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Formulation of the CBC-model for modelling the contaminants and footprints in natural attenuation of BTEX

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Abstract

This paper provides the details of the Coupled Biological and Chemical (CBC) model for representing *in situ* bioremediation of BTEX. The CBC model contains novel features that allow it to comprehensively track the footprints of BTEX bioremediation, even when the fate of those footprints is confounded by abiotic reactions and complex interactions among different kinds of microorganisms. To achieve this comprehensive tracking of all the footprints, the CBC model contains important new biological features and key abiotic reactions. The biological module of the CBC-model includes these important new aspects: (1) it separates BTEX fermentation from methanogenesis, (2) it explicitly includes biomass as a sink for electrons and carbon, (3) it has different growth rates for each biomass type, and (4) it includes inhibition of the different reactions by other electron acceptors and by sulfide toxicants. The chemical module of the CBC-model includes abiotic reactions that affect the footprints of the biological reactions. In particular, the chemical module describes the precipitation/dissolution of $CaCO_3$, Fe_2O_3, FeS, FeS_2, and S°. The kinetics for the precipitation/dissolution reactions follow the critical review in Maurer & Rittmann (2004).

Introduction

Monitoring and its interpretation are the keys for assessing *in-situ* bioremediation, especially for natural attenuation. The biological conversion of a pollutant is connected with a stoichiometric consumption and production of several other compounds, called *footprints* of the biodegradation reaction. This knowledge, properly quantified in a mathematical model, creates a body of evidence documenting the efficiency of bioremediation (NCR 1993, 2000; Maurer & Rittmann 2004). To be able to exploit this information, the chemical fate, as well as the biological fate, of these substances has to be known. For example, precipitation and dissolution of solids, like iron sulfide or calcium carbonate, affect the fate of the biodegradation footprints Fe(II), inorganic carbon, and alkalinity (Maurer & Rittmann 2004). Furthermore, the biological model must provide an accurate representation of the types of microbially catalysed reactions.

Currently available models (BIOPLUME III, Rafai et al. 1998; RT3D, Clement 1997; UTCHEM, Pope et al. 1999; TBC, Schäfer et al. 1998a, b) concentrate mainly on groundwater transport and have simplified models of the biological processes and no chemical processes. They do not provide the biological or chemical features needed to accurately represent the fate of footprints of BTEX bioremediation. To overcome this limitation, we present here the Coupled Biological and Chemical (CBC) model for the comprehensive quantification of BTEX bioremediation. The CBC model includes the key abiotic chemical reactions that affect the fate of the footprints for BTEX biodegradation, and it also provides a realistic representation of the microbiological reactions. The scientific foundation for the new features in the CBC model is summarized in Maurer & Rittmann (2004). We demonstrate im-

portant predictions of the CBC model in a subsequent work (Rittmann and Maurer, in preparation).

The goal of the CBC model is to have an explicit coupling of the key microbiological and chemical reactions that can affect the fate of BTEX and its biodegradation footprints during natural attenuation. Achieve this goal makes possible a quantitative connection between loss of BTEX and the appearance of BTEX-biodegradation footprints, even when the footprints are affected by other reactions.

Model overview

The CBC model focuses on the saturated zone of the groundwater system and assumes that it is a closed system (no gas exchange). In this paper, we describe all of the reaction processes. Transport processes are not described here.

Model reactions

The CBC model contains the following kinetically controlled microbiological processes:

- BTEX (hydrocarbon) biodegradation by bacteria that respire the following electron acceptors: oxygen (O_2), nitrate (NO_3^-), iron(III) (Fe_2O_3), and sulfate (SO_4^{2-}).
- Fermentation of BTEX to acetate and hydrogen (H_2).
- Oxidation of acetate by bacteria using the same four electron acceptors.
- Methanogenesis (methane formation) by acetate cleavage.
- Methanogenesis by oxidation of H_2 and reduction of inorganic carbon.
- Decay of the microorganisms

The CBC model also contains five kinetically controlled precipitation/dissolution reactions:

- Dissolution and precipitation of calcium carbonate ($CaCO_{3(s)}$), which buffers the pH, changes the alkalinity and inorganic-carbon concentrations, and affects the dissolved calcium (Ca^{2+}) concentration.
- Conversion of FeS into the thermodynamically stable pyrite (FeS_2), which brings about loss of H_2S, but is an abiotic source of H_2.
- Reductive dissolution of solid iron(III) by oxidation of sulfide, which consumes H_2S while producing dissolved Fe^{2+} and SO_4^{2-} or S_8 (elemental sulfur).

The CBC model also contains a set of equilibrium reactions needed to describe the aqueous chemistry. They involve speciation of the carbonate and sulfide systems and the precipitation and dissolution of iron(II) and sulfide as amorphous iron monosulfide (FeS). These processes decrease the biodegradation footprints Fe^{2+} and HS^-.

Model components

The CBC model contains 16 dissolved components: BTEX, acetate, oxygen, nitrate, ammonium, sulfate, methane, hydrogen, calcium, iron(II), inorganic carbon, and inorganic sulfide. Inorganic carbon and sulfide are partitioned into different species according to equilibrium: H_2CO_3, HCO_3^-, CO_3^{2-}, H_2S, HS^-, and H^+. BTEX, S_{HC}, is lumped together and treated as a single organic substance, while acetate ($CH_3CO_2^-$), S_F, is assumed to be the organic product from BTEX fermentation.

The model distinguishes five unique biomass types:

- Fast growing heterotrophic bacteria that respire oxygen and nitrate, X_{BO}, are able to utilise BTEX and acetate as electron donors and carbon sources. When oxygen and nitrate are absent, these microbes are able to ferment BTEX to acetate and H_2.
- Sulfate-reducing bacteria, X_{BS}, are able to reduce sulfate and use BTEX or acetate as electron donor and carbon source.
- Iron-reducing bacteria, X_{BF}, are able to access solid iron(III) and use it as an electron acceptor. BTEX and acetate serve as electron donors and carbon sources.
- Acetoclastic methanogens, X_{BAc}, cleave acetate to methane and inorganic carbon.
- Hydrogen-oxidizing methanogens, X_{BH_2}, produces methane by using hydrogen as an electron donor and inorganic carbon as electron acceptor and carbon source.

The CBC model has five inorganic solid phases: calcium carbonate ($CaCO_3$), X_{CaCO_3}; iron(III) oxide (Fe_2O_3), $X_{Fe_2O_3}$; iron(II) monosulfide (FeS), X_{FeS}; pyrite (FeS_2), X_{FeS_2}; and elementary sulfur (S^0), X_S.

Model kinetics expressions

The rate terms for microbiological reactions are formulated in a way that includes how the rates of biomass synthesis and electron-donor utilization are

controlled by the concentrations of the cells themselves, their electron donor and electron acceptor, their nitrogen source, and any inhibitory chemical species. The rates of production or consumption of other chemical species involved in the microbiological reactions (e.g., inorganic carbon, ammonium nitrogen, Fe(II), and sulfide) are computed from stoichiometry.

The rate terms for abiotic precipitation or dissolution of the inorganic solid species are taken directly from the review by Maurer & Rittmann (2004).

The CBC model does not include the re-oxidation of these terminal reduced products: methane, sulfide, and iron(II). Re-oxidation might occur if groundwater containing these reduced products was mixed with oxygenated water.

The next section describes the details of how the CBC model is formulated. Appendix 1 lists all the variables, their symbols, and their definitions. Appendix 2 lists the standard values for all the parameters mentioned in the next section.

