ORIGINAL PAPER

Mathematical modeling of the biodegradation of residual hydrocarbon in a variably-saturated sand column

Xiaolong Geng · Michel C. Boufadel · Brian Wrenn

Received: 10 September 2011/Accepted: 12 June 2012/Published online: 4 July 2012 © Springer Science+Business Media B.V. 2012

Abstract The biodegradation of heptadecane in five sand columns was modeled using a multiplicative Monod approach. Each column contained 1.0 kg of sand and 2 g of heptadecane, and was supplied with an artificial seawater solution containing nutrients at a flow rate that resulted in unsaturated flow through the column. All nutrients were provided in excess with the exception of nitrate whose influent concentration was 0.1, 0.5, 1.0, 2.5, or 5.0 mg N/L. The experiment was run around 912 h until no measurable oxygen consumption or CO₂ production was observed. The residual mass of heptadecane was measured at the end of the experiments and the biodegradation was monitored based on oxygen consumption and CO₂ production. Biodegradation kinetic parameters were estimated by fitting the model to experimental data of oxygen, CO₂, and residual mass of heptadecane obtained from the two columns having influent nitrate-N concentration of 0.5 and 2.5 mg/L. Noting that the oxygen and CO₂ measurements leveled off at around 450 h, we fitted the model to these data for that

X. Geng · M. C. Boufadel (⊠) · B. Wrenn Department of Civil and Environmental Engineering, Center for Natural Resources Development and Protection, New Jersey Institute of Technology, Newark, NJ 07102, USA e-mail: boufadel@gmail.com

X. Geng

Department of Mathematics, Anshan Normal University, Anshan, Liaoning 114001, China range. The estimated parameters fell in within the range reported in the literature. In particular, the half-saturation constant for nitrate utilization, K_N , was estimated to be 0.45 mg N/L, and the yield coefficient was found to be 0.15 mg biomass/mg heptadecane. Using these values, the rest of experimental data from the five columns was predicted, and the model agreed with the observations. There were some consistent discrepancies at large times between the model simulation and observed data in the cases with higher nitrate concentration. One plausible explanation for these differences could be limitation of biodegradation by reduction of the heptadecane–water interfacial area in these columns while the model uses a constant interfacial area.

Keywords Biodegradation · Multiple-Monod kinetics · Parameter estimation · Mathematical model

Abbreviations

- μ Gross growth of the active biomass (day⁻¹)
- μ_{max} Maximum growth rate (day⁻¹)
- ρ_{sand} True density of the sand (mg/cm³)
- σ^2 Squared error of estimation
- ε Constant fraction of the decayed biomass
- ω_{ij} Weights
- φ Shape factor
- A_s Specific surface area of sand (cm²/g)
- C CO₂ production
- $d_{\rm dvg}$ Average particle size (mm)

_						
F	Objective function					
Η	Hessian matrix					
k_d	Endogenous biomass decay rate (day^{-1})					
K_S	Half-saturation concentration (mg/cm ²)					
K_N	Half-saturation concentration for nitrogen					
	consumption (mg N/L of pore water)					
n	Number of observations					
Ν	Nitrate concentration (mg N/L of pore water)					
N_K	Number of measurements of dependent					
	variable					
O ₂	Oxygen					
р	Number of estimated parameters					
S	Substrate					
Sarea	Surface-area normalized concentration of					
	heptadecane (mg/cm ²)					
Smass	Concentration of heptadecane					
	(mg/kg of dry sand)					
Χ	Active biomass concentration (mg/cm ²)					
X_i	Inert biomass concentration (mg/cm ²)					
X_0	Initial biomass concentration (mg/cm ²)					
Y_{CS}	Stoichiometric coefficient for CO ₂ production					
	from substrate (mg C/mg S)					
Y_{CX}	Stoichiometric coefficient for CO ₂ produced					
	during complete mineralization of biomass					
	(mg C/mg X)					
Y_{OS}	Stoichiometric coefficient for oxygen					
	consumption based on complete					
	mineralization of substrate (mg of O ₂ /mg of S)					
Y_{OX}	Stoichiometric coefficient for oxygen					
	consumption during the complete					
	mineralization of biomass (mg of O ₂ /mg of X)					
Y_X	Biomass yield coefficient for growth on					
	substrate (mg X/mg S)					
u_S	Simulated result for the dependent variable					

- u_O Observed result for the dependent variable
- V_x Covariance matrix

Introduction

Contamination of beaches by accidental oil spills constitutes immediate and long lasting problems because the toxic oil components can persist for relatively long time in the subsurface of shorelines (Taylor and Reimer 2008; Esler 2010; Guo et al. 2010; Li and Boufadel 2010; Xia et al. 2010; Atlas and Hazen, 2011). One available alternative for cleanup is bioremediation, which involves the addition of

nutrients (such as nitrate and phosphate) to a contaminated environment to stimulate the growth of indigenous microbes and subsequent biodegradation of the hydrocarbons. Successful bioremediation is achieved by maximizing the contact time between the hydrocarbon and a sufficiently high concentration of nutrients. Previous research (Venosa et al., 1996; Boufadel et al. 1999; Du et al. 1999) has shown that 2–10 mg nitrate–N/L is sufficient to support maximum hydrocarbon biodegradation rates in open systems. Such concentrations are not commonly found in the environment (Venosa et al. 1996; Boufadel et al. 2010; Boufadel et al. 2011), and thus the addition of nutrient is required for optimal hydrocarbon biodegradation.

