

Sorption of Trichloroethylene onto a Zeolite Accompanied by Methanotrophic Biotransformation

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The rate and extent of trichloroethylene (TCE) sorption from aqueous phase onto a synthetic hydrophobic zeolite, silicalite, were measured. Equilibrium sorption fit the Langmuir isotherm with parameters of $Q_0 = 201$ mg of TCE/g of silicalite and $b = 0.52$ L/mg. Rate studies showed that TCE uptake by silicalite was rapid (equilibrium approached within 25 min) as was desorption following perturbation of equilibrium by chloroform (CF) addition to the aqueous phase. The availability of sorbed TCE for cometabolic transformation by methanotrophic bacteria was evaluated, and a model was developed based upon the hypothesis that the TCE transformation rate is proportional to the concentration of TCE in solution and independent of the mass of sorbed TCE. A comparison between experimental and model results for concurrent sorption/biotransformation supported the validity of the hypothesis and model assumptions. In the presence of silicalite, the methanotrophic transformation of solution-phase TCE induced desorption of TCE from the silicalite, resulting in bioregeneration of the sorption sites. The potential for using silicalite in an advanced waste treatment process incorporating bioregeneration is discussed.

Introduction

The sorption of solutes onto porous media is a ubiquitous phenomenon in both natural and man-made systems. The fate and movement of contaminants in porous media and, consequently, the effectiveness of *in-situ* biological remediation of groundwater are substantially affected by the concurrent processes of sorption and biotransformation. These processes are also of particular interest in systems such as lake and ocean sediments, digestive tracts, oral cavities (plaque buildup on teeth), activated carbon (AC) adsorbers, solid media filters, plant roots, and soils. Whereas extensive research has been conducted on both sorption and biotransformation individually and the understanding of their relative effects on compound fate has improved, basic aspects of their interrelationships have yet to be examined adequately.

As a means of exploring the interrelationship between sorption and biodegradation, experiments were conducted to examine the rate and extent of trichloroethylene (TCE) sorption onto silicalite, a highly sorptive synthetic zeolite, and subsequently to evaluate the effect of the presence of silicalite on the cometabolic transformation rate of TCE by methanotrophic bacteria. Experimental results are compared to predictions made by incorporating sorption into a model describing cometabolic transformation kinetics. Fortuitously, this study also illuminated the potential of silicalite for use in advanced waste treatment processes as a sorbent amenable to complete bioregeneration.

The selection of TCE for this study was based upon indications that it is the most commonly encountered groundwater contaminant (1), is a possible carcinogen (2), and as a hydrophobic halogenated compound, is highly persistent in the groundwater environment (3). In many cases TCE has been transported into water supplies where it becomes a potential health risk. In addition, oxidation of TCE by methanotrophic bacteria is well known (4-9) and *in-situ* biodegradation by this process has been demonstrated at the field scale (10).

Background

Combined Sorption and Biodegradation Studies. Previous studies on the effects of organic sorption on biotransformation were reviewed by van Loosdrecht *et al.* (11). Such studies have been conducted with a variety of sorbing materials including soils (12-16), clays (17, 18), sediments (19), activated carbon (20-28), and hydroxyapatite (29).

Gordon and Millero (29) evaluated the correlation between the extent of substrate sorption and its degradation rate using a porous solid (hydroxyapatite) and a series of substrates with differing sorption affinities. They concluded that the availability of sorbed substrate to microbial transformation is an inverse function of the sorption affinity of the substrate. Ogram *et al.* (12) studied 2,4-dichlorophenoxyacetic acid degradation in the presence of soils and concluded that both suspended and attached bacteria were capable of degrading dissolved substrate and that sorbed substrate was unavailable for degradation until desorbed. Miller and Alexander (18) were able to model the degradation of benzylamine in the presence of montmorillonite by assuming that benzylamine was readily desorbed from the solids and that only dissolved benzylamine was degradable.

Although the majority of research supports the unavailability of sorbed substrates until desorbed, several studies have dissented from this view and maintained that not only are sorbed structures available for direct microbial attack but also that sorption causes substrates to be concentrated at surfaces, enhancing their microbial uptake (21, 25, 30, 31). However, results showing sorption-enhanced degradation in the presence of glass beads (30) were not reproducible by van Loosdrecht *et al.* (11) and were presumed by them to have been due to organic contamination. Additional reports of sorption-enhanced degradation have involved activated carbon (AC) as the sorbent (21, 25, 31). Contradictory results suggest that although AC acts as a good support medium for enhanced microbial growth, sorbed material is unavailable for degradation unless it desorbs into solution (23, 24). Lowry and Burkhead (23) further suggested that nonsteady-state conditions resulting in slow additional sorptive uptake by the AC are responsible for the observed organic removal previously attributed to sorption-enhanced degradation.

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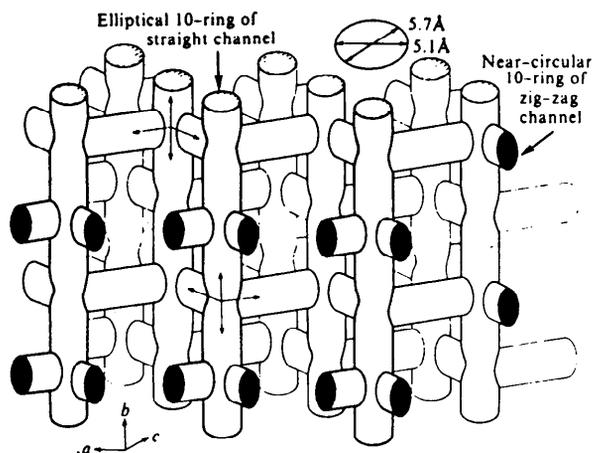


Figure 1. Silicalite microporous cage structure (reproduced with permission from ref 33).

