ROLE OF BIODEGRADATION IN THE ATTENUATION OF ETHYLBENZOATE IN AQUIFERS
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ABSTRACT
Experiments were conducted to investigate the fate of ethylbenzoate and soil microorganisms in shallow aquifers. Biodegradation and volatilization have been identified to be the major mechanisms in attenuating ethylbenzoate in contaminated soils. The parameters of an available model have been obtained by fitting it to the experimental data. Various facets of biodegradation, including the effects of mass transfer resistance and initial distribution of microorganisms, have been numerically analyzed on the basis of the model.

KEY WORDS
bioremediation, aquifer, volatilization, mass transfer

INTRODUCTION
Many aromatic organic chemicals, substituted or unsubstituted, have been on the EPA list of priority pollutants. Once released into the environment, these chemicals pose acute or chronic threats to the health of humans as well as animals. Long-term exposure to benzene causes hemopoietic tissue changes in the form of anemia or leukopenia [1]. Numerous aromatics have been reported to be teratogenic, mutagenic or carcinogenic [1]. Among the technologies for remediating soils contaminated by aromatic chemicals, several involve the principles of biodegradation and volatilization, or the interplay of these two. To quantitatively evaluate the magnitude of biodegradation and volatilization is, therefore, essential for the design and implementation of technologies such as biocleaning, air-sparging and bio-wall [2-4].

Ethylbenzoate is chosen as a representative contaminant in this study because of its semi-volatile and water-immiscible characteristics. Ethylbenzoate has a density of 1.05 g/cm³, a water-solubility of 820 mg/L at 25°C, and a vapor pressure of 1 mm Hg at 44°C; it is used primarily as an organic solvent. A dense non-aqueous phase liquid (DNAPL) is formed when ethylbenzoate is present in excess of its water-solubility. One of the objectives of this work is to experimentally assess the relative significance of biodegradation and volatilization in attenuating ethylbenzoate in aquifers. Other objectives are to estimate the parameters in an available model based on the experimental study, to simulate the processes of transport and biotransformation in aquifers, and to numerically analyze the factors affecting the fate of ethylbenzoate.

EXPERIMENTAL METHODS
Experiments entail sample preparation, calibration and measurement of ethylbenzoate concentrations, and biodegradation of ethylbenzoate in bench-scale, shallow-bed reactors. These are delineated below.

Materials
Ethylbenzoate with a grade of 99% (Aldrich Chemical) served as the sole substrate in the experiments; it was used as received. A salt solution prepared for supplying nutrients and pH buffering contained 1000 mg of K₂HPO₄, 200 mg of KH₂PO₄, 300 mg of...
(NH₄)₂SO₄, and 100 mg of MgSO₄·7H₂O per liter of distilled water; the pH of the solution was measured to be 7.1. Rhizospheric surface soil near Durland Hall, Kansas State University, Manhattan, was collected as the source of microorganisms. The soil, from which visible plant roots and tissues of the soil were removed by forceps, was stored in a refrigerator. Plate Count Agar (Difco Laboratories, Detroit, MI) served as the culturing medium for counting the bacterial population.

Apparatus and equipment

The experimental setup is illustrated in Figure 1. Each reactor was a 2 L flask with a rubber stopper through which one or more glass tubes, each with a length of 10 cm and an I.D. of 3 mm, were inserted. All reactors were placed in an incubator (New Brunswick Scientific Co., New Brunswick, NJ). The aqueous concentration of ethylbenzoate was measured with a spectrophotometer (Hewlett Packard 8452A). Gas composition in the reactors was monitored by a mass-spectrometer (Dycor, Ametek, PA).