Modelling the microbiological reactions

Table 1 lists all the kinetic expression used in the microbiological model. Table 2 is a matrix that shows the stoichiometry that links the different components involved in the microbiological reactions. The following sections describe the key features of the kinetics and stoichiometric expressions. All nomenclature used in the CBC is defined in Appendix 1, while Appendix 2 gives the numerical values and units for all parameters.

Kinetic format

All rates of biomass synthesis and donor utilization are based on a modified Monod equation that takes into account the effects of multiple substrates, nutrients, and inhibitors (Henze et al. 2000; Bae & Rittmann 1996; Rittmann & McCarty 2001). For biomass synthesis (processes B1–B5 in Table 1), the rate expression is of the form

$$\rho_i = \mu_i \cdot [\text{modified Monod terms}]_i \cdot X_i$$

in which ρ_i = the growth rate of biomass type i [$M_C d^{-1}$], μ_i = the maximum specific growth rate of biomass type i (d^{-1}), X_i = the concentration of biomass type i (M_C), and [modified Monod terms]$_i$ = the set of hyperbolic terms needed to describe how substrates, nutrients, and inhibitors control the overall rate for biomass type i [dimensionless]. The modified Monod terms are described below and shown in Table 1. In order to calculate a specific substrate utilization rate, the rate from Table 1 is multiplied with the corresponding stoichiometric expression in Table 2. For example, the oxygen consumption rate by the fast-growing heterotrophs, ρ_O [$M_{O_2} d^{-1}$], is given with:

$$\rho_O = \underbrace{-\left(\frac{i_{HC}}{Y_O} - i_{XB}\right)}_{\substack{\text{Stoichiometric coefficient} \\ \text{(Table 2 for } S_{O_2} \text{ and B1}}} \cdot \mu_{O,F} \cdot \frac{S_O}{K_O + S_O} \cdot \frac{S_{HC}}{K_{HC} + S_{HC}} \cdot \underbrace{\frac{(S_{NH} + S_{NO})}{K_N + (S_{NH} + S_{NO})} \cdot \frac{S_{HC}}{S_F + S_{HC}} \cdot X_{BO}}_{\text{Rate expression for B1 (aerobic growth) in Table 1}}$$

The following types of hyperbolic functions are multiplied together make up the modified Monod term:

- Limitation by an electron-donor or electron-acceptor substrate S: $S/(K_S + S)$. This is the common Monod expression for saturation kinetics by a donor and acceptor (Bae & Rittmann 1996).
- Inhibition by an inhibitor I: $K_I/(K_I + I)$. This form represents inhibition that is non-competitive and reversible. For example, $K_O/(K_O + S_O)$ in process B2 (Table 1) shows how oxygen inhibits anaerobic BTEX degradation, where S_O is the oxygen concentration and K_O is the inhibition coefficient.
- Modulation between two donor substrates (S_1 and S_2) by the same type of microorganisms: $S_1/(S_1 + S_2)$. For example, $S_{HC}/(S_F + S_{HC})$ in process B2 shows how acetate (S_F) reduces the utilisation rate of BTEX (S_{HC}) and correspondingly, the rate of B14 is limited with the modulation term $S_F/(S_F + S_{HC})$. Modulation guarantees that the growth rate of biomass growing on two substrates cannot exceed the microorganism's maximum specific growth rate.

Decay of biomass

The loss of active biomass is a first-order process for each biomass type (X_i) and with a rate coefficient b_{Bi} (d^{-1}). Processes B6–B10 in Table 1 are the decay terms. A fraction of the decayed biomass (γ_i in Table 2) is recalcitrant and produces inactive biomass solid. Inactive biomass is no longer tracked by the model.

Table 1. Kinetics of the biological processes. The corresponding stoichiometry is given in Table 2

Microbial BTEX degradation	
B1 Aerobic growth	$\mu_{O,F} \cdot \frac{S_O}{K_O+S_O} \cdot \frac{S_{HC}}{K_{HC}+S_{HC}} \cdot \frac{S_{HC}}{S_F+S_{HC}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot X_{BO}$
B2 Denitrification	$\eta_{NO}\mu_{O,F} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_{HC}}{S_{HC}+S_{HC}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot \frac{S_{HC}}{S_F+S_{HC}} \cdot X_{BO}$
B3 Iron reduction	$\mu_{Fe,F} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_{HC}}{K_{HC}+S_{HC}} \cdot \frac{S_{HC}}{K_{HC}+S_F} \cdot \frac{X_{Fe_2O_3}}{K_{Fe_2O_3}+X_{Fe_2O_3}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot X_{BF}$
B4 Sulfate reduction	$\mu_{SO,F} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_{SO}}{K_{SO}+S_{SO} \cdot \left(1+\frac{S_{H_2S}+S_{HS}}{K_{iS_4}}\right)} \cdot \frac{S_{HC}}{K_{HC}+S_{HC}} \cdot \frac{S_{HC}}{K_F+S_{HC}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot X_{BS}$
B5 Fermentation	$\eta_{an}\mu_{O,F} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{K_{H_2,an}}{K_{H_2,an}+S_{H_2}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot \frac{S_{HC}}{K_{HC}+S_{HC} \cdot \left(1+\frac{S_{H_2S}}{K_{iS_5}}\right)} \cdot X_{BO}$
Decay of biomass	
B6 Lysis of X_{BO}	$b_{BO} \cdot X_{BO}$
B7 Lysis of X_{BFe}	$b_{BFe} \cdot X_{BFe}$
B8 Lysis of X_{BSO}	$b_{BSO} \cdot X_{BSO}$
B9 Lysis of X_{BAc}	$b_{BAc} \cdot X_{BAc}$
B10 Lysis of X_{BH_2}	$X_{BH_2} \cdot X_{BH_2}$
Methanogenesis	
B11 Cleavage of Ac	$\mu_{Ac} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_F}{K_F+S_F \cdot \left(1+\frac{S_{H_2S}}{K_{iS_1}}\right)} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot X_{BAc}$
B12 Methanogenesis H_2	$\mu_{H2} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_{H_2}}{K_{H_2}+S_{H_2} \cdot \left(1+\frac{S_{H_2S}}{K_{iS_1}}\right)} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot X_{BH_2}$
Oxidation of the organic fermentation product (acetate)	
B13 Aerobic growth, S_F	$\mu_O \cdot \frac{S_O}{K_O+S_O} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot \frac{S_F}{S_F+S_{HC}} \cdot X_{BO}$
B14 Anoxic growth, S_F	$\eta_{NO}\mu_O \cdot \frac{K_O}{K_O+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot \frac{S_F}{S_F+S_{HC}} \cdot X_{BO}$
B15 Iron reduction, S_F	$\mu_{Fe} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{X_{Fe_2O_3}}{K_{Fe_2O_3}+X_{Fe_2O_3}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot \frac{S_F}{S_{HC}+S_F} \cdot X_{BF}$
B16 Sulfate reduction	$\mu_{SO} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{K_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_{SO}}{K_{SO}+S_{SO} \cdot \left(1+\frac{S_{H_2S}+S_{HS}}{K_{iS_3}}\right)} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_F}{S_F+S_{HC}} \cdot \frac{(S_{NH}+S_{NO})}{K_N+(S_{NH}+S_{NO})} \cdot X_{BS}$