Table I. Physical Characteristics of Silicalite (33, 35, 38)

silicalite characteristics	
pore volume, cm ³ /g	0.19
void fraction, %	33
density, g/cm ³	1.8
surface area, m ² /g	300
pore diameter, nm	0.6
crystal diameter, μm	1-10
structural formula	SiO ₂
melting temperature, °C	1300

In this study, the availability of sorbed substrates is further explored experimentally by comparing the biotransformation of TCE in the absence and presence of a high-capacity sorbent.

Silicalite. Natural and synthetic zeolites are crystalline materials with high internal surface areas, good thermal stability, and uniform pore sizes. Zeolites are widely used as catalysts and in separation technology to isolate chemicals from liquid or gas phases (32). Silicalite is a synthetic zeolite developed by Union Carbide (U.S. Patent 4 061 724), which is composed of silicon and oxygen arranged in a tetrahedral framework of consistent microporous crystalline structure (Figure 1). The micropores are arranged in two-dimensional cross connections with straight elliptical channels intersecting nearly circular sinusoidal channels (33, 34). Although silicalite decomposes to glass at 1300 °C, it is thermally stable to temperatures of 1100 °C (33, 35), allowing thermal regeneration with minimal material loss.

Due to the absence of cations or polar groups in the silicalite crystal structure together with the absence of sites for hydrogen bonding or acid-base interactions, silicalite crystals are chemically inert and hydrophobic, making them unique among zeolites (33, 35, 36). Further, the high internal surface area of silicalite (Table I) and the abundance of uniformly sized (0.6-nm) micropores in the diameter range of low molecular weight organic molecules are characteristic of a high capacity sorbent.

The sorption of organics onto silicalite occurs by a pore-filling process involving only van der Waals forces (33) and is a function primarily of micropore geometry rather than the chemical characteristics of the sorbent (34, 37). Consequently, small nonpolar organics, which are hydrophobic, have an affinity for the micropores which results in their concentration within the cage structure of the crystal (37, 38). Additionally, since the primary sorption

mechanism within silicalite is induced dipole interactions, the silicalite surface is organophilic (33, 37). Hence polar organic molecules which are smaller than the silicalite pores are also concentrated within the crystal due to their high polarizability. Therefore, silicalite is able to isolate a broad range of small organic molecules from water, such as aldehydes, acids, esters, ethers, alcohols, ketones, nitriles, phenols, and halogenated species while excluding large diameter organics (33, 35, 38, 39). The magnitude of van der Waals forces acting on solutes within the silicalite micropores increases with molecular size (37). Hence, while both TCE and CF molecules are sufficiently small to sorb onto silicalite, the larger TCE molecules (0.56 nm) can be expected to sorb to a greater extent than CF (0.44 nm).

Additionally, since the silicalite crystal is formed of pores which interconnect in two dimensions (promoting freedom of sorbate movement) and since sorbates are held only by van der Waals forces, the small molecular diameter compounds used in this study would be expected to sorb and desorb readily with changes in equilibrium conditions. Rapid sorptive uptake has been reported for a range of compounds (35, 39, 40), and rapid desorption has been reported using both solvent and thermal elution (35).

The homogeneous nature and hydrophobicity of silicalite renders in an ideal sorbent for this study, as equilibrium coefficients and sorption rates can be expected to be undeviating, allowing accurate determination of sorption parameters and thus promoting reproducible results. Although silicalite is available in pellet form, the powder form (pure crystals) was used in this study in order to observe sorption behavior unhindered by the presence of binders.

Model Description

Sorption Isotherm. The application of the Langmuir sorption isotherm to TCE uptake onto silicalite is justified by the homogeneous surface of the silicalite, by the lack of electrostatic binding sites, and by a micropore diameter which necessitates monolayer sorption behavior for most organics (33, 34, 39, 41). The Langmuir isotherm is expressed as follows (42):

$$q_{eq} = \frac{Q_o b S_{eq}}{1 + b S_{eq}} \quad (1)$$

where q_{eq} is the mass of solute sorbed per mass of silicalite at equilibrium (mg/g), S_{eq} is the aqueous concentration of solute at equilibrium (mg/L), Q_o is the maximum solute sorption capacity of silicalite (mg/g), and b is the constant related to energy of sorption (L/mg). The total mass of solute partitioning between the sorbed and aqueous phases could then be expressed by:

$$M = S_{eq} V_{aq} + q_{eq} m_s = S_{eq} V_{aq} + \frac{m_s Q_o b S_{eq}}{1 + b S_{eq}} \quad (2)$$

where M is the total mass of solute (mg), V_{aq} is the volume of aqueous phase (L), and m_s is the mass of silicalite (g).

When M is known, computation of the aqueous solute concentration (S_{eq}) for specific Langmuir coefficients can be achieved by rearranging eq 2 as a quadratic with respect to S_{eq} and solving for the positive root.

