Biodegradation **14:** 105–121, 2003.

Slow complexation kinetics for ferric iron and EDTA complexes make EDTA non-biodegradable

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Accepted 1 January 2003

Key words: aerobic, biodegradation, biogeochemical modeling, BNC1, EDTA

Abstract

Published experimental data on ethylenediaminetetraacetic acid (EDTA) biodegradation in the presence of ferric iron (Fe(III)) showed that rapid biodegradation of EDTA suddenly stopped, leaving a residual of unbiodegraded EDTA that was equal to the concentration of dissolved Fe(III). We hypothesize that slow kinetics for the dissociation of two iron-EDTA complexes - $FeEDTA^-$ and $FeOHEDTA^{2-}$ – sequestered the EDTA in a form that is biologically unavailable. To evaluate this hypothesis, we added to the biogeochemical model CCBATCH a new sub-model for kinetically controlled complexation. CCBATCH simulations with kinetically controlled complexation for $FeEDTA^-$ and $FeOHEDTA^{2-}$ and the observed concentration of total dissolved Fe(III) accurately predicted the sudden cessation of EDTA biodegradation at the exact time shown experimentally. Our simulations also correctly predicted the observed residual EDTA concentration and the amounts of biomass and NH_4^+. Alternate explanations for the experimental results – strong equilibrium complexation of ferric iron and EDTA and precipitation of calcium and magnesium solids – could not capture the observed trends. This analysis using CCBATCH's new sub-model for kinetically controlled complexation shows that EDTA, once it becomes complexed with Fe(III), becomes biologically unavailable.

Abbreviations: EDTA – ethylenediaminetetracetic acid; M – molarity, mole/liter; NTA – nitrilotriacetic acid; atm – atmosphere; K – Kelvin

Introduction

Ethylenediaminetetraacetic acid (EDTA) is a synthetic, organic, polyprotic aminocarboxylic acid with the ability to form strong, water-soluble complexes with a variety of metal cations (Stumm & Morgan 1996). Metal-EDTA complexes are formed when electron donor atoms on EDTA, such as the amino nitrogen and carboxyl oxygen, form coordinate, electrostatic bonds with metal cations, which serve as electron acceptors. EDTA is referred to as a chelating agent (from the Greek work *chele*, or "claw"), because EDTA tightly wraps around the metal ion. In the United States, EDTA has been commercially produced since 1948 (Potos 1965) and is the mostly widely used chelating agent (Kroschwitz 1993). EDTA is used in a variety of industrial processes, as well as in consumer and food products, because it prevents the negative effects free-metal ions have on products or processes. Additionally, EDTA was extensively used by the United States government for over 50 years to control the concentrations of radionuclides and heavy metals in nuclear weapons manufacturing and material processing (Riley & Zachara 1992).

With its wide use, significant quantities of EDTA-containing waste have been discharged to the environment. EDTA is often not removed by biological treatment in typical wastewater treatment facilities (Alder

et al. 1990; Pavlostathis & Morrison 1994; Hinck et al. 1997) and, once in the environment, it seems to persist. For example, EDTA is the synthetic organic chemical with the highest concentration in one part of the Rhine river in Europe (Haberer 1991), and it has been used as an indicator for the presence of wastewater effluent in ground water (Fujita et al. 1996). However, the greatest EDTA contamination occurs at sites where nuclear weapons were manufactured (Riley & Zachara 1992).

Although EDTA is not toxic to humans, its persistence in the environment is a concern. EDTA is implicated in the mobilization and transport of radionuclides and metal ions from disposal sites (Toste et al. 1983), and its presence in surface waters could remobilize heavy metals from contaminated sediments (Nowack et al. 2001).

Although EDTA was once thought recalcitrant to biodegradation, an EDTA-degrading microorganism named BNC1 (German Collection of Microorganisms and Cell Cultures - DSM - 6780) was isolated by Nörtemann (1992) from an EDTA-degrading mixed culture. The other member of the mixed culture was a non-EDTA degrading, morphologically different companion organism. The growth rate of the mixed culture on EDTA was greater than that of BNC1 alone, but the role of the companion organism is not known. BNC1 is an aerobic, Gram-negative member of the α-subclass of Proteobacteria (Nörtemann 1992) and can use EDTA as its sole carbon, nitrogen, and energy source (Henneken et al. 1995, 1998; Klüner et al. 1998).

Several studies with pure BNC1, or the mixed culture with 80% of the cells identified as BNC1, showed that EDTA biodegradation depends on the amounts and types of metal cations present in the culture medium. BNC1 cannot transport EDTA into the cell when it is complexed with Fe(III), Co(II), Cd, Pb, Ni, or Cu(II), but $MgEDTA^{2-}$ and $CaEDTA^{2-}$ are able to be taken up and the EDTA metabolized (Henneken et al. 1995, 1998; Klüner et al. 1998). For EDTA biodegradation to occur, the resistant complexes must disassociate, so that $CaEDTA^{2-}$ and/or $MgEDTA^{2-}$ complexes can form. Thus, the presence or absence of a particular complex of EDTA can greatly affect its biodegradation rate, as some forms are biologically active, while others are not. This phenomenon occurs during biodegradation of a related chelating agent, nitrilotriacetic acid (NTA), by *Chelatobacter heintzii*. Experimental (Bolton et al. 1996) and modeling (VanBriesen et al. 2000) studies showed that the NTA biodegradation was controlled by the concentration of $CaNTA^{-}$. Therefore, the key issue for predicting the fate of EDTA in the environment is to understand EDTA's chemical speciation.

The primary objective of this paper is to show that the kinetically controlled release of EDTA from $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ complexes hinders EDTA biodegradation. Specifically, it is the slow dissociation rate of these complexes that causes EDTA to be sequestered as non-biologically available Fe(III) complexes. The result is incomplete EDTA biodegradation when Fe(III) is present. Our approach is to use computer simulations of experimental data from Henneken et al. (1995, 1998) for EDTA biodegradation with the BNC1 mixed culture to test this hypothesis, as well as the hypothesis that the high equilibrium constants for the formation of $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ prevent EDTA from biodegraded. To achieve these objectives, we expanded the biogeochemical model CCBATCH to include kinetically controlled complexation.

Description of experiments from Henneken et al. (1995, 1998)

Henneken et al. (1995, 1998) report experimental results for EDTA biodegradation by the mixed BNC1 culture. The experiments were performed in the dark in 6-liter, stirred, batch reactors containing EDTA, culture broth, and the mixed culture. Eighty percent of the mixed culture cells were BNC1, and other species in the culture could not degrade EDTA, although it may support BNC1's metabolism of EDTA (Nörtemann 1992). The initial total EDTA concentration was 3.81×10^{-3} M as measured by high-performance liquid chromatography on acidified samples. The mineral medium contained millimolar amounts of Ca, Mg, Fe(III), and K and micromolar amounts of Na, Zn, Co(II), Ni, Cu(II), Mn(II), phosphate, and sulfate. The initial total biomass concentration was 0.025 g cell dry weight/L. During the experiment, biomass was monitored by measuring the culture broth absorbance at 546 nm, which was previously correlated to g cell dry weight. The culture broth pH was maintained by the automatic addition of H_2SO_4 or NaOH by a pH controller. The cultures were incubated at 35 °C and sparged with air providing a dissolved oxygen concentration of greater than 90% saturation (around 6.1 mg/L at 35 °C) throughout the experiment. The ammonium concentration in acidified samples

was measured spectrophotometrically throughout the experiment.

The experimental data show accelerating degradation of EDTA concurrent with biomass and ammonium production until 66 hours. At 66 hours and a total EDTA concentration of 0.16 mM, the EDTA removal rate suddenly became zero, and biomass started to decrease. Although this residual EDTA is only 4% by mass of the starting EDTA concentration, its resistance to biodegradation is significant for two reasons. First, concentrations of EDTA in the environment are in the 10^{-5} M or lower range (Cleveland & Rees 1981; Barber et al. 1997; Fujita et al. 1996), making 0.16 mM a significant amount to remain not biodegraded. Second, and most relevant to this work, is that the biodegradation of EDTA abruptly ceased.

Model description

CCBATCH (Rittmann & VanBriesen 1996; VanBriesen & Rittmann 1999) is a biogeochemical computer model that comprehensively couples microbial reactions with aqueous geochemistry in batch systems. It predicts the effects of equilibrium aqueous speciation on kinetically controlled biodegradation and the effects of biodegradation on the aqueous speciation. Please refer to the Appendix for a tabulation of equations.

Equilibrium reactions

Most complexation and all acid/base reactions rapidly reach thermodynamic equilibrium in aqueous solutions and are mathematically described in CCBATCH using traditional multicomponent equilibrium modeling techniques (Morel & Morgan 1972). To solve the equilibrium speciation problem, a set of chemical components is selected and used to form all possible equilibrium complexes and acid/base species. For the EDTA culture medium of Henneken et al. (1995, 1998), the chemical components are: EDTA (as the primary electron donor and carbon source), BNC1 mixed culture (represented as $C_5H_7O_2N$ following Rittmann & McCarty (2001)), H^+, $H_2CO_3^*$, O_2, NH_4^+, Fe(III), Ca, Mg, Na, Zn, K, Co(II), Ni, Cu(II), Mn(II), SO_4^{2-}, and PO_4^{3-}. As shown in (1), an equilibrium mass-action expression is written for each equilibrium complexation reaction (j) using critically-reviewed values for equilibrium constants (NIST 1997). In (1) and the following text, M refers to molarity in moles/liter.

$$\prod_i \left(\frac{\{a\}_i}{\gamma_i}\right)^{\nu_i} = \beta_j^c \quad \frac{\{a_i\}}{\gamma_i} = \frac{[c_i]}{([c_0] = 1)} \qquad (1)$$

where $\prod$ refers to the product of the terms that follow: a_i are the unitless equilibrium activities of species in reaction j; c_0 is the standard state concentration for an ideal aqueous solution, $c_0 = 1$ M (Stumm & Morgan 1996); c_i are the equilibrium concentrations of species in reaction j in M; ν_i is the unitless stoichiometric coefficient for each species in reaction j; β_j^c is the unitless concentration-scale equilibrium constant for reaction j; γ_i are the unitless activity coefficients for each species in reaction j.

