A Procedure to Calculate Biodegradation during Preferential Flow through Heterogeneous Soil Columns

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ABSTRACT

Pesticides are found in groundwater sooner than commonly is predicted with traditional management models. These models do not account for preferential flow and assume uniform biodegradation. For more accurate modeling, the interaction of preferential flow and nonuniform biodegradation needs to be addressed. In this paper, we tested techniques to measure the rate of biodegradation in preferential flow paths by continuously applying p-nitrophenol (PNP) to the surface of packed soil columns that contained two types of preferential flow paths. The first type was an open macropore in a uniform soil matrix (macropore column), and the second type was a channel of coarser soil in a finer soil matrix (channel column). Breakthrough curves were obtained from separate small areas of the macropore column with fiberglass wicks to collect and separate macropore flow from soil matrix flow. Breakthrough curves were also obtained from the channel columns for comparison to curves from homogeneous columns. Average biodegradation rates were calculated from the breakthrough curves. The PNP biodegradation occurred in both matrix and macropore flow regions of the macropore column, but the time and rate of biodegradation differed in the two flow regions, as well as between the channel columns and the homogeneous columns. The PNP was degraded rapidly despite short travel times through the path of preferential flow, indicating that rapid biodegradation may occur in macropores and other preferential flow paths. The data provide a basis for improved modeling of fate and transport of chemicals through vadose soil to groundwater.

Preferential flow is the movement of water in preferred pathways such as channels of higher conductivity material or macropores formed by cracks, earthworm burrows, or root channels. Preferential flow has been studied in detail (Kanchanasuit et al., 1978; Beven and Germann, 1982; Singh and Kanwar, 1991). Recent field studies have shown that preferential flow in the unsaturated zone is widespread (Richard and Steenhuis, 1988; Czupar and Kanwar, 1991) and that it greatly reduces the time for transport of contaminants to groundwater (Steenhuis and Parlane, 1991). Although only 1 to 2% of flow is carried by macropores, this preferential flow is significant if a contaminant is toxic at low concentrations (Steenhuis and Parlane, 1991).

In several field experiments, breakthrough of solutes to groundwater was observed earlier than predicted (Pivetz and Steenhuis, 1989; Shalit et al., 1992). In these experiments and in subsequent investigations, the observed concentrations of pesticides unexpectedly decreased rapidly after the initial breakthrough or did not increase as expected, indicating that chemical loss occurred at depth in the unsaturated zone. Large populations of microorganisms have been found below the rootzone (Webster et al., 1985), suggesting the possibility of biodegradation at depth.

Studies of biodegradation are usually conducted in batch systems, and it is often assumed that biodegradation occurs uniformly throughout the soil (Federle and Pastwa, 1988; Klecka et al., 1990; Madsen, 1991). The type of kinetics and the rates of biodegradation depend on substrate concentration and initial microbial population (Simkins and Alexander, 1984), and since microbial processes are also affected by the availability of electron acceptors and inorganic nutrients—all of which vary spatially in a soil—the presence of a macropore can be of considerable importance in affecting the fate of toxicants. Thus, if preferential flow occurs, biodegradation should be evaluated with soils containing these flow paths.

Therefore, a study was conducted to quantify the rate of biodegradation in soil containing macropores and to determine the relationship between biodegradation and preferential flow in the macropores. Since batch studies are not as appropriate as column flow experiments to represent in situ field conditions (Bazin et al., 1976), techniques were developed to study biodegradation in different flow paths. The PNP was used as the test compound because it is rapidly biodegraded, only slightly adsorbed, and easily analyzed.