Cometabolic Transformation. The cometabolic transformation of TCE by batch cultures of methanotrophic resting cells can be modeled by incorporating the following

expression for cell inactivation due to product toxicity:

$$X = X_0 - \frac{1}{T_c}(S_0 - S) \quad (3)$$

into traditional Monod kinetics:

$$-\frac{dS}{dt} = \frac{kXS}{(K_s + S)} \quad (4)$$

resulting in the following equation for contaminant disappearance over time (43):

$$-\frac{dS}{dt} = \frac{k\left(X_0 - \frac{1}{T_c}(S_0 - S)\right)S}{(K_s + S)} \quad (5)$$

where X is the active microbial concentration at time t (mg/L), X_0 is the initial active microbial concentration (mg/L), S is the aqueous concentration of cometabolized contaminant at time t (mg/L), S_0 is the initial aqueous concentration of cometabolized contaminant (mg/L), k is the maximum rate of contaminant transformation (mg of S/mg of cells day⁻¹), K_s is the half-velocity constant for contaminant (mg/L), and T_c is the transformation capacity of cometabolized contaminant (mg of S/mg of cells) with initial conditions $S = S_0$ and $X = X_0$ at $t = 0$.

This model has been shown to adequately describe the kinetics of TCE transformation in the absence of methane by a mixed culture of methane oxidizers (43, 44).

Combined Sorption and Biotransformation. Rate-Limiting Reaction. Examining relative reaction rates is useful in order to determine the rate-limiting reaction when modeling the simultaneous processes of sorption and biotransformation. For a well-mixed batch reactor, the mass transfer rate of solute or sorbate from the bulk solution to a sorbent is defined as J_a (mg/L day⁻¹). The relative significance of the maximum mass transfer rate (J_a') and the maximum solute transformation rate (kX_0) can be evaluated using the Damköhler number (45):

$$Da = \frac{kX_0}{J_a'} \quad (6)$$

When $Da \gg 1$ the transformation rate is high compared to the sorption mass transfer, hence the reaction can be considered mass transfer limited. When $Da \ll 1$, transformation is slow relative to sorptive transfer and becomes the rate-limiting reaction. In the latter case it is possible to assume that the aqueous-phase solute is virtually in equilibrium with the sorbed phase, allowing the following approximation:

$$S \cong S_{eq} \quad (7)$$

Sorption/Biotransformation Model. In order to model transformation behavior in batch reactors in the presence of silicalite, the following assumptions were made: (1) the partitioning of TCE between sorbed and aqueous phases is described by the Langmuir isotherm (eq 2); (2) instantaneous equilibrium between sorbed and aqueous TCE is maintained because Da is expected to be much less than 1 (justification to follow); and (3) only the TCE in aqueous solution is available for biotransformation, i.e., transformation rates are independent of the amount of TCE sorbed.

The model calculations were based on a time-step series of TCE mass balances within the batch reactors. The following series of calculations was repeated for each time

step ($\Delta t = 0.1$ h), with the subscripts i and j referring to consecutive steps and with initial conditions of $M_i = M_0$ and $X_i = X_0$: (Calculation 1) M_i , the total mass of TCE present in the reactor, is used along with eqs 2 and 7 to compute the aqueous TCE concentration, S_i . (Calculation 2) The following expression derived from eq 4 is used along with S_i to compute the concentration of TCE biotransformed during a single time step:

$$\Delta S_i = \frac{kX_i S_i}{K_s + S_i} \Delta t \quad (8)$$

(Calculation 3) The concentration of active cells for the next time step is computed using the following expression derived from eq 3:

$$X_j = X_i - (\Delta S_i / T_c) \quad (9)$$

(Calculation 4) The total mass of TCE remaining in the reactor for the next time step is given by

$$M_j = M_i - \Delta S_i V_{aq} \quad (10)$$

The desorption of TCE from silicalite, which results in a constant release of contaminant into solution, makes it difficult to directly determine the contaminant disappearance with time. Hence, in this study the accumulation of ¹⁴C-labeled transformation products (carbon dioxide and nonvolatile intermediates) rather than TCE disappearance was used as a measure of reaction speed and to estimate kinetic parameters. However, the use of product accumulation for estimation of kinetic parameters necessitates the assumption that little of the cometabolized product is incorporated into cells or sorbed onto the silicalite. As part of this study, this assumption was experimentally validated for the appropriate conditions.

Methods

Experimental Methods. Solutions. Saturated aqueous solution of trichloroethylene (99+ % pure ACS reagent, Aldrich Chemicals Co., Milwaukee, WI) and chloroform (99.5% pure "Photrex" Baker Reagent, J. T. Baker Chemical Co., Phillipsburg, NJ) were maintained as described previously (44). An aqueous solution of uniformly ¹⁴C-labeled TCE (Sigma Chemical Co., St. Louis, MO) with a reported specific activity of 4.1 mCi/mmol and a measured purity of 95% was used, and the activity was detected using a Tricarb 4530 scintillation spectrometer. Samples were analyzed for ¹⁴C-labeled volatile, nonvolatile, and carbon dioxide fractions using the acid/base/neutral method described previously (46). In order to ensure consistency with the sorption/biotransformation studies, all experiments were conducted in mineral salts medium used for growth of the methanotrophic cells described previously (46).

Silicalite. Silicalite was donated by the Molecular Sieves Division of Union Carbide (Union Carbide Corp., Danbury, CT), type S-115 powder (1–10- μ m cubes), lot no. 96188661033-S, and was maintained in a 500 °C oven prior to use.