Oil-contaminated shorelines are open environments, where dissolved nutrients that are added to the beach surface to enhance oil biodegradation are washed out of the bioremediation zone by the action of tide and waves (Wrenn et al. 1997; Boufadel et al. 1998). Therefore, successful bioremediation of coastal oil spills is more dependent on the kinetics of nutrient consumption than on its stoichiometry, but guidance on oil-spill bioremediation is often given in terms of the total mass of nutrients required to remediate the oil that is present (Gibbs 1975; Floodgate 1979; Bragg et al. 1994). More useful guidance could be provided to spill responders in the form of an effective numerical model based on the kinetics of nutrient consumption rather than stoichiometry. Such a model should demonstrate the relationship between the rate of biodegradation and nutrient addition and could be used to design successful bioremediation projects. Several kinetics-based models-ranging from those where the kinetics are represented as first-order decay (Venosa et al. 1996; Venosa et al. 2010) to more complicated models where Monod expressions are used (Nicol et al. 1994; Essaid et al. 1995; Schirmer et al. 2000; Sarioglu and Copty 2008)-have been developed to describe the rate of hydrocarbon biodegradation. Because the latter approach is more general and can reduce to first-order when the hydrocarbon concentration is small, it was used in this study. In addition, these previous models were extended herein to include the effects of nutrient concentration and to predict the rates of the coupled processes of oxygen consumption and carbon dioxide production. Our model was validated using microcosm studies of heptadecane (C17H36) biodegradation conducted by Boufadel et al. (1999).

Because model validation relies on data from a previously published study, the methods and results of that study are described and are followed by a description of the kinetic model of hydrocarbon biodegradation in porous media. The inverse problem (i.e., parameter estimation from experimental data) is then described. Eight kinetic parameters and initial biomass concentration were estimated by fitting the model to experimental data, which consisted of oxygen, CO_2 , and the mass of heptadecane remaining at the end of the experiments. The model was evaluated by comparing the estimated kinetic parameters to values reported in the literature, and the robustness of the model was assessed by error quantification.

Biodegradation in experimental microcosms

Heptadecane biodegradation rate was studied in continuous-flow microcosm reactors that consisted of glass tubes 7.6 cm (ID) by 38.7 cm long packed with 1.0 kg of sand. The sand used in this study has a very narrow grain-size distribution (0.6–0.85 mm), and the bulk (dry) density was about 1.43 kg/L. The porosity was measured at 0.38. The sand was retained in the microcosms by a screen placed 5.7 cm from the bottom. An artificial seawater solution containing nutrients and micro-nutrients was fed to each microcosm at a rate of 7.2 L/day. All nutrients except nitrate were provided at concentrations that ensured that they did not limit microbial growth (Boufadel et al. 1999). The flow rate through the systems resulted in a specific flux of 158.7 cm/day, which was lower than the saturated hydraulic conductivity of the sand. Therefore, unsaturated flow conditions prevailed in the microcosms. The average soil moisture ratio (soil moisture divided by porosity) in the microcosms was equal to 0.65 (Boufadel et al. 1999).

The hydrocarbon substrate, *n*-heptadecane, was immobilized on the sand at a concentration S_{mass} of 2 mg/g of dry sand before the sand/hydrocarbon mixture was packed into the microcosms (Boufadel et al. 1999). (The designation "S" reflects that we are treating heptadecane as substrate (i.e., food) for biodegradation). For convenience, the concentration of heptadecane is represented as mass of heptadecane per unit area of sediments (mg/cm²). The conversion

between mg/g, S_{mass} , and mg/cm², S_{area} , is done according to:

$$S_{\text{area}} = \frac{S_{\text{mass}}}{A_{\text{S}}} \tag{1}$$

Where A_S is the specific surface area of sand per mass of sediments (cm²/g) and can be estimated by:

$$A_{\rm S} = \frac{6}{\varphi d_{\rm avg} \rho_{\rm sand}} \tag{2}$$

Where φ is the shape factor, d_{avg} is the average particle size, and ρ_{sand} is the true density of the sand. Here, the shape factor, φ , is taken as 0.75, which is a reasonable value for sand (Reynolds and Richards 1996). The density and the average particle size of sand grains were found to be 2.65 g/cm³ and 0.725 mm (Boufadel et al. 1999). The initial surface-area normalized concentration of heptadecane, S_{area} (t = 0) was thus estimated to be 0.048 mg/cm².

Each microcosm was isolated from the atmosphere and connected to a respirometer (N-Con System, Larchmont, NY), which provided oxygen whenever the pressure in the system dropped due to oxygen consumption (due to aerobic biodegradation of the heptadecane). A KOH tap was used to capture the carbon dioxide (CO_2) . The heptadecane biodegradation rate in each microcosm was monitored by continuously recording the amount of oxygen that was supplied by the respirometer. Dissolved inorganic carbon (i.e., CO₂) and nitrate concentrations were periodically measured in the reactor effluent. Five different influent nitrate concentrations were used. They were 0.1, 0.5, 1.0, 2.5, and 5.0 mg of nitrate-N/L of influent solution. The experiment was run around 912 h until no measurable oxygen consumption or CO₂ production was observed.

