Sorption of 2,4,6Trichlorophenol by Bacillus subtilis

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · MARCH 1998

Impact Factor: 5.33 · DOI: 10.1021/es970295p

Environ. Sci. Technol. **1998**, *32,* 749−752

Sorption of 2,4,6-Trichlorophenol by *Bacillus subtilis*

CHRISTOPHER J. DAUGHNEY*,†,‡ AND JEREMY B. FEIN§

Earth and Planetary Sciences, McGill University, 3450 University Street, Montréal, Quebec, H3A 2A7 Canada, and Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, Indiana 46556-0767

The mobility of 2,4,6-trichlorophenol (TCP) in groundwater may be controlled, in part, by its sorption onto bacterial surfaces. In this study, we perform batch experiments as a function of pH, time, and solid-to-solution ratio to investigate the sorption of TCP by the common soil bacterium *Bacillus subtilis*. We first describe the experimental data with a hydrophobic partitioning model, in which sorption is controlled by the speciation of the TCP and the concentration of bacteria present. This model provides an adequate description of the data below pH 8 but overpredicts sorption in more basic solutions, where negligible sorption is observed for all solid-to-solution ratios. TCP-*B. subtilis* sorption is better described by a surface complexation model in which both the negative and the neutral forms of TCP form 1:1 surface complexes with the neutral hydroxyl functional groups of the bacteria: R-OH^0 + TCP$^-$ ↔ R-OH-TCP$^-$ (log $K = 3.01 \pm 0.50$) and R-OH^0 + HTCP^0 ↔ R-OH-HTCP^0 (log $K = 3.77 \pm 0.50$). These stability constants may be incorporated into thermodynamic models to predict the fate of TCP in complex systems.

Introduction

Chlorinated phenols, including 2,4,6-trichlorophenol (TCP), have been widely used in agriculture, wood preservation, and pulp bleaching (*1*−*3*). Due to widespread occurrence (*4, 5*), toxicity (*6, 7*), and persistence in the environment, TCP is an E.P.A. priority pollutant (*8*). To evaluate the environmental risk associated with TCP contamination and to develop effective strategies for remediation, the chemical reactions affecting TCP in the natural environment must be quantified.

Sorption by bacterial cell walls may affect the fate of TCP in many natural environments, but at present this interaction is poorly understood. Bacteria are ubiquitous in near-surface fluid-rock systems, and they may coat mineral surfaces, representing a significant fraction of the surface area exposed to natural waters. Sorption of TCP by organic surfaces is extensive (*9*), while sorption by mineral surfaces is limited or undetectable (*10, 11*). In soils, TCP sorption is well correlated with organic carbon content (*3, 4, 12*). Many workers have reported that TCP sorption is pH dependent (*3, 4, 10, 11*). TCP is ionizable within the pH range of natural waters ($pK_a = -5.99$; *13*) and thus can exist as either a neutraly (HTCP^0) or a negatively (TCP$^-$) charged species. Both species of TCP are hydrophobic, though the negative form is less so, and as a result, sorption is generally observed to decrease where pH > pK_a. In this study, we investigate the sorption of TCP by *Bacillus subtilis*, a common soil bacterium (*14*).

Many researchers have described TCP sorption with a hydrophobic partitioning model (HPM) (*3, 4, 11*−*13*). The HPM predicts sorption through a set of distribution coefficients (D) that relate the aqueous activities of HTCP^0 and/or TCP$^-$ (a, mol/kg of solution) to their sorbed concentrations (represented by square brackets, mol/kg of solution) and the sorbent weight (Γ, g/kg of solution):

$$\mathrm{TCP^-_{aq}} \leftrightarrow \mathrm{TCP^-_{adsorbed}} \tag{1}$$

$$\frac{[\mathrm{TCP^-_{adsorbed}}]}{a_{\mathrm{TCP^-}}\Gamma} = D_{\mathrm{TCP^-}} \tag{2}$$

$$\mathrm{HTCP^0_{aq}} \leftrightarrow \mathrm{HTCP^0_{adsorbed}} \tag{3}$$

$$\frac{[\mathrm{HTCP^0_{adsorbed}}]}{a_{\mathrm{HTCP^0}}\Gamma} = D_{\mathrm{HTCP^0}} \tag{4}$$

Here, a linear isotherm relates the D values to Γ (*15*). Once normalized with respect to Γ, the D values may be applied to model sorption over the range of solid-to-solution ratios described by the isotherm.