Isotherm Measurement. Aqueous-phase isotherm studies were conducted using 9-mL glass vials containing three glass beads to promote mixing and sealed with 90-mil (2.3-mm) Teflon-lined silicone septa and sample-hole caps. [¹⁴C]TCE was used to simplify quantification, and volatilization loss was avoided by the exclusion of headspace.

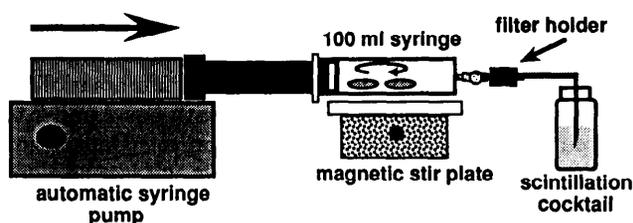


Figure 2. Experimental apparatus used to measure rates of TCE sorption onto silicalite.

Vials were weighed before and after silicalite addition to determine solids added, and again after medium addition to determine precise liquid volume. Aqueous solvent solutions were added by gas-tight Pressure-lok syringes immediately prior to sealing (septa were not pierced), and the vials were incubated at 4 rpm mixing in a 20 °C chamber. Vials were centrifuged for 15 min at 4500 rpm (1100g), and the supernatant was filtered through Whatman GF/F microfiber filters (Whatman International Ltd., Maidstone, England) held in stainless steel filter holders to ensure removal of residual silicalite before being analyzed for [¹⁴C]TCE.

Sorption Rate Measurements. A 100-mL gas-tight gas syringe (Glenco Scientific, Inc., Houston, TX) with a Teflon plunger, and fitted with a Teflon sampling valve (Figure 2), was filled with 10 mg of silicalite, 100 mL of mineral medium, and two Teflon-coated stir bars. A magnetic stir plate provided high agitation, and an automatic syringe pump (Model 35, Sage Instruments, Cambridge, MA) was manually activated at discrete intervals for sampling. To commence the experiment, [¹⁴C]TCE was added to the syringe; the first sample was taken after 1 min. Supernatant was force-filtered through a 0.2- μ m Nylon 66 filter (Alltech, Inc., Deerfield, IL) and glass fiber AP prefilter (Millipore Corp., Bedford, MA) housed in a 25-mm stainless syringe filter holder (Allied Corp., Fisher Scientific, Pittsburgh, PA) to remove the silicalite from the liquid before it was added to scintillation cocktail for counting of the [¹⁴C]TCE. TCE losses due to uptake onto the filter apparatus were found to be insignificant, and TCE losses due to incident sorption or volatilization over time were measured using controls containing no silicalite.

Desorption Rate Measurements. The desorption rate of TCE and CF from silicalite following perturbation of equilibrium conditions was determined using a time series of headspace analyses. CF was used in conjunction with the TCE because its similar molecular structure would cause it to compete with the TCE for sorption sites on the silicalite, causing desorption of the initially sorbed species. A preweighed mass of silicalite was added to 62-mL glass vials containing 20 mL of mineral medium and sealed with Mininert valves. TCE or CF in saturated aqueous solution was added to the vials, which were then shaken at 300 rpm at 21 °C, and the first sample was taken after 15 s. The TCE and CF concentration in the vial headspace was periodically analyzed by a gas-tight syringe. Control bottles without silicalite were used to measure the total mass of TCE or CF added and to monitor possible solvent losses throughout the experiment.

Concurrent Sorption/Biotransformation Studies. The biological availability of sorbed TCE was determined using [¹⁴C]TCE and 8.8-mL glass vials which were devoid of headspace, contained three glass beads to promote mixing, and were sealed with two Teflon-lined septa and a screw

cap with a sampling hole. For preequilibration with TCE, each vial was weighed, amended with silicalite, reweighed, filled with mineral salts medium, amended with [¹⁴C]TCE using a Pressure-lok gas-tight syringe, sealed with cap and septa, reweighed, and incubated for 1 day at 20 °C and 4 rpm by inversional mixing. A mixed culture of methanotrophs which were grown in mineral salts medium in a completely mixed flow reactor with methane as sole carbon and energy source (46), and which were capable of cometabolically transforming TCE in the absence of methane, were used for the biotransformations. Either 0.8 mL of 1.0 mL of freshly harvested cells was injected through the septa of a preequilibrated vial with a Multifit glass syringe, while an equivalent volume of medium was simultaneously withdrawn with a second syringe. The vials were then incubated in the dark at 20 °C and mixed on a 12 rpm vertical bottle rotator for various time intervals before analysis for ¹⁴C-labeled products and TCE dissolved in the supernatant was performed. A vial was sacrificed for each analysis.

The amount of TCE-carbon incorporated into cellular material or sorbed onto silicalite following transformation was determined by using 8.8-mL vials containing 3.8 mL headspace and sealed with 90-mil (2.3-mm) Teflon-lined silicone septa. Some cells were inactivated to methane and TCE transformation by shaking with a 20% acetylene headspace for 1.5 h prior to the experiment (47). The addition of 4.5 mL of either active or inactivated cells to the vials was followed by the addition of 0.5 mL of [¹⁴C]-TCE without piercing the septa. Vials were incubated at 23 °C and 400 rpm for 43 h. Reactions were stopped, and ¹⁴CO₂ was fixed in solution by the addition of 100 μ L of 1.0 N NaOH. A 0.2-mL aliquot of a 50 g/L slurry of silicalite in mineral medium was added to half the vials yielding 1900 mg/L of silicalite, after which the vials underwent a further 1.5-h incubation. Duplicate vials were sacrificed for analysis of ¹⁴C-labeled products and TCE in the supernatant.

