form, albeit with initially slower rates. Figure 6 shows the model predictions with and without the Fe when $HCit^{2-}$ is the degradable form. It also shows results when iron is present and either $FeOHCit^{-}$ or $Fe(OH)_2Cit^{2-}$ is degradable. CCBATCH predicts citrate degradation in the presence of iron to be incomplete at 50 hours when $HCit^{2-}$ is the degradable form, even though complete degradation within 20 hours is predicted when Fe is absent. Instead of simply slowing the loss rate with eventual removal of the citrate as we might expect, CCBATCH predicts that the presence of Fe^{3+} will stop the degradation of citrate when $HCit^{2-}$ is the degradable form, even though ample citrate-degrading organisms are present and adequate citrate concentrations remain.

A closer look at the citrate speciation in the system (Figure 7) provides a possible explanation for the persistence of citrate in the system if $HCit^{2-}$ is assumed to be the degradable form. While the initial speciation has 3% $HCit^{2-}$ (a percentage of degradable form

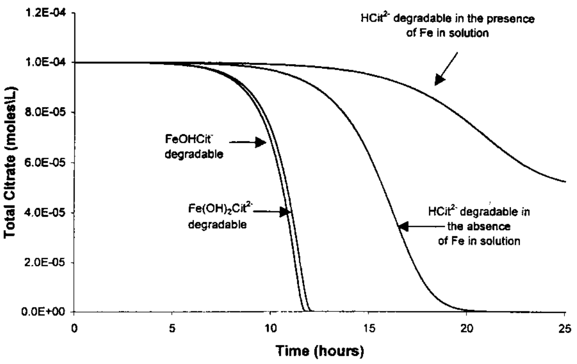

Figure 6. Total citrate for the case where $HCit^{2-}$ or $FeOHCit^{-}$ or $Fe(OH)_2Cit^{2-}$ are the degradable form for citrate degradation in the presence of a complexing Fe^{3+} concentration equal to half the starting citrate concentration (fixed pH 7). The case where $HCit^{2-}$ is the degradable form in the absence of Fe^{3+} is shown for comparison.

that can support growth and complete degradation as shown in Figure 2 previously), degradation reduces the total citrate concentration, and the ratio of total citrate to total iron in the solution changes. As the total concentration of citrate approaches 5×10^{-5}, or equimolar with the iron (at approximately 25 hours), the citrate speciation is 52% $FeOHCit^{-}$, 43% $Fe(OH)_2Cit^{2-}$, 5% Cit^{3-}, and less than 1% $HCit^{2-}$. This low concentration of degradable form produces a very low substrate utilization rate. Experimentally, this effect has been observed by Joshi-Tope and Francis (1995) who report citrate degradation in some metal citrate systems (U, Cu, Fe(II)) initially, followed by no degradation once the metal concentration ratio in solution reaches 1 : 1. For the model system, when either $FeOHCit^{-}$ or $Fe(OH)_2Cit^{2-}$ is degradable, citrate degradation is predicted to be rapid and complete by 12 hours (Figure 6) with no residual citrate remaining in the solution. The speciation (Figure 7) shows that the degradable species always remains the dominant form of the citrate in this case, and thus, degradation goes to completion.

Model verification and limitations

The modeling example above shows the potential for CCBATCH to be used in conjunction with experimentation to provide insight into the behavior of complex mixtures of metals and organic compounds undergoing biotransformation reactions. Verification that the model is predictive under a variety of experimental conditions has been demonstrated elsewhere (VanBriesen, 1998; VanBriesen et al. 1999a, b, c, d; Banaszak et al. 1996, 1997a, b, 1998a, b, 1999; Willett et al. 1999). In addition to verifying that the model results are predictive of experimental behavior, these researchers demonstrate the use of CCBATCH to explore hypotheses suggested by experimental results.