We also describe TCP sorption with a surface complexation model (SCM). The SCM describes distinct adsorption reactions between functional groups on the bacterial surface and species in solution through mass action laws having fixed stoichiometries and stability constants (*16, 17*). We characterize the protonation of carboxyl, phosphate, and hydroxyl functional groups on *B. subtilis* as described by Fein et al. (*18*):

$$\mathrm{R\text{-}COOH^0 \leftrightarrow R\text{-}COO^- + H^+} \qquad \log K = -4.82 \tag{5}$$

$$\mathrm{R\text{-}PO_4H^0 \leftrightarrow R\text{-}PO^- + H^+} \qquad \log K = -6.9 \tag{6}$$

$$\mathrm{R\text{-}OH^0 \leftrightarrow R\text{-}O^- + H^+} \qquad \log K = -9.4 \tag{7}$$

R represents the bacterium to which the functional groups are attached. *B. subtilis* has a surface area of 140 m^2/g and 1.2×10^{-4} mol of carboxyl sites, 4.4×10^{-5} mol of phosphate sites, and 6.2×10^{-5} mol of hydroxyl sites/g (*18*). Several adsorption reactions may be required to describe the adsorption of TCP by *B. subtilis*. For example, the adsorption of HTCP^0 onto the protonated carboxyl surface sites may be represented by the following chemical reaction, mass action law, and stability constant (K):

$$\mathrm{R\text{-}COOH^0 + HTCP^0 \leftrightarrow R\text{-}COOH\text{-}HTCP^0} \tag{8}$$

$$\frac{[\mathrm{R\text{-}COOH\text{-}HTCP^0}]}{[\mathrm{R\text{-}COOH^0}]a_{\mathrm{HTCP^0}}} = K \tag{9}$$

The square brackets represent the concentration of the enclosed surface species in moles per kilogram of solution. Similar mass action laws with discrete stability constants describe adsorption reactions involving HTCP^0 or TCP$^-$ and the protonated or deprotonated carboxyl, phosphate, or hydroxyl surface sites. The bacterial surface develops a negative electric potential due to deprotonation of its surface

* Corresponding author tel: 604-822-2449; fax: 604-822-6088; e-mail: daughney@eos.ubc.ca.
† McGill University.
‡ Present address: Earth and Ocean Sciences, University of British Columbia, 6339 Stores Rd., Vancouver, BC, V6T 1Z4 Canada
§ University of Notre Dame.

S0013-936X(97)00295-2 CCC: $15.00
Published on Web 02/03/1998

functional groups. This potential affects the hydrophobicity of the surface, and it influences interactions between the surface sites and charged species in solution. As such, experimentally determined stability constants, $K_{experimental}$, must be adjusted by the following relationship:

$$K = K_{experimental} \exp(zF\psi/RT) \quad (10)$$

The stability constant K (eq 9) is thus referenced to the condition of zero surface charge (*19*). The variables z, F, ψ, R, and T represent the charge of the adsorbing ion, Faraday's constant, the electric potential of the bacterial surface, the gas constant, and the absolute temperature, respectively. The surface electric potential (ψ) can be related to the solid surface charge (σ) by a constant capacitance double-layer model (*20*):

$$C = \frac{\sigma}{\psi} \quad (11)$$

The capacitance (C) of the *B. subtilis* surface is 8.0 F/m^2 (*18*).

Materials and Methods

B. subtilis cells were obtained from T. J. Beveridge (University of Guelph, Ontario) and cultured and washed as described by Fein et al. (*18*). TCP and $NaNO_3$ were obtained from Aldrich and used without further purification. Distilled, deionized water (18 MΩ) was used for all solutions.