Analytical Procedures. ¹⁴C Analysis. Liquid samples were centrifuged at 4500 rpm for 15 min to remove cells and silicalite; and acid, base, and neutral purgeable and nonpurgeable fractions of ¹⁴C were measured in aliquots of the supernatant as described previously (46).

TCE and CF Analysis. Headspace analyses were used to determine both TCE and CF concentrations using a Tracor MT-220 gas chromatograph equipped with a linearized electron capture detector (46). Since both targeted compounds are volatile, Henry's law, $H_c = S_g/S_l$, was used with headspace measurements of TCE and CF to compute liquid concentrations and total mass present using the following relation:

$$M_{l,g} = S_l V_l + S_g V_g = S_l (V_l + H_c V_g) = S_g (V_l/H_c + V_g) \quad (8)$$

where $M_{l,g}$ is the total mass of solute partitioning between gas and liquid phases (μ g), V_l is the volume of liquid in a bottle (mL), V_g is the volume of gas in a bottle (mL), S_l is the concentration of solute in liquid (mg/L), S_g is the concentration of solute in gas (mg/L), and H_c is the Henry's constant for solute ([mg of solute/L of gas]/ [mg of solute/L of liquid]). H_c values of 0.31 and 0.11 at 20–21 °C were used for TCE and CF, respectively (48).

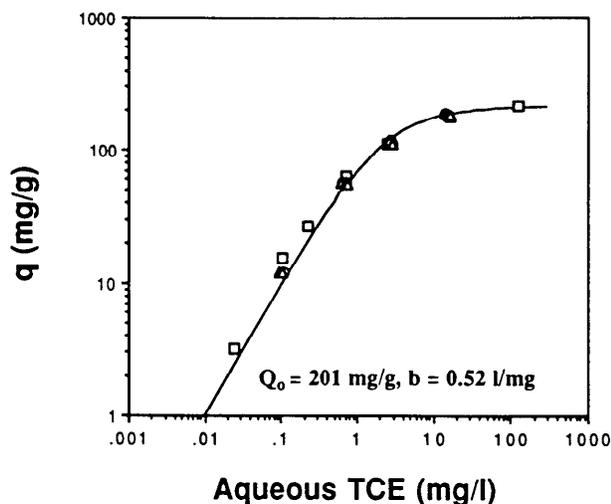


Figure 3. Equilibrium isotherm measured for TCE sorption onto 1–2 mg of silicalite. Data for sorption from aqueous solution after incubations of 1 day (\square), 5 days (\circ), and 10 days (Δ) are presented together with the best fit of the Langmuir equation (line).

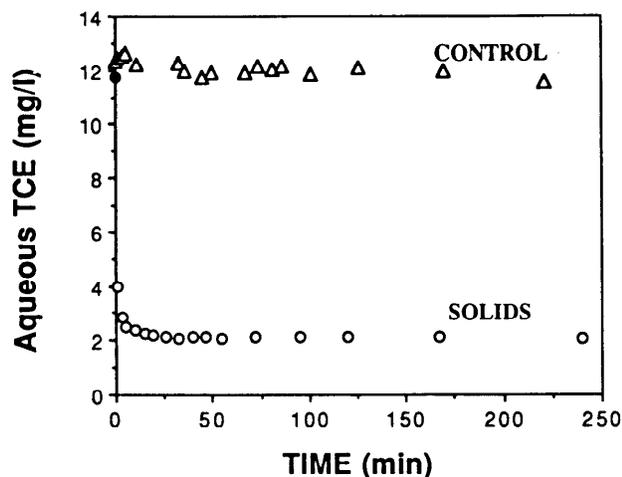


Figure 4. TCE uptake by 100 mg/L silicalite (\circ) plotted along with the control (Δ , no silicalite). Time zero data point (\bullet) is a computed value.

Results

Sorption Studies. The equilibrium TCE sorption capacity of silicalite was determined from measurement of the amount of TCE in solution after periods of contact with silicalite. TCE over a concentration range from 0.4 to 150 mg/L was contacted for periods of 1, 5, or 10 days with 1 or 2 mg of silicalite (110 or 230 mg/L), resulting in final TCE concentrations between 0.02 and 127 mg/L (Figure 3). A nonlinear regression analysis (Systat 5.0, Systat Inc., Evanston, IL) of the Langmuir isotherm yielded values of $Q_0 = 201$ mg/g and $b = 0.52$ L/mg ($r^2 = 0.994$). The similarity of results for the 1-, 5-, and 10-day incubation times indicates that equilibrium was reached in less than 1 day.