MATERIALS AND METHODS

Experimental Procedure

Studies of solute transport and biodegradation were conducted by dripping an aqueous solution of PNP at a constant application rate onto the surface of unsaturated soil columns containing preferential flow paths and measuring PNP breakthrough. Three types of packed columns were used in two sets of experiments. The first set of experiments used one column that contained a macropore, which will be referred to as a macropore column, and the second set of experiments used two types of columns, homogeneous columns and columns that contained a vertical channel of higher conductivity soil within a lower conductivity soil matrix, which will be called channel columns. The change in biodegradation rates during the course of each experiment and the difference in biodegradation rates between the soil matrix and macropore in the macropore column, or between a channel column and a homogeneous column, were investigated at several water velocities and proportions of matrix and macropore flow. Small columns were used as a simple model to understand the effect of the physical structure and variable water flux on rate of biodegradation. The experiments were conducted to test the experimental techniques and to determine if biodegradation in macropores warranted further study. A variety of materials to construct the columns and a range of experimental conditions were used since the intent was to observe if preferential flow had the same impact.

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Abbreviations: PNP, p-nitrophenol; BTC, breakthrough curves; CDE, convection-dispersion equation; D, dispersion; V, velocity; R, retardation.
under widely varying conditions, rather than to make quantitative conclusions.

A salt solution of low ionic strength was used to flush the columns to minimize formation of a colored effluent that would interfere with the analysis of PNP. The macropore column was flushed with a 10 mM CaSO₄ solution, and the channel and homogeneous columns were flushed with a 0.7 mM CaCl₂, 0.1 mM MgCl₂, and 1.5 mM NaCl solution. The columns were flushed with the salt solution at the same application rate to be used during PNP application for at least 2 d or until the effluent was colorless. Effluent was collected for use as a blank for spectrophotometric analysis.

The salt solution was then amended with PNP at 10 mg L⁻¹ and applied continuously to the surface of the soil columns during the biodegradation experiments at the application rates specified below for each experiment, and the loss due to biodegradation was measured in the column effluent. The PNP in the effluent was determined by measurement of the absorbance at 400 nm with a Hitachi (San Jose, CA) U-2000 spectrophotometer. Samples of the effluent were first adjusted to pH = 10 to intensify the yellow color of the PNP.

**Macropore Column**

The macropore column (5 cm diameter, 5 cm long) was constructed with Teel silt loam (coarse-silty, mixed, mesic Fluvaquentic Eutrochrept) collected in Freeville, NY, and had a vertical 0.7-cm-diameter artificial macropore. The macropore consisted of an open cylindrical channel through a homogeneous soil matrix of the <1.0-mm fraction (pₚ = 1.18 g cm⁻³). The macropore was constructed by packing the soil around a vertical tube, wetting the column from below, and removing the tube.

To investigate biodegradation in two distinct regions of the soil, the macropore and matrix regions of the column were sampled individually. Separate fiberglass wicks (50 cm long, 0.6 cm diameter) were used to collect effluent samples by suction from a small region of the soil matrix around the macropore (macropore region), centered on and including the actual macropore, and from the remaining soil matrix area (matrix region). A diagram of the column and wick sampling system is given in Fig. 1. The wicks were placed in contact with the bottom of the soil and allowed to hang free. Effluent was drawn into the wick by suction and then collected as it dripped from the wick. Fiberglass wicks have been used to collect water from unsaturated soils (Boll et al., 1990). The wick ensured that unsaturated conditions were maintained at the bottom of the soil column.

To compare effluents from the macropore and matrix regions, two experiments were conducted sequentially in the macropore column, the variables being Darcy velocity (also called specific flux), microbial acclimation, and temperature. The intent was to see if preferential flow had an impact under any given set of conditions, not to determine the effect of each experimental condition. The PNP biodegradation was first studied at a high PNP application rate (10 mg L⁻¹ at 1.49 cm h⁻¹ for the entire column). Apparent Darcy velocities for each region were estimated by dividing the average discharge from each wick by the wick sampler area. However, these apparent Darcy velocities can only be rough approximations of the actual flow rates through the two regions of the column, since there could have been mixing between the two flow paths, although the calculations assumed that this did not occur. The first, high, application rate resulted in apparent Darcy velocities of 2.52 cm h⁻¹ for the macropore region and 1.26 cm h⁻¹ for the matrix region with the higher apparent Darcy velocity for the macropore due to preferential flow. After a 10-d interval, the column was used at a low PNP application rate (10 mg L⁻¹ at 0.67 cm h⁻¹ for the entire column), resulting in apparent Darcy velocities of 0.50 cm h⁻¹ for the macropore region and 0.69 cm h⁻¹ for the matrix region, with the lower Darcy velocity in the lower macropore figure due to the absence of preferential flow near the soil matrix–macropore interface. The second experiment was done to assess the effect of microbial acclimation for PNP biodegradation. The temperature was 25°C during the first experiment and 21°C during the second. The breakthrough of Br⁻ was determined in the macropore column for each experiment.