The sorption of TCP by *B. subtilis* was studied in 0.1 M $NaNO_3$ electrolyte solutions. Batch experiments were conducted at 25 ± 1 °C, as a function of pH, solid-to-solution ratio, and equilibration time. Each reaction vessel contained a suspension of 0.1 M $NaNO_3$, 0.0001 M TCP, and *B. subtilis* (6.0, 9.0, 12.0, or 30.0 g/L). Reagent grade HNO_3 and NaOH were used to adjust the pH of the experimental solutions. During equilibration, the reaction vessels were placed on a rotary shaker. The equilibrium pH was recorded, and the suspensions were passed through a 0.45-μm cellulose acetate filter. The filtrate was basified and analyzed for TCP by ultraviolet spectrophotometry (*21*).

Desorption experiments were conducted to evaluate the reversibility of the sorption reactions. The sorbent was placed in contact with aqueous TCP as described above. The suspensions were adjusted to a pH value where significant sorption was expected to occur and equilibrated for 90 min. The suspension pH was measured, the solid present in each reaction vessel was pelleted by centrifugation (6000 rpm for 15 min), and the supernatant was analyzed for TCP and discarded. The solid was resuspended in 0.1 M $NaNO_3$; the solution pH was adjusted to a value favoring TCP desorption. After 30−230 min, the final pH of each suspension was measured, and the samples were filtered, basified, and analyzed for TCP.

Results and Discussion

TCP displays a strong affinity for the cell walls of *B. subtilis* (Figure 1). Solution pH and solid-to-solution ratio strongly affect sorption. There is excellent agreement between the sorption and desorption experiments, indicating that the reactions are reversible and rapid (equilibration occurs in approximately 30 min).

We first consider the possibility that these data result from anything other than the sorption of TCP by *B. subtilis*. Blank sorption runs were performed in the absence of the bacteria, and no sorption of TCP by the walls of the reaction vessels occurred. An examination of the TCP-*B. subtilis* suspensions (by HPLC and optical microscopy) both before and after the experiments gave no evidence of organic exudates or of cell disruption, and so the sorption of TCP was not affected by