The results of an experiment to measure the rate of TCE sorption onto silicate are presented in Figure 4, including a control run (experiment repeated in the absence of silicalite), and a time zero data point computed from the known mass of TCE added. The initial TCE solution concentration of 12 mg/L was reduced to 4 mg/L within the first minute, to 2.5 mg/L within 5 min, and to an equilibrium concentration of 2.1 mg/L within 25 min, yielding a computed initial TCE mass transfer rate (J_a)

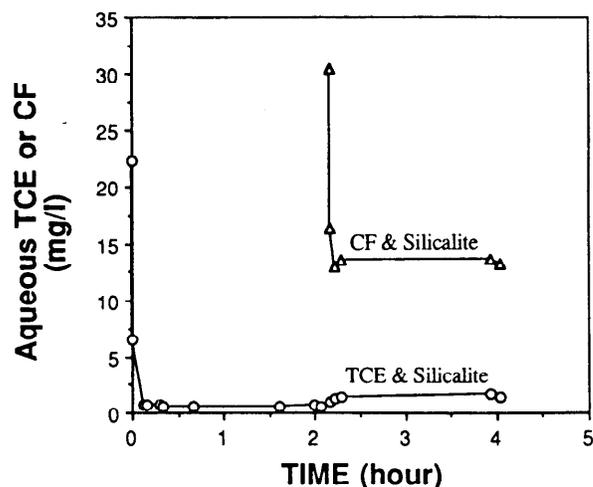


Figure 5. Rapid uptake of 22 mg/L TCE (\circ) by 500 mg/L silicalite resulting in an equilibrium aqueous concentration of 0.6 mg/L TCE. Addition of 30 mg/L CF at 2 h (Δ) resulted in the desorption of TCE from the silicalite and uptake of CF yielding aqueous concentrations of 13 mg/L CF and 1.4 mg/L TCE.

of 11 000 mg/L day⁻¹. Here, Da can be computed by assuming that the rates of sorption and desorption are equivalent and by using transformation rates measured for this study ($k = 0.3$ day⁻¹, $X = 200$ mg/L). The resulting value, $Da = 0.006$, indicates that the transformation reaction is rate-limiting and, hence, validates the instantaneous equilibrium assumption.

Headspace analysis of TCE and CF in the presence of silicalite was used to evaluate the rate at which sorbed solutes would desorb due to equilibrium perturbation. That is, after sorption equilibrium was achieved between a single sorbate and silicalite, a second sorbate was added to perturb the equilibration, and the rate of equilibrium reestablishment was observed. CF was chosen as the second sorbate since it is molecularly similar to TCE. Figure 5 displays the TCE solution concentrations when 22 mg/L TCE is added to a bottle containing the 500 mg/L (10 mg) silicalite. About 70% of the added TCE was sorbed within 15 s after the TCE addition, and shortly after that, an equilibrium concentration of 0.6 mg/L was attained. After 2.1 h, 30 mg/L CF was added to the vial, resulting in the rapid desorption of a fraction of the sorbed TCE and uptake of CF onto the silicalite. About 50% of this TCE desorption occurred within 15 s, eventually resulting in TCE and CF solution concentrations of 1.4 and 13 mg/L, respectively. Results of a similar experiment in which CF was added prior to the TCE are shown in Figure 6. Here 29 mg/L added CF decreased to an equilibrium concentration of 7.4 mg/L (50% of the sorption occurred within 15 s). Addition of 24 mg/L TCE caused desorption of a fraction of the CF (CF desorption 75% complete within 15 s) to a final solution concentration of 13 mg/L, and a final TCE solution concentration of 1.5 mg/L. The rapidity of the exchange of the sorbed compounds along with the similarity of final TCE and CF concentrations regardless of the order in which the compounds were applied (Figures 5 and 6) suggests that desorption was rapid and that displacement occurred readily with little steric interference.

Effect of Sorption on Biotransformation. The availability of TCE sorbed onto silicalite to microbial transformation was explored using ¹⁴C-labeled TCE and the methanotrophic mixed culture.

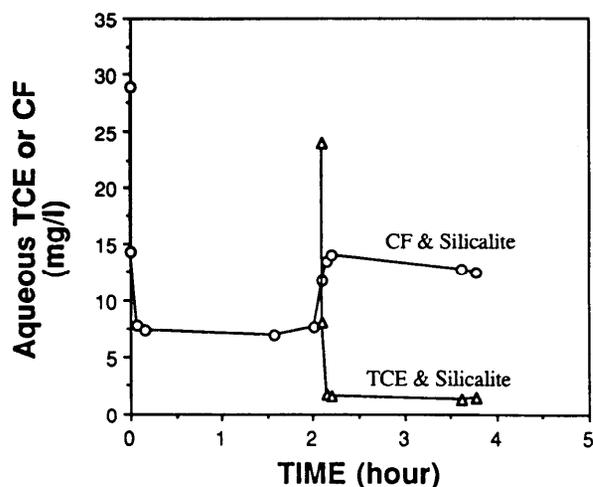


Figure 6. Rapid uptake of 29 mg/L CF (O) by 500 mg/L silicalite resulting in an equilibrium aqueous concentration of 7.4 mg/L CF. Addition of 24 mg/L TCE at 2 h (Δ) resulted in the desorption of CF from the silicalite and uptake of TCE yielding aqueous concentrations of 1.5 mg/L TCE and 13 mg/L CF.

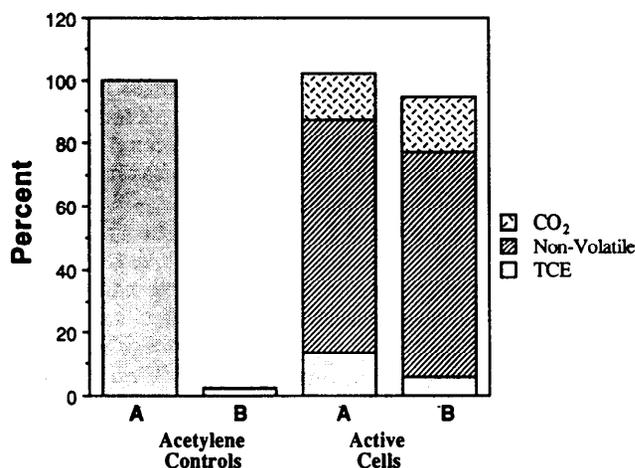


Figure 7. Percent of ^{14}C from 81 mg/L TCE found in various fractions 43 h after being added to 3000 mg/L acetylene-treated or resting cells. After reactions were stopped by the addition of NaOH, one set (A) from each treatment was sampled while the second set (B) was incubated with 1900 mg/L silicalite for 1.5 h before sampling. Each column represents an average of duplicates.