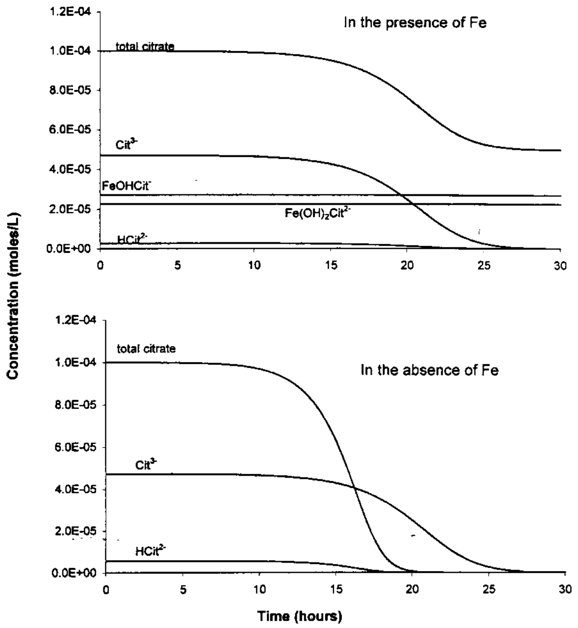

Figure 7. Citrate speciation for fixed pH 7 for citrate degradation in the presence of a complexing Fe^{3+} concentration equal to half the starting citrate concentration. The case in the absence of Fe^{3+} is shown for comparison.

VanBriesen et al. (1999a, b) evaluate the experimental results of Bolton et al. (1996) for NTA degradation in the absence of heavy metals. Their analysis demonstrates that the persistence of dissolved organic carbon during the degradation of NTA by *Chelatobacter heintzii* (observed by Bolton et al. 1996 and Quinn 1996) is related to the formation of intermediates in the NTA degradation pathway rather than to the production of cell decay by-products. These intermediates sequester carbon and reduce the expected cell yield and CO_2 production. VanBriesen et al. (1999b) couple model predictions to experimental work and determine the stoichiometry representative of the full mineralization of NTA stepwise through its intermediates, imminodiacetic acid (IDA), glycine and glyoxylate. Monod kinetic parameters for *C. heintzii* utilization of NTA and its degradation intermediates were developed by fitting model simulations to experimental results. Model simulations of the concentration of unmeasured intermediates allowed VanBriesen et al. (1999a) to conclude NTA degradation in this system resulted in the transient formation of IDA and the persistence of glyoxyate following removal of NTA from solution. VanBriesen et al. (1997, 1999d) further analyzed NTA degradation in growing systems under nutrient limited conditions (Quinn 1996) and again identified the formation of intermediates as the cause of the low cell yield observed.

VanBriesen et al. (1999c) considered the results of Bolton et al. (1996) and Bolton and Girvin (1996) related to the degradation of NTA in the presence of metals and at different pHs. The experimental results show different rates and extent of degradation of NTA systems containing equimolar NTA and Mg, Ca, Al, Fe, Co, Ni, Cu and Zn, and at different fixed pHs. CCBATCH was able to predict the observed behavior in all these systems using the kinetic parameters and stoichiometry determined by VanBriesen et al. (1999b) from an independent experimental system. The coupling of the modeling with the experimental results allowed VanBriesen et al. (1999c) to identify the rate-controlling, transportable form of NTA.

Banaszak et al. (1997b, 1998a) present experimental and modeling analysis for the degradation of NTA in the presence of neptunium and plutonium. CCBATCH was utilized to differentiate between growth inhibition due to actinide toxicity and decreased substrate utilization caused by chemical speciation effects. For neptunium, the model accurately accounted for aqueous speciation during chelate degradation under uncontrolled pH conditions, chemical toxicity of the radionuclide, and formation of a Np-phosphate precipitate. For plutonium, coupled experimental and modeling work suggests chemical speciation effects may contribute to slower NTA degradation, but Pu radiotoxicity also plays a role in controlling residual NTA concentrations. Banaszak et al. (1998b) further explore the interconnected reactions of radiological and chemical toxicity, precipitation, and biodegradation in the neptunium/NTA/*C. heintzii* system. They were able to link speciation changes predicted by CCBATCH during NTA degradation to Np-carbonate complex formation demonstrated by VIS–NIR adsorption spectra.

