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SCALE-UP IMPLICATIONS OF RESPIROMETRICALLY DETERMINED MICROBIAL KINETIC PARAMETERS

P. J. Sturman, R. R. Sharp, J. B. DeBar, P. S. Stewart, A. B. Cunningham, and J. H. Wolfram

INTRODUCTION

Successful scale-up of remediation processes requires an understanding of the extent to which they are influenced by scale-dependent phenomena such as mass transport and interfacial transfer limitations. Such effects may introduce rate limitations in field scale bioremediation which were not present in the laboratory. Thus, what appears to be a viable bioremediation strategy in the lab may be unsuccessful in the field (Goldstein et al. 1985). A better understanding of the requirements of the scale-up process is necessary to successfully predict which information translates across scales.

The goal of this research was to assess the adequacy of respirometrically determined kinetic parameters to predict field-scale biotransformation via bioprocess modeling. Electrolytic respirometry was used to determine kinetic parameters for an indigenous microbial consortium at a site contaminated with dissolved alkylbenzenes at concentrations up to 10 mg/L. Mean values and 95% confidence intervals (CIs) were determined for kinetic parameters. These parameters were used in a bioprocess model to estimate the active zone of biotransformation for a field remediation system. To assess the effects of kinetic parameter variation on the contaminant mass biotransformed predicted by the model, the values of two important parameters (μ_{max} and K_s) were varied through their CIs in the input to the bioprocess model. The μ_{max} (maximum specific growth rate) is the consortia growth rate (μ) under optimal conditions for a given substrate (S), whereas K_s (half-saturation coefficient) is the substrate concentration at which μ is one-half μ_{max}. Where $K_s >> S$, μ responds in first-order fashion to changes in S. Where $S >> K_s$, μ responds in zero-order fashion to changes in S.

METHODS

The microbial consortium used in the respirometry experiments was collected from a site that has been exposed to dissolved benzene, toluene, ethylbenzene, and xylenes (BTEX) continuously for the last 15 years. Hydrocarbon degraders were isolated by plating on selective (BTEX-rich) media. Biosciences electrolytic respirometers (500-mL capacity) were filled with 400 mL of mineral salts solution,

1 mL of concentrated cell inoculum, and 4.4 to 22.8 mg total BTEX (equal quantities of each compound). The final contaminant concentration in the reaction vessel varied from 11 to 57 mg/L. Total oxygen demand from each respirometry run was automatically recorded. A typical accumulated O_2 demand curve is shown in Figure 1.

The O_2 demand curve generated in each respirometry experiment was fitted with a 5-parameter Monod model with cell decay and least sum squares regression techniques. The model equation used for the change in accumulated O_2 demand with time was:

$$\frac{dO_2}{d_t} = Y_{o/x}\left(\frac{\mu_{max}S}{K_s + S}\right)X + 1.42bX \qquad (1)$$

where $Y_{o/x}$ = mass O_2 utilized per mass biomass produced. Other parameters as defined in Table 1. Kinetic parameters (μ_{max}, K_s, decay rate, and biomass yield) and initial active biomass concentration (X_o) were estimated by continuously varying the input parameters until a curve of best fit was generated. Parameter means and 95% CIs were thus obtained (Table 1).

Consortia kinetic parameters obtained from respirometry and physical data obtained from the actual contaminated site (Table 1) were entered as inputs into a two-dimensional porous medium bioprocess model (Ewing et al. 1984). This deterministic model considers advection, diffusion, dispersion, and biological reaction kinetics. Bulk fluid flow is described by Darcy's law and bioreactions by double Monod kinetics (both O_2 and substrate potentially limit biodegradation