In order to evaluate whether TCE transformation products were incorporated into cellular material or could be sorbed onto silicalite, the recovery of ^{14}C in the supernatant liquid was measured following [^{14}C]TCE transformation by active and inactivated methanotrophs in the absence of silicalite, and again after equilibration of the final mixture with silicalite. Figure 7 shows results when 81 mg/L TCE was incubated for 43 h with 3000 mg/L cells. After reactions were stopped by base addition, the supernatant of one set for each cell treatment was sampled while the second set was incubated with 2000 mg/L silicalite for 1.5 h prior to sampling. When 100% ^{14}C recovery is defined as that in the silicalite-free, acetylene-treated controls in which no TCE transformation occurred, a total ^{14}C recovery of 94–105% was obtained for the active cells, both with and without silicalite addition. Here, most of the TCE was converted into a nonvolatile fraction and CO_2 , neither of which tended to sorb significantly to silicate or to be incorporated into cell particulate matter. This is confirmed by the high recovery

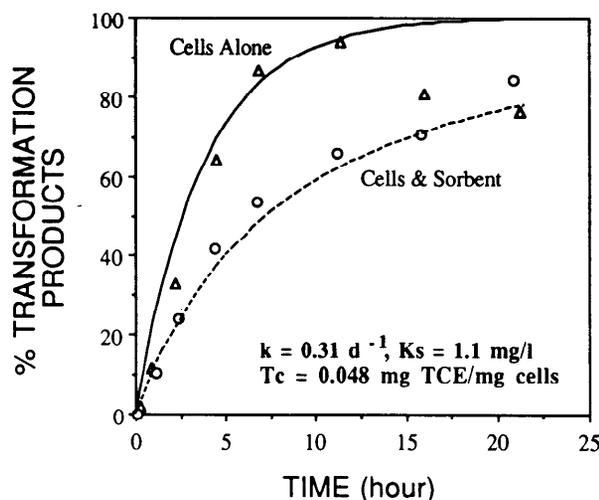


Figure 8. Appearance in the supernatant of ^{14}C -labeled transformation products produced by active cells from 7 mg/L TCE in the absence of silicalite (Δ) and in the presence of 110 mg/L silicalite (O). The best fit of the cometabolic transformation model (solid line) to the cells alone experimental data, and the prediction for the cells and sorbent case resulting from the coupling of the cometabolic transformation model with the Langmuir isotherm (dashed line).

observed with the active cells both with and without silicalite.

Transformation of 62 μg of TCE by 1.5 mg of cells (170 mg/L) was measured using two sets of vials: "cells alone", to which no silicalite was added, and "cells and sorbent" in which 1.0 mg of silicalite (110 mg/L) had been equilibrated with TCE for 1 day prior to active cell addition. Measured TCE solution concentrations prior to cell addition were 7.0 mg/L in the absence of silicalite and 0.8 mg/L with silicalite present. Medium controls, with and without silicalite addition, demonstrated that in the absence of cells no transformation occurred. In the absence of silicalite, 94% of the TCE was transformed within 11.3 h (cells alone, Figure 8). The reason for the decline in transformation product accumulation after 11.3 h is unknown; however, the diminished recovery of total ^{14}C -labeled compounds in the supernatant of those samples compared to the earlier samples suggests that transformed carbon was being accumulated into a separate phase (e.g., the cells) where it was not measured. Cellular carbon uptake may be due to CO_2 fixation by the starved methanotrophs or other carbon metabolism under conditions of low TCE addition, when cells would not be significantly inactivated by TCE transformation products (in contrast to conditions of the previous experiment). In the presence of silicalite (cells and sorbent), transformation products accumulated slower than for cells alone. Non-linear fit of the cells alone product accumulation data (excluding data after 11.3 h) to eq 5 along with an estimated T_c of 0.048 mg of TCE/mg of cells yielded values of $k = 0.31 \text{ day}^{-1}$, and $K_s = 1.1 \text{ mg/L}$ ($r^2 = 0.990$). These coefficients were used along with the sorption/biotransformation model and the previously determined Langmuir coefficients to estimate the rate of product accumulation for cells and sorbent. Experimental data and model prediction (dashed line) shown in Figure 8 indicate good agreement.