The microcosms were inoculated with a mixed culture of oil-degrading microorganisms that were originally isolated from a sandy beach near the mouth of Delaware Bay. The initial biomass concentration of the inoculum was determined by measuring lipid phosphate (Findlay et al. 1989). This method assumes that the measured phospholipids originate only from the cell membranes of living microorganisms because phospholipids are turned over rapidly upon cell death, but the conversion factor relating lipid phosphate to biomass has been reported to vary between of 1,920 and 10,000 ng of C/nmol lipid phosphate (Findlay

et al. 1989; Frostegard et al. 1991; Köster and Meyer-Reil 2001). Therefore, the initial biomass concentration was estimated during the optimization procedure using these bounds. Further details on the experimental procedures are reported in Boufadel et al. (1999).

Mathematical model

Model description

The objective of this study was to develop a relatively simple model to describe hydrocarbon degradation in variably saturated porous media (i.e., where the moisture content is less than 100 % and varies with position). The stoichiometric equation for the complete mineralization of heptadecane is:

$$C_{17}H_{36} + 26O_2 \rightarrow 17CO_2 + 18H_2O$$
 (3)

The biomass was assumed to consist of active and inert microbial cells. The growth kinetics of the active biomass was assumed to be first-order with respect to the biomass (Kindred and Celia 1989; Godeke et al. 2008; Mohamed and Hatfield 2011):

$$\frac{dX}{dt} = \mu X - k_d X \tag{4}$$

Where X is the surface-area normalized concentration of active biomass (mg/cm²) and k_d is the endogenous biomass decay rate (day⁻¹). The decay coefficient was assumed to be constant (Metcalf and Eddy 1991). The gross growth of the active biomass, μ (day⁻¹), was assumed to be limited by the availability of substrate and nitrogen, which is expressed herein as a multiple Monod formulation (e.g., Bailey and Ollis 1986; Molz et al. 1986; Schirmer et al. 2000):

$$\mu = \mu_{\max} \frac{S_{\text{area}}}{K_S + S_{\text{area}}} \frac{N}{K_N + N} \tag{5}$$

where μ_{max} is the maximum growth rate (day⁻¹), S_{area} is the surface-area normalized concentration of substrate (mg/cm²) defined in Eq. 2, K_S is the halfsaturation concentration for substrate consumption (mg/cm²), N is the nitrate concentration (mg N/L of pore water), and K_N is the half-saturation concentration for nitrogen consumption (mg N/L of pore water). Equation 5 does not have a term that accounts for the effect of dissolved oxygen on the growth rate, because the concentration of dissolved oxygen was assumed to be in equilibrium with the head space, and therefore it was considered high enough to be non-limiting (say 8.0 mg/L).

The inert biomass consists of biological molecules that degrade relatively slowly and accumulate in the system. Accumulation of inert biomass has been shown to impact biodegradation rate adversely (Moussa et al. 2005). The inert biomass X_i (mg/cm²) in this paper was assumed to be a constant fraction, ε , of the decayed biomass (Laspidou and Rittmann 2002):

$$\frac{dX_i}{dt} = \varepsilon \, k_d X \tag{6}$$

Substrate consumption was assumed to be a function of the active biomass concentration (Metcalf and Eddy 1991; Rittmann and McCarty 2001):

$$\frac{dS_{\text{area}}}{dt} = -\frac{\mu}{Y_X}X\tag{7}$$

where Y_X is the biomass yield coefficient for growth on substrate (mg X/mg S).

Oxygen consumption was assumed to depend on the rate of substrate degradation, the decay rate of the degradable fraction of the biomass, and the total amount of biomass formed (Cao and Alaerts 1996; Rittmann and McCarty 2001). In essence, oxygen consumption was modeled by performing a mass balance on electrons, where electrons released by oxidation of substrate and decaying biomass are taken up by reduction of oxygen and synthesis of new biomass (Sperandio and Paul 1997):

$$\frac{dO_2}{dt} = -Y_{\rm OS}\frac{dS_{\rm area}}{dt} + Y_{\rm OX}(1-\varepsilon)k_{\rm d}X - Y_{\rm OX}\mu X \quad (8)$$

where Y_{OS} (mg O₂/mg S) is the stoichiometric coefficient for oxygen consumption based on complete mineralization of substrate and Y_{OX} is the stoichiometric coefficient for oxygen consumption during the complete mineralization of biomass.

Similarly, CO_2 production (expressed as mg of C) is assumed to depend on the substrate consumption rate, the gross rate of biomass growth, and the biomass decay rate (Grady and Lim 1980; Rittmann and McCarty 2001). Carbon dioxide production was modeled using a mass balance on carbon, assuming that carbon is released by oxidation of substrate and decaying biomass and is consumed by synthesis of new biomass with the remainder ending up in CO_2 (Sperandio and Paul 1997):

$$\frac{dC}{dt} = -Y_{CS}\frac{dS_{\text{area}}}{dt} + Y_{CX}(1-\varepsilon)k_dX - Y_{CX}\mu X \qquad (9)$$

where Y_{CS} (mg C/mg S) is the stoichiometric coefficient for CO₂ production from substrate, and Y_{CX} (mg C/mg X) is the amount of CO₂ produced by complete mineralization of biomass.