Additional verification work has been done in the citrate system with *Pseudomonas fluorescens* using the data of Francis et al. (1992) and Joshi-Tope and Francis (1995) by Banaszak et al. (1996, 1997a, 1999) and in the EDTA system with BNC1 using the data of Nortemann (1992), Henneken et al. (1995), and Kluner et al. (1998) by Willett et al. (1999). These results confirm the utility of CCBATCH to predict chemical speciation and its interaction with biological degradation for a variety of systems.

As with any modeling tool, correct usage of CCBATCH requires attention to its many capabilities (demonstrated in section 3.0 and more fully exemplified in the additional works cited above) and to its limitations. There are three kinds of model limitations we consider: those related to the numerical solution techniques employed, those related to the complexity the model attempts to capture, and those related to the development stage of the model.

The coupling of equilibrium and kinetic effects achieved in CCBATCH requires iterating between solution to the system of mass balance equations that define the equilibrium speciation and solution to the dual-Monod formulated kinetic biodegradation equation. Solution of the system of mass balance equations is based on the Newton-Raphson method (Press et al. 1992) that has previously been implemented for batch chemical speciation modeling (Westall et al. 1976; Allison et al. 1991; Parkhurst et al. 1980; Bethke 1992). The numerical method is an excellent way to identify the root of a system of non-linear equations when the initial guess values are “in the neighborhood” of the solution (Press et al. 1992). The Newton-Raphson method is well known for its exceptional local convergence and poor global convergence properties. Thus, success with the method depends on the user-input starting values for the aqueous concentration of the components. Since convergence to an appropriate solution is enhanced by selecting an appropriate initial ‘guess’ for the solver, CCBATCH includes a separate routine for improving the initial guess sent to the solver routine. If the numerical solution method does not converge on a solution with the user-input starting values, this routine successively (and systematically) changes the initial guess until a solution is found for the first speciation. Subsequent calls to the speciation routine use the solution for the previous timestep as a guess value and therefore converge more quickly and rarely require the successive approximation routine.

Another common limitation of the Newton-Raphson approach is related to its rapid convergence. Near the solution, the number of significant digits approximately doubles with each step. This causes the method to have a high sensitivity to round-off errors if the elements of the matrix are of very different magnitudes. CCBATCH includes a routine to scale the system of mass balance equations to improve the accuracy of the solution by making sure all the elements of the matrix to be solved are of the same order of magnitude (Meintjes and Morgan 1985). Additional details of the numerical procedures used in CCBATCH are provided in Tebes -Stevens and Valocchi (1998).

Unlike the equilibrium solution, the dual-Monod formulation to represent biodegradation does not have significant numerical limitations since it is a single algebraic equation. Once it is solved for the rate of substrate utilization, all other rates are predicted based on the stoichiometry of the degradation reaction in simple algebraic equations.

In addition to the limitations typical of computer-based equilibrium solutions, there are also limitations introduced by the complexity of the system we are trying to model. Chelate/metal systems involve the formation of many aqueous complexes. As with all equilibrium solvers, equilibrium formation and dissociation constants must be known for all chemical complexes that may form in the system. While many equilibrium constants are known (Martell and Smith 1974–1989; NIST 1997), chelate systems require an unusually large number of these constants and, in some cases, include complexes for which reliable constants do not exist. Since complexation constants involve a degree of uncertainty, Tebes-Steves and Valocchi (1998) evaluated the sensitivity of their reactive transport code, FEREACT, to variability in the equilibrium constants. FEREACT and CCBATCH share the same equilibrium solver, thus the results of this analysis are relevant for CCBATCH. Tebes-Steves and Valocchi (1998) found that the solver is sensitive to equilibrium complexation constants but the sensitivity was limited to a few key complexes in each system they evaluated. This analysis suggests that improved model predictions can be attained by validating a limited subset of the equilibrium constants in a system.