Table 2. Stoichiometric matrix for the CBC module BTEX

Process	S_{HC} [M_C]	S_F [M_C]	S_{O2} [M]	S_{NO} [M]	S_{NH} [M]	S_{SO} [M]	S_{CH4} [M_C]	S_{H2} [M]	S_{Fe} [M]	S_{H2CO3} [M]	S_{HCO3} [M]	S_{HS} [M]	X_{Fe2O3} [M_{Fe}]	X_{BIO} [M_C]	X_{BF} [M_C]	X_{BS} [M_C]	X_{BAc} [M_C]	X_{BH2} [M_C]
	S1	S2	S3	S4	S5	S6	S7	S8	S10	S13	S14	S17	X6	X1	X2	X3	X4	X5
Microbial BTEX degradation																		
B1 Aerobic growth	$-\frac{1}{Y_O}$		$-\left(\frac{i_{HC}}{Y_O}-i_{XB}\right)$	$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$					σ_C				1				
B2 Anoxic growth	$-\frac{1}{Y_{NO}}$			$-\left(\frac{i_{HC}}{Y_{NO}}-i_{XB}\right)\cdot\frac{4}{5}-(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$					σ_C	$\sigma_{e\text{-}}$			1				
B3 Iron reduction	$-\frac{1}{Y_{Fe}}$			$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$				$\left(\frac{i_{HC}}{Y_{Fe}}-i_{XB}\right)\cdot\frac{4}{1}$	σ_C	[a]$\sigma_{e\text{-}}$		$-\left(\frac{i_{HC}}{Y_{Fe}}-i_{XB}\right)\cdot\frac{4}{1}$		1			
B4 Sulfate reduction	$-\frac{1}{Y_{SO}}$			$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$	$-\left(\frac{i_{HC}}{Y_{SO}}-i_{XB}\right)\cdot\frac{4}{6}$				σ_C	$\sigma_{e\text{-}}$	$\left(\frac{i_{HC}}{Y_{SO}}-i_{XB}\right)\cdot\frac{4}{6}$				1		
B5 Fermentation	$-\frac{1}{Y_{an}}$	[b]v_F		$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$			$\frac{Y_{an,H2}}{Y_{an}}$		σ_C	[a]$\sigma_{e\text{-}}$			1				
Decay of biomass																		
B6 Lysis of X_{BO}		1-γ_i			σ_N									-1				
B7 Lysis of X_{BFe}		1-γ_i			σ_N										-1			
B8 Lysis of X_{BSO}		1-γ_i			σ_N											-1		
B9 Lysis of X_{BCH1}		1-γ_i			σ_N												-1	
B10 Lysis of X_{BCH2}		1-γ_i			σ_N													-1
Methanogenesis																		
B11 Cleavage of Ac		$-\frac{1}{Y_{CH1}}$		$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$		$\frac{i_F-Y_{CH1}\cdot i_{XB}}{i_{CH4}\cdot Y_{CH1}}$			σ_C	$\sigma_{e\text{-}}$						1	
B12 Methanogenesis H2				$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$		$\frac{i_{H2}-Y_{CH2}\cdot i_{XB}}{i_{CH4}\cdot Y_{CH2}}$	$-\frac{1}{Y_{CH2}}$		σ_C								1
Oxidation of the organic fermentation product (acetate)																		
B13 Aerobic growth, S_F		$-\frac{1}{Y_O}$	$-\left(\frac{i_F}{Y_O}-i_{XB}\right)$	$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$					σ_C				1				
B14 Anoxic growth, S_F		$-\frac{1}{Y_{NO}}$		$-\left(\frac{i_F}{Y_{NO}}-i_{XB}\right)\cdot\frac{4}{5}-(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$					σ_C	$\sigma_{e\text{-}}$			1				
B15 Iron reduction, S_F		$-\frac{1}{Y_{Fe}}$		$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$				$\left(\frac{i_F}{Y_{Fe}}-i_{XB}\right)\cdot\frac{4}{1}$	σ_C	[a]$\sigma_{e\text{-}}$		$-\left(\frac{i_F}{Y_{Fe}}-i_{XB}\right)\cdot\frac{4}{1}$		1			
B16 Sulfate reduction		$-\frac{1}{Y_{SO}}$		$(1-f_{nh})\cdot\sigma_N$	$f_{nh}\cdot\sigma_N$	$-\left(\frac{i_F}{Y_{SO}}-i_{XB}\right)\cdot\frac{4}{6}$				σ_C	$\sigma_{e\text{-}}$	$\left(\frac{i_F}{Y_{SO}}-i_{XB}\right)\cdot\frac{4}{6}$				1		
Mass balances																		
MC Carbon	1	1					1			1	1			1	1	1	1	1
MN Nitrogen	0	0		1	1									$i_{N,XB}$	$i_{N,XB}$	$i_{N,XB}$	$i_{N,XB}$	$i_{N,XB}$
Me- Charge		-1		-1	1	-2			2		-1	-1						

The decay of biomass is not connected directly to consumption of an electron acceptor. Instead, $(1 - \gamma_i)$ of the lost biomass is assumed to be released as acetate, which is a donor substrate.

Temperature corrections

The maximum growth rates (μ_I) and the decay rates (b_B) are corrected for the temperature according to a modified Arrhenius equation:

$$\mu(T_2) = \mu(T_1) \cdot e^{\lambda \cdot (T_2 - T_1)} \quad (1)$$

where $\mu(T_2)$ and $\mu(T_1)$ are the maximum growth rate at temperature T_2 and T_1 respectively, and λ is the rate specific temperature coefficient [$^\circ C^{-1}$].

Stoichiometry

Table 3 presents the stoichiometry for all of the catabolic reactions, along with standard Gibbs free energy values. Toluene represents BTEX, while acetate is assumed to be the only organic fermentation product.

- Growth yields: No growth yields are reported in the literature for mixed cultures degrading BTEX. Pure-culture studies for benzene and toluene show little variations between the two substrates, but significant differences among various microbial cultures (Reardon et al. 2000). The suggested aerobic growth yield $Y_O = 0.5$ $mol_C mol_C^{-1}$ is an average value from several pure culture studies. For nitrate respiration, the yield is between 60 and 80% of the yield determined with oxygen as electron acceptor (e.g. Orhon et al. 1996; Kuba et al. 1996), and we use 80% ($Y_{NO} = 0.8 \cdot Y_O$). The yield for iron reduction ($Y_{Fe} = 0.07$ $mol_C mol_C^{-1}$) is taken from a study with a pure culture of *Shewanella oneidensis* strain MR-1 (Kostka et al. 2002). The growth yield for sulfate reduction (Y_{SO}) is assumed to be proportional to the Gibbs free energies (ΔG_f) for sulfate and oxygen reductions in Table 3. The growth yield for fermentation (Y_{an}) is 0.076 and deduced from Schink (1997) and Thauer et al. (1977).
- Oxygen demand (stoichiometric parameters i_{HC} and i_{XB} in Table 2): To determine the amount of electron acceptor, the reduction equivalent of BTEX and the biomass have to be known. The values in Table 4, expressed as mole oxygen per mole carbon, reflect the theoretical chemical oxygen demand (COD) of the compounds. These values can be obtained by applying the methods described by Reichert et al. (2001) or Henze et al. (2000). Because the CBC model does not distinguish among the BTEX components, i_{HC} is calculated from the fractions of the BTEX mixture. (i.e. 60% benzene and 40% toluene: $i_{HC} = 0.6 \cdot 1.25 + 0.4 \cdot 1.29$ $mol_{O_2} mol_C^{-1}$).
- Nitrogen uptake (stoichiometric parameter f_{nh} in Table 2): In order to ensure that synthesis of biomass can consume NO_3^- or NH_4^+ (or both) as the N source, the stoichiometric coefficient f_{nh} is used to modulate the flow of synthesis N according to the availability of NH_4^+–N. The coefficient is defined as $f_{nh} = S_{NH}/(K_N + S_{NH})$, where S_{NH} is the ammonium concentration and K_N the half saturation coefficient for nitrogen. When the NH_4^+ concentration is high, all of the synthesis N comes from NH_4^+, the more favourable N source. NO_3^- is used as the N source only when NH_4^+ is at a low concentration.