Tests of Br⁻ and PNP breakthrough conducted with the wicks indicated that the PNP was not adsorbed by or biodegraded in the wick material, even after the wick was removed from a column with an active PNP biodegrading population. However, the BTC data for soil columns were corrected for travel times through the wicks. The BTC data from tests on the wicks were fit to the CDE with the curve-fitting program CXTFIT (Parker and van Gemuchten, 1984). The average travel times in the wicks were subtracted from the BTC data for the soil columns.

After adjustment for travel time through the wick, the BTC were used in combination with the CDE to characterize the differences between the macropore and matrix regions of the soil. The assumption that flow paths did not intermix meant that the CDE could be applied to each region separately, although with different parameters.

After the biodegradation experiment was completed, 0.1 M Br⁻ was added to the salt solution, and breakthrough of Br⁻ was measured in the column effluent. Bromide, which was used as a conservative tracer, was determined with an ion analyzer (Orion Research, Inc., Boston, MA) fitted with a Br⁻-specific electrode.

**Channel and Homogeneous Columns**

The channel columns (4.1 cm diameter, 15 cm long) were constructed with dried sieved Lima loam (fine-loamy, mixed, mesic Glossoboric Hapludalf) collected in Freeville, NY. The columns contained a 0.5-cm-diameter vertical channel composed of the 1.7- to 2.0-mm fraction of Lima loam embedded within a matrix consisting of either the 0.6- to 1.0-mm fraction or the <0.6-mm fraction. The channel was constructed by packing the finer soil around a vertical hollow tube, wetting the column from below, and removing the tube while pouring.
the coarser soil through it. Effluent from each entire column was collected as it dripped through a coarse ceramic porous plate at the bottom of the column. Homogeneous columns of the same size were also constructed with the 0.6- to 1.0-mm fraction or the <0.6-mm fraction, and effluent collected from each of these was compared with effluent collected from the appropriate matching channel column to assess the impact of the channel.

Four experiments were conducted with pairs of channel and homogeneous columns, the variables being particle size and application rate and (resulting Darcy velocity). Darcy velocities of 1.2, 2.1, and 3.4 cm h⁻¹ were used with columns of the 0.6- to 1.0-mm soil fraction (ρₚ = 0.87 g cm⁻³). A Darcy velocity of 2.0 cm h⁻¹ was used with a column of the <0.6-mm fraction (ρₚ = 1.09 g cm⁻³).

**Theoretical Development**

In a homogeneous soil, water and solute applied at the surface are transported downwards according to the CDE while the microbial populations degrade the substrate and increase in number. Biodegradation in soil can be studied by infiltrating water and solute and plotting BTC showing the outflow concentration with time. The D, biodegradation, and V or R of a solute can be quantified by fitting a BTC to a solution of the CDE. Analytical solutions of the CDE are only available for first and zero order biodegradation rates with or without dispersion and/or homogeneous soils. Another difficulty in using BTC is distinguishing the effects of D and biodegradation, especially if macropores are present. Our technique does not assume homogeneity or any type of biodegradation rates. Rather, we use the CDE for its ability to describe the transport process and determine the biodegradation rates. The extent of biodegradation ΔC at any time without the confounding effects of D and sorption can be approximated by subtracting the concentration of an experimental nonsterile PNP BTC (Cₑxperimental) from the concentration of a PNP BTC of a sterile soil (Cₘicrobial) in which no biodegradation took place. Because such a sterile curve is not always easily obtained, it can be approximated by a theoretically calculated BTC (Cₘicrobial), that is based on the CDE without degradation and with appropriate values for the V, D coefficient, and R. Thus, ΔC is found by

\[ ΔC = Cₘicrobial - Cₑxperimental \]

Throughout the text and figures, the shorthand C will be used to indicate C/C₀, where C₀ is the input concentration.