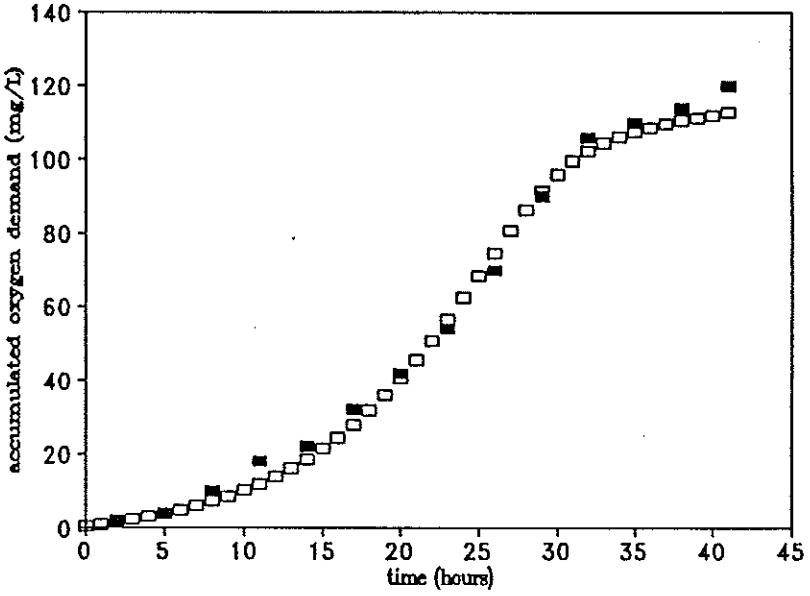

FIGURE 1. Typical oxygen demand curve from respirometry (■), and model-generated curve (□).

TABLE 1. Kinetic parameters from respirometry and bioprocess model inputs.

		Mean	Std.Dev.
Microbial Parameters			
$\mu_{max}^{(a)}$	maximum specific growth rate	0.23 hr^{-1}	0.01 hr^{-1}
$K_S^{(a)}$	substrate half-saturation coefficient	16.9 mg L^{-1}	1.9 mg L^{-1}
$Y_{x/s}^{(a)}$	biomass (X) yield from substrate	0.67 gX gS^{-1}	0.31 gX gS^{-1}
$Y_{x/e}$	biomass (X) yield from e^- acceptor	0.32 gX gE^{-1}	
$b^{(a)}$	endogenous respiration rate (decay)	0.02 hr^{-1}	0.004 hr^{-1}
K_E	e^- acceptor half-saturation coefficient	0.1 mg L^{-1}	
Site Parameters			
ϕ	porosity	0.3	
k	permeability	0.32 Darcy	
d_l	longitudinal dispersivity	4.5 m	
d_t	transverse dispersivity	0.45 m	
C_S	initial substrate concentration	10 mg L^{-1}	
C_X	initial biomass concentration	1.24 mg L^{-1}	
X	formation length	152 m	
Y	formation width	152 m	
Transport Parameters			
Q	injection rate	2,04 m^3 d^{-1} m^{-1} aquifer thickness	
C_E	background e^- acceptor concentration	8 mg L^{-1}	
μ	viscosity	1.31 centipoise	

(a) Respirometrically determined parameters.

rate). The model assumes that the contaminant is fixed in place within the aquifer, while the electron acceptor is transported with the bulk fluid flow. This mimics a field situation where a sorbed contaminant desorbed at a rate similar to that at which biodegradation occurs (i.e., desorption continuously replenishes the aqueous phase with contaminant until no sorbed contaminant remains). Dissolved O_2 at 8 mg/L is transported into the formation through a single injection well (see Figure 2), whereas BTEX is transformed in situ. Numerical simulation of the model uses mixed finite elements, the Modified Method of Characteristics, and methods for solving stiff ordinary differential equations. Model runs were performed with nine combinations of the kinetic coefficients μ_{max} and K_s. Minimum, maximum, and mean values for μ_{max} and K_s were used in combination (minimums and maximums determined by CIs).

RESULTS AND DISCUSSION

Respirometry experiments were performed at initial total BTEX concentrations of 11, 33, 47, and 57 mg/L. Each experiment generated an oxygen demand curve,

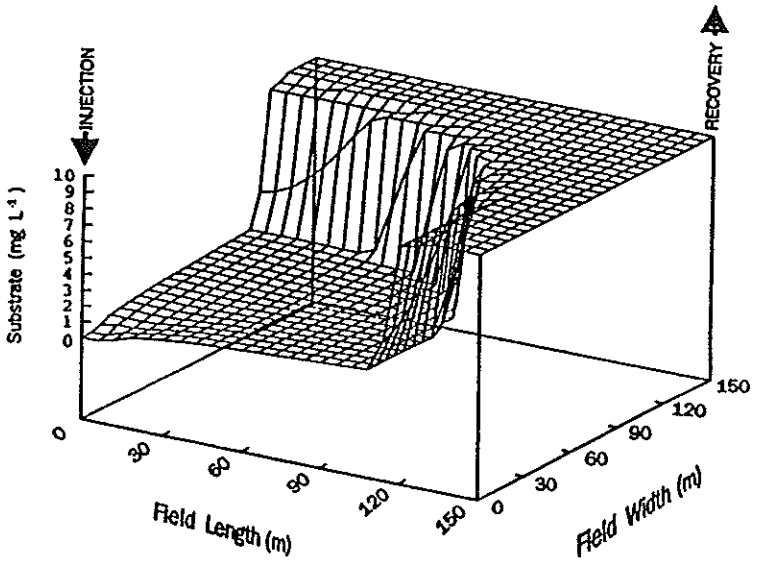

FIGURE 2. Substrate (contaminant) concentration within flow field of bioprocess model. Run time 4,800 days.