Procedure

For calibrating the spectrophotometer, standard aqueous solutions of ethylbenzoate were prepared. Each sample was extracted twice with equal amounts of cyclohexane; two peaks, at 274 nm and 280 nm, were identified to be the absorbance of ethylbenzoate in the UV spectrum [5]. The absorbance is plotted against the aqueous concentration of ethylbenzoate in Figure 2; the solid lines in the figure are linear regression of the measurements. The bacteria in the original soil cultured on Plate Count Agar yielded 8.8 x 10⁷ cells per gram of wet soil by plate counting [6]. A drop of Tween 80 (Difco) was added to disperse the microorganisms in the measurement. The moisture content of the soil was determined to be 9.24% by the conventional oven-drying method. A 200 g sample of the soil was mixed with 1 L of the salt solution, blended for 1 minute in a household blender, and then centrifuged for 3 minutes at 9000 rpm. The centrifuged solution was passed through a Whatman no. 1 filter paper to further remove particulate solid matter. About 900 ml of final liquid were collected per liter of the salt solution. This liquid had a pH of 8.0 and contained 4.9 x 10⁸
cells/ml. To one liter of the liquid, 0.82 ml of ethylbenzoate was added with a 1 ml syringe, and the mixture was shaken thoroughly for at least 3 minutes until no ethylbenzoate blobs were visible.

Each of the reactors was filled with 250 ml of aqueous solution to form a 1.5 cm high shallow pool. These reactors were shaken at 80 rpm in the incubator maintained at 30ºC. Liquid and gas samples were withdrawn through hypodermic needles. The reactors were divided into three sets. The first set, containing a solution of soil filtrate without ethylbenzoate, was to determine the rate of cell decay. The second set was to quantify the rate of volatilization; it contained a solution with ethylbenzoate and was sterilized in an autoclave for 30 minutes at 250°F and 15 psig. The third set, containing aqueous solution of nutrients and ethylbenzoate, was to measure the rate of biodegradation. Each stopper on the first and third sets of reactors had only one glass tube while some stoppers on the second-set of reactors had one tube, and others had two. The glass tubes provided openings connecting the gas phase above the simulated aquifer solution with air in the atmosphere; they represent tortuous channels in soils above the aquifer where gas phase diffusion is present.

MODELING

Several distinct processes are involved in the attenuation of contaminants in subsurface environments; these processes include volatilization, dissolution, convection, dispersion, adsorption, and chemical and biological degradation. The present study, resorting to batch experiments in well-mixed reactors, focuses on the processes of volatilization, dissolution and biological degradation.

Derivation of governing equations

It is considered that equilibrium is established almost instantaneously between the concentration of ethylbenzoate in the air chamber of a reactor and that in the aqueous solution. This is attributable to the large liquid-air interfacial area and continuous mixing by incubator shaking. The rate of volatilization loss of ethylbenzoate to air, determined by the mass flux through the tubes, is considered to obey a first-order expression; it has the following form [7]:

$$j_{sc} = k_{m}C_{s}$$  \hspace{1cm} (1)

Various models are available to describe the growth of microorganisms through assimilation of substrates. The suitability of each model depends on a variety of factors such as substrate inhibition, substrate competition, microbial acclimation, and oxygen or electron acceptor limitation [8]. The present study adopts the well-known Monod model without neglecting the decay of cells, which accounts for the loss of viable cells. It is assumed that the rate of cell decay is of the first order; furthermore, the rate constant of decay, $k_{d}$, is averaged over the whole community of microorganisms.

A mass balance on a reactor gives the governing equation for substrate, i.e., the contaminant, which is ethylbenzoate in this work,

$$-\frac{dC_{s}}{dt} = \frac{\mu_{m}C_{b}}{Y_{s}} \left( \frac{C_{s}}{K_{s} + C_{s}} \right) + k_{m}C_{s} \frac{A}{V}$$  \hspace{1cm} (2)

and also that for biomass,

$$\frac{dC_{B}}{dt} = \mu_{m}C_{B} \left( \frac{C_{s}}{K_{s} + C_{s}} \right) - k_{C}C_{B}$$  \hspace{1cm} (3)

Complete degradation of an organic chemical usually requires the interplay among various species of microorganisms. It is common for a soil to harbor complex indigenous microbial communities; nonetheless, not all microorganisms in the soil participate in the biodegradation. In this study, therefore, the microorganisms in the soil are divided into two categories. One includes those involved in the biodegrada-
tion, whose concentration is governed by Equation 3; the other is those inactive in the biodegradation, whose concentration is governed by the following equation provided that energy sources are unavailable.

\[
\frac{dC_{bi}}{dt} = -k_d C_{bi}
\]  (4)