As with the equilibrium modeling, the biological modeling requires key kinetic constants to be provided by the user. With simple first order kinetics and an interest only in the loss of the primary substrate in solution, a single constant would be needed. However, CCBATCH encompasses the more complicated and predictive dual-Monod methodology, and tracks all chemicals affected the the biological reactions. Here the complexity we are trying to capture requires additional model input and thus is a limitation we accept in order to achieve improved predictions and additional insight into system behavior. For each biological reaction involving a distinct microbial species growing on a distinct carbon-source-electron-donor substrate, a full stoichiometry for the degradation must be developed as a model input. McCarty (1969, 1971, 1972a, b, 1975) details a methodology for developing stoichiometric biological reactions when the electron-donor is mineralized. VanBriesen and Rittmann (1999b) discuss development of stoichiometric equations when the primary electron donor substrate is not mineralized but results in the production of intermediates that are then available for subsequent degradation, The Monod parameters, K_s, K_A, q, and b (e.g. Table 2) are also required to adequately represent the degradation kinetics. These parameters may be available in the literature; however, they can be highly dependent on system conditions, previous growth conditions for the organism, and even, as suggested here for citrate, on the aqueous chemical form of the substrate. Thus, determination of these parameters is not trivial, and they are required a priori in the modeling. This limitation can be overcome by using CCBATCH to fit appropriate kinetic parameters from within literature reported ranges (as demonstrated by VanBriesen et al. 1999b). This requires additional experimental results independent of those under study.

CCBATCH is a developing model and as such has some limitations that do not represent inherent numerical or complexity limits, but rather reflect that it continues to be improved and expanded. Presently, CCBATCH considers only reactions in batch (non-transport) systems. This reflects its main development goal and present implementation for use in experimental batch systems. This limitation can be overcome by using the FEREACT model that includes a limited version of the CCBATCH biodegradation and coupling routines (Tebes-Steves et al. 1998). However, implementation of the full complexity of microbial modeling handled by CCBATCH within FEREACT results in severe time investments for the model simulations. This is due, in part, to CCBATCH modeling being limited to a single user-selected time step size for each simulation rather than implementation of a time-step splitting methodology. Addition of this methodology into the coupled CCBATCH/FEREACT code is under consideration. Likewise, presently the model considers all reactions other then biodegradation to be equilibrium-controlled. Additional kinetic routines including precipitation and sorption are currently under development.

Conclusions

Predicting the chemical and biological behavior of complex mixtures of contaminants requires careful consideration of the interactions between chemistry and biology. In this paper, we introduce a model, CCBATCH, designed to couple biological degradation reactions with chemical equilibrium reactions in experimental batch systems. CCBATCH utilizes an equilibrium formulation for acid/base and complexation reactions in aqueous solution. It uses a dual-Monod kinetic model for biological reactions and links the effects of biological reactions to the equilibrium reactions by using stoichiometry to determine changes in all component concentrations. The proton condition and its implementation are essential for accurately linking acid/base effects of kinetically controlled reactions to the equilibrium pH.

CCBATCH is specifically designed to capture pH and speciation effects on biological reactions, and this capability is demonstrated by a series of examples in a model system. The substrate available to the bacteria may be all or only some chemical forms of a degradable component, leading to different rates of degradation at different fixed pHs. When the degradable form is abundant as a percentage of the total substrate, we expect degradation to be rapid. However, when the percentage of the degradable substrate is low due to complexation or acid/base speciation, we expect degradation rates may be slow despite high concentrations of total substrate. As demonstrated here, CCBATCH accurately predicts these expected effects of pH on biological chelate degradation reactions. Furthermore, when pH is not fixed in a system, it can change during the course of degradation due to utilization or production of acidic hydrogen in the biological reactions of substrate utilization and cell decay. This change in pH may increase or decrease the degradable substrate concentration as a percentage of the total substrate. The effect of these speciation changes on biodegradation is captured in CCBATCH, and thus may offer an explanation for systems where biodegradation begins rapidly and then stops before complete substrate utilization.

Acknowledgments

This research was funded by the U.S. Department of Energy. The continued support of Dr. Frank Wobber is gratefully acknowledged.

References

Allison JD, Brown DS & Novo-Gradac KJ (1991) MINTEQA2/PRODEFA2, A geochemical assessment model for environmental systems: Version 3.0 User's Manual. EPA/600/3-91/021 U.S. Environmental Protection Agency, Athens, GA.