To find the biodegradation rate for the channel and homogeneous columns and for the macropore column, two slightly different procedures are used to find the Cₘicrobial because of differences in experimental technique. In both cases, it is assumed that when an elemental volume of PNP solution travels with a V, an average biodegradation rate (averaged across the time that the elemental volume is in the column) is a useful characterization of the biodegradation process.

**Channel and Homogeneous Columns**

To quantify biodegradation for the channel and homogeneous columns, flow was collected and the PNP concentration measured. Determination of the theoretical breakthrough involved two steps: First, D and V for PNP were fitted with CXTFIT (Parker and van Genuchten, 1984) using the first portion (up to 75% of the maximum observed concentration) of the nonsterile PNP curve. Then, the Cₘicrobial was calculated with the fitted values of V and D in the CDE. Biodegradation, ΔC, was found with Eq. [1].

**Macropore Column**

For the macropore column, biodegradation in a macropore and in the soil matrix was determined by approximating the macropore column as two (homogeneous) flow-through systems, one representing the macropore flow path and the other, the soil matrix flow path. In this case, because of some uncertainty of the nature of the flow region, the Br⁻ BTC was used to fit the values (with CXTFIT) of V and D with R = 1. The moisture contents, θ, were calculated for the soil matrix region and the macropore region by dividing the region's outflow flux, as captured by the wicks in the region by the V. Then, for the Teel silt loam with an organic matter content of 4.5% and estimated adsorption partition coefficient, kₐ, value of 1.5 cm³ g⁻¹, a retardation value was obtained for each region depending on the θ value. The kₐ value was obtained with the method of Rao and Davidson (1980) with an octanol water partition coefficient of 81.3 (U.S. Environmental Protection Agency, 1983). The fitted values of V and D and the calculated R value were then used to generate the PNP Cₘicrobial for each flow region. Biodegradation was again calculated with Eq. [1] for the macropore and soil matrix flow paths.

To demonstrate and test the procedure of determining the amount of biodegradation, we performed an experiment with a 10-cm-long homogeneous column similar to the homogeneous columns described previously. In this experiment, we determined BTC for Br⁻, PNP with biodegradation (nonsterile PNP column) and PNP without biodegradation (sterile PNP column). The Darcy flux for this experiment was 1.5 cm h⁻¹. The sterile PNP BTC was obtained by using a solution of PNP with 0.05 g L⁻¹ NaN₃ added as a germicide.

With data from the nonsterile PNP column, a PNP velocity of 1.4 cm h⁻¹ and a D coefficient of 0.22 cm h⁻¹ were found to be a good fit. Figure 2 shows that the Cₘicrobial so-obtained (dashed line) was comparable with the Cₘicrobial measured in the sterile PNP column (pluses). Slight discrepancies between the PNP Cₘicrobial and Cₘicrobial were caused by small nonuniformities in packing and minor differences in the flow rates in the two columns. In Figure 3, the biodegradation was calculated by subtraction of the experimental PNP BTC (Cₑxperimental) from both the theoretical BTC (Cₘicrobial) and the sterile BTC (Cₘicrobial). Although, at early times, there were small differences in biodegradation, it was identical for either calculation method after 10 h.

![Fig. 2. Comparison of p-nitrophenol (PNP), breakthrough curve (BTC) from sterile soil with the predicted curve developed with the nonsterile PNP BTC data. Predicted and observed Br⁻ BTC are also shown.](image-url)
Fig. 3. Comparison of \( p \)-nitrophenol biodegradation with the sterile and the calculated theoretical breakthrough curves.