Numerical implementation of the direct problem

The 'direct' problem consists of solving Eqs. 4 through 9 for known values of the kinetic parameters and the initial biomass. The output is the values of oxygen, CO_2, X, X_i , and S as functions of time. Equations 4–7 present a coupled nonlinear system of equations. This system was solved by the Picard iteration scheme (Atkinson 1978). Although the convergence rate of this scheme is first-order and, therefore, slower than the Newton iteration scheme (which is second order), it is generally more stable (Paniconi and Putti 1994). Equations 8 and 9 were solved after finding the solutions to Eqs. 4-7. Integration through time was done using the unconditionally stable implicit Euler scheme (Atkinson1978). However, the nonlinear nature of the equations required the use of a very small time step ($\Delta t = 0.01$ h) to ensure convergence of the Picard scheme. Therefore, the implicit scheme was less helpful than it is for linear systems where the time step can be made large.

The inverse problem

The inverse problem involves estimating the parameter values that provide the best fit of the kinetic model to the observed data. The model has eleven adjustable parameters (Table 1). Two of these, Y_{OS} and Y_{CS} , were given by the stoichiometric coefficients for complete oxidation of heptadecane (Eq. 3). Thus, nine parameters, μ_{max} , K_S , K_N , k_d , Y_X , ε , Y_{OX} , Y_{CX} , and X_0 , were estimated by minimizing a weighted least squares objective function involving the differences between the simulated and the observed results for the columns that were provided with 0.5 mg nitrate–N/L and 2.5 mg nitrate–N/L. For these two columns, noting that the oxygen and CO₂ measurements leveled off at around 450 h, we fitted the model to these data for that range, and the estimated parameters were then used to predict the rest of experimental data in the five columns. A weighted least square objective function was used. It was formulated as:

$$F = \sum_{i=2,2}^{4} \sum_{j=1}^{3} \sum_{k=1}^{N_{K}} \omega_{ij} (u_{S,i,j,k} - u_{O,i,j,k})^{2}$$
(10)

Where *u* is a generic form for the dependent variables and the indices j = 1, 2, 3 represent oxygen (O), carbon (C), and heptadecane (S). The terms u_S and u_O represent the simulated and the observed results for the dependent variables, respectively; i = 2, 4 represents the sand columns that received 0.5 and 2.5 mg N/L, respectively. The notation "i = 2,2" in the equation implies a step of 2, thus only columns 2 and 4 are used in the objection function. The index $k = 1,..., N_K$ represents the number of measurements of dependent variable $u_{O,i,j}$ and ω_j represents weights discussed below.

The weights in Eq. 10 are intended to quantify the confidence of the researcher in the data. A large weight in a term of Eq. 10 implies that higher confidence is put on the data in that term. This is because the minimization algorithm proceeds in the direction that causes the largest change in the objective function (between the initial estimate and the optimum) thereby forcing the model to match the observed data of terms with the highest weight. In the special case of equal weights, the weighted least square reduces to the ordinary least square (OLS), where equal confidence is put on the data. The weights were selected to be:

$$\omega_{ij} = \frac{1}{\left(N_K u_{O,i,j,k}\right)^2} \tag{11}$$

This type of weighting factor puts equal emphasis on the data of each dependent variable and from each column. The term N_K in the denominator accounts for the number of data points to avoid giving more weight for data sets with high number of measurements (i.e., residual heptadecane concentration measured once per microcosm, was given equal weight to oxygen consumption and CO₂ production, which were represented by 10 data points per microcosm). The weighting by the inverse of the observed value was intended to normalize the data to account for measurements of different magnitudes and/or units. Such an approach is common in environmental applications (Mishra and Parker 1989; Boufadel et al. 1998).

The minimization algorithm used in this study is a generalized reduced gradient technique named GRG2

Parameter	Units	Lower bound	Upper bound	Literature values	Optimal value	Variance
Y _{OS}	mg of O2/mg of S	Parameter is	fixed ^a	Bailey and Ollis (1986)	3.47	
Y_{CS}	mg of C/mg of S	Parameter is	fixed ^a	Bailey and Ollis (1986)	0.85	
$\mu_{\rm max}$	day^{-1}	0.01	10	7.6, Nicol et al. (1994); 0.05 ∼ 2.15, Schirmer et al. (2000)	1.57	0.002
k _d	day^{-1}	0.01	0.1	0.01, Essaid et al. (1995); 0.05 ∼ 0.76, Nicol et al. (1994)	0.01	1.3×10^{-5}
Y_X	mg X/mg S	0.1	4	$0.01 \sim 1.33$, Essaid et al. (1995); $0.25 \sim 1.24$, Schirmer et al. (2000)	0.15	2.2×10^{-5}
K_S	mg S/cm ²	0.0	12		0.88	6.3×10^{-4}
K_N	mg of N/L of solution	0.02	5	0.1, Essaid and Bekins (1997)	0.45	9.5×10^{-4}
Y_{CX}	mg of C/mg of X	0.47	0.6	0.47 \sim 0.6, Bailey and Ollis (1986)	0.6	0.14
Y_{OX}	mg of O2/mg of X	0.68	2.72	0.68 ~ 2.72, Bailey and Ollis (1986)	1.56	1.15
X_0	mg X/cm ²	4.8×10^{-7}	4.8×10^{-3}	$4.8 \times 10^{-7} \sim 4.8 \times 10^{-3}$, Kopke et al. (2005)	1.7×10^{-3}	1.5×10^{-4}
3	Dimensionless	0.05	0.3	0.05 ~ 0.3, Bailey and Ollis (1986)	0.3	0.18