Bae W & BE Rittmann (1996) A structured model of dual-limitation kinetics. Biotechnol. Bioeng. 49: 683–689.

Baer JM (1990) Kinetically influenced terms for solute transport affected by heterogeneous and homogeneous classical reactions. Water Resour. Res. 26(1): 21–34.

Banaszak JE, VanBriesen JM, Rittmann BE, Joshi-Tope G & Francis AJ (1996) Speciation dependent degradation of citrate by *Pseudomonas fluorescens*. Presented at the 19th Annual Environmental Chemistry Workshop, Purdue University, 12–13 October 1996.

Banaszak JE, VanBriesen JM & Rittmann BE in collaboration with Joshi-Tope G & Francis AJ (1997a) Mathematical modeling of speciation dependent biodegradation. Presented at Mathematical Issues in Bioremediation, Los Alamos National Laboratory, 11–13 June 1997.

Banaszak JE, VanBriesen JM, Rittmann BE & Reed DT (1997b) Effects of aerobic and anaerobic chelate biodegradation on actinide speciation. Presented at MIGRATION '97, Sixth International Conference on the Chemistry and Migration Behavior of Actinides and Fission Products in the Geosphere, Sendai, Japan, 26–31 October 1997.

Banaszak JE, VanBriesen JM, Rittmann BE & Reed DT (1998a) Mathematical modeling of the effects of aerobic and anaerobic chelate biodegradation on actinide speciation, Radiochimica Acta 82: 445–451.

Banaszak JE, Reed DT & Rittmann BE (1998b) Speciation-dependent toxicity of neptunium(V) toward *Chelatobacter heintzii*. Environ. Sci. Technol. 32: 1085–1091.

Banaszak JE, Krause T, VanBriesen JM, Rittmann BE, Joshi-Tope G, Francis AJ & Reed DT (1999) Experimental and modeling determination of the form of citric acid degraded by *Pseudomonas fluorescens* (in preparation).

Bethke CM (1994) The geochemist's workbench, a user's guide to Rxn, Act 2, Tact, React & Ttplot. University of Illinois.

Bolton H Jr & Girvin DC (1996) Effect of adsorption on the biodegradation of nitrilotriacetate by *Chelatobacter heintzii*. Environ. Sci. Technol. 30: 2057–2065.

Bolton H Jr, Girvin C, Plymale AE, Harvey SD & Workman DJ (1996) Degradation of metal-nitrilotriacetate (NTA) complexes by *Chelatobacter heintzii*. Environ. Sci. Technol. 30(3): 931–938.

Cherry RS & Thompson DN (1997) Shift from growth to nutrient-limited maintenance kinetics during biofilter acclimation. Biotech. Bioeng. 56: 330–339.

DeSilva VJ & Rittmann BE (1998) Interpreting the response to loading changes in a mixed culture CSTR, Water Environ. Res. (forthcoming).

Engesgaard P. & Kipp KK (1992) A geochemical transport model for redox-controlled movement of mineral fronts in groundwater flow systems: a case of nitrate removal by oxidation of pyrite. Water Resour. Res. 28(10): 2829–2843.

Francis AJ, Dodge CJ & Gillow JB (1992) Biodegradation of metal citrate complexes and implications for toxic metal mobility. Nature 356(6365): 140–142.

Hamm RE, Shull CMJ & Grant DM (1954) Citrate complexes with iron(II) and iron(III). J. Am. Chem. Soc. 76: 2111–2114.

Horn H & Hempel DC (1997) Growth and decay in an auto-/heterotrophic biofilm. Water Research 31(9): 2243–2252.

Henneken LB, Nortemann B & Hempel DC (1995) Influence of physiological conditions on EDTA degradation. Appl. Microbiol. Biotechnol. 44: 190–197.

Joshi-Tope G & Francis AJ (1995) Mechanisms of biodegradation of metal-citrate complexes by *Pseudomonas fluorescens*. J. Bacteriol. 177(8): 1989–1993.

Kinzelbach W, Schafer W & Herzer J (1991) Numerical modeling of natural and enhanced denitrification processes in aquifers. Water Resour. Res. 27(6): 1123–1135.