To establish that the procedure to find \( C_{\text{theoretical}} \) also has validity for the macropore column, it is necessary to show that theoretical PNP and Br\(^-\) breakthrough are identical except for the R factor. Dispersion coefficients are independent of the R coefficient. Because we already fitted the nonsterile PNP curve and compared it with the sterile PNP concentrations, we will show that we can obtain the Br\(^-\) curve with an R value that falls within the range of published values in the literature. With the values for PNP of \( D = 0.22 \, \text{cm}^2 \, \text{h}^{-1} \) and \( V = 1.4 \, \text{cm} \, \text{h}^{-1} \), it was found with CXTFIT that the R coefficient was 0.4 \( \text{cm}^2 \, \text{g}^{-1} \) for Br\(^-\) (or 2.5 \( \text{cm}^2 \, \text{g}^{-1} \) for PNP). The moisture content (\( \theta = 0.43 \)) in the column was obtained by dividing the water flux (1.5 cm h\(^{-1}\)) by the Br\(^-\) velocity (3.5 cm h\(^{-1}\)). Figure 2 shows that the Br\(^-\) curve fitted the data points well. Based on this data and a bulk density of 1.2 \( \text{g cm}^{-3} \), the adsorption partition coefficient, \( k_a \), for PNP in the Lima soil was 0.53. This is equivalent to an organic matter content of 15 g kg\(^{-1}\) based on the method proposed by Rao and Davidson (1980).

Biodegradation rates were calculated by dividing \( \Delta C \) by the PNP travel time through the column (\( \Delta T \)). The biodegradation rate, \( \Delta C/\Delta T \), is an effluent biodegradation rate that described the biodegradation that occurred in one particular elemental volume of PNP solution (the one which, at time \( t \), is found at the end of the flow path) while it moved through the soil column. It was more useful to know the average column biodegradation rate occurring across the entire length of the column at an instant in time since biodegradation was occurring at other rates in many other elemental volumes of solution, each at different depths in the flow path. The average biodegradation rate for all depths throughout the flow path for the instant in time \( t \) was obtained by averaging all values of \( \Delta C/\Delta T \) across \( \Delta T \).

Relative biodegradation or dimensionless biodegradation for each flow path was calculated by dividing the biodegradation rate by the maximum possible rate of biodegradation in that flow path, where the maximum (possible) biodegradation rate is defined to be the expected outflow concentration from a sterile column divided by the travel time \( \Delta T \), i.e., \( C_{\text{max}}/\Delta T \). Thus, the dimensionless biodegradation is given by \( (\Delta C/\Delta T)/C_{\text{max}}/\Delta T \), or simplified, \( \Delta C/C_{\text{max}} \). This allows comparison of the relative biodegradation rates at different flow rates, by removing the effect of flow rate.

Fig. 4. \( p \)-nitrophenol (PNP) breakthrough curves in the macropore region (squares) and in the matrix region (triangles). Open symbols indicate nonacclimated soil and 25°C. Closed symbols indicate acclimated soil and 21°C.

RESULTS AND DISCUSSION

Macropore Column

The same macropore column was used for two experiments, first at a high flow rate and then at a low flow rate, so that during the second experiment, the microorganisms were acclimated for PNP metabolism. Comparisons may be made between the macropore and matrix region BTC within each experiment to establish the difference between macropore and matrix biodegradation, and it is instructive to observe the influence of variable Darcy flux, acclimation, and temperature on the BTC and biodegradation in the two experiments.

The BTC from the matrix and macropore regions of the macropore column are shown in Fig. 4, with data from the column exposed to either high or low flow. Faster initial breakthrough reflects the higher flow rate during the first experiment with this column. The BTC at the higher flow for the matrix and macropore regions (apparent Darcy velocities of 1.26 and 2.52 cm h\(^{-1}\), respectively) were similar. Breakthrough of PNP from the macropore region occurred slightly before breakthrough from the matrix, and the peak concentration of PNP was attained several hours sooner. Peak PNP concentrations were from 0.3 to 0.35 \( C/C_0 \), and the samples from the macropore region had the higher concentrations.