Thus, the total biomass concentration, \( C_{bT} \), is

\[
C_{bT} = C_b + C_{bi}
\]  (5)

The effectiveness factor, \( f_b \), is defined in such a way that at \( t = 0 \),

\[
C_b = f_b C_{bT}
\]  (6)

Since the quantity of a contaminant in subsurface may exceed the limit of its water solubility, or the rate of mass transfer may be restrained, a distinct non-aqueous phase is frequently present. Under such a circumstance, the rate of dissolution plays an important role in the bioremediation [9]. The non-aqueous phase in soils can exist as discrete or continuous entities with various sizes and shapes, e.g., blobs, channels and films. An organic phase dispersed in water, however, tends to form spheres because of the surface tension. The structures of soils may reshape these spheres by the forces of adhesion, coalescence and emulsification.

By assuming that there are \( \lambda \) non-aqueous organic entities per unit volume of a soil, the mass flux from dissolution of the non-aqueous phase, \( j_n \), is obtained as [9]

\[
j_n = \lambda \gamma \sigma v k_m (C_{sat} - C_s)
\]  (7)

To determine \( j_n \) according to this equation requires the values of the volume of an entity, \( v \), the surface area-to-volume ratio, \( \sigma \), and the ratio of the aqueous contacting area to the surface area, \( \gamma \), but it does not require explicit knowledge of the entity's shape. To determine \( j_n \) also requires the value of the mass transfer coefficient, \( k_m \), which can be obtained in many cases from available expressions for estimating it [10].

### Parameter estimation

The parameters to be determined are the mass transfer coefficient of volatilization, yield factor, maximum specific growth rate, saturation constant, decay constant and biomass effectiveness factor. In accordance with the experimental design, the following procedure has been established.

When \( C_b = 0 \), Equation 2 is integrated to be

\[
\ln \left( \frac{C_s}{C_{s0}} \right) = -k_m t, \quad \left( k_m = \frac{k_m A}{V} \right)
\]  (8)

Similarly, Equation 4 may be integrated to obtain

\[
\ln \left( \frac{C_{bi}}{C_{bi0}} \right) = -k_d t
\]  (9)

The values of \( k_m \) and \( k_d \) are obtained by fitting Equations 8 and 9 to the experimental data by minimizing the objective function

\[
\sum_{t_i} \left[ \phi(t_i) - \Psi(t_i) \right]^2
\]  (10)

where \( \Phi(t_i) \) is the value of \( C_s \) or \( C_{bi} \) at time \( t_i \) predicted by Equation 8 or 9, respectively; and \( \Psi(t_i) \) is the corresponding measured value of either variable.

The values of \( Y_s \), \( \mu_m \), \( K_s \) and \( f_b \) are estimated by minimizing the following objective function by the Adaptive Random Search method (see, e.g., [11]);

\[
\sum_{k} \sum_{t_i} \left[ \phi_k(t_i) - \Psi_k(t_i) \right]^2, \quad (k = s,b)
\]  (11)
where \( \Phi_i(t_i) \) is the value of \( C_s \) or \( C_b \) at time \( t_i \), predicted by simultaneously solving Equations 2 and 3 by the second-order improved Euler method [12], and \( \Psi_i(t_i) \) is the corresponding experimental data.

**RESULTS AND DISCUSSION**

In Figure 3, the measured concentrations of ethylbenzoate are compared with the values predicted by Equation 8. The area for mass transfer through the stopper of the reactors, each with two tubes, was twice that of the reactors, each with a single tube. The mass transfer coefficient, \( k_m' \), of the former has been determined to be 0.0102 hr\(^{-1}\), which is exactly twice that of the latter, i.e., 0.0051 hr\(^{-1}\). Since \( k_m' = k_m A/V \), this verifies the assumption of first-order mass transfer. During the experiment, bacteria in the second-set of reactors were undetectable, thereby indicating that sterilized environments were achieved throughout the experiment and that the loss of ethylbenzoate was solely due to volatilization. The value of \( k_d \) has been estimated to be \( 3.06 \times 10^{-2} \) hr\(^{-1}\) from the data obtained with the first set of reactors in which microorganisms survived on nutrients without the carbon source (ethylbenzoate); see Figure 4.