CCBATCH computes activity coefficients for all ions at the specified ionic strength (Stumm & Morgan 1996). It then computes β_j^c values using an input value of the equilibrium constant (β_j) at an ionic strength of zero and these activity coefficients.

All mass-action expressions are then solved simultaneously with mass balances on all total component concentrations (which are initially user input and then adjusted over time) by a Newton–Raphson numerical technique (VanBriesen & Rittmann 1999). The result is the concentration of all equilibrium complexes and aqueous ion species for the set of total component concentrations.

Equilibrium precipitation and dissolution reactions are modeled as described in Rittmann et al. (2002). In brief, the precipitation/dissolution reaction and equilibrium constant are input to CCBATCH. The amount of solid that must precipitate or dissolve to reach equilibrium with the aqueous phase species is calculated at each time step using CCBATCH's equilibrium speciation routines and the precipitation/dissolution equilibrium constant.

Biological reactions

Biological reactions, such as the consumption of EDTA by the BNC1 mixed culture and the production of biomass, are kinetically controlled and mathematically represented in CCBATCH using the multiplicative-Monod equation (Bae & Rittmann 1996). The rate of substrate utilization is (In (2) M refers to molarity in moles/liter.)

$$r_S = \frac{\mathrm{d}S_{\mathrm{tot}}}{\mathrm{d}t} = -q_{\max} X \frac{S}{S + K_s} \frac{\mathrm{O}_2}{\mathrm{X}_2 + K_{\mathrm{O}_2}} \qquad (2)$$

where r_S is the substrate utilization rate in M_S h^{-1}; q_{max} is the maximum specific rate of substrate utilization by the BNC1 mixed culture in $M_S M_X$ h^{-1}; X is the concentration of active biomass in M_X; S_{tot} is the total concentration of substrate (total EDTA) in M_S; S is the concentration of the electron-donor substrate (the biologically available EDTA complex) in M_S; K_S is the half-maximum rate concentration for the electron-donor substrate in M_S; O_2 is the concentration of molecular oxygen, the electron-acceptor substrate, in M_{O_2}; K_{O_2} is the half-maximum rate concentration for molecular oxygen in M_{O_2}.

The rate of active biomass (X) production is

$$r_X = \frac{dX}{dt} = Y_{true}(-r_S) - bX \qquad (3)$$

where r_X is the active biomass production rate in M_X h^{-1}; Y_{true} is the true yield coefficient for the BNC1 mixed culture in M_X/M_S; b is the biomass endogenous decay coefficient in h^{-1}.

Chelating agents like EDTA have many acid/base and metal complexed forms. Typically, one of these forms is biologically active, and this form can be actively transported into the microbial cell, while the others cannot (Joshi-Topé & Francis 1995; Bolton et al. 1996; VanBriesen et al. 2000). The dependence of EDTA biodegradation on the concentration of a specific metal-complexed form is reflected in the substrate utilization rate (2) by the value of S. In (2), S is the concentration of the biologically available EDTA complex, not the total concentration of EDTA. However, it is the total concentration of EDTA (S_{tot} in (2)) that is decreased by biodegradation; thus, the substrate utilization rate (2) describes changes in S_{tot}, the total concentration of EDTA, not only the concentration of the biologically available form of EDTA (S).

The biologically active species for EDTA biodegradation by the BNC1 mixed culture most likely is $CaEDTA^{2-}$, although $MgEDTA^{2-}$ cannot be ruled out. Klüner et al. (1998) showed that EDTA was biodegraded when equimolar amounts of Ca or Mg and EDTA were present, but EDTA was not biodegraded when equimolar amounts of Fe(III), Zn, Cu(II), Co(II), or Ni and EDTA were present. Klüner et al. (1998) also found that the addition of 0.6 mM Ca and 0.6 mM Mg to experiments with 0.6 mM each of Fe(III) and EDTA significantly increased the biodegradation rate. Further evidence for $CaEDTA^{2-}$ as the biologically available form comes from VanBriesen et al. (2000) and Witschel et al. (1999). VanBriesen et al. (2000) found the that biologically available form for nitrilotriacetic acid (NTA), a chelating agent similar to EDTA, was $CaNTA^{-}$. Witschel et al. (1999) found that uptake of Ca greatly increased during EDTA biodegradation by strain DSM 9103, a microorganism with an EDTA metabolism similar to that of BNC1. Because the evidence points to $CaEDTA^{2-}$ being the biologically available form of EDTA, all simulations presented were performed with S in (2) equal to $CaEDTA^{2-}$. However, simulations with S in (2) equal to $MgEDTA^{2-}$ gave similar results and are not presented. Our results show that the experimental data from Henneken et al. (1995, 1998) can be modeled with either $CaEDTA^{2-}$ or $MgEDTA^{2-}$ as the biologically available form because the EDTA utilization rate is not sensitive to S for the first part of the simulation, and it is controlled by the release of $EDTA^{4-}$ from $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ at the end of the simulation.

EDTA biodegradation produces and consumes species like oxygen, acidic hydrogen, NH_4^+, and $H_2CO_3^*$, (where $H_2CO_3^*$ represents the sum of the dissolved carbon dioxide concentration and the carbonic acid concentration) all of which are in the component basis of CCBATCH. Changes in the concentrations of these components during EDTA biodegradation are quantified using the total EDTA utilization rate (2) and the following biological stoichiometry

$$\begin{aligned} &C_{10}H_{12}O_8N_2^{2-}(\text{EDTA}) + 5.465\ O_2 + 3.179\ H_2O \\ &\quad +5.393\ H^+ \rightarrow \\ &\quad 0.6071\ C_5H_7O_2N + 1.393\ NH_4^+ + 6.965\ H_2CO_3^* \end{aligned} \qquad (4)$$

The EDTA ($C_{10}H_{12}O_8N_2^{4-}$) biological stoichiometry was calculated using the experimental value for the yield coefficient (Henneken et al. 1995) given in Table 1 and by assuming the BNC1 mixed culture is described by $C_5H_7O_2N$ (Rittmann & McCarty 2001).

Monod parameters for all modeling runs, summarized in Table 1, were taken from Henneken et al. (1995, 1998).

Reactions involving dissolved gases

$H_2CO_3^*$ is produced by biodegradation of EDTA and can exchange with CO_2(g) in the atmosphere of the culture medium. $H_2CO_3^*$ and CO_2(g) were assumed to be at equilibrium and were modeled as an open system using Henry's Law at 35 °C, the temperature of the experiments in Henneken et al. (1995, 1998).

$$a_{H_2CO_3^*} = \gamma \frac{c_{H_2CO_3^*}}{(c_0 = 1)} = \frac{P_{CO_2}}{(P_0 = 1)} K_H \qquad (5)$$

Table 1. Monod parameters for EDTA biodegradation by the BNC1 mixed culture (adapted from Henneken et al. (1995, 1998)). Mole VSS (volatile suspended solids), the unit of biomass used in CCBATCH, was calculated from the experimental units of grams cell dry weight (g cdw) by assuming each g cdw is 10% by mass non-volatilizable material (ash) and each mole of VSS has an empirical formula of $C_5H_7O_2N$. The conversion factor is $125.6 \frac{\text{g cdw}}{\text{mole VSS}}$.

	Parameter	Value	Units
Y_{true}	True yield	0.607	$\frac{\text{mole VSS}}{\text{mole EDTA}}$
$\mu_{\max}$	Maximum specific growth rate	0.044	h^{-1}
$q_{\max} = \frac{\mu_{\max}}{Y_{\text{true}}}$	Maximum specific substrate utilization rate	0.725	$\frac{\text{mole EDTA}}{\text{mole VSS h}}$
b	Endogenous decay coefficient	0.0029	h^{-1}
K_S	Monod half-maximum rate coefficient for biologically available substrate	7.87×10^{-6}	$\frac{\text{mole EDTA}}{\text{L}}$
K_{O_2}	Monod half-maximum rate coefficient for electron acceptor	1.563×10^{-5}	$\frac{\text{mole O}_2}{\text{L}}$

where $a_{H_2CO_3^*}$ is the unitless activity of $H_2CO_3^*$ in M; c_0 is the standard state concentration for an ideal aqueous solution, $c_0 = 1$ M (Stumm & Morgan 1996); $c_{H_2CO_3^*}$ is the concentration of $H_2CO_3^*$ in M; P_0 is the standard state pressure for an ideal gas, $P_0 = 1$ atm (Stumm & Morgan 1996); γ is the unitless activity coefficient for neutrally charged molecules; P_{CO_2} is the atmospheric partial pressure of CO_2(g) ($10^{-3.47}$ atm, Morel & Hering (1993)); K_H is the Henry's Law coefficient for CO_2(g) (2.67×10^{-2} M/atm at 35 °C, International Critical Tables (1928)), note units are added to K_H when the standard state reference is omitted).