At the lower flow rate, macropore flow did not occur. However, flow did occur in the vicinity of the macropore, and effluent was collected from this region. The apparent Darcy velocity was slightly higher in the matrix (0.69 cm h\(^{-1}\)) than in the macropore region (0.50 cm h\(^{-1}\)). The overall application rate of 0.67 cm h\(^{-1}\) was lower than the saturated conductivity of the matrix (which was >1.26 cm h\(^{-1}\), as indicated by the velocity in the matrix at the higher flow rate); therefore, macropore flow could not be initiated, and flow was restricted to the soil matrix. The BTC were different, with a peak PNP concentration of 0.55 \( C/C_0 \) for the macropore region and 0.4 \( C/C_0 \)
for the matrix region (Fig. 4). The PNP in the effluent decreased to zero in less time in the macropore region than in the soil matrix.

The curves depicting biodegradation in the macropore column during the course of an experiment are presented in Fig. 5. The numerical value of the column biodegradation rate at any given point in time during the experiment is given by the height of the curve at that time. At the high application rate, when the apparent Darcy velocity through the macropore region was greater than in the matrix region and preferential and faster flow down the macropore walls dominated, the biodegradation rate was higher in the macropore region (Fig. 5). The biodegradation rate increased faster in the macropore region than in the matrix region. However, because of the greater flux of PNP, the macropore region required greater time until PNP was no longer present in the effluent.

The first application of PNP resulted in acclimation to PNP by the indigenous microbial populations. This is suggested by a comparison of the BTC in acclimated and nonacclimated soils (Fig. 4); i.e., the PNP concentrations decreased more rapidly in acclimated soil upon reapplication of PNP. However, this interpretation is complicated by the fact that the flow rate and temperature also varied between the two experiments. The importance of temperature is suggested by the higher peak concentrations of PNP that were reached during the second study, which was conducted at 21°C rather than at 25°C. It is possible that the initial growth rate was slower at the lower temperature, allowing an initially higher concentration of PNP to pass through the soil. The biodegradation curves indicate that flow rate and temperature are more significant than acclimation in determining the rate of loss of PNP (Fig. 5). At the high flow rate and higher temperature, the biodegradation rates are greater and increase more rapidly than at the low flow rate and lower temperature, despite the presence of acclimated microorganisms under the low flow rate and low temperature conditions.

Although the exact interaction between flow rate, temperature, and microbial acclimation cannot be determined from these experiments, it is clear that there is a difference in biodegradation rates between the macropore and matrix regions. The macropore may provide better conditions for biodegradation than is found in the matrix. As application rate increases, the soil matrix water content increases, resulting in lower oxygen content, whereas the macropore is still open for exchange of atmospheric gases. The microbial populations may be different, not only in population but in variety of microorganisms in the two regions. Microbial growth may occur along the entire length of the macropore resulting in microbial activity deeper in the soil profile. However, under some conditions, very fast flow in the macropore might have adverse effects on biodegradation because of detachment of attached microbial cells and because the cells may be unable to utilize all the substrate that rapidly moves past. The walls of the macropore may also allow growth of only a thin film of microorganisms instead of a thicker film that might result in the soil matrix. These hypothetical adverse effects probably did not occur in these ex-
Fig. 7. Biodegradation in homogeneous columns and channel columns. Numbers indicate Darcy velocities. (A) Biodegradation rates with matrix soil of 0.6- to 1.0-mm fraction (coarse matrix). (B) Biodegradation rates for matrix soil of <0.6-mm fraction (fine matrix). (C) Nondimensional biodegradation rates for matrix soil of 0.6- to 1.0-mm fraction (coarse matrix). (D) Nondimensional biodegradation rates for matrix soil of <0.6-mm fraction (fine matrix).

experiments, and are mentioned only for purposes of discussion.