The values of other parameters estimated are as follows: the yield factor, \( Y_s \), 1.56 g cell/g substrate; the maximum specific growth rate, \( \mu_m \), 0.49 hr\(^{-1}\); the saturation constant, \( K_s \), 0.062 g/L; and the effectiveness factor of biomass, \( f_b \), 0.34. Figure 5 compares the experimentally measured data from the third set of reactors with those predicted by Equations 2 and 3 under the assumption that a cell had a mass of \( 1 \times 10^{-12} \) g [8, 13].

In aerobic environments, the availability of oxygen affects appreciably the rate of biodegradation [14]. The maximum oxygen demand can be calculated from the following stoichiometric formula.

\[
C_9H_{10}O + 10.5O_2 \rightarrow 9CO_2 + 5H_2O
\]  

(12)

This indicates that 0.205 g of ethylbenzoate in each reactor requires 0.015 mol of \( O_2 \), which is equivalent to 1.11 L of air. The air chamber in a reactor is 1.75 L; furthermore, oxygen transfer is allowed through the tube with a relatively small opening. Thus, the shallow bed reactors are capable of maintaining satisfactory aerobic environments.
Figure 5 shows that the oxygen concentration in the air chamber was stable throughout each experiment.

**Effect of initial biomass concentration**

For bioremediation to be effective, participating microorganisms must be genetically robust. The rate of biodegradation depends directly on the concentration of biomass. A sharp increase in microorganism population was observed at a certain moment in each experiment. This phenomenon is demonstrated in Figure 6 where the process is numerically simulated by Equations 2 and 3 with different initial biomass concentrations. Note that microorganisms propagate very rapidly as long as the contaminant is available as the food source and nutrients are sufficient.

Nevertheless, a sufficiently high concentration of biomass does not necessarily imply that it is distributed homogeneously; in fact, subsurface soils often exhibit heterogeneities. Unevenly distributed microorganisms can easily grow into a biofilm as illustrated in Figure 7. In this scenario, the bioavailability of the contaminant is low, and the rate of biodegradation is limited by the rate of mass transfer. Thus, adequate mixing is vital in bioremediation.

**Dissolution and biodegradation**

The water-solubility of an aromatic hydrocarbon is usually low. Suppose that 1% of the liquid volume is occupied by the non-aqueous phase, i.e., pure ethylbenzoate. As such, the total concentration is 11.3 g/L, of which 7 percent is in the aqueous solution, while 93 percent is present as a non-aqueous phase.

It is well known that microorganisms can only survive in environments with low concentrations of some xenobiotic chemicals. An aqueous environment with high concentrations of these chemicals may inhibit or even prevent biotransformation. If the volume fraction of a non-aqueous phase is
small, biotransformation of readily biodegradable compounds at the liquid interface or within the non-aqueous phase is insignificant compared to that in the aqueous phase. The rates of microbial assimilation for two different biomass concentrations under the condition that biotransformation in the aqueous phase prevails are presented in terms of the aqueous concentration of ethylbenzoate in Figure 8. This figure also plots the rates of dissolution for two different blob sizes simulated under the assumption that the non-aqueous phase comprises discrete sphere blobs of the same sizes. The dissolution rate is obtained from Equation 7 in which $k_n$ is calculated by the following two equations [7, 15].

\[
\frac{k_n(2r)}{D_{ab}} = 2
\]  
\[
D_{ab} = 7.4 \times 10^{-8} \left(\frac{\psi_B M_B}{\mu_B \nu_A}\right)^{1/2} \tau
\]  

The estimated values of the mass transfer coefficient, $k_n$, are $1.83 \times 10^{-4}$ cm/s for $r = 0.5$ mm and $0.915 \times 10^{-4}$ cm/s for $r = 1$ mm. It can be discerned from Figure 8 that when stable biodegradation is established, the rate of biodegradation is always determined by the rate of dissolution. The dissolution rate depends on the mass transfer coefficient and liquid interfacial area, which, in soils, are determined by factors such as hydrodynamic shear stress and pore structure. Surface tension and adhesive force may cause the non-aqueous phase to emulsify with solid particles and water drops to form large ganglia which can substantially reduce the mass transfer area.