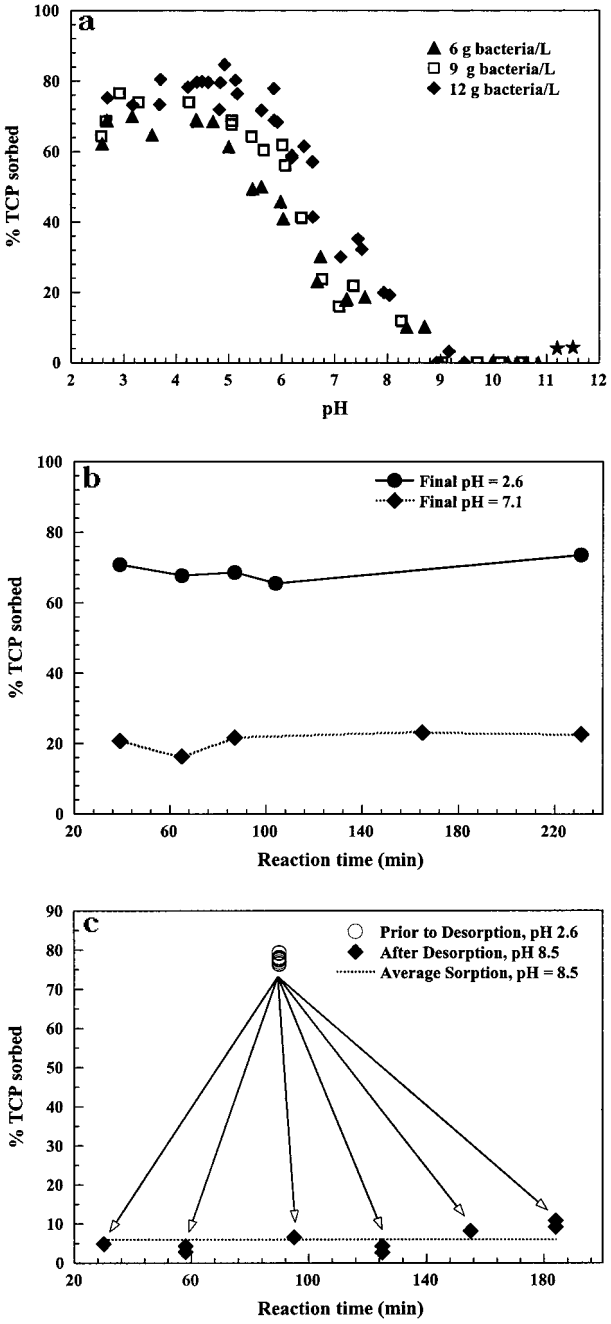

FIGURE 1. (a) Percent sorption of TCP by *B. subtilis* as a function of pH. Solutions contain $10^{-4.00}$ M TCP, $10^{-1.00}$ M $NaNO_3$, and either 6, 9, or 12 g of bacteria/L. Data points represented by stars have 30.0 g of bacteria/L. (b) Percent sorption of TCP by *B. subtilis* as a function of time. Solutions contain $10^{-4.00}$ M TCP, $10^{-1.00}$ M $NaNO_3$, and 12 g of bacteria/L. Initial pH ≈ 4.5. (c) Percent desorption of TCP from *B. subtilis* as a function of time. Initial solutions contained $10^{-4.00}$ M TCP, $10^{-1.00}$ M $NaNO_3$, and 12 g of bacteria/L. Circles show percent TCP initially sorbed after 90 min at pH 2.6. The pH 2.6 solutions were discarded; the bacteria were resuspended in $10^{-1.00}$ M $NaNO_3$, adjusted to a pH of 8.5, and allowed to equilibrate for varied times as indicated by the arrows. The dashed line represents the average amount of sorption observed in panel a at pH 8.5, corrected for the loss of TCP that occurs in the desorption experiments when the pH 2.6 solutions are discarded.

aqueous complexation by any dissolved organic. Our kinetic and desorption experiments show that the changes in aqueous TCP concentration are rapid and reversible and therefore not representative of bacterial consumption or degradation. We conclude that all observed changes in

aqueous TCP concentration can be attributed completely to sorption by *B. subtilis*.

Scatter in the data arises from two sources. First, due to the nature of batch experiments, the mass of solid in each reaction vessel differs slightly, though we account for this in our modeling. Second, a ± 3% error is associated with the analysis of TCP concentration by UV spectrophotometry. The bacteria do not multiply, lyse, or sporulate during the experiments, and so variations in cell concentration and surface chemistry do not contribute appreciably to the observed scatter.

We use FITEQL 2.0 (*22, 23*), as modified by Johannes Lützenkirchen (Department of Inorganic Chemistry, Umea University, Sweden; Personal communication) to develop and compare models involving a variety of TCP sorption reactions, to identify the model that best describes the experimental data. We process the data from the 6.0, 9.0, and 12.0 g of bacteria/L experiments simultaneously. For both the HPM and SCM, we compare models invoking the sorption of only $HTCP^0$ to those considering the sorption of $HTCP^0$ and TCP^- in combination. For the SCM, we compare models invoking sorption onto specific combinations of protonated and/or deprotonated surface sites. We use FITEQL to solve for distribution coefficients (HPM) or equilibrium constants (SCM) for each model reaction, and we use the variance calculated by the program as a quantitative measure of the goodness of fit of each model (Table 1). All model calculations apply conventional standard states, and all stability constants and distribution coefficients are referenced to 25 °C and zero ionic strength (adjusted with Davies equation activity coefficients).

For both the SCM and HPM, models considering only the sorption of $HTCP^0$ do not effectively match the data. We observe 10−20% sorption even at pH 8, where less than 1% of the TCP is present as $HTCP^0$. This is most likely due to the sorption of TCP^-, a conclusion in general agreement with previous research (*13*). We therefore disregard all models that do not consider the sorption of both $HTCP^0$ and TCP^-.