Types of inhibition

Sulfide is relatively toxic due to its ability to form strong complexes with essential metal-containing compounds in the cell (e.g., iron in cytochromes). Sulfide can inhibit fermenters, sulfate reducers, and acetoclastic and hydrogen-oxidising methanogens. Following the experimental results and discussion of Maillacheruvu & Parkin (1996), the CBC model assumes that H_2S inhibits methanogens and fermenters, whereas total sulfide concentration is toxic to sulfate-reducing organisms. The inhibition is implemented into the rate expression by using a non-competitive, reversible approach (see Table 1 the hyperbolic terms for acetate (S_F) and hydrogen (S_{H_2}) in the processes for 'Methanogenesis').

The other growth processes are assumed to be inhibited according to the following scheme: nitrate respiration (B12) is inhibited by oxygen; iron reduction (B3) is inhibited by oxygen and nitrate; sulfate reduction (B4) is inhibited by oxygen, nitrate, and sulfide ($H_2S + HS^-$); and fermentation (B5) is inhibited by oxygen, nitrate, hydrogen, and dihydrogen sulfide (H_2S).

Kinetics for iron(III) as electron acceptor

It is well established that the rate of the iron(III) reduction is microbially catalysed (Kostka & Nelson 1995). Mineralogy, crystal structure, and surface area influence the availability of solid-phase iron(III) (Burdige

Table 3. Stoichiometries for the microbiological reactions and the Gibbs Free energies for the reactions as written. ΔG_f^0 = under standard conditions; ΔG_f for pH = 7. Reaction (4) uses hematite as iron(III) source and assumes that free aqueous iron(II) is released. All data are taken from CRC, 1988

		ΔG_f^0 [kJ]	ΔG_f [kJ]	
Oxygen reduction	$C_7H_8 + 9\,O_2 + 3H_2O \rightarrow 7H_2CO_3$	−3798.9	−3798.9	(2)
Nitrate reduction	$C_7H_8 + 7.2NO_3^- + 7.2H^+ \rightarrow 7H_2CO_3 + 3.6N_2 + 0.6H_2O$	−3820.5	−3542.6	(3)
Iron(III) reduction	$C_7H_8 + 18Fe_2O_3 + 72H^+ \rightarrow 7H_2CO_3 + 36Fe^{2+} + 33H_2O$	−1500.3	+1278.6	(4)
Sulfate reduction	$C_7H_8 + 4.5SO_4^{2-} + 9H^+ + 3H_2O \rightarrow 7H_2CO_3 + 4.5\,H_2S$	−573.8	−226.4	(5)
Fermentation	$C_7H_8 + 13H_2O \rightarrow 3H_2CO_3 + 2CH_3COOH + 10H_2$	+362.7	+362.7	(6)
Methanogenesis from acetate	$2CH_3COOH + 2H_2O \rightarrow 2H_2CO_3 + 2CH_4$	−97.6	−97.6	(7)
Methanogenesis from hydrogen	$10H_2 + 2.5H_2CO_3 \rightarrow 2.5CH_4 + 7.5H_2O$	−459.5	−459.5	(8)

et al. 1992; Stumm & Sulzberger 1992; Postma 1993). Ryan & Gschwend (1991) suggest that the microbially accessible iron ($X_{fe_2O_3}$) can be measured by a reductive extraction with a Ti(III) solution. The CBC-model assumes that all Fe(III) is equally accessible and can be utilized by the microorganisms from its solid form, which has a mol/L concentration as if it were a dissolved species.

Fermentation stoichiometry

The fermentative stoichiometry is characterised by two coefficients: the synthesis yield Y_{an} of the fermenting organisms and the yield of hydrogen per mole of converted substrate, Y_{an,H_2} (in $mol_{H_2} mol_C^{-1}$). These two parameters determine, based on carbon and COD balances, the oxidation state of the average fermentation product, i_F. Currently, no experimental data supply us with indisputable values for these coefficients. Based on the overall biomass yield of fermentative toluene degradation, we use $Y_{an} = 0.076\ mol_C mol_C^{-1}$. From a carbon and COD balance, we can calculate the coefficient Y_{an,H_2} with:

$$Y_{an,H_2} = \frac{-i_F \cdot (1 - Y_{an}) + i_{HC} - i_{XB} Y_{an}}{i_{H_2}}. \quad (9)$$

The i coefficients can be found in Table 4. A reasonable assumption is that acetate is the final C-fermentation product of all possible fermentative processes (Maurer & Rittmann 2004). From Table 4, we see that in this case i_F is 1.0 $mol_{O_2} mol_C^{-1}$, giving $Y_{an,H_2} = 0.502\ mol_{H_2} mol_C^{-1}$.

Similar considerations lead to the stoichiometric coefficient ν_F for the stoichiometry of the fermentation product from BTEX fermentation:

$$\nu_F = \frac{i_{HC} - Y_{an,H_2} \cdot i_{H_2} - Y_{an} \cdot i_{XB}}{i_F \cdot Y_{an}}. \quad (10)$$

Methanogenesis stoichiometry

Growth yields (Y_{CH_1} and Y_{CH_2}) for acetoclastic and hydrogen-oxidising methanogens can be found in the literature (e.g., Maillacheruvu & Parkin 1996): $Y_{CH_1} = 0.09\ mol_C mol_C^{-1}$; $Y_{CH_2} = 0.04\ mol_C mol_{H_2}^{-1}$.

Oxidation of the fermentation product

Acetate, the organic fermentation products (S_F), is oxidized by oxygen-, nitrate-, and sulfate-reducing bacteria that degrade BTEX. Different are only the maximum growth rates and the half-saturation coefficients for acetate versus BTEX. The re-oxidation of the other reduced species are not considered in this model.

Mass balances (coefficients σ_N, σ_C, σ_{e-})

The stoichiometric coefficients σ are gained from a mass balance, either for carbon (σ_C), nitrogen (σ_N), or charge (σ_{e-}). They are calculated with the help of the coefficients in Table 2, in the last three rows labled MC, MN, and Me-, according to the following formulas:

$$\sigma_{C,a,b} = -\frac{1}{\nu_{MC,b}} \left(\sum_{i=1}^{b-1} \nu_{a,i} + \sum_{i=b+1}^{N} \nu_{a,i} \cdot \nu_{MC,i} \right) \quad (11)$$

where $\sigma_{Ca,b}$ is the stoichiometric coefficient at row a and column b based on a carbon mass balance; a, b

Table 4. COD of the CBC model compounds. [a]: Biomass $CH_{2.09}O_{0.54}N_{0.2}$ (Smolders et al. 1995)

Compound		COD (i) $mol_{O_2} mol_C^{-1}$
Benzene	i_{HC}	1.25
Toluene	i_{HC}	1.29
Ethylbenzene	i_{HC}	1.31
Xylenes	i_{HC}	1.31
Acteate	i_F	1.00
Methane	i_{CH_4}	2.00
Biomass[a]	i_{XB}	1.25
Hydrogen	i_{H_2}	0.5
$mol_{O_2} mol_H^{-1}$		

row # and column #; N is the total number of column; $\nu_{a,I}$ is the stoichiometric constant at row a and column i (in Table 2); $\nu_{MC,I}$ is the stoichiometric constant in mass balance row MC and column i (in Table 2).