Henneken et al. (1995) sparged the culture medium with air and maintained an oxygen concentration in the liquid of 90% of the saturation concentration for atmospheric oxygen in water (6.1 mg/L at 35 °C). This oxygen concentration was used in CCBATCH and was maintained for the duration of all simulations.

Significant amounts of NH_4^+ and NH_3 were produced during EDTA biodegradation (there are 2 moles of nitrogen per mole of EDTA), and the NH_3 was stripped from solution by the air sparging (Henneken et al. 1998). The loss of reduced nitrogen from the culture medium was modelled using the following equation for the gas film controlled mass transfer rate

$$\left(\frac{\mathrm{d}c_{NH_3(aq)}}{\mathrm{d}t}\right)_{\text{stripping}} = K_L a(c_{NH_3(aq)} - c^*_{NH_3(aq)}) \quad (6)$$

where $K_L a$ is the overall mass-transfer coefficient for the liquid in h^{-1}; $c_{NH_3(aq)}$ is the concentration of NH_3 in the culture medium in M; $c^*_{NH_3(aq)}$ is the equilibrium concentration of NH_3 in M corresponding to the atmospheric pressure of NH_3 (g).

The Henry's Law constant for NH_3 is 56.234 M/atm (Stumm & Morgan 1996), and the atmospheric partial pressure of NH_3 (g) is 10^{-9}–10^{-10} atm (Manahan 1994), giving a $c^*_{NH_3(aq)}$ value of no greater than $10^{-7.3}$ M. Compared to experimental $c_{NH_3(aq)}$ of around 10^{-4} M, the value of $10^{-7.5}$ M can be neglected. The transfer of NH_3 across the water-gas interface is gas film controlled, and

$$K_L a = \frac{k_G a}{K_H R T} \quad \text{(Wankat 1988)}$$

where $K_L a$ is the overall mass-transfer coefficient for the liquid in h^{-1}; $k_G a$ is the gas film mass transfer coefficient for NH_3 in h^{-1}; K_H is the Henry's Law coefficient for NH_3 (g) in M/atm; R is the universal gas constant in atm/(M K); T is the temperature of the system in K.

The value of $k_G a$ is in the range 180–2.2×10^4 h^{-1} (Schwarzenbach et al. (1993)). To fit the experimental data for total ammonium, $k_G a$ was varied from 180–2.2×10^4 h^{-1}. A value of 432 h^{-1} fit the aqueous ammonium data best.

Kinetic complexation

Kinetic complexation, a new feature of CCBATCH, is modeled using complexation rate equations that are solved over time for the concentrations of kinetically controlled complexes. The formation and dissociation of $FeEDTA^-$ and $FeOHEDTA^{2-}$ are considered kinetically controlled (Xue et al. 1995) and are used here to illustrate the kinetic complexation feature of CCBATCH. To model $FeEDTA^-$ and $FeOHEDTA^{2-}$ as kinetically controlled, these species were removed from the list of complexes available for equilibrium speciation in CCBATCH, and, instead, their concentrations are solved for over time, along with the biological rate equations, using a disjunctive mechanism and rate equation (Morel & Hering 1993). The disjunctive mechanisms for $FeEDTA^-$ and $FeOHEDTA^{2-}$ are

$$\begin{aligned} Fe^{3+} + EDTA^{4-} &\xrightarrow{k_1} FeEDTA^- \\ FeEDTA^- &\xrightarrow{k_{-1}} Fe^{3+} + EDTA^{4-} \\ FeOH^{2+} + EDTA^{4-} &\xrightarrow{k_{1OH}} FeOHEDTA^{2-} \\ FeOHEDTA^{2-} &\xrightarrow{k_{-1OH}} FeOH^{2+} + EDTA^{4-} \end{aligned} \tag{7}$$

where k_1 is the $FeEDTA^-$ formation rate constant in M^{-1} h^{-1}; k_{-1} is the $FeEDTA^-$ dissociation rate constant in h^{-1}; k_{1OH} is the $FeOHEDTA^{2-}$ formation rate constant in M^{-1} h^{-1}; k_{-1OH} is the $FeOHEDTA^{2-}$ dissociation rate constant in h^{-1}.

The two pairs of reactions in (7) have equilibrium reactions and constants defined by

$$\begin{aligned} &Fe^{3+} + EDTA^{4-} \overset{K_{FeEDTA}}{\longleftrightarrow} FeEDTA^- \\ &K_{FeEDTA} = \frac{k_1}{k_{-1}} \\ &FeOH^{2+} + EDTA^{4-} \overset{K_{FeOHEDTA}}{\longleftrightarrow} FeOHEDTA^{2-} \\ &K_{FeOHEDTA} = \frac{k_{1OH}}{k_{-1OH}} \end{aligned} \tag{8}$$

where K_{FeEDTA} is the equilibrium constant for $FeEDTA^-$ in M^{-1}; $K_{FeOHEDTA}$ is the equilibrium constant for $FeOHEDTA^{2-}$ in M^{-1}.

At the ionic strength of the culture medium in Henneken et al. (1995, 1998), approximately 0.3 M, K_{FeEDTA} is equal to 10^{24} M^{-1} and $K_{FeOHEDTA}$ is equal to $10^{19.7}$ M^{-1} (NIST 1997). The equilibrium constants are written in terms of concentrations for direct use in CCBATCH, and have units of M^{-1}. The rate equations for the mechanisms in (7) are

$$\begin{aligned} \frac{d[FeEDTA]}{dt} &= k_1[Fe^{3+}][EDTA^{4-}] \\ &\quad -k_{-1}[FeEDTA^-] \\ \frac{d[FeOHEDTA]}{dt} &= k_{1OH}[FeOH^{2+}][EDTA^{4-}] \\ &\quad -k_{-1OH}[FeOHEDTA^{2-}] \end{aligned} \tag{9}$$

The formation rate of Fe(III) and EDTA complexes proceeds by a two-step mechanism that involves hydrated Fe^{3+}. The EDTA molecule first forms an outer sphere complex with the hydrated metal cation ($Fe(H_2O)_6^{3+}$) and then slowly displaces the water molecules associated with the cation (Morel & Hering 1993).

$$\begin{aligned} Fe(H_2O)_6^{3+} + EDTA^{4-} &\overset{K_{OS}}{\longleftrightarrow} Fe(H_2O)_6EDTA^- \\ Fe(H_2O)_6EDTA^- &\xrightarrow{k_w} FeEDTA^- + 6H_2O \end{aligned} \tag{10}$$

where K_{OS} is the concentration-scale equilibrium constant for the outer-sphere complex formation in M^{-1}; k_w is the first-order rate constant for the water loss reaction in s^{-1}.

The initial complexation of EDTA with the hydrated cation occurs rapidly and is modeled as an equilibrium reaction with a concentration-scale equilibrium constant (K_{OS}). The loss of the bound water molecules is typically taken as the rate-limiting step and modeled as a first order reaction. Assuming equilibrium of the first reaction and omitting the coordinated water molecules yields the rate equation

$$\frac{d[FeEDTA^-]}{dt} = k_1[Fe^3][EDTA^{4-}] \quad k_1 = K_{OS}k_w \tag{11}$$

where k_1 is the overall rate constant for the formation of $FeEDTA^-$ in M^{-1} h^{-1}.

The value of K_{OS} depends on the ionic strength of the medium and the charges of the metal and ligand. It can be calculated from statistical thermodynamic theory by considering electrostatic attraction between ions and the effects of the ionic strength of the medium on the reaction. K_{OS} has been measured for some slow-reacting species, but most species react too fast for accurate data to be collected. Morel & Herring (1993) give a calculated K_{OS} value for the complexation of +3 cations with −4 ligands (e.g., Fe^{3+} and

$EDTA^{4-}$) equal to 2.5×10^3 M^{-1} and for the complexation of +2 cations and −4 ligands (e.g., $FeOH^{2+}$ and $EDTA^{4-}$) equal to 5.8×10^2 M^{-1}. Both values are for an ionic strength of 0.1 M, which was the highest value of ionic strength for which information was given.