to which a curve of best fit was applied via least sum squares error analysis. A typical curve fit is indicated in Figure 1. Each of the four respirometry-generated O_2 demand curves resulted in an estimate of four kinetic parameters. The means and 95% CIS are shown in Table 1. Although only four data points are considered in this initial experimentation, the CIs for μ_{max}, K_s, and b are relatively small. The calculated $Y_{x/s}$ has a considerably larger CI (0.039 to 1.3), although this did not cause excessive variation in the other kinetic coefficients. The average μ_{max} and K_s predicted were 0.23 hr^{-1} and 19.9 mg/L, respectively. Few literature values for alkylbenzene compounds exist, but the above values fall within the ranges of 0.18 to 0.78 hr^{-1} for μ_{max} and 6 to 500 mg/L for K_s reported by Grady and Lim (1980) for mixed organic wastes.

Each bioprocess model run resulted in predictions of the percent of total contaminant mass biotransformed. The volume biotransformed is shown in Figure 2 for a typical model run. O_2-rich water is injected at the left side of the figure and removed via a recovery well at the right. Similar plots were generated for nine combinations of μ_{max} and K_s through the respirometrically determined CIs (Figure 3, inset). As expected, the highest μ_{max} and lowest K_s resulted in the greatest mass biotransformed in 4,800 days. As μ_{max} decreased and K_s increased, the contaminant mass biotransformed decreased by only 3% from 47 to 45% of the total mass present (Figure 3).

This result is somewhat surprising in light of the dramatic impact the maximum specific growth rate has on microbial utilization of substrate in batch situations, even where small variations in μ_{max} are used. Several factors may account

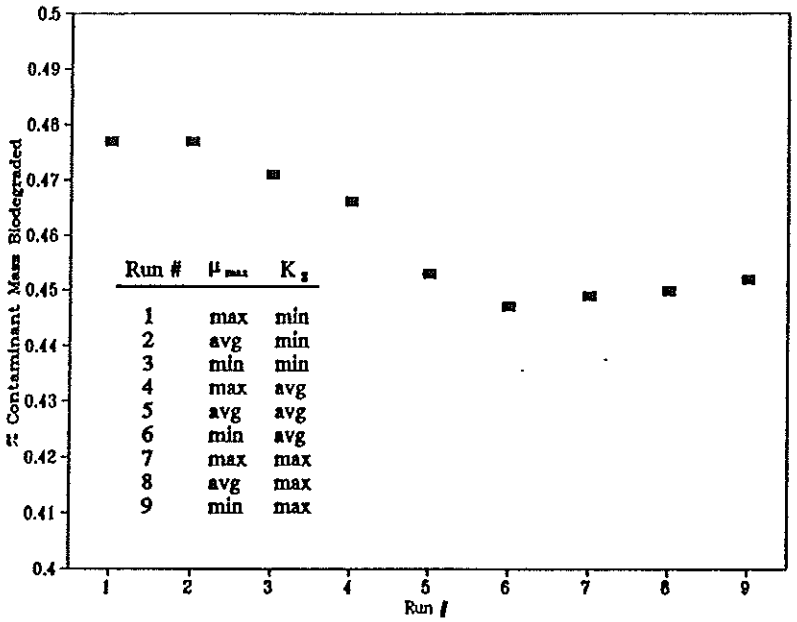

FIGURE 3. Comparison of the percent of the total contaminant mass biodegraded in 4,800 days with various kinetic parameter inputs. Maximum and minimum μ_{max} and K_s are defined by the 95% confidence interval determined from respirometry.

for the observed lack of sensitivity to variation in kinetic parameters. The influence of kinetics may be overshadowed by the importance of advective transport of electron acceptor to the reaction site. The steep substrate front in Figure 2 indicates a very narrow zone of reaction, which supports this idea. Substrate degradation appears to be limited by the presence of oxygen, rather than by the intrinsic kinetics of the consortium present. The implications of this result to scale-up are twofold: (1) efforts to maximize bioremediation should focus on increasing O_2 transport to the reaction site, and (2) small changes in the intrinsic kinetics of the site consortium may have little impact on the rate of biodegradation.

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