 Table 1
 Parameters fixed or estimated in the model

^a These parameters were fixed at the stochiometric values obtained from Eq. 3

(Lasdon et al. 1979). Lower and upper bounds on decision variables are quite easy to handle with GRG2. The GRG2 requires a user-supplied subroutine GCOMP to compute the objective function and the constraints for values of the decision variables. More information on the software can be found in Lasdon et al. (1980), and applications of GRG2 in hydrology can be found in the works by Unver and Mays (1984) and Boufadel (1998).

Bound and constraints

To eliminate the possibility of obtaining unrealistic parameter estimates, lower and upper bounds (Table 1) were imposed on the estimated parameters by examination of the scientific literatures. Furthermore, to avoid oscillation in the numerical solution, oxygen consumption, CO_2 production, and heptadecane remaining in the sand columns at the end of the experiment were forced to be positive through a constraint function. The constraints rely on "penalty functions", to correct for unrealistic results.

Results

columns at the end of the experiment. The observed results indicate that the amount of heptadecane remaining from the 1.0, 2.5 and 5.0 mg N/L columns was essentially the same. The quasi-constant distribution of the heptadecane remaining within the column for these treatments suggests that the rate-limiting factor for biodegradation is the oil–water interfacial area or processes occurring there; it seems that the 500 mg of heptadecane that remained at the end of the experiments was probably not available to the alkane-degrading bacteria within the time scale of the experiment. This is probably due to entrapment of oil within dead-end pores where transport occurs only by Knudsen diffusion (Clark 1970).

Figure 2 reports the average concentration in each column and the modeling results, where the agreement is good considering that only one set of parameter values was used, and that other data (CO_2 and oxygen) were also used in the fitting.

The oxygen uptake that was observed in each of the microcosm reactors is shown in Fig. 3, along with modeling results. The data of the first 450 h were fitted in the columns with influent nitrate–N concentration of 0.5 and 2.5 mg/L, and the rest of experimental data were predicted by the model including the data of the latter 450 h in the two fitted columns and the data of the total 912 h in other three columns.



Fig. 1 Heptadecane remaining in the sand columns for various influent nitrated–N concentrations (*layer 1 is the top layer*)

Data for CO_2 production are shown in Fig. 4. Reasonable agreement between the model predictions and the experimental observations was obtained for both of the response variables for all five microcosms. The model captured the rapid rise at early times, but overestimated the observed oxygen and CO₂ at latter times, especially for the cases of higher nitrate concentration. However, the overall behavior of the model is better in this case, because the model in Figs. 3 and 4 underestimated the observed oxygen and CO_2 data in the early stage and overestimated them later. One plausible explanation for this systematic discrepancies between prediction and observations is that the model does not allow the interfacial area between oil and water (Eq. 2) to decrease as the oil gets consumed, as done, for example, by Nicol et al. (1994). This investigation is left for future work.

Discussion

heptadecane in the sand

nitrate-N concentrations

columns for various influent

The parameter estimates that were obtained by fitting this model to the microcosm data are consistent with previously reported values collected in very different experimental systems. In particular, the maximum specific growth rate, μ_{max} , that was estimated by this model (1.57 day^{-1}) is within the range of values from 0.13 to 9.6 day⁻¹ that have been previously reported for bacteria growing on normal hydrocarbons (Chen et al. 1992; Nicol et al. 1994; Essaid et al. 1995; Schirmer et al. 1999; Schirmer et al. 2000). Estimates of endogenous decay coefficients vary widely: ranging from about 0.03 to 1.6 day^{-1} in wastewater treatment systems and slowly growing mixed cultures (Grady CPL Jr. and Lim 1980; Nicol et al. 1994). The estimate obtained in this study (0.01 day⁻¹) is similar to decay coefficients that were used in previous studies (Borden and Bedient 1986; Malone et al. 1993; Essaid, et al. 1995).

The coefficients for biomass elemental composition Y_{CX} and Y_{OX} , reflect the elemental composition of the labile biomass, and therefore, the range of acceptable values was relatively narrow because the reported elemental composition of biomass is relatively consistent (Bailey and Ollis 1986). The coefficient for carbon, Y_{CX} , went to the upper limit of acceptable values (0.6 mg C/mg X), but the optimal value for the oxygen coefficient, Y_{OX} (1.56 mg O₂/mg X) was not limited by the predetermined constraints.

The optimal biomass yield coefficient, Y_X , was 0.15 mg biomass/mg heptadecane, which seems reasonable for aerobic biodegradation of an organic compound. Previous estimates ranged from about 0.05 to 1.56 mg biomass/g carbon (MacQuarrie et al. 1990; Alvarez et al. 1991; Chen et al. 1992; Essaid et al. 1995).