The value of k_w, the rate constant for water loss from the outer sphere complex, is directly related to the electrostatic interactions between the cation and water molecules and decreases as the charge on the cation increases. It has been measured for many metal cations and is 3.6×10^7 h^{-1} for Fe^{3+} and 3.6×10^8 h^{-1} for FeOH2+ (Morel & Herring 1993). Based on the numbers from Morel & Herring (1993), the overall complex formation rate constant (k_1 and k_{1OH}) for $FeEDTA^-$ is equal to 9.0×10^{10} M^{-1} h^{-1} and for $FeOHEDTA^{2-}$ is equal to 2.1×10^{11} M^{-1} h^{-1}. The reverse rate constants were calculated from the values of the equilibrium constants for the equilibrium reactions for $FeEDTA^-$ and $FeOHEDTA^{2-}$. In summary, values for all complexation rate constants are

$$\begin{aligned} k_1 &= 9.0 \times 10^{10}\ M^{-1}\ h^{-1} \\ k_{-1} &= 9.0 \times 10^{-14}\ h^{-1} \\ k_{1OH} &= 2.1 \times 10^{11}\ M^{-1}\ h^{-1} \\ k_{-1OH} &= 4.0 \times 10^{-9} h^{-1}. \end{aligned}$$

The initial concentrations of the kinetic $FeEDTA^-$ and $FeOHEDTA^{2-}$ species were calculated by assuming that the aqueous solution of metals and EDTA was pre-equilibrated. Thus, at the start of the simulation, all complexes are at their equilibrium concentrations for the initial total component concentrations in the desired system. The pre-equilibration is implemented in CCBATCH by omitting the kinetic complexation routines and modeling $FeEDTA^-$ and $FeOHEDTA^{2-}$ complexation as equilibrium reactions. Given that Henneken et al. (1995) made the EDTA culture media with the free acid of EDTA and metal salts and that the formation rate of Fe(III) and EDTA complexes are extremely rapid (Margerum 1959), the pre-equilibration assumption is a valid one. After the initial concentrations for $FeEDTA^-$ and $FeOHEDTA^{2-}$ are calculated using the equilibrium speciation routines, $FeEDTA^-$ and $FeOHEDTA^{2-}$ are removed from the list of equilibrium complexes, and the kinetic complexation routines are activated. The initial total concentrations of the components $EDTA^{4-}$ and Fe(III) are decreased by the amounts of $FeEDTA^-$ and $FeOHEDTA^{2-}$ that are present initially. This step accounts for the amounts of $EDTA^{4-}$ and Fe(III) that were used to form $FeEDTA^-$ and $FeOHEDTA^{2-}$ during the pre-equilibration.

Coupling equilibrium and kinetic reactions

In CCBATCH, the solution of the equilibrium speciation is coupled with the solution of the kinetic rate equations. Initial total concentrations for all components are input and an initial speciation using equilibrium acid/base, complexation, and precipitation/dissolution is calculated. Then, the kinetic reaction rate equations for biodegradation, kinetic complexation, and gas-liquid mass transfer are solved, using concentrations of aqueous species from the solution of the speciation routines from the current time step, to get the total component concentrations for the next time step. These new total component concentrations are then used to re-equilibrate the system and calculate the new aqueous speciation of the system. This sequential iteration between equilibrium and kinetic routines is repeated for a user-specified number of time steps or until a stop criterion, such as a lack of an essential substrate, occurs.

Simulations and discussion

CCBATCH simulations of the experimental data from Henneken et al. (1995, 1998) under four different conditions are presented in Figures 1–4. In all figures, the top plot displays the experimental data and CCBATCH simulations for total EDTA, total ammonium, and biomass. Biomass has been converted from g cell dry weight to mole volatile suspended solids (VSS) as detailed in Table 1. The bottom plot gives the CCBATCH-predicted EDTA speciation over time. Initial concentrations for the simulations presented in Figures 1–4 are given in Table 2.

Effects of equilibrium versus kinetic complexation

Figure 1 shows CCBATCH simulations with equilibrium complexation for $FeEDTA^-$ and $FeOHEDTA^{2-}$; the new kinetic complexation routines were not used for this simulation. The simulations show that the model-predicted concentrations of FeEDTA- and $FeOHEDTA^{2-}$ decrease to zero, and all EDTA is biodegraded. Therefore, equilibrium complexation for $FeEDTA^-$ and $FeOHEDTA^{2-}$ does not predict the residual EDTA concentration.

Table 2. Initial total concentrations for CCBATCH simulations.

Parameter	Figure 1 & Figure 4	Figure 2	Figure 3
Biomass (X)	1.99×10^{-4} M	1.99×10^{-4} M	1.99×10^{-4} M
EDTA	3.81×10^{-3} M	3.42×10^{-3} M	3.65×10^{-3} M
pH	8.20	8.20	8.20
$H_2CO_3^*$	8.51×10^{-6} M	8.51×10^{-6} M	8.51×10^{-6} M
O_2	6.07 mg/L	6.07 mg/L	6.07 mg/L
NH_4^+	3.75×10^{-4} M	3.75×10^{-4} M	3.75×10^{-4} M
Fe(III)	4.10×10^{-4} M	2×10^{-5} M	4.1×10^{-6} M
Ca(II)	1.89×10^{-3} M	1.89×10^{-3} M	1.89×10^{-3} M
Mg(II)	4.52×10^{-3} M	4.52×10^{-3} M	4.52×10^{-3} M
Na	4.30×10^{-8} M	4.30×10^{-8} M	4.30×10^{-8} M
Zn(II)	3.90×10^{-8} M	3.90×10^{-8} M	3.90×10^{-8} M
K	2.50×10^{-3} M	2.50×10^{-3} M	2.50×10^{-3} M
Co(II)	9.25×10^{-8} M	9.25×10^{-8} M	9.25×10^{-8} M
Ni(II)	8.41×10^{-9} M	8.41×10^{-9} M	8.41×10^{-9} M
Cu(II)	8.41×10^{-9} M	1.62×10^{-8} M	1.62×10^{-8} M
Mn(II)	2.37×10^{-8} M	2.37×10^{-8} M	2.37×10^{-8} M
SO_4^{2-}	4.57×10^{-3} M	4.57×10^{-3} M	4.57×10^{-3} M
PO_4^{3-}	2.50×10^{-3} M	2.50×10^{-3} M	2.50×10^{-3} M
$FeEDTA^-$	0 M	4.0×10^{-5} M	1.59×10^{-5} M
$FeOHEDTA^{2-}$	0 M	3.5×10^{-4} M	1.4×10^{-4} M

Figure 2 shows CCBATCH simulations for the same conditions as Figure 1, except that the complexation of $FeEDTA^-$ and $FeOHEDTA^{2-}$ is modeled as kinetically controlled. Modeling with kinetic complexation predicts the observed sharp decrease in the EDTA biodegradation rate and the persistence of EDTA, although it does not predict the exact concentration of residual EDTA or the time when biodegradation stops. CCBATCH predicts that 0.4 mM of EDTA remains at the end of the experiment, instead of 0.16 mM. For time less than 66 hours, the CCBATCH-predicted biomass and total ammonium concentrations in Figures 1 and 2 are similar and correspond to the experimental data. At 66 hours, CCBATCH over predicts the experimental biomass and total ammonium peaks in Figure 1, but under predicts the peaks in Figure 2.

For time less than 40 hours, the EDTA speciation of the equilibrium system (Figure 1, bottom) is similar to the speciation of the kinetic system (Figure 2, bottom). The main difference for time less than 40 hours is the slight decrease in $FeEDTA^-$ seen only in Figure 1, bottom. $FeEDTA^-$ decreases slightly in the equilibrium system, because the total EDTA concentration is reduced by biodegradation and the equilibrium state of the system requires that $FeEDTA^-$ decrease as well, according to its mass action expression. In the kinetic system, neither complex responds to changes in other species because of slow kinetics for dissociation from the equilibrium state that was established at the start of the simulation.

At 40 hours, $Fe(OH)_3(s)$ precipitates in the equilibrium system (Figure 1), but not in the kinetic system (Figure 2). In the equilibrium system, the Fe^{3+} concentration increases to maintain equilibrium as EDTA is biodegraded (decreasing $FeEDTA^-$ is the source of the Fe^{3+}). In the kinetic system, Fe^{3+} does not rise as EDTA is degraded, because slow dissociation kinetics allow $FeEDTA^-$ and $FeOHEDTA^{2-}$ to hold Fe^{3+} and prevent it from rising above its solubility concentration. Thus, precipitation of $Fe(OH)_3(s)$ does not occur in the kinetic system. After 40 hours, precipitation in the equilibrium system causes a rapid decrease in Fe^{3+} concentration, which drives significant reductions in the $FeEDTA^-$ and $FeOHEDTA^{2-}$ concentrations. The kinetic system has no such driving force, and the $FeEDTA^-$ and $FeOHEDTA^{2-}$ concentrations remain constant for the rest of the simulation. In both systems, $MgEDTA^{2-}$, $CaEDTA^{2-}$ are initially depleted at a greater rate than $FeEDTA^-$ and $FeOHEDTA^{2-}$. The equilibrium constants for the formation of the