Volatilization and biodegradation

The rate of volatilization is linearly proportional to the mass transfer area and vapor pressure of a contaminant. Figure 9 plots the rates of measured volatilization and biodegradation of ethylbenzoate. Note that volatilization is much slower than biodegradation because the area available for mass exchange between the reactors and an outside environment was highly constrained. The experiments mimic a scenario in soils with air channels: the contaminant vapor must disperse through tortuous pore paths to reach these channels. In remediation technologies involving volatilization, channeling and bypassing in the soil will dramatically reduce air-contaminant contacting area, thereby lowering their efficiency.
The vapor pressure of an organic chemical is sensitive to temperature. The Antoine equation correlates the vapor pressure with temperature as follows [16]:

\[ \log P = A - \frac{B}{C + T} \]  

(15)

where A, B and C are constants. The vapor pressure approximately increases exponentially with temperature. Thus, the higher the temperature, the higher the rate of volatilization.

**CONCLUDING REMARKS**

Experiments were performed in shallow-bed aerobic reactors in which homogeneous environments with constant concentration of oxygen are maintained for microbial growth. The rates and extent of volatilization and biodegradation were measured in the experiments. The kinetic parameters in a model for simulating attenuation of ethylbenzoate in a mixed culture have been estimated from the resultant experimental data. Parameters obtained include the mass transfer coefficient of volatilization, maximum specific growth rate, cell decay constant, yield factor, saturation constant, and the effectiveness factor of microorganisms involved in biotransformation.

The results of experiments and subsequent numerical simulation indicate that when microorganisms become acclimated to the environment, they propagate very rapidly as long as food sources are sufficient. When a non-aqueous phase exists and the biodegradation in the aqueous phase prevails, the rate of contaminant attenuation is determined by the rate of dissolution of the non-aqueous phase. Finally, volatilization can be significant if air-liquid contacting is allowed to maximally increase the mass transfer area; furthermore, elevated temperature will enhance the rate of volatilization.

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NOMENCLATURE

\( A \) area for volatilization, \( L^2 \)

\( C_b \) biomass concentration involved in the contaminant degradation, \( M/L^3 \)

\( C_{bi} \) biomass concentration not involved in the contaminant degradation, \( M/L^3 \)

\( C_{b0} \) initial biomass concentration, \( M/L^3 \)

\( C_{bT} \) total biomass concentration, \( M/L^3 \)

\( C_s \) contaminant concentration, \( M/L^3 \)

\( C_{sat} \) contaminant solubility in water, \( M/L^3 \)

\( C_{s0} \) initial contaminant concentration, \( M/L^3 \)

\( D_{AB} \) diffusion coefficient, \( L^2/t \)

\( f_b \) effectiveness factor of the biomass concentration

\( j_{A0} \) volatilization flux, \( M/(L^2t) \)

\( j_n \) dissolution flux, \( M/(L^3t) \)

\( k_d \) decay constant, \( 1/t \)

\( k_m \) mass transfer coefficient, \( L/t \)

\( k_m' \) mass transfer coefficient (= \( k_mA/V \)), \( 1/t \)

\( K_s \) saturation constant, \( M/L^3 \)

\( M_B \) molecular weight

\( P \) vapor pressure, \( M/(Lt^2) \)

\( r \) radius of the non-aqueous phase liquid blob, \( L \)

\( t \) time, \( t \)

\( T \) temperature, \( K \)

\( V \) volume of the non-aqueous phase liquid blob, \( L^3 \)

\( V \) volume of liquid in a reactor, \( L^3 \)

\( V_A \) specific volume of a compound, \( L^3/M \)

\( Y_s \) yield factor

\( \gamma \) ratio of the aqueous contacting area to the surface area of the non-aqueous phase liquid blob

\( \lambda \) number distribution of non-aqueous phase liquid blobs, \( 1/L^3 \)

\( \mu_B \) viscosity, \( M/(Lt) \)

\( \mu_m \) maximum specific growth rate of biomass, \( 1/t \)

\( \sigma \) surface area-to-volume ratio of the NAPL blob, \( 1/L \)

\( \Phi \) predicted value of a variable

\( \Psi \) measured value of a variable

\( \psi_B \) constant

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