Fig. 2 Observed (*symbols*) and simulated (*curves*) concentration of



The half-saturation coefficient for nitrate was the parameter of most interest in this study, and unfortunately, few previous studies are available for comparison





to this estimate. In fact, this study was motivated by the absence of good previous estimates of the half-saturation constant for nitrogen utilization by hydrocarbondegrading bacteria. The relatively low value observed $(K_N = 0.45 \text{ mg N/L})$ is consistent with the results of a bioremediation field study that was conducted on the shore of Delaware Bay (Venosa et al. 1996). In that study, increasing the average nitrate concentration in the interstitial pore water from the background value of 0.8 mg N/L to over 6 mg N/L doubled the alkane biodegradation rate. This suggests that the background nitrate concentration was close to the half-saturation concentration for indigenous alkane degraders.

The physiological interpretation of the half-saturation concentration for heptadecane, K_S , is uncertain because it is affected by many factors, including substrate transport into the microbial cells and biodegradation through multistep metabolic pathways in which free intermediates sometimes occur. In addition to the factors that determine K_S for soluble substrates, the half-saturation concentration K_S for insoluble substrates may also be affected by the dissolution rate, the interfacial area between bacteria and substrate, and the production of microbial products that enhance biological availability (Aichinger et al. 1992). The half-saturation concentration estimated for heptadecane in this study (0.88 mg C/cm^2) might also depend on the heptadecane-water interfacial area and the thickness of the substrate coating on the sand particles. Nevertheless, the estimated K_S value in this study may still be useful for groundwater models, where due to heterogeneity, many of the physical factors (such as the surface area) affecting K_S cannot be measured throughout the porous domain. The relatively large value of K_S with respect to the highest substrate concentration (0.048 mg C/cm²) in this paper indicates that the rate of biodegradation of heptadecane was approximately first order, which is consistent with the good fit that Venosa et al. (1996; 2010) obtained when they used a first-order rate law to describe their experimental data of oil biodegradation.

Model assessment

The squared error of the estimation, σ^2 , can be expressed by the following formula (Bard 1974):

$$\sigma^2 = \frac{F}{n-p} \tag{12}$$

Where F is the value of the objective function, n is the number of observations, p is the number of estimated parameters. The value of Eq. 12 at the optimum was $\sigma^2 = 0.071$. The sensitivity of the model to the parameter estimates is shown in Fig. 5, where the value of σ^2 is shown for variations of ± 50 % in the value of each parameter. The quantity σ^2 of the error stays very flat as parameters K_d , Y_{CX} , ε , Y_{OX} vary by ± 50 % around their estimated values. However, it changes relatively rapidly when parameters K_S , Y_X , $\mu_{\rm max}$, X_0 , K_N vary by ± 50 % around their estimated values, which indicates that the model is sensitive to these parameters. Thus, more attention should be paid to calibrating these values in future's simulation of hydrocarbon biodegradation. Notice that the value of the objective function (as expected) increases going away from the optimum, which confirms (visually) that the search algorithm was robust.

To quantify the findings of Fig. 5, we evaluated the covariance matrix, V_x , of the parameters according to the equation (Bard 1974):

$$V_x = \sigma^2 H^{-1} \tag{13}$$

Where H is the Hessian matrix whose terms are the second derivative of the objective function with respect to the parameters. To estimate these values, we fitted a quadratic function to the value of the objective function near the optimum as given by Fig. 5. We used only the diagonal terms in this work for simplicity.

161

The last column of Table 1 reports the variances of the parameters as given by Eq. 13. The variances of the parameters Y_{CX} , ε , Y_{OX} are large, which is due to the small curvature of the objective function. This indicates that the model is not sensitive to these parameters, which is due to the fact that no information on the biomass was used as input. Thus, when biomass information is used in the model, we suggest that one does not estimate these parameters, rather use their values from the literature. This is a welcome news for oil spill responders who need to decide on the fate of oil and where time constraints precludes obtaining information on the microbial community.

Summary and conclusion

A kinetic model was established to describe the biodegradation of residual hydrocarbons in variablysaturated porous media. Multiple-Monod kinetics was used to represent the dependence of microbial growth and hydrocarbon biodegradation on the substrate and nutrient. Eight kinetic parameters and the initial biomass concentration were estimated by fitting the model to experimental data of oxygen, CO₂, and remaining heptadecane obtained from columns having influent nitrate-N concentration of 0.5 and 2.5 mg/L. The estimates fell in the range reported previously in the literature despite the fact that many of them were allowed to cover wider ranges (Table 1). Using the estimated parameters, the model was able to predict the biodegradation of heptadecane in the remaining columns. The fitting showed some consistent discrepancies



Fig. 5 The error of estimation σ^2 while varying one parameter at a time

between the model simulation and observed data in the cases of higher nitrate concentration. One plausible explanation of this mismatch is that the oil–water interfacial area may have limited the rate and extent of biodegradation in these columns. Further model improvements or experimental verification may be able to resolve this issue.