The HPM involving the independent sorption of $HTCP^0$ and TCP^- fits the data well below pH 8 (Figure 2), where a linear isotherm effectively relates the D values to Γ (eqs 2 and 4). The single set of D values listed in Table 1 can be used to predict sorption for all solid-to-solution ratios investigated here, where pH < 8. The large variance associated with this HPM (Table 1) arises from a discrepancy between the model and the experimental data above pH 8. The HPM predicts increased TCP sorption with increased Γ, for all pH values, whereas the experimental data indicate that negligible sorption occurs above pH 9, regardless of Γ. At 30.0 g of bacteria/L, the HPM (using the Γ-normalized D values from Table 1) predicts approximately 30% sorption at pH 11.5, though less than 5% adsorption actually occurs under these conditions. The linear isotherm may not apply for Γ as high as 30.0 g of bacteria/L, but the HPM overpredicts sorption above pH 9 for Γ as low as 6.0 g of bacteria/L. Because there is essentially no change in the speciation or hydrophobicity of TCP above pH 8, the discrepancy between the HPM and the experimental data suggests that sorption is controlled by more than the speciation of the TCP.

The SCM relates sorption to the speciation of the TCP and the speciation of the bacterial surface. The SCM that best describes the data involves the sorption of $HTCP^0$ and TCP^- to the neutral hydroxyl surface groups of the bacteria (Figure 2). This model is chemically reasonable because (a) $HTCP^0$ and the neutral hydroxyl functional groups coexist at low pH, (b) electrostatic repulsion may limit the adsorption of TCP^- onto negatively charged sites, (c) a decline in sorption is observed above pH 9, where the majority of the hydroxyl surface sites become negatively charged, and (d) less than 5% sorption is observed even at pH 11.5 and 30.0 g of bacteria/L, which suggests that TCP sorption is controlled more by the speciation of the bacterial surface than by the sorbent concentration.

TABLE 1. Comparison of TCP-*B. Subtilis* Adsorption Models

Surface Complexation Models

model[a]	log K^b	V^c
R-COOH-$HTCP^0$	4.83	134
R-PO_4H-$HTCP^0$	4.07	50.7
R-OH-$HTCP^0$	3.86	29.8
R-COOH-$HTCP^0$ R-COOH-TCP^-	3.40 5.06	28.4
R-COOH-$HTCP^0$ R-PO_4H-TCP^-	3.72 4.06	25.3
R-COOH-$HTCP^0$ R-OH-TCP^-	4.16 3.33	60.4
R-PO_4H-$HTCP^0$ R-COOH-TCP^-	3.80 4.86	36.9
R-PO_4H-$HTCP^0$ R-PO_4H-TCP^-	3.90 3.84	15.6
R-PO_4H-$HTCP^0$ R-OH-TCP^-	3.96 3.12	11.3
R-OH-$HTCP^0$ R-COOH-TCP^-	3.74 4.51	28.8
R-OH-$HTCP^0$ R-PO_4H-TCP^-	3.72 3.69	16.1
R-OH-$HTCP^0$ *R-OH-TCP^-*	*3.77* *3.01*	*9.5*
R-OH-$HTCP^0$ R-$COO\text{-}TCP^{2-}$	3.77 2.94	10.8
R-OH-$HTCP^0$ R-$PO_4\text{-}TCP^{2-}$	3.81 3.44	12.7
R-OH-$HTCP^0$ R-$O\text{-}TCP^{2-}$	3.86 3.56	28.6

Bulk Partitioning Models

model[d]	D^e	V^c
$HTCP^0$	0.38	43.9
$HTCP^0$ TCP^-	0.31 0.016	25.5

[a] Models consider formation of surface complexes due to adsorption of $HTCP^0$ and/or TCP^- onto carboxyl, phosphate, and/or hydroxyl surface sites; best fitting model is in italics. [b] Log K values calculated for the various surface complexes, referenced to the condition of zero ionic strength and zero surface charge. Log K values have relative 2σ errors of ±15%. [c] Variance as calculated by FITEQL. [d] Models consider sorption of $HTCP^0$ and/or TCP^- onto the surface. [e] D values for the various aqueous species, referenced to zero ionic strength and normalized with respect to sorbent mass (g/L) by a linear isotherm. D values have relative 2σ errors of ±15%.