In order to enhance the observed sorption effect, a similar experiment was conducted using a 30-fold increase in silicalite. Transformation of 132 μg of TCE by 2.4 mg of cells (280 mg/L) was measured using cells alone vials

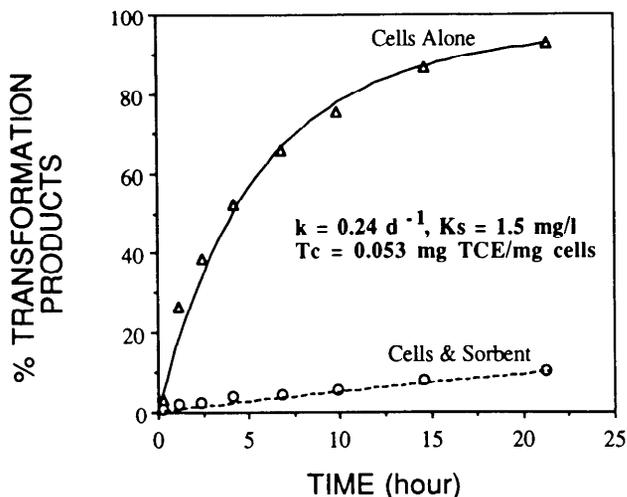


Figure 9. Appearance in the supernatant of ^{14}C -labeled transformation products produced by active cells from 15 mg/L TCE in the absence of silicalite (Δ) and in the presence of 3400 mg/L silicalite (\circ). The best fit of the cometabolic transformation model (solid line) to the cells alone experimental data and the prediction for the cells and sorbent case resulting from the coupling of the cometabolic transformation model with the Langmuir isotherm (dashed line).

and cells and sorbent vials which had been equilibrated with 30 mg of silicalite (3400 mg/L) for 4 days prior to cell addition. TCE solution concentrations prior to cell addition were 15 mg/L in the absence of silicalite and 0.07 mg/L with silicalite present. After 21 h, 93% of the ^{14}C was detected in transformation products in the cells alone vials while only 10% transformation products accumulated with cells and sorbent (Figure 9). Coefficients obtained from fitting the cometabolic model to the cells alone data of $T_c = 0.053$ mg of TCE/mg of cells, $k = 0.24$ day $^{-1}$ and $K_s = 1.5$ mg/L ($r^2 = 0.998$), were in good agreement with results from the previous experiment. Predictions using the sorption/biotransformation model (dashed line) and experimental results were again in good agreement (Figure 9), suggesting that model assumptions were reasonable.

Discussion

The sorptive uptake of TCE by silicalite was rapid as was found with other hydrophobic compounds (35, 39, 40, 49). The multisolute-induced desorption study reported here suggests a rapid reestablishment of equilibrium upon perturbation, similar to that observed with the aluminum-rich analog of silicalite, ZSM-5 (50). The inert nature of the silicalite surface, its high sorptive capacity, and its rapid approach to sorption equilibrium suggest its suitability for use in a sorption/biodegradation treatment system. Nearly complete bioregeneration of silicalite also appears possible. TCE transformation in the presence of a low concentration of silicalite was shown to proceed at a rate only slightly reduced from that in the absence of silicalite (Figure 8). Also, since only 11% of the total added TCE was initially in solution with silicalite present, whereas 84% of the total added TCE was transformed, bioregeneration by transformation-induced desorption is indicated.

The validity of the hypothesis that the TCE transformation rate was a function of TCE in solution only and was unaffected by the sorbed TCE is further indicated by the experimental data in Figure 9. Here, in the absence of sorbent, the initial TCE solution concentration was well

above K_s , and transformation occurred at the maximum rate. In the presence of silicalite, the TCE solution concentration was well below K_s , and the transformation rate was predictable from TCE solution concentration alone, i.e., independent of the sorbed TCE.

A possible design for a combined sorption/biodegradation treatment system for low molecular weight organics is a two-stage process. Stage one would entail the accumulation of the contaminants onto silicalite from either a gas or an aqueous waste stream, requiring a short detention time (rapid uptake) and yielding a high-quality effluent (high sorptive partitioning). Due to the rapid desorption characteristic of silicalite, concentration fluctuations in the influent waste stream could result in the release of sorbed contaminants decreasing the quality of the effluent. Therefore, a reactor configuration such as a plug flow column, which would minimize the effects of influent fluctuations and produce a consistent effluent quality should be chosen for stage one. Intermittently, the silicalite from stage one would be removed to stage two where it would be contacted with an appropriate microbial consortium capable of bioregeneration. Considering the range of biodegradable compounds which sorb onto silicalite, many accumulation/transformation combinations are possible. Compounds which are likely to be accumulated and transformed effectively with this process include commonly found groundwater contaminants such as chlorinated and brominated ethylenes, ethanes, and methanes, as well as other low molecular weight organics.

Conclusions

- (1) The sorptive uptake of TCE onto silicalite, a hydrophobic silicon oxide crystal, can be adequately described using a Langmuir isotherm, with a maximum TCE uptake of 20% (mass/mass).
- (2) The sorption and desorption rate of TCE was found to be rapid (equilibrium approached within 25 min) and inducible by perturbations in equilibrium solution concentrations.
- (3) Methanotrophic transformation of solution-phase TCE induced desorption of TCE from the silicalite, resulting in bioregeneration of the sorption sites.
- (4) In the presence of silicalite, the rate of TCE transformation was proportional to the concentration of TCE in solution and independent of the mass of TCE sorbed.

Acknowledgments

This research was supported in part by fellowships from the Switzer Foundation and the American Water Works Association (Larson Aquatic Research Support Scholarship), and by NIEHS Grant P42-ES04705 and by the Office of Research and Development, U.S. Environmental Protection Agency, through the Western Region Hazardous Substance Research Center, under Agreement R-815738. The contents of this article do not necessarily represent the views of these agencies.

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Received for review January 11, 1993. Revised manuscript received June 7, 1993. Accepted June 15, 1993.*

* Abstract published in *Advance ACS Abstracts*, August 15, 1993.