Kluner T, Hempel DC & Nortemann B (1998) Metabolism of EDTA and its metal chelates by whole cells and cell-free extracts of strain BNC1. Appl. Microbiol. Biotechnol. 49: 194–201.

Lauff JJ, Bernie Steele D, Coogan LA & Breitfeller JM (1990) Degradation of the ferric chelate of EDTA by a pure culture of an *Agrobacterium* sp. Appl. Environ. Microbiol. 56(11): 3346–3353.

Lichtner PC (1985) Continuum model for simultaneous chemical reactions and mass transport in hydrothermal systems. Geochemica Cosmochim Acta 49: 779–800.

Lichtner PC (1996) Continuum formulation of multi-component-multiphase reactive transport. Reviews in minerology, Vol 34: Reactive transport in porous media, PC Steefel, CI Oelkers & EH Lichtner (eds.) 1. Minerological Society of America.

Lovley DR & Phillip EJP (1988) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. 54: 1472–1480.

McCarty PL (1969) Energetics and bacterial growth. The Fifth Rudolf Research Conference.

McCarty PL (1971) Energetics and bacterial growth. Organic compounds in aquatic environments. SD & Hunter JV Faust (eds). Marcel Dekker, New York.

McCarty PL (1972a) Energetics of organic matter degradation. Water pollution microbiology. Ralph Mitchell (ed.). Wiley-Interscience, New York.

McCarty PL (1972b) Stoichiometry of biological reactions. Paper presented at the International Conference Toward a Unified Concept of Biological Waste Treatment Design.

McCarty PL (1975) Stoichiometry of biological reactions. Progress in Water Technol. 7: 157–172.

Martell AE & Smith RM (1974–1989) Critical stability constants. Plenum Press, New York.

Meintjes K & Morgan A (1985) A methodology for solving chemical equilibrium systems, General Motor Research Laboratories Research Publication No. GMR-4971.

Monod J (1946) The growth of bacterial cultures. Ann. Rev. Microbiol. 3: 371–394.

Morel F & Morgan J (1972) A numerical method for computing equilibria in aqueous chemical systems. Environ. Sci. Technol. 6(1): 58–67.

Morel FMM & Hering JG (1993) Principles and applications of aquatic chemistry. John Wiley, New York.

NIST Critically Selected Stability Constants of Metal Complexes Database, Version 4.0. (1997). Smith RM & Martell AF (collected and selected data); RJ Motekaitis (developed program). NIST: Standard Reference Data Program: Gaithersburg, MD.

Nortemann B (1992) Total Degradation of EDTA by mixed cultures and a bacterial isolate. Appl. Environ. Microbiol. 58(2): 671–676.

Nowack B, Xe H & Sigg L (1997) Influence of natural and anthropogenic ligands on metal transport during infiltration of river water to groundwater. Environ. Sci. Technol. 31: 866–872.

Odencrantz JE (1992) Modeling the biodegradation kinetics of dissolved organic contaminants in saturated heterogeneous two-dimensional aquifer. Ph.D dissertation, University of Illinois.

Palumbo AV, Lee SY & Boerman P (1994) The effect of media composition on EDTA degradation by *Agrobacterium* sp. Appl. Biochem. Biotechnol. 45/46: 811–822.

Parkhurst D, Thorstenson DC & Plummer LN (1980) PHREEQE – a computer program for geochemical calculations: U.S Geological Survey Water Resources Investigations 80–96. National Technical Information Services Report PB81-167801, Springfield, VA.

Pirt SJ (1965) The maintenance energy of bacteria in growing cultures. Proc. Roy. Soc. Lond. 163B: 224–231.

Pohlmeier A & Knoche W (1996) Kinetics of the complexation of Al^{3+} with aminoacids, IDA, and NTA. Inter. J. Chem. Kinet. 28: 125–136.

Press, WH, Teukolsky SA, Vettering WT & Flannery BP (1992) Numerical recipes in FORTRAN: the art of scientific computing. Cambridge University Press, New York.

Quinn JW (1996) Monitoring reactants and products of the aerobic degradation of nitrilotriacetic acid by *Chelatobacter heintzii* Strain ATCC 29600. Northwestern University Master Research Project Report.