Row MC, MN, and Me- are indicating the contribution of a state variable to the corresponding mass balance (for further details, see Larsen & Gujer 1995). This procedure guarantees that no process violates any mass balance.

The model assumes that all biomass maintains a constant N:C ratio. A typical value for $i_{N,XB}$ is 0.2 $mol_N mol_C^{-1}$ (Smolders et al. 1995).

Model module: Chemistry

Overview

Integrating chemical reactions that affect the fate of biological footprints is at the heart of the CBC model. The key chemical reactions, relevant to freshwater conditions, were reviewed in Maurer & Rittmann (2004) and are compiled in Table 5 and Table 6.

Activity coefficients

Although the reactions are relevant to freshwater, salt concentrations often are high enough to require activity corrections so that molar concentrations can be used in equilibrium expressions. To account for ionic strength ($0 < I < 0.1$), we use the Güntelberg approximation (Stumm & Morgan 1996):

$$\log(f_i) = -0.5 \cdot i^2 \cdot \frac{\sqrt{I}}{1+\sqrt{I}} \tag{12}$$

$$I = 0.5 \cdot \sum_j (S_j \cdot z_j^2) \tag{13}$$

where S_j is the (measured) concentration of substance j [M], z_j is the charge of substance j, and f_i is the activity coefficient for a ion with the charge $|i|$.

Temperature correction

The rate constants of the chemical reactions are corrected for the temperature according to the Arrhenius equation (Stumm & Morgan 1996):

$$k(T_2) = k(T_1) \cdot e^{-\frac{E_a}{R} \cdot \left(\frac{1}{T_2+273} - \frac{1}{T_1+273}\right)}, \tag{14}$$

where $k(T_2)$ and $k(T_1)$ are the rate constant at temperatures T_2, and T_1 [°C], respectively, E_a is the activation energy coefficient of the reaction according to Arrhenius [J mol^{-1}], and R is the molar universal gas constant (8.3144 J mol^{-1} K^{-1}).

Equilibrium constants are adapted to the ambient temperature with the van't Hoff relationship (Stumm & Morgan 1996):

$$-\log(K(T_K)) = \log(K(T = 298\,\mathrm{K})) - \frac{\Delta H_{298}}{R \cdot \log(10)} \cdot \left(\frac{1}{298} - \frac{1}{T_K}\right) \tag{15}$$

where K is the equilibrium constant at T_K or 298 [K], respectively, ΔH_{298} is the heat of formation at 298 K [J mol^{-1}], and R is the molar universal gas constant.

Calcium carbonate (calcite)

As discussed in Maurer & Rittmann (2004), the net rate ρ for the formation or dissolution of calcite ($CaCO_{3,s}$) close to the equilibrium ($1.2 > \Omega > 0.8$) is given with:

$$\begin{aligned} \rho &= a_{CaCO_3} \cdot k_1 \cdot \left(1 - \frac{f_2 S_{Ca} \cdot f_2 S_{CO_3}}{10^{-pKsp1}}\right)^n \\ &= a_{CaCO_3} \cdot k_1 \cdot (1 - \Omega_1)^n \end{aligned} \tag{16}$$

where ρ is the net reaction rate [M d^{-1}], a_{CaCO_3} the specific surface of calcite [$m^{-2}lt^{-1}$], k_1 the rate constant [mol m^{-2} d^{-1}], $f_2 \cdot S_{Ca}$ and $f_2 \cdot S_{CO_3}$ are the activities of Ca^{2+}, and CO_3^{2-} respectively [M], pK_{SP1} is the negative logarithm of the solubility constant of calcite [−], n a constant, Ω_1 is called the saturation

Table 5. Stoichiometric matrix and the corresponding kinetics of the rate determined chemical reactions

Process	S_{SO} [M] S6	S_{H2} [M] S8	S_{Ca} [M] S9	S_{Fe} [M] S10	S_{H} [M] S11	S_{CO3} [M] S15	S_{H2S} [M] S16	X_{Fe2O3} [M_{Fe}] X6	X_{CaCO3} [M_{Ca}] X7	X_{FeS} [M_{Fe}] X8	X_{FeS2} [M_{Fe}] X9	X_{S} [M_{S}] X10	Process
I1 Precipitation calcite			-1			-1			1				$k_1 \cdot a_{CaCO3} \cdot (1-\Omega_1)^n$
I2 Formation of pyrite		1					-1			-1	1		$k_5 \cdot X_{FeS} \cdot S_{H2S}$
I3 Conversion to pyrite										-1	1	-1	$k_8 \cdot X_{FeS} \cdot X_S$
I4 Reductive dissolution				1	-2		-½	-1				½	$a_{Fe2O3} \cdot (k_{e1} \cdot A_{FeS} + k_{e2} \cdot A_{FeSH})$
I4b Reductive dissolution	$\frac{1}{8}$			1	$-\frac{14}{8}$		$-\frac{1}{8}$	-1					$a_{Fe2O3} \cdot (k_{e1} \cdot A_{FeS} + k_{e2} \cdot A_{FeSH})$

state, and $(1 - \Omega_1)$ is the thermodynamic difference from equilibrium. Parameter values are listed in Appendix 2.

Iron monosulfide (FeS)

The formation of amorphous iron monosulfide from Fe^{2+} and sulfides is fast and treated as an equilibrium process, as reviewed in Maurer & Rittmann (2004). The equation and the thermodynamic properties are given in Table 6.

Formation of pyrite (FeS2) from FeS

The rate of pyrite formation from amorphous iron sulfide (FeS) follows the findings of Rickard (1995, 1997; Rickard & Luther 1997), as reviewed in Maurer & Rittmann (2004). Amorphous FeS seems to be the main intermediate in natural aquatic environments, and its presence is a prerequisite for the formation of pyrite (FeS_2) by the following rate expression:

$$\rho = k_5 \cdot X_{\mathrm{FeS}} \cdot S_{\mathrm{H_2S}} \tag{17}$$

where ρ is the formation rate of pyrite [M d^{-1}], X_{FeS} is the concentration of iron sulfide, treated as if it were a dissolved species ($mol_{FeS}\ l_{fluid}^{-1}$) [M], S_{H_2S} is the aqueous H_2S concentration [M], and k_5 is the rate constant [M^{-1} d^{-1}] at temperature T.

This formulation of the rate assumes that the ratio of iron monosulfide to total surface area is constant. This assumption is based on the observation that iron monosulfide (FeS) is an intermediate that under natural conditions will not accumulate in large quantities (Davison 1991).

Because of its thermodynamic and kinetic stability, pyrite is assumed to be a final product, and therefore no dissolution reaction is considered (Maurer & Rittmann 2004).

Formation of pyrite (FeS_2) from elemental S and FeS

Various experiments document that elemental sulfur and iron monosulfide react together to form pyrite (Maurer & Rittmann 2004). Elemental sulfur and iron monosulfide are products of the reductive dissolution of iron(III), described below. Little is known about the mechanisms and the reaction rates, although Rickard et al. (1995) report from a lab experiment that the reaction forms pyrite within days. The CBC-model uses simple first-order kinetics with respect of X_{FeS} and X_S.

$$\rho = k_8 \cdot \mathrm{X_{FeS}} \cdot \mathrm{X_S} \tag{18}$$

where ρ is the formation rate of pyrite [M d^{-1}], X_{FeS} is the concentration of iron monosulfide [M], X_S is the concentration of elemental sulfur [M], and k_8 is the rate constant [M^{-1} d^{-1}] at temperature T.