Under the conditions of this investigation, the sorption of TCP by *B. subtilis* is better described by the SCM than the HPM. Although there is abundant literature evidence that hydrophobicity is the governing control in the sorption of organics by biological surfaces (*15, 19*), the HPM cannot describe all of the experimental data. We note that some experimental conditions are not environmentally relevant, but they better constrain the choice of model. The SCM selected here may not reflect all pertinent reaction mechanisms. It is possible that TCP also adsorbs onto the neutral carboxyl and phosphate sites on the bacterial surface, but because there is no noticeable decrease in sorption as these sites deprotonate, these reactions are not resolvable. It is also possible that TCP sorbs as a mulitdentate complex when the solid-to-solution ratio is very large. Until spectroscopic techniques are applied to directly observe the sorbed TCP,

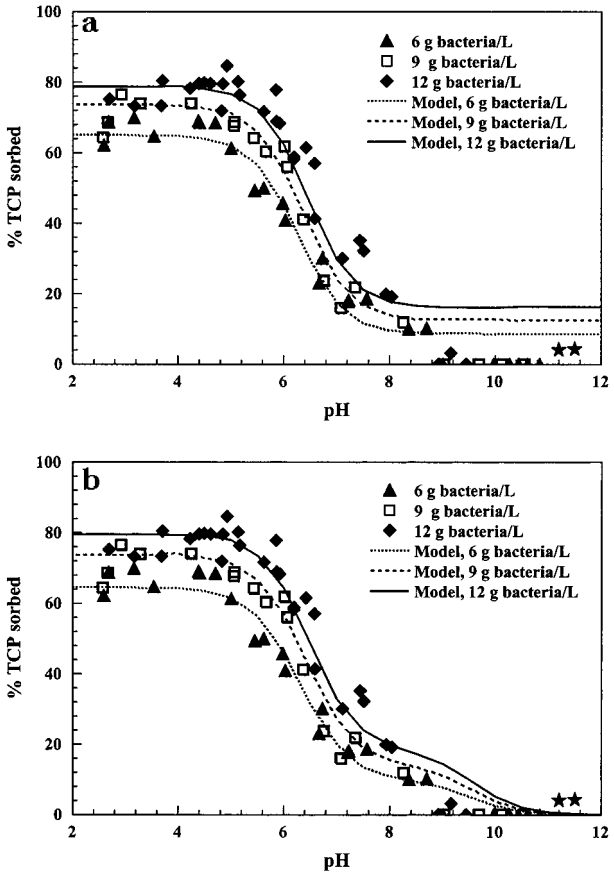

FIGURE 2. (a) HPM curves generated by FITEQL, assuming nonspecific partitioning of TCP^- and $HTCP^0$ onto the bacterial surface. (b) SCM curves generated by FITEQL, assuming site-specific adsorption of TCP^- and of $HTCP^0$ onto the neutral hydroxyl sites. Data points represented by stars have 30.0 g of bacteria/L.

the SCM selected here provides an adequate description of sorption.

Both the SCM and the HPM described here are difficult to apply to the prediction of TCP sorption in a natural soil. We have only investigated adsorption at 25 °C, making it impossible to adjust the model constants to other temperatures. The SCM is only effective if all pertinent stability constants have been determined, and the HPM *D* values apply only to the chemical conditions in which they were measured. In a natural soil, many species of bacteria may be present, cell characteristics are dependent on growth conditions, and a significant fraction of cells may exist as spores. Literally thousands of equilibria must be examined before an accurate SCM can be developed, and HPM *D* values must be measured for each soil. However, the SCM offers three advantages over the HPM: (a) the *K* values are constant regardless of solution composition or solid-to-solution ratio, (b) the SCM stability constants can be utilized with other previously measured stability constants, and (c) recent research suggests that it may be possible to estimate stability constants describing adsorption onto different bacterial surfaces from a limited experimental data set (*24*). Therefore, we suggest that additional research concerning the application of the SCM to organic bacteria sorption is warranted.