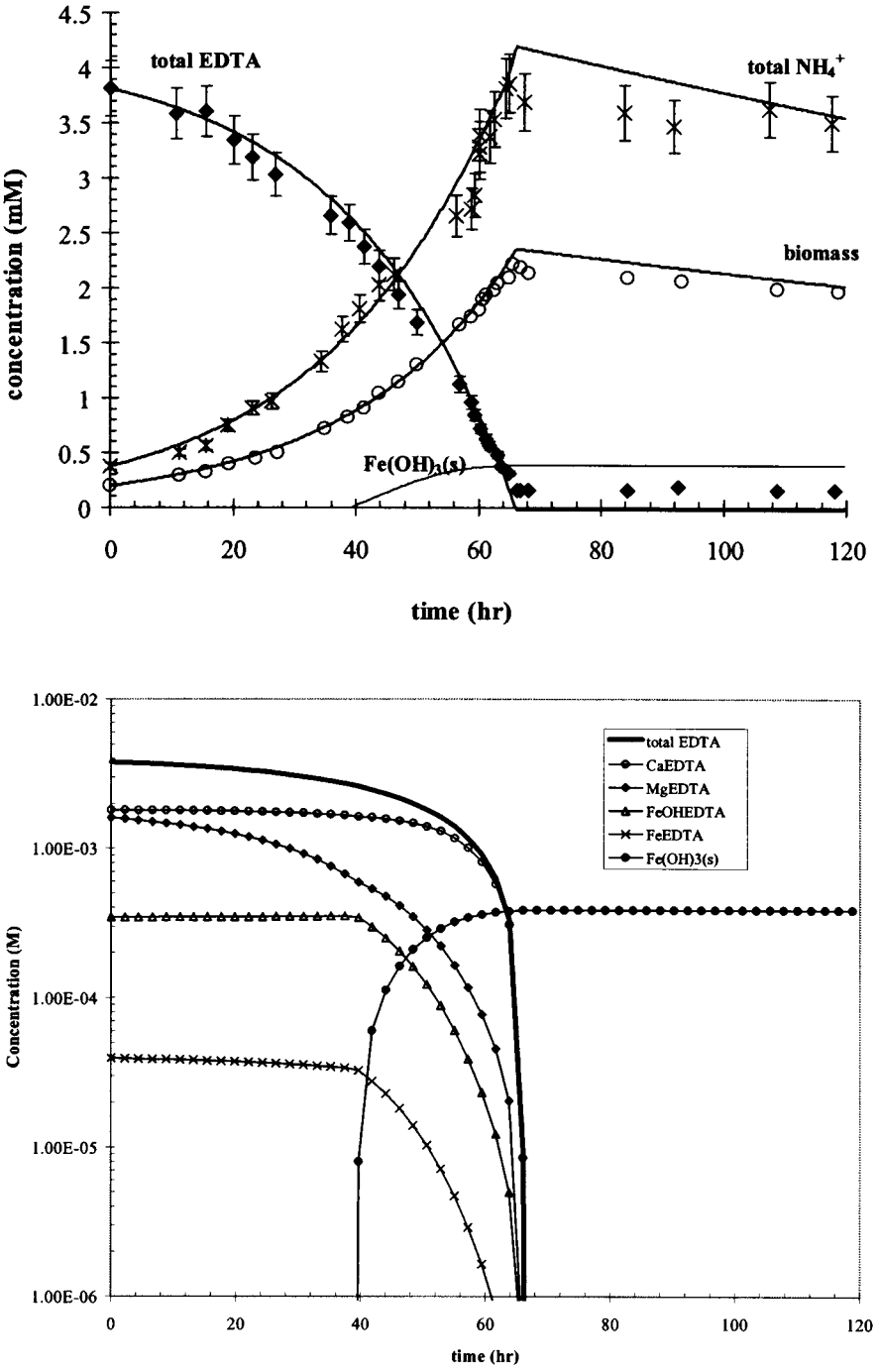

Figure 1. CCBATCH simulation with equilibrium $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ complexation and with $Fe(OH)_3(s)$ precipitation using the initial total Fe(III) concentration given in Henneken et al. (1995, 1998) ($Fe(III)_{tot}$ = 0.4 mM).

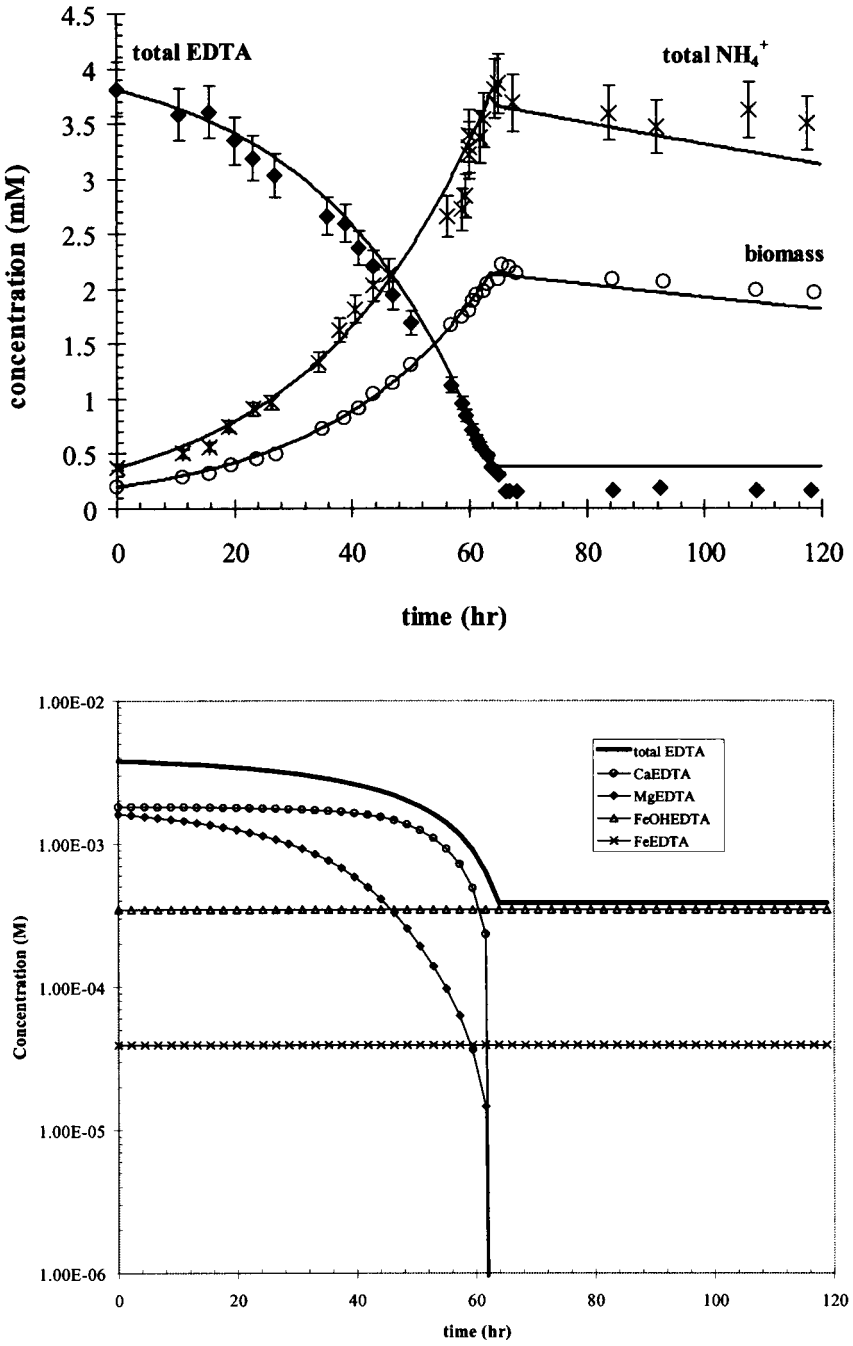

Figure 2. CCBATCH simulation with kinetic $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ complexation and with $Fe(OH)_3(s)$ precipitation using the initial total Fe(III) concentration given in Henneken et al. (1995, 1998) ($Fe(III)_{tot}$ = 0.4 mM).

Mg and Ca complexes are lower (log K equals 10.6 for $MgEDTA^{2-}$ and 12.4 for $CaEDTA^{2-}$ at 0 M ionic strength) than those for $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ (log K equals 27.7 for $FeEDTA^{-}$ and 22.2 for $FeOHEDTA^{2-}$ at 0 M ionic strength). As the total EDTA concentration decreases via biodegradation, strong complexation in the equilibrium system or slow dissociation kinetics holds EDTA as ferric iron complexes. In contrast, weaker equilibrium complexation allows calcium or magnesium EDTA complexes to decrease, as EDTA is removed.

In Figure 2, biodegradation stops around 64 hours, because the concentration of $CaEDTA^{2-}$, the biodegradable substrate in the simulations, is decreased to a tiny value by the sequestering of EDTA as $FeEDTA^{-}$ and $FeOHEDTA^{2-}$. In the CCBTACH simulation in Figure 2, $CaEDTA^{2-}$ decreases from 4.2×10^{-4} M at 60 hours to 10^{-15} M at 64 hours. After 64 hours, the biodegradation rate is imperceptible, and the concentration of $CaEDTA^{2-}$ remains at 10^{-15} M for remainder of the simulation.

Kinetic complexation with a revised total Fe(III) concentration

Although CCBATCH with the kinetic complexation sub-model correctly simulated the sudden stopping of EDTA biodegradation, it did not exactly predict the time of stoppage, the residual EDTA concentration, or the peaks in biomass and ammonium. Henneken et al. (1995) report that, for the experiment modeled here, dissolved Fe(III) at the end of the experiment was equal to 0.16 mM and was not equal to the added concentration of 0.4 mM. It is quite likely that a portion of the missing Fe(III) was removed from solution via precipitation of $Fe(OH)_3(s)$ before the beginning or during the first few hours of the experiment, given the insolubility of Fe(III) in alkaline systems (the pH is 8.2). To more accurately simulate the experimental data, we decreased the total dissolved Fe(III) concentration by 60 % from the added concentration of 0.4 mM to the reported dissolved Fe(III) concentration at the end of the experiment of 0.16 mM. The results of this simulation are shown in Figure 3.

The simulation with 0.16 mM total Fe(III) (Figure 3) correctly predicts the residual EDTA concentration and the time when biodegradation stops. It also matches the peaks for biomass and ammonium. The good match between CCBATCH simulations and experimental data for all experimentally measured parameters suggests that the mechanisms and the total Fe(III) concentration used to generate Figure 3 are correct. When the initial dissolved total concentration of Fe(III) is decreased to 0.16 mM, the initial concentrations of $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ also decrease. Since slow dissociation kinetics keep the concentrations of $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ complexes constant during the simulation, the final concentrations of these complexes are equal to their initial concentrations and also to the residual EDTA concentration. Thus, setting total Fe(III) equal to the measured dissolved Fe(III) concentration (0.16 mM) allows CCBATCH to predict the observed residual EDTA concentration (also 0.16 mM).