Reductive dissolution of Fe(III)

The rate for the reductive dissolution of iron(III) derives from the approach developed by Dos Santos Afonso & Stumm (1992). The rate of reductive dissolution is proportional to the concentration of the two surface complex ≡FeSH (A_{FeSH}) and ≡FeS^- (A_{FeS}) and is given in Table 5 (processes I4 and I4b). The concentration of the surface complexes can be derived from a set of equilibrium reactions under the assumption that the concentration of the surface complex ≡FeO^- can be neglected:

Table 6. Reversible chemical reactions and thermodynamic equilibrium constants in the CBC model at 25 °C and I = 0. Legend: log K = log of equilibrium constant at 298 °K; ΔH_{298} = heat of formation at 298 °K; * = sum of $H_2CO_{3,aq}$ and $CO_{2,aq}$; References: b: Davison et al. (1999), d: Davison (1991), e: Stumm & Morgan (1996), f: Plummer & Busenberg (1982), Dos Santos Afonso & Stumm (1992)

Equation	log K		Lit	ΔH_{298} (J mol^{-1})	Lit
$H_2O \leftrightarrows OH^- + H^+$	−14.0		e		
$H_2S_{(aq)} \leftrightarrows HS^- + H^+$	−6.98 ± 0.02		d		
$H_2CO_3^* \leftrightarrows H^+ + HCO_3^-$	−6.36		e	9113	e
$HCO_3^- \leftrightarrows H^+ + CO_3^{2-}$	−10.33		e	14907	e
$Fe^{2+}_{aq} + 2HS^- \leftrightarrows Fe(HS)_2$	6.45 ± 0.12		b		
$Fe^{2+} + HCO_3^- \leftrightarrows [FeHCO_3]^+$	2.0		e		
$Fe^{2+} + CO_3^{2-} \leftrightarrows [FeCO_3]^0_{aq}$	4.38		e		
$Fe^{2+} + CO_3^{2-} \leftrightarrows [FeCO_3]^0_s$	10.45		e		
$Fe^{2+} + HS^- \leftrightarrows H^+ + [FeS]_{s,amorph}$	2.95		e	32500	d
$Ca^{2+} + HCO_3^- \leftrightarrows [CaHCO_3]^+$	2.69		e		
$Ca^{2+} + CO_3^{2-} \leftrightarrows [CaCO_3]^0_{aq}$	3.22		e		
Thermodynamic properties of kinetic processes					
$\equiv FeOH + H^+ \leftrightarrows \equiv FeOH_2^+$	7.25	$-pK_{A1}$	g		
$\equiv FeOH + H_2S_{(aq)} \leftrightarrows \equiv FeS^- + H_3O^+$	−1.72	$-pK_{A2}$	g		
$\equiv FeOH + H_2S_{(aq)} \leftrightarrows \equiv FeSH + H_2O$	3.80	−pKA3	g		
$Ca^{2+} + CO_3^{2-} \leftrightarrows [CaCO_3]_s$	8.48	$-pK_{SP1}$	e	−3071.4	f
				9615	e

$$A_{FeS} = \frac{A_{FeSH}}{\lambda} \cdot 10^{pKA3-pKA2+pH} \quad (19)$$

$$A_{FeSH} = A_{FeOH,tot} \cdot \left(1 + \xi + \frac{\xi}{\lambda} \cdot 10^{pKA3-pKA2+pH}\right)^{-1} \quad (20)$$

$$A_{FeOH_2} = (A_{FeOH,tot} - A_{FeSH} - A_{FeS}) \cdot \lambda \cdot \frac{10^{-pH-pKA1}}{1 + \lambda \cdot 10^{-pH-pKA1}} \quad (21)$$

where all values for the thermodynamics and kinetics are taken from Dos Santos Afonso & Stumm (1992) and are listed in Table 6. All other values are given in:

$$\xi = \frac{S_{H_2S} \cdot 10^{-pKA3}}{(1 + \lambda \cdot 10^{-pKA1-pH})} \quad (22)$$

$$\lambda = e^{\frac{-F \cdot \psi}{R \cdot T_K}} \quad (23)$$

$$\psi = \frac{0.438 \cdot F}{\sqrt{I}} \cdot (A_{FeOH_2} - A_{FeS}) \quad (24)$$

A_{FeS}, A_{FeSH} and A_{FeOH_2} are the concentration of the surface complexes ≡FeS-, ≡FeSH and $\equiv FeOH_2^+$, respectively in [mol m^{-2}], and $A_{FeOH,tot}$ is the density of sites on the iron-oxide surface. Table 5 presents two possible stoichiometries for the process: Elemental sulfur as the dominant product is described with $Fe(OH)_3$ and FeOOH as iron minerals (process no. I4), whereas sulfate is the major product with hematite (Fe_2O_3; process no. I4b).

Equilibrium reactions

The equilibrium reactions in Table 6 are needed to represent the chemical state of the aquatic environment. Because the subsurface environment normally is carbonate-buffered, it is possible to implement the equilibrium part of the CBC-model relatively simply. In particular, it is often possible to assume that the formation of carbonate complexes (e.g. $[FeHCO_3]^+$, $[FeCO_3]^0_{aq}$, $[CaHCO_3]^+$, $[CaCO_3]^0_{aq}$) does not influence the total carbonate concentration; therefore, the concentrations of free Fe^{2+} and Ca^{2+} and the pH can be computed analytically. This simplification needs to be evaluated for a specific case; however it usually is valid and avoids the need for solving the system of linear equations for the equilibrium reactions and makes the implementation of the model with a transport model (e.g., into RT3D, Clement 1997) tractable.

Summary

The CBC model contains novel features that allow it to comprehensively track the footprints of BTEX

bioremediation, even when the fate of those footprints is confounded by abiotic reactions and complex interactions among different kinds of microorganisms. To achieve this comprehensive tracking of all the footprints, the CBC model contains important new biological features and key abiotic reactions.

The biological module of the CBC-model includes these important new aspects that are needed to track important footprints.

- The CBC model separates BTEX fermentation from methanogenesis. This introduces two fermentation products, acetate and hydrogen gas, both of which can be footprints. Frequently reported measurements of hydrogen and acetate in real sites and experiments indicate that fermentation occurs and therefore should be considered in a quantitative simulation model (Landmeyer et al. 1996; Cozzarelli et al. 1994; Chaudhuri & Wiesmann 1996; Grbic-Galic & Vogel 1987; Grbic-Galic 1991; Revesz et al. 1995).
- It explicitly include biomass as a sink for electrons and carbon. Ignoring synthesis as a sink for electrons can lead to significant overestimates of reduced products (such as sulfide and methane) and inorganic carbon.
- The CBC model has different growth rates for each biomass type. Due to the different growth rates of the several types of microorganisms, the various biodegradation pathways usually have significantly different startup times. This model feature corresponds well with laboratory experiments, which generally report long lag and startup phases, particularly for methanogenesis. For example Edwards & Grbic-Galic (1991) observed anaerobic adaptation periods in chemostats of 100 to 120 days for toluene degradation and 200 to 255 days for o-xylene. Baedecker et al. (1993) reported that a plume from a crude oil spill became more reducing over a 5-year period.
- The model includes inhibition of the different reactions by other electron acceptors and by sulfide toxicants.

The chemical module of the CBC-model includes abiotic reactions that affect the footprints of the biological reactions. In particular, the chemical module describes the precipitation/dissolution of $CaCO_3$, Fe_2O_3, FeS, FeS_2, and S°. The kinetics for the precipitation/dissolution reactions follow the critical review in Maurer & Rittmann (2004). In addition, the CBC model includes equilibrium speciation of the carbonate and sulfide species, a feature necessary to describe the abiotic kinetics and inhibition of the biological reactions.