Rittmann BE & VanBriesen JM (1996) Microbiological processes in reactive modeling. Reviews in mineralogy, Vol. 34: Reactive transport in porous media. PC Teefel, CI & Oclkers EH Lichtner (eds.), 311 Mineralogical Society of America.

Steefel CI & MacQuarrie KTB (1996) Approaches to modeling of reactive transport in porous media. Reviews in mineralogy, Vol. 34: Reactive transport in porous media. P. C Steefel CI & Oelkers EH Lichtner (eds.), Mineralogical Society of America.

Stumm W & Morgan JJ (1996) Aquatic chemistry, 3rd edn. Wiley Interscience, New York.

Tebes-Stevens C, Valocchi AJ, VanBriesen JM & Rittmann, BE (1998) Multicomponent transport with coupled geochemical and microbiological reactions: model description and example simulations. J. Hydrology 209: 8–26.

Tebes-Stevens C & Valocchi AJ (1998) Numerical solution techniques for reaction parameter sensitivity coefficients in multicomponent subsurface transport models. Hydraulic Engineering Studies, No. 59. University of Illinois at Urbana-Champaign, Urbana, IL.

Valocchi AJ & Malmstead M (1992) A note on the accuracy of operator splitting for advection-dispersion-reaction problems. Water Resour. Res. 28(5): 1471–1476.

VanBriesen JM, Banaszak JF, Quinn J, Reed DT & Rittmann BF (1997) A systematic study of coupled chemical and biological reactions in the aerobic degradation of nitrilotriacetic acid by *Chelatobacter heintzii*. Presented at the Symposium on the Influence of Coupled Processes on Contaminant Fate and Transport at the Soil Science Society of America Annual Meeting, Anaheim CA, 26–30 October 1997.

VanBriesen JM (1998) Modeling coupled biogeochemical processes in mixed waste systems, Ph.D dissertation. Northwestern University.

VanBriesen, JM & Rittmann BE (1998) Mathematical description of microbiological reactions involving intermediates. Biotech. Bioeng. (in press).

VanBriesen JM, Rittmann BE, Bolton Jr H & Girvin DC (1999a) Intermediate formation in the biodegradation of nitrilotriacetic acid by *Chelatobacter heintzii*. Submitted to Environ. Sci. Technol.

VanBriesen JM, Rittmann BE, Bolton Jr H & Girvin DC (1999b) Kinetic parameters for biodegradation of nitrilotriacetic acid by *Chelatobacter heintzii*. Submitted to Environ. Sci. Technol.

VanBriesen JM, Rittmann BE, Bolton Jr H & Girvin DC (1999c) The rate-controlling substrate form for the biodegradation of nitrilotriacetic acid by *Chelatobacter heintzii* Submitted to Environ. Sci. Technol.

VanBriesen JM, Quinn J, Reed D & Rittmann BE (1999d) NTA degradation intermediate formation under growth conditions with *Chelatobacter heintzii*. (in preparation).

Van Uden N (1967) Transport-limited growth in the chemostat and its competitive inhibition: a theoretical treatment. Arcive Für Mikrobioiogie 58: 145–154.

Westall JC, Zachary JL & Morel FMM (1976) MINEQL, a computer program for the calculation of chemical equilibrium, 91pp. Hydrodynamics Laboratory Technical Note 18, MIT, Cambridge, MA.

Widdowson MA, Motz FJ & Benefield LD (1988) A numerical model for oxygen an nitrate-based respiration linked to substrate and nutrient availability in porous media. Water Resour. Res. 24(9): 1553–1565.

Willett A, VanBriesen JM & Rittmann BE (1999) Modeling EDTA degradation by BNC1 (in preparation).

Xue H, Sigg, L & Kau FG (1995) Speciation of EDTA in natural waters: exchange kinetics of FeEDTA river water. Environ. Sci. Technol. 29: 59–68.

Yeh GT & Tripathi VS (1989) A critical evaluation of recent developments in hydrogeochemical transport models for reactive multichemical components. Water Resour. Res. 25(1): 93–108.