Acknowledgments

This study was funded by an NSERC Operating Grant and a FCAR Nouveaux Chercheurs Grant to J.B.F. while he was at McGill University. We thank Dave Yiptong, Krystyne Blaikie, Christian Gravel, and Pascal Benalil for conducting some of the experiments. We also thank Alfonso Mucci for the use of his spectrophotometer. Five anonymous reviewers provided comments and suggestions which greatly improved this paper.

Literature Cited

(1) Makinen, P. M.; Theno, T. J.; Ferguson, J. F.; Ongerth, J. E.; Puhakka, P. M. *Environ. Sci. Technol.* **1993**, *27*, 1434−1439.
(2) Suntio, L. R.; Shiu, W. Y.; Mackay, D. *Chemosphere* **1988**, *17*, 1249−1290.
(3) Yoshida, K.; Shigeoka, T.; Yamamuchi, F. *Chemosphere* **1987**, *16*, 2531−2544.
(4) You, C. N.; Liu J. C. *Water Sci. Technol.* **1996**, *33*, 263−270.
(5) Mueller, J. G.; Chapman, P. J.; Pritchard, P. H. *Environ. Sci. Technol.* **1989**, *23*, 1197−1201.
(6) Kishino, T.; Kobayashi, K. *Water Res.* **1995**, *29*, 431−442.
(7) Niimi, A. J.; Palazzo, V. *Water Res.* **1985**, *19*, 205−207.
(8) Keith, L. H.; Telliard, W. A. *Environ. Sci. Technol.* **1979**, *13*, 416−423.
(9) Smejtek, P.; Blochel, A.; Wang, S. *Chemosphere* **1996**, *33*, 177−201.
(10) Kung, K.-H. S.; McBride, M. B. *Environ. Sci. Technol.* **1991**, *25*, 702−709.
(11) Schellenberg, K.; Leuenberger, C.; Schwartzenbach, R. P. *Environ. Sci. Technol.* **1984**, *18*, 652−657.
(12) Lee, L. S.; Rao, P. S.; Nkedi-Kizza, P.; Delfino, J. J. *Environ. Sci. Technol.* **1990**, *24*, 654−661.
(13) Westall, J.; Leuenberger, C.; Schwartzenbach, R. P. *Environ. Sci. Technol.* **1985**, *19*, 193−198.
(14) Duncan, K. E.; Ferguson, N.; Kimura, K.; Zhou, X.; Istock, C. A. *Evolution* **1994**, *48*, 2002−2025.
(15) Langmuir, D. *Aqueous Environmental Geochemistry*; Prentice Hall: Englewood Cliffs, NJ, 1997.
(16) Davis, J. A.; Kent, D. B. In *Mineral-Water Interface Geochemistry*; Hochella, M. F., White, A. F., Eds.; Reviews in Mineralogy; Mineralogical Society of America: Washington, DC, 1990; Vol. 23, pp 177−260.
(17) Schindler, P. W.; Stumm, W. In *Aquatic Surface Chemistry*; Stumm, W., Ed.; John Wiley and Sons: New York, 1987; pp 83−110.
(18) Fein, J. B.; Daughney, C. J.; Yee, N.; Davis, T. *Geochim. Cosmochim. Acta* **1997**, *61*, 3319−3328.
(19) Stumm, W.; Morgan, J. J. *Aquatic Chemistry*; Wiley-Interscience: New York, 1981.
(20) Westall, J.; Hohl, H. *Adv. Colloid Interface Sci.* **1980**, *12*, 265−294.
(21) Daughney, C. J.; Fein, J. B. *Geochim. Cosmochim. Acta* **1997**, *61*, 719−729.
(22) Westall, J. *FITEQL. A Computer Program for Determination of Chemical Equilibrium Constants from Experimental Data*; Department of Chemistry, Oregon State University; Report 82-01.
(23) Westall, J. *FITEQL. A Computer Program for Determination of Chemical Equilibrium Constants from Experimental Data*; Department of Chemistry, Oregon State University; Report 82-02.
(24) Daughney, C. J.; Fein, J. B.; Yee, N. *Chem. Geol.* In press.

Received for review March 31, 1997. Revised manuscript received November 17, 1997. Accepted November 18, 1997.

ES970295P