In conclusion, the CBC model explicitly couples the chemical and biological reactions needed to understand the fate of BTEX and its biodegradation footprints, even when the footprints are affected by other reactions. The CBC model makes it possible to evaluate the multiple biological and chemical processes that may affect the footprints from natural attenuation of BTEX.

Appendix 1 – Variables, symbols, and definitions

Program variables (state variables)

Dissolved components

		Unit	
S_{HC}	S1	M_C	Hydrocarbons (BTEX)
S_F	S2	M	Organic fermentation products
S_{O_2}	S3	M	Dissolved oxygen ($O_{2,aq}$)
S_{NO}	S4	M	Nitrate (NO_3^-) and nitrite equivalents (=0.6*NO_2^-)
S_{NH}	S5	M	Ammonium (NH_4^+)
S_{SO_4}	S6	M	Sulfate (SO_4^{2-})
S_{CH_4}	S7	MC	Dissolved methane ($CH_{4,aq}$)
S_{H_2}	S8	M	Dissolved hydrogen-gas ($H_{2,aq}$)
S_{Ca}	S9	M	Calcium (Ca^{2+})
S_{Fe}	S10	M	Iron(II) (Fe_{aq}^{2+})
S_H	S11	M	Proton-ion (H_{aq}^+ or H_3O^+)
S_{OH}	S12	M	Hydroxyl-ion (OH^-)
$S_{H_2CO_3}$	S13	M	Dihydrogen carbonate ($H_2CO_{aq}^{3*}$)
S_{HCO_3}	S14	M	Hydrogen carbonate (HCO_3^-)
S_{CO_3}	S15	M	Carbonate (CO_3^{2-})
S_{H_2S}	S16	M	Dihydrogen sulfide (H_2S_{aq})
S_{HS}	S17	M	Hydrogen sulfide (HS^-)
Dissolved Complexes			
S_{CaCO_3}	S18	M	Calcium carbonate ($CaCO_{3,aq}$)
S_{CaHCO_3}	S19	M	Calcium hydrogen carbonate ($CaHCO_{3,aq}^+$)
S_{FeCO_3}	S20	M	Iron(II) carbonate ($FeCO_{3,aq}$)
S_{FeHCO_3}	S21	M	Iron(II) hydrogen carbonate ($FeHCO_{3,aq}^+$)
S_{FeHS_2}	S22	M	Iron(II) dihydrogen sulfide ($Fe(HS)_{2,aq}$)

Particulate components

X_{BO}	X1	M_C	Het. biomass using oxygen and nitrate as electron acceptors
X_{BF}	X2	M_C	Het. biomass using iron(III) as electron acceptors
X_{BS}	X3	M_C	Het. biomass using sulfate as electron acceptors
X_{BAc}	X4	M_C	Methanogenic biomass (splitting acetate)
X_{BH_2}	X5	MC	Methanogenic biomass (using hydrogen)
$X_{Fe_2O_3}$	X6	M_{Fe}	Iron(III)oxide
X_{CaCO_3}	X7	M_C	Calcium carbonate (calcite, $CaCO_3$)
X_{FeS}	X8	M_{Fe}	Iron monosulfide (FeS_{amorph})
X_{FeS_2}	X9	M_{Fe}	Pyrit (FeS_2)
X_S	X10	M_S	Elementary sulfur (S_8)

General parameters

	Units	*Value*	*Remark*
F	C mol^{-1}	96484.6	Faraday constant
f_i	–	see eq. (12)	Activity coefficient
I	–	see eq. (13)	Ionic strength
pH	–	-log(SH)	pH definition
R	J mol^{-1} K^{-1}	8.3144	Molar gas constant
T	°C		Temperature in °C
T_K	K		Temperature in K

Appendix 2. Standard values of stoichiometric and kinetic parameters

Stoichiometric parameters

Param.	Value	Unit	Lit	Description, remarks
ν_F	12.16	–	{t}	Stoichiometric coefficient for the production of fermentation products (see eq. (10))
ν_i	0.02	–	{1}	Fraction of inert Carbon after decay of biomass
f_{nh}		–		Description: distribution factor for the use of nitrate or ammonium as N-source. $f_{nh} = S_{NH}/(K_N + S_{NH})$
i_{CH_4}	2.0	$mol_{O_2} mol_C^{-1}$	{t}	COD of methane
i_f	1.0	$mol_{O_2} mol_C^{-1}$	{t}	COD of SF, if assumed to be acetate only
i_{H_2}	0.5	$mol_{O2} mol_{H_2^{-1}}$	{t}	COD of hydrogen
i_{HC}	1.27	$mol_{O2} mol_C^{-1}$	{t}	COD of BTEX (hydrocarbons); see Table 4
$i_{N,XB}$	0.2	$mol_N mol_C^{-1}$	{2}	Nitrogen content of biomass
i_{XB}	1.25	$mol_{O_2} mol_C^{-1}$	{2}	COD of biomass
Y_{an}	0.076	$mol_C mol_C^{-1}$	{5}	Growth yield of heterotrophic organisms on fermentation
Y_{an,H_2}	0.502	$mol_{H_2} mol_C^{-1}$	{T}	Hydrogen production in anaerobic fermentation (mole hydrogen per mole of substrate); see eq. (9)

Param.	Value	Unit	Lit	Description, remarks
Y_{Fe}	0.07	$mol_C mol_C^{-1}$	{T}	Growth yield of heterotrophic organisms using Fe(III)
Y_{CH_1}	0.09	$mol_C mol_C^{-1}$	{3}{6}	Growth yield of acetoclastic methanogens
Y_{CH_2}	0.044	$mol_C mol_H^{-1}$	{3}{6}	Growth yield of hydrogen oxidising methanogens
Y_{NO}	$0.8 \cdot Y_O$	$mol_C mol_C^{-1}$	{T}	Growth yield of heterotrophic organisms using nitrate as electron acceptor
Y_O	0.51	$mol_C mol_C^{-1}$	{14}	Growth yield of heterotrophic organisms using oxygen as electron acceptor. Averaged value from 9 different pure culture studies with benzene and toluene and converted with a molecular weight for biomass of 26 g mol_C^{-1}.
Y_{SO}	$0.13 \cdot Y_O$	$mol_C mol_C^{-1}$	{T}	Growth yield of heterotrophic organisms using sulfate as electron acceptor