Precipitation of dolomite

Another possibility for the sharp decrease in the EDTA utilization rate and for the persistence of EDTA is the precipitation of a Ca or Mg phosphate, carbonate, or sulfate solid. As EDTA is removed from the culture medium by biodegradation, much of the 1.89 mM total Ca and 4.52 mM total Mg in the experiment are released from EDTA complexes (e.g., see the bottom panel of Figures 1–4). The result is an increase in aqueous Ca^{2+} and Mg^{2+} concentrations, possibly to values greater than the solubility limit for a number of phosphate, carbonate, or sulfate solids. Total phosphate, carbonate, and sulfate were present in the culture medium in millimolar amounts, making precipitation with free cations possible. Decreases in the total Ca and Mg concentrations via precipitation would cause a re-speciation of the system and a decrease in the concentration of $CaEDTA^{2-}$ and $MgEDTA^{2-}$. If $CaEDTA^{2-}$ is the biologically available form of EDTA, its reduction by precipitation of calcium solids would slow the rate of EDTA biodegradation.

To determine the possible effects of Ca and Mg precipitation on EDTA biodegradation during the experiments of Henneken et al. (1995, 1998), we examined the equilibrium precipitation of several Ca and Mg phosphate, carbonate, or sulfate solids.

Table 3 shows a list of solids that can form from components in the culture medium in Henneken et al. (1995, 1998). Using CCBATCH-predicted speciation from the bottom panel of Figure 3, we determined which solids were above their solubility limit by comparing each ion product coefficient, Q, with the value of the dissolution constant, K, at the ionic strength of the culture medium (0.3 M). The value of Q changes over time as EDTA is biodegraded. We computed Q

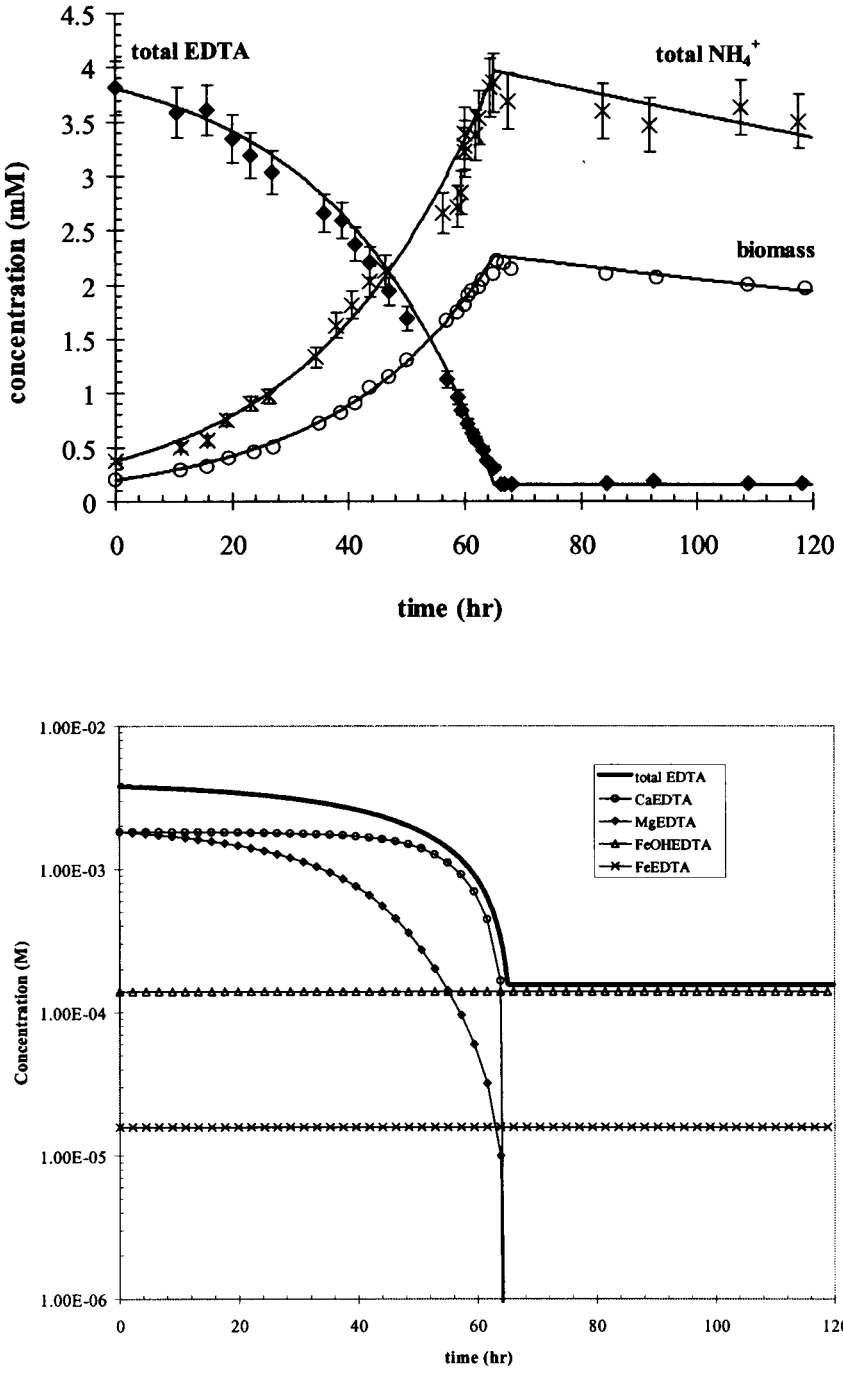

Figure 3. CCBATCH simulation with kinetic $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ using a revised total Fe(III) concentration that is based on the initial Fe(III) concentration given in Henneken et al. (1995, 1998), but adjusted to account for the precipitation of $Fe(OH)_3(s)$ prior to inoculation with the BNC1 mixed culture ($Fe(III)_{tot}$ = 0.16 mM).

for each solid throughout the entire simulation in Figure 3. For example, Q for the first reaction in Table 3 is

$$MgCO_3(s) \longleftrightarrow Mg^2 + CO_3^{2-} \quad Q = [Mg^{2+}][CO_3^{2-}]. \tag{12}$$

If $Q > K$, the system is oversaturated and solid can precipitate. The last column of Table 3 shows which solids were oversaturated at any time during the simulation. The five solids that became oversaturated during the simulations are $CaMg(CO_3)_2(s)$, $Ca_4H(PO_4)_3(s)$, $Ca_3(PO_4)_2(s)$, $Ca_5OH(PO_4)_3(s)$, and $Ca_{10}(OH)_2(PO_4)_6(s)$.

We then modeled the experimental data from Henneken et al. (1995, 1998) using CCBATCH's equilibrium precipitation routine for each of the oversaturated solids, on a one-by-one basis. We set CCBATCH to model equilibrium FeEDTA- and $FeOHEDTA^{2-}$ complexation with the initial concentrations in Table 2 for Figure 1 and Figure 4 (first column). The biologically available form for EDTA biodegradation was $CaEDTA^{2-}$. CCBATCH predicted that four of the five oversaturated solids precipitated, but to such a minor extent ($< 10^{-8}$ M solid) that they had no impact on EDTA biodegradation. The only oversaturated solid in Table 3 that precipitated to a significant extent during CCBATCH simulation was $CaMg(CO_3)_2(s)$, dolomite.

CCBATCH simulations with equilibrium dolomite precipitation and equilibrium $FeEDTA^-$ and $FeOHEDTA^{2-}$ complexation are shown in Figure 4. The precipitation of dolomite slows the simulated EDTA removal around 65 hours when compared to the equilibrium simulation with $Fe(OH)_3(s)$ precipitation in Figure 1, but the sudden halt in EDTA biodegradation and the EDTA plateau are not predicted. The precipitation of dolomite, which occurs at around 58 hours, consumes Ca and drastically decreases the $CaEDTA^{2-}$ concentration. Because $CaEDTA^{2-}$ is the biologically available form for this simulation, EDTA biodegradation slows as $CaEDTA^{2-}$ decreases. Although, most of the total Ca (1.89×10^{-3} M) precipitated as $CaMg(CO_3)_2(s)$ at 58 hours, the bottom panel of Figure 4 shows that about 10^{-6} M $CaEDTA^{2-}$ was present as 58 hours, and this was enough to support significant EDTA biodegradation past 65 hours.

Correlation between dissolved Fe(III) and residual EDTA

Henneken et al. (1995) gave experimental results showing a linear relationship between the residual EDTA concentration and the final concentration of dissolved Fe(III) in the culture medium. Their results are shown as data points (squares) in Figure 5. We simulated each experimental data point in Figure 5 using CCBATCH with kinetic $FeEDTA^-$ and $FeOHEDTA^{2-}$ complexation and total initial concentrations in Table 2 for all species, except for Fe(III), EDTA, $FeEDTA^-$, and $FeOHEDTA^{2-}$. We used total EDTA equal to 3.81×10^{-3} M and total Fe(III) equal to the dissolved Fe(III) concentration for each experimental data point in Figure 5 to calculate the pre-equilibrated amounts of $FeEDTA^-$ and $FeOHEDTA^{2-}$ that are initially present, as described previously. To get starting concentrations for all species, the total amounts of EDTA and Fe(III) were decreased by the initial amounts of $FeEDTA^-$ and $FeOHEDTA^{2-}$. Because total Fe(III) in the system changes for each experimental data point in Figure 5, the starting concentrations of EDTA and Fe(III) also vary. In all cases, CCBATCH predicted that $Fe(OH)_3(s)$ did not precipitate, making the total Fe(III) concentration equal to the dissolved Fe(III) concentration throughout the simulation.