The kinetic model developed in this work simulated the consumption of substrate, consumption of oxygen, and production of CO₂. Models that rely primarily on substrate consumption are experimentally cumbersome and expensive when applied to biodegradation of nonaqueous phase substrates due to the difficulty of collecting representative subsamples for analysis. Typically, one would need to sacrifice the column to get the concentration of non aqueous hydrocarbon concentration. This is particularly true of the variably saturated porous-medium microcosms used in this study. So, basing parameter estimates on abundant data of surrogates obtained through stoichiometry, such as oxygen and carbon dioxide, results in estimates that are physically based. Indeed, this was confirmed when the parameters estimated from two columns were able to predict the biochemical reactions in other columns, for the corresponding period. Ultimately, we hope to use these parameters to simulate the rate of hydrocarbon biodegradation under a variety of scenarios for two purposes: (1) better understand the limitations on biodegradation at the macroscopic scale, and (2) use the results to provide guidance to spill responders who need to evaluate specific response alternatives in real time.

Acknowledgments This work was supported in part by funding from the Exxon Valdez Trustee Council under project no. 11100836. This article does not necessarily reflect the views of the funding agency and no official endorsement should be inferred.

Reference

- Aichinger G, Grady CPL, Tabak HH (1992) Application of respirometric biodegradability testing protocol to slightly soluble soluble organic compounds. Wat Env Res 64(7): 890–900
- Alvarez PJJ, Anid PJ, Vogel TM (1991) Kinetics of aerobic biodegradation of benzene and toluene in sandy aquifer material. Biodegradation 2:43–51
- Atkinson KE (1978) An introduction to numerical analysis. Wiley, New York, p 587
- Atlas RM, Hazen TC (2011) Oil biodegradation and bioremediation: a tale of the two worst spills in US history. Environ Sci Technol 45(16):6709–6715

- Bailey JE, Ollis DF (1986) Biochemical engineering fundamentals. McGraw-Hill, New York
- Bard Y (1974) Nonlinear parameter estimation. Academic Press, New York
- Borden RC, Bedient PB (1986) Transport of dissolved hydrocarbons influenced by oxygen-limited biodegradation. 1. Theoretical development. Water Resour Res 22(13): 1973–1982
- Boufadel MC (1998) Unit hydrographs derived from the Nash model. J Am Water Resour Assoc 34:167–177
- Boufadel MC, Suidan MT, Venosa AD, Rauch CH, Biswas P (1998) 2D variably saturated flows: physical scaling and Bayesian estimation. J Hydrol Eng 3(4):223–231
- Boufadel MC, Reeser P, Suidan MT, Wrenn BA, Cheng J, Du X, Venosa AD (1999) Optimal nitrate concentration for the biodegradation of *n*-heptadecane in a variably-saturated sand column. Environ Technol 20(2):191–199
- Boufadel MC, Sharifi Y, Van Aken B, Wrenn BA, Lee K (2010) Nutrient and oxygen concentrations within the sediments of an Alaskan beach polluted with the Exxon Valdez oil spill. Environ Sci Technol 44(19):7418–7424
- Boufadel MC, Wrenn BA, Moore BE, Boda KJ, Michel J (2011) A biodegradation assessment tool for decision on beach response. In: Proceeding of the 2011 International Oil Spill Conference. Portland, pp 2011–2348
- Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation of the Exxon Valdez oil spill. Nature 368:413–418
- Cao YS, Alaerts GJ (1996) A model for oxygen consumption in aerobic heterotrophic biodegradation in dual-phase drainage systems. Water Res 30(4):1010–1022
- Chen YM, Abriola LM, Alvarez PJJ, Anid PJ, Vogel TM (1992) Modeling transport and biodegradation of benzene and toluene in sandy aquifer material-comparisons with experimental measurements. Water Resour Res 28(7):1833–1847
- Clark A (1970) The theory of adsorption and catalysis. Academic Press, New York
- Du XM, Reeser P, Suidan MT, Huang TH, Moteleb M, Boufadel MC, Venosa AD (1999) Optimum nitrogen concentration supporting maximum crude oil biodegradation microcosms.
 In: Proceedings 1999 International oil spill conference, American Petroleum Institute, Washington, DC, pp 485–488
- Esler D (2010) Exxon Valdez effect goes. Trac-Trend Anal Chem 29(SI6):V–VI
- Essaid HI, Bekins BA (1997) BIOMOC, a multispecies solutetransport model with biodegradation. US Geological Survey, Water-Resources Investigations Report 97–4022
- Essaid HI, Bekins BA, Godsy EM, Warren E, Baedecker MJ, Cozzarelli IM (1995) Simulation of aerobic and anaerobic biodegradation processes at a crude oil spill site. Water Resour Res 31(12):3309–3327
- Findlay RH, King GM, Watling L (1989) Efficacy of phospholipid analysis in determining microbial biomass in sediments. Appl Environ Microbiol 55:2888–2893
- Floodgate GD (1979) Nutrient limitation. In: Bourquin AW, Pritchard PH (eds) Microbial degradation of pollutants in marine environments. EPA-66019-79-012, Environmental Research Laboratory, Gulf Breeze, pp 107–119
- Frostegard A, Tunlid A, Baath E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. J Microbiol Meth 14(3):151–163