Kinetic parameters

Param.	Value	Unit	Lit	Description, remarks
η_{an}	0.1	–	{7}	Reduction coefficient for the fermentative max. growth rate
η_{no}	0.8	–	{8}	Reduction coefficient for the anoxic max. growth rate
$\mu_{Ac,25}$	0.09	d^{-1}	{6}	Growth rate constant of acetoclastic organisms at 25 °C
$\mu_{Fe,25}$	0.08	d^{-1}	{t}	Heterotrophic growth rate constant growing on iron(III) at 25 °C
$\mu_{Fe,F,25}$	0.1	d^{-1}	{t}	Heterotrophic growth rate constant with acetate at 25 °C for iron(III)
$\mu_{H_2,25}$	0.8	d^{-1}	{6}	Growth rate constant of methanogens at 25 °C
$\mu_{O,25}$	0.16	d^{-1}	{4}	Heterotrophic growth rate constant growing on oxygen at 25 °C
$\mu_{O,F,25}$	6.0	d^{-1}	{7}	Aerobic heterotrophic growth rate constant with acetate at 25 °C
$\mu_{SO,25}$	0.08	d^{-1}	{t}	Heterotrophic growth rate constant growing on sulfate at 25 °C
$\mu_{SO,F,25}$	0.1	d^{-1}	{t}	Heterotrophic growth rate constant with acetate at 25 °C for sulfate
λ_{rfe}	0.069	$°C^{-1}$	{t}	Temperature coefficient for r_{Fe} (growth on iron(III))
λ_{rCH_1}	0.069	$°C^{-1}$	{t}	Temperature coefficient for r_{CH1} (methanogenesis by cleavage of acetate)
λ_{rCH_2}	0.069	$°C^{-1}$	{t}	Temperature coefficient for r_{CH2} (methanogenesis by cleavage of acetate)
λ_{rO}	0.069	$°C^{-1}$	{t}	Temperature coefficient for r_O (aerobic growth)
λ_{rSO}	0.069	$°C^{-1}$	{t}	Temperature coefficient for r_{SO} (growth on sulfate)
Ω_1		–	{T}	Saturation state of calcite, see eq. (16)
$A_{FeOH,tot}$	$1.66 \cdot 10^{-6}$	mol m^{-2}	{11}	Density of total active sites on a $X_{Fe_2O_3}$ surface, typically: 10^{18} sites per m^2
A_{FeS}		mol m^{-2}	{11}	Concentration of surface complex $\equiv FeS^-$ (see eq. (19))
A_{FeSH}		mol m^{-2}	{11}	Concentration of surface complex A_{FeSH} (see eq. (20))
$a_{Fe_2O_3}$		M^2 lt^{-1}		Specific surface area of $X_{Fe_2O_3}$
a_{CaCO3}		M^2 lt^{-1}		Specific surface area of X_{CaCO_3}
$b_{BAc,25}$	$0.15 \cdot r_{CH_1,25}$	d^{-1}	{3}{6}	Lysis rate constant for X_{BAc} at 25 °C
$b_{BFe,25}$	$0.1 \cdot r_{Fe,25}$	d^{-1}	{t}	Lysis rate constant for X_{BF} at 25 ^{c}ircC
$b_{BH_2,25}$	$0.15 \cdot r_{CH_2,25}$	d^{-1}	{3}{6}	Lysis rate constant for XBH2 at 25 °C
$b_{BO,25}$	$0.1 \cdot r_{O,25}$	d^{-1}	{t}	Lysis rate constant for XBO at 25 °C
$b_{BSO,25}$	$0.1 \cdot r_{SO,25}$	d^{-1}	{t}	Lysis rate constant for X_{BSO} at 25 °C
$E_{a,k1}$	45500	J mol^{-1}	{9}	Arrhenius 'Activation energy' for calcite formation
$E_{a,k5}$	35000	J mol^{-1}	{10}	'Activation energy' for FeS2 formation
$E_{a,k8}$	47787	J mol^{-1}	{t}	'Activation energy; for FeS + S conversion (283–293 K = factor 2)
$k_{1,25}$	0.156	mol m^{-2} d^{-1}	{12}	Reaction rate constant for precipitation of calcite at 25 °C
$k_{5,25}$	8.90	M^{-1} d^{-1}	{10}	Reaction rate constant for precipitation of FeS_2 at 25 °C
$k_{8,25}$	0.01	M^{-1} d^{-1}	{t}	Reaction rate constant for conversion of FeS and S to FeS_2
$k_{e1,25}$	720	d^{-1}	{11}	Reaction rate constant for the reductive dissolution of $X_{Fe_2O_3}$ at 25 °C
$k_{e2,25}$	9600	d^{-1}	{11}	Reaction rate constant for the reductive dissolution of Xfe_2O_3 at 25 °C

Param.	Value	Unit	Lit	Description, remarks
K_{F1}	$230 \cdot 10^{-6}$	$mol_C\ lt^{-1}$	{13}	Half saturation coefficient for acetate and acetoclastic organisms (B11)
K_F	10^{-5}	$mol_C lt^{-1}$	{t}	Half saturation coefficient for fermentation products
$K_{Fe_2O_3}$	10^{-3}	$mol_C mol_{Fe}^{-1}$	{t}	Half saturation coefficient for iron(III) (Xfe_2O_3)
K_{H_2}	$0.15 \cdot 10^{-6}$	$mol_{H_2} lt^{-1}$	{3}	Half saturation coefficient for hydrogen utilisation of methanogens
$K_{H2,an}$	$5 \cdot 10^{-6}$	$mol_{H_2} lt^{-1}$	{t}	Inhibition coefficient for hydrogen and fermentation
K_{HC}	$0.25 \cdot 10^{-6}$	$mol_C lt^{-1}$	{4}	Half saturation coefficient for BTEX; $K_{Benzene} = 0.2\ \mu M_C$; $K_{TEX} = 0.3\ \mu M_C$
K_{IS1}	$3.4 \cdot 10^{-3}$	$mol_S lt^{-1}$	{3}	Inhibition coefficient for sulfide (H_2S) of acetoclastic methanogens
K_{IS2}	$19.5 \cdot 10^{-3}$	$mol_S lt^{-1}$	{3}	Inhibition coefficient for sulfide (H_2S) of hydrogenotrophic methanogens
K_{IS3}	$1.1 \cdot 10^{-3}$	$mol_S l^{-1}$	{3}	Inhibition coefficient for sulfide ($HS^- + H_2S$) of acetate SBR (35 mg/l S_{tot})
K_{IS4}	$21.2 \cdot 10^{-3}$	$mol_S lt^{-1}$	{3}	Inhibition coefficient for sulfide ($HS^- + H_2S$) of SBR
K_{IS5}	$1.0 \cdot 10^{-3}$	$mol_S lt^{-1}$	{3}	Inhibition coefficient for sulfide (H_2S) of fermentation
K_N	$5.0 \cdot 10^{-6}$	$mol_N lt^{-1}$	{t}	Half saturation coefficient for nitrogen and biomass growth
K_{NO}	$3.0 \cdot 10^{-6}$	$mol_N lt^{-1}$	{t}	Half saturation coefficient for nitrate
K_O	$3.0 \cdot 10^{-6}$	$mol_{O_2} lt^{-1}$	{t}	Half saturation coefficient for oxygen
K_{SO}	$1.0 \cdot 10^{-6}$	$mol_S lt^{-1}$	{t}	Half saturation coefficient for sulfate
n	3.3	–	{12}	Exponential factor for the calcite dissolution rate and conditions close to the equilibrium ($1.2 > \Omega_1 > 0.8$), see eq. (16)
pK_{A1}	7.25	–	{T}	Equilibrium constant for $\equiv FeOH_2^+$, see Table 6
pK_{A2}	−1.72	–	{T}	Solubility product of $\equiv FeS^-$, see Table 6
pK_{A3}	3.80	–	{T}	Solubilty product of $\equiv FeSH$, see Table 6
pK_{SP1}	8.48	–	{T}	Solubility product of calcite, see Table 6

Legend: {t}: typical value, {T}: see text, {1}: Rittmann et al. (1987), {2}: Smolders et al. (1995), {3}: Maillacheruvu & Parkin (1996), {4}: Bielefeld & Stensel (1999), {5}: Schink (1997); Thauer et al. (1977), {6}: Gujer & Zehnder (1983) and Zehnder et al. (1982), {7}: Henze et al. (2000), {8}: Kuba et al. (1996), {9}: Dawe & Zhang (1997); White (1997); Kralj & Brecevic (1995); Kralj et al. (1997), {10}: Rickard (1997), {11}: Dos Santos Afonso & Stumm (1992), {12}: Svensson & Dreybrodt (1992), {13}: Smith & Mah (1980), {14}: Reardon et al. (2000).

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