The CCBATCH-predicted EDTA residuals are shown by the line in Figure 5. The residual concentration of EDTA and the dissolved Fe(III) concentration are correlated at a nearly 1 : 1 mole ratio in the CCBATCH simulations and the experimental data. According to our simulations, this 1 : 1 relationship occurs because nearly all of the Fe(III) forms $FeEDTA^-$ and $FeOHEDTA^{2-}$ complexes, which are slow to dissociate and inert to biodegradation.

Conclusions

Addition of a kinetic complexation sub-model to CCBATCH makes it possible to evaluate the reason for incomplete EDTA biodegradation in experiments from Henneken et al. (1995, 1998). Our modeling evaluation with CCBATCH indicates that the "trapping" of EDTA as $FeEDTA^-$ and $FeOHEDTA^{2-}$, which are slow to dissociate, causes the abrupt cessation of EDTA biodegradation and the persistence of EDTA at the end of the experiment. Equilibrium complexation of $FeEDTA^-$ and $FeOHEDTA^{2-}$ fails to predict

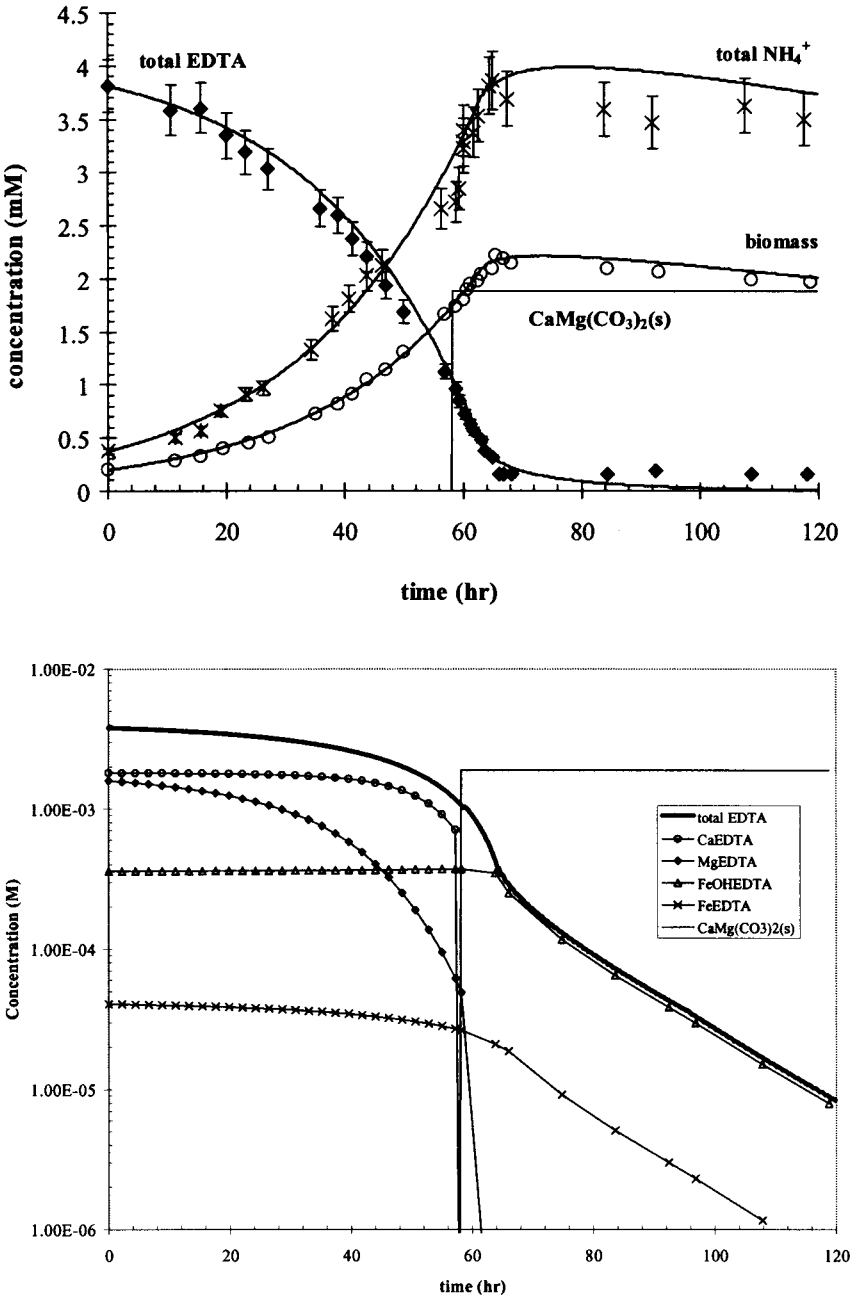

Figure 4. CCBATCH simulation with equilibrium $FeEDTA^{-}$ and $FeOHEDTA^{2-}$ complexation and with $CaMg(CO_3)_2(s)$ (dolomite) precipitation using the initial total Fe(III) concentration given in Henneken et al. (1995, 1998) ($Fe(III)_{tot}$ = 0.4 mM).

Table 3. Precipitation and dissolution reactions for the culture medium from Henneken et al. (1995, 1998). Solubility products are from Stumm and Morgan (1996) and NIST (1997).

Reaction	log K ($I = 0$ M)	log K ($I = 0.3$ M)	Over saturated? ($Q > K$)
$MgCO_3(s) \leftrightarrow Mg^{2+} + CO_3^{2-}$	−7.45	−6.15	N
$Mg(OH)_2(s) \leftrightarrow Mg^{2+} + 2OH^-$	−11.16	−10.18	N
$Mg_3(PO_4)_2(s) \leftrightarrow 3Mg^{2+} + 2PO_4^{3-}$	−23.28	−18.40	N
$MgHPO_4(s) \leftrightarrow Mg^{2+} + HPO_4^{2-}$	−5.85	−4.55	N
$CaMg(CO_3)_2 \leftrightarrow Ca^{2+} + Mg^{2+} + 2CO_3{}^{2-}$	−16.54	−13.94	Y
$CaSO_4(s) \leftrightarrow Ca^{2+} + SO_4^{2-}$	−4.62	−3.32	N
$CaCO_3(s) \leftrightarrow Ca^{2+} + CO_3^{2-}$	−8.22	−6.92	N
$CaHPO_4(s) \leftrightarrow Ca^{2+} + HPO_4^{2-}$	−6.6	−5.30	N
$Ca_4H(PO_4)_3(s) \leftrightarrow 4Ca^{2+} + H^+ + 3PO_4^{3-}$	−46.9	−39.9	Y
$Ca_3(PO_4)_2(s) \leftrightarrow 3Ca^{2+} + 2PO_4^{3-}$	−28.92	−24.04	Y
$Ca_5OH(PO_4)_3(s) \leftrightarrow 5Ca^{2+} + OH^- + 3PO_4^{3-}$	−58.63	−50.2	Y
$Ca_{10}(OH)_2(PO_4)_6(s \leftrightarrow 10Ca^{2+} + 2OH^- + 6PO_4^{3-}$	−114	−98.38	Y

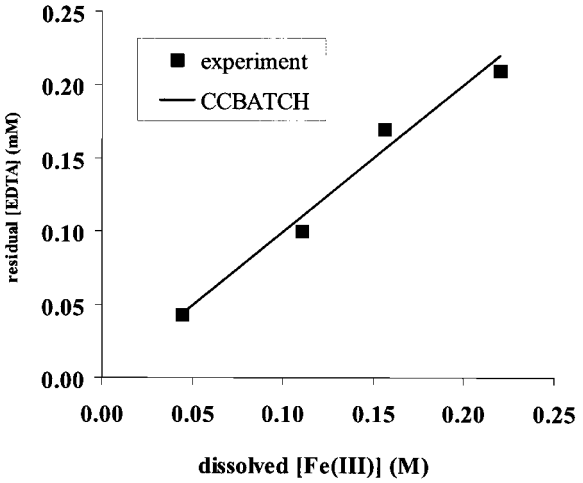

Figure 5. Experimental (Henneken et al. 1995) and CCBATCH-predicted residual EDTA concentration as a function of dissolved Fe(III) concentration. A linear fit of the experimental data gives a slope of 0.98 mole residual EDTA/mole dissolved Fe(III). The slope of the CCBATCH simulation is 1 mole residual EDTA/mole dissolved Fe(III).

the EDTA residual, and the precipitation of calcium and magnesium solids only slows the EDTA biodegradation. Our simulation of the EDTA biodegradation data shows that once EDTA becomes complexed with Fe(III), it becomes unavailable for biodegradation. Thus, treatment methods for EDTA, when it is present as iron complexes (or possibly as other heavy metal complexes), should focus on increasing the rate of dissociation via large changes in temperature, pH, redox potential, and/or addition of metals like calcium that may drive the dissociation forward.