- Gibbs CF (1975) Quantitative studies on marine biodegradation of oil. 1. Nutrient limitation at 14 °C. Proc Royal Soc Lond B 188:61–82
- Godeke S, Voqt C, Schirmer M (2008) Estimation of kinetic Monod parameters for anaerobic degradation of benzene in groundwater. Environ Geol 55(2):423–431
- Grady CPL Jr., Lim HC (1980) Biological wastewater treatment: theory and applications. Mardet Dekker, New York
- Guo QN, Li HL, Boufadel MC, Sharifi Y (2010) Hydrodynamics in a gravel beach and its impact on the Exxon Valdez oil. J Geophys Res-oceans. doi:10.1029/2010JC006169
- Kindred JS, Celia MA (1989) Contaminant transport and biodegradation. 2. Conceptual model and test simulations. Water Resour Res 25(6):1149–1160
- Kopke B, Wilms R, Engelen B, Cypionka H, Sass H (2005) Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. Appl Environ Microbiol 71(12): 7819–7830
- Köster M, Meyer-Reil L (2001) Characterization of carbon and microbial biomass pools in shallow water coastal sediments of the southern Baltic Sea (Nordrugensche Bodden). Mar Ecol-Prog Ser 214:25–41
- Lasdon LS, Warren AD, Jain A, Ratner M (1979) Design and testing of a generalized reduced gradient code for nonlinear programming. ACM Trans Math Software 4:34–50
- Lasdon LS, Warren AD, Jain A, Ratner M (1980) GRG2's user's guide report. Dept of General Business, University of Texas, Austin
- Laspidou CS, Rittmann BE (2002) A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. Water Res 36(11):2711–2720
- Li HL, Boufadel MC (2010) Long-term persistence of oil from the Exxon Valdez spill in two-layer beaches. Nat Geosci 3(2):96–99
- MacQuarrie KTB, Sudicky EA, Frind EO (1990) Simulation of biodegradable organic contaminants in groundwater. 1. Numerical formulation in principal directions. Water Resour Res 26(2):207–222
- Malone DR, Kao CM, Borden RC (1993) Dissolution and biorestoration of nonaqueous phase hydrocarbons: model development and laboratory evaluation. Water Resour Res 29(7):2203–2213
- Metcalf Eddy (1991) Wastewater engineering: treatment, disposal, and reuse, 3rd edn. McGraw-Hill, New York
- Mishra S, Parker JC (1989) Parameter estimation for coupled unsaturated flow and transport. Water Resour Res 25:385–396
- Mohamed M, Hatfield K (2011) Dimensionless parameters to summarize the influence of microbial growth and inhibition on the bioremediation of groundwater contaminants. Biodegradation 22(5):877–896
- Molz FJ, Widdowson MA, Benefield LD (1986) Simulation of microbial growth dynamics coupled to nutrient and

oxygen transport in porous media. Water Resour Res 22(8):1207-1216

- Moussa MS, Hooijmans CM, Lubberding HJ, Gijzen HJ, Van Loosdrecht MCM (2005) Modeling nitrification, heterotrophic growth and predation in activated sludge. Water Res 39(20):5080–5098
- Nicol JP, Wise WR, Molz FJ, Benefield LD (1994) Modeling biodegradation of residual petroleum in a saturated porous column. Water Resour Res 30:3313–3325
- Paniconi C, Putti M (1994) A comparison of Picard and Newton iteration in the numerical solution of multidimensional variably saturated flow problems. Water Resour Res 30:3357–3374
- Reynolds Tom D, Paul Ricards (1996) Unit operations and processes in environmental engineering. PWS, Boston
- Rittmann BE, McCarty PL (2001) Environmental biotechnology: principles and applications. McGraw-Hill, Boston
- Sarioglu MS, Copty NK (2008) Modeling the enhanced bioremediation of organic contaminants in pyrite-containing aquifers. Transport Porous Media 72(2):203–221
- Schirmer M, Butler BJ, Roy JW, Frind EO, Barker JF (1999) A relative-least-squares technique to determine unique Monod kinetic parameters of BTEX compounds using batch experiments. J Contam Hydrol 37:69–86
- Schirmer M, Molson JW, Frind EO, Barker JF (2000) Biodegradation modeling of a dissolved gasoline plume applying independent laboratory and field parameters. J Contam Hydrol 46:339–374
- Sperandio M, Paul E (1997) Determination of carbon dioxide evolution rate using on-line gas analysis during dynamic biodegradation experiments. Biotechnol Bioeng 53(3): 243–252
- Taylor E, Reimer D (2008) Oil persistence on beaches in Prince William Sound-A review of SCAT surveys conducted from 1989 to 2002. Mar Pollut Bull 56(3):458–474
- Unver O, Mays LW (1984) Optimal determination of loss rate functions and unit hydrograph. Water Resour Res 20:203–214
- Venosa AD, Suidan MT, Wrenn BA, Strohmeier KL, Haines JR, Eberhart BL, King D, Holder E (1996) Bioremediation of an experimental oil spill on the shoreline of Delaware bay. Environ Sci Technol 30(5):1764–1775
- Venosa AD, Campo P, Suidan MT (2010) Biodegradability of lingering crude oil 19 years after the Exxon Valdez oil spill. Environ Sci Technol 44:7613–7621
- Wrenn BA, Suidan MT, Strohmeier KL, Eberhart BL, Wilson GJ, Venosa AD (1997) Nutrient transport during bioremediation of contaminated beaches: evaluation with lithium as a conservative tracer. Water Res 31:515–524
- Xia YQ, Li HL, Boufadel MC, Sharifi Y (2010) Hydrodynamic factors affecting the persistence of the Exxon Valdez oil in a shallow bedrock beach. Water Resour Res. doi: 10.1029/2010WR009179