Acknowledgements

This first author of this research paper was supported by an EPA STAR Graduate Fellowship. The assistance of Dr. Jeanne VanBriesen and Dr. Jim Banaszak is gratefully acknowledged.

Appendix of equations

$$\prod_i \left(\frac{\{a\}_i}{\gamma_i}\right)^{\nu_i} = \beta_j^c \quad \frac{\{a_i\}}{\gamma_i} = \frac{[c_i]}{([c_0] = 1)} \tag{13}$$

$$r_S = \frac{\mathrm{d}S_{\mathrm{tot}}}{\mathrm{d}t} = -q_{\max} X \frac{S}{S + K_s} \frac{\mathrm{O}_2}{\mathrm{X}_2 + K_{\mathrm{O}_2}} \tag{14}$$

$$r_X = \frac{\mathrm{d}X}{\mathrm{d}t} = Y_{\mathrm{true}}(-r_S) - bX \tag{15}$$

$$\begin{aligned} &C_{10}H_{12}O_8N_2^{2-}(\mathrm{EDTA}) \\ &\quad +5.465\ O_2 + 3.179\ H_2O \\ &\quad +5.393\ H^+ \rightarrow 0.6071\ C_5H_7O_2N \\ &\quad +1.393\ NH_4^+ + 6.965\ H_2CO_3^* \end{aligned} \tag{16}$$

$$a_{H_2CO_3^*} = \gamma \frac{c_{H_2CO_3^*}}{(c_0 = 1)} = \frac{P_{CO_2}}{(P_0 = 1)} K_H \tag{17}$$

$$\left(\frac{\mathrm{d}c_{\mathrm{NH_3(aq)}}}{\mathrm{d}t}\right)_{\text{stripping}} = K_L a(c_{\mathrm{NH_3(aq)}} - c^*_{\mathrm{NH_3(aq)}}) \tag{18}$$

$$\begin{aligned} \mathrm{Fe^{3+} + EDTA^{4-}} &\xrightarrow{k_1} \mathrm{FeEDTA^-} \\ \mathrm{FeEDTA^-} &\xrightarrow{k_{-1}} \mathrm{Fe^{3+} + EDTA^{4-}} \\ \mathrm{FeOH^{2+} + EDTA^{4-}} &\xrightarrow{k_{1\mathrm{OH}}} \mathrm{FeOHEDTA^{2-}} \\ \mathrm{FeOHEDTA^{2-}} &\xrightarrow{k_{-1\mathrm{OH}}} \mathrm{FeOH^{2+} + EDTA^{4-}} \end{aligned} \tag{19}$$

$$\mathrm{Fe^{3+} + EDTA^{4-}} \overset{K_{\mathrm{FeEDTA}}}{\longleftrightarrow} \mathrm{FeEDTA^-}$$

$$K_{\mathrm{FeEDTA}} = \frac{k_1}{k_{-1}}$$

$$\mathrm{FeOH^{2+} + EDTA^{4-}} \overset{K_{\mathrm{FeOHEDTA}}}{\longleftrightarrow} \mathrm{FeOHEDTA^{2-}}$$

$$K_{\mathrm{FeOHEDTA}} = \frac{k_{1\mathrm{OH}}}{k_{-1\mathrm{OH}}} \tag{20}$$

$$\begin{aligned} \frac{\mathrm{d[FeEDTA]}}{\mathrm{d}t} &= k_1[\mathrm{Fe^{3+}}][\mathrm{EDTA^{4-}}] \\ &\quad -k_{-1}[\mathrm{FeEDTA^-}] \\ \frac{\mathrm{d[FeOHEDTA]}}{\mathrm{d}t} &= k_{1\mathrm{OH}}[\mathrm{FeOH^{2+}}][\mathrm{EDTA^{4-}}] \\ &\quad -k_{-1\mathrm{OH}}[\mathrm{FeOHEDTA^{2-}}] \end{aligned} \tag{21}$$

$$\begin{aligned} \mathrm{Fe(H_2O)_6^{3+} + EDTA^{4-}} &\overset{K_{OS}}{\longleftrightarrow} \mathrm{Fe(H_2O)_6EDTA^-} \\ \mathrm{Fe(H_2O)_6EDTA^-} &\xrightarrow{k_w} \mathrm{FeEDTA^- + 6H_2O} \end{aligned} \tag{22}$$

$$\mathrm{MgCO_3(s)} \longleftrightarrow \mathrm{Mg^2 + CO_3^{2-}} \quad Q = [\mathrm{Mg^{2+}}][\mathrm{CO_3^{2-}}]. \tag{23}$$

References

Alder AC, Siegrist H, Gujer W & Giger W (1990) Behavior of NTA and EDTA in biological wastewater treatment. Water Res 24(6): 733–742

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

Barber LB, Brown GK, Kennedy KR, Leenheer JA, Noyes TI & others (1997) Organic constituents that persist during aquifer storage and recovery of reclaimed water in Los Angeles County, California. Amer. Water Resour. Assoc. J. 1997: 261–272

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

Cleveland JM & Rees TF (1981) Characterization of plutonium in maxey flats radioactive trench leachate. Sci. 212: 1507–1509

Fujita Y, Ding W-H & Reinhard M (1996) Identification of wastewater dissolved organic carbon characteristics in reclaimed wastewater and recharged groundwater. Water Environ. Res. 68(5): 867–876

Haberer K (1991) Rheinwasser Als Rohstoff Für Die Grundwasseranreicherung in Wiesbaden. Wasser Abwasser 132(2): 60–64

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

Henneken L, Klüner B, Nörtemann B & Hempel DC (1998) Biological degradation of EDTA: Reaction kinetics and technical approach. J. Chem. Technol. Biotechnol. 73: 144–152

Hinck ML, Ferguson J & Puhaakka J (1997) Resistance of EDTA and DTPA to aerobic biodegradation. Water, Sci. Technol. 35(2–30): 25–31

International Critical Tables of Numerical Data, Physics, Chemistry and Technology (1928) E.W. Washburn (editor) McGraw-Hill Book Company, Inc. for the National Research Council: Washington, D.C

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

Klüner T, Hempel, DC & Nörtemann 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

Kroschwitz JI (1993) Chelating agents in Kirk-Othmer encyclopedia of chemical technology (Vol 5, pp 764–795), 4th edn. John Wiley and Sons, New York

Manahan SE (1994) Environmental chemistry, 6th edn. Lewis Publishers, Boca Raton

Margerum DW (1959) Coordination kinetics of ethylenediaminetetraacetate complexes. J. Phys. Chem. 63: 336–339

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

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

NIST (1997) Critically selected stability constants of metal complexes database, Version 4.0. Smith RM & Martell AE (collected and selected data); RJ Motekaitis (developed program) NIST: Standard Reference Data Program: Gaithersburg, MD

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

Nowack B, Kari FG & Krüger HG (2001) The remobilization of metals from iron oxides and sediments by metal-EDTA complexes. Water, Air, and Soil Pollut. 125: 243–257

Pavlostathis SG & Morrison D (1994) Response of continuous-flow activated sludge reactors to photoprocessing waste waters. Water Res. 28(2): 269–276

Potos C (1965) Effects of EDTA on wastewater treatment. J. Water Pollut. Contr. Feder. 37(9): 1247–1255

Riley RG & Zachara JM (1992) Chemical contaminants on DOE lands and selection of contaminant mixtures for subsurface science research. DOE/ER 0547; U.S. Department of Energy/OER, Washington, D.C

Rittmann BE & Van Briesen JM (1996) Microbiological processes in reactive modeling. Chapter 7 in reactive transport in porous media. In Lichtner PC, Steefel CI, Oelkers EH (eds) Reviews

in mineralogy (Vol 34, pp 311–334). Mineralogical Society of America

Rittmann BE & McCarty PL (2001) Environmental biotechnology: Principles and Applications. McGraw-Hill, New York, NY

Rittmann BE, Banaszak JE, VanBriesen JM & Reed DT (2002) Mathematical modeling of precipitation and dissolution reactions in microbiological systems. Biodegrad., submitted

Schwarzenbach RP, Gschwend PM & Imboden DM (1993) Environmental organic chemistry, 6th edn. John Wiley & Sons, Inc., New York

Stumm W & Morgan JJ (1996) Aquatic chemistry: Chemical equilibria and rates in natural waters, 3rd edn. John Wiley & Sons, Inc., New York

Toste AP, Kirby LJ, Robertson DE, Abel KH & Perkins RW (1983) Characterization of radionuclide behavior in low-level waste sites. IEEE Trans. Nuc. Sci. NS-30(1): 580–585

VanBriesen JM & Rittmann BE (1999) Modeling speciation effects of biodegradation in mixed metal/chelate systems. Biodegrad. 10: 315–330

VanBriesen JM, Rittmann BE, Xun L, Girvin DC & Bolton H Jr. (2000) The rate-controlling substrate of nitrilotriacetate for biodegradation by chelatobacter heintzii. Environ. Sci. Technol. 34: 3346–3353

Wankat PC (1988) Equilibrium staged separations. Prentice Hall, New Jersey

Witschel M, Egli T, Zehnder AJB, Wehrli E & Spycher M (1999) Transport of EDTA into cells of the EDTA-degrading strain DSM 9103. Microbiol. Rev. 154: 973–983

Xue H, Sigg L & Kari LG (1995) Speciation of EDTA in natural waters: Exchange kinetics of Fe-EDTA in river water. Environ. Sci. Technol. 29(1): 59–68