Under low flow conditions, although there is not any flow in the macropore itself, biodegradation of PNP flowing in the matrix near the macropore could still be affected by the presence of the macropore. An apparent beneficial impact of the macropore would be to allow diffusion of O₂ into the surrounding soil matrix. This increased aeration may have contributed to the greater biodegradation rates and the faster increase in rates noted for the macropore region compared with the matrix at the low flow rates (Fig. 5).

Channel and Homogeneous Columns

To allow comparison of these columns, the concentration and biodegradation rates are plotted against pore volumes because of slight differences in travel time through the channel columns and associated homogeneous columns. Pore volume is the volume of water in each column, and the number of pore volumes is given by dividing the effluent volume by the pore volume in the column. The channel columns and homogeneous columns have almost the same total water volume, so that pore volumes may be used for comparative purposes since effluent is collected from the entire column. Since these columns used gravity drainage, there was a thin saturated layer at the bottom of the column; however, this did not affect the results since effluent was collected from an entire column and not from discrete regions of the column as in the macropore column.

The BTC for the columns with and without a high conductivity channel are shown in Fig. 6. The concentration of PNP in each instance increased during the course of the experiment, reached a peak value, and then, as biodegradation occurred, started to decline. Figure 6A shows that as Darcy velocity increased, a greater number of pore volumes was required before the effluent was free of PNP and the difference in BTC between the channel columns and the homogeneous columns increased. It is important to examine this difference in behavior in terms of pore volumes since they control the amount of contaminant that enters groundwater. Comparison of the BTC for a Darcy velocity of 2.1 cm h⁻¹ (Fig. 6A) to the BTC for an approximately equal Darcy velocity of 2.0 cm h⁻¹ (Fig. 6B) indicates that the column with the coarser matrix (Fig. 6A) requires significantly
fewer pore volumes than the column with a finer matrix (Fig. 6B) for biodegradation to begin and to be completed.

The curves depicting biodegradation are presented in Fig. 7. These curves indicate that in each experiment and at each flow rate, biodegradation was more rapid in columns with a channel than in columns that were homogeneous, at a given pore volume of flow (except for the lowest flow rate, when there was no discernible difference). As the flow rate increased, the difference in rates of biodegradation between the channel columns and the homogeneous columns became more pronounced. This is shown most clearly in Fig. 7C, in which the dimensionless biodegradation is compared at different flow rates. The increase in flow rate appears to increase the effect of the channel on biodegradation, although further experimentation and replicates would be required before a quantitative expression could be proposed.

The data show that the channel promotes biodegradation. Despite ever more rapid flow through the channel, the rate of biodegradation is faster in the soil with channels than in homogeneous soil. This greater activity in soil with a channel may be a result of the channel providing more favorable conditions for biofilm development than the matrix and a greater supply of O₂ for microbial metabolism. The coarse material in the channel may allow a thicker mass of microorganisms to grow than does the material in the matrix. In addition, the faster flow in channels initially will transport substrate and perhaps microorganisms to the lower portions of the channel, allowing for growth to occur at deeper levels of the soil at earlier times.

In all cases, under many different experimental conditions, the biodegradation rate increased during the course of an experiment. Thus, any one constant value for biodegradation rate will result in errors if it is used to predict the amount of chemical metabolized in a period of time. The biodegradation rate depends on the delivery of substrate to the microorganisms, substrate concentration, temperature, and the microbial population, which are determined by the Darcy velocity of substrate, the length of the application period, and the conditions under which biodegradation occurs. The importance of the last factor is shown by the difference in rates between the macropore and matrix flow paths; thus, the impact of preferential flow paths on biodegradation warrants further investigation. The preferential flow path, whether a macropore or a coarsely-filled channel, increased the rate of biodegradation across a homogeneous matrix, despite different soil fractions used, different soil bulk densities, different temperatures, acclimation, or different acclimation rates, suggesting that merely the presence of a preferential flow path can increase biodegradation rates. In addition, this study investigated only the physical aspects of macropore preferential flow (the presence of a large open pore through the soil or smaller pores in a yet finer matrix). Under natural conditions, these openings would be modified by biological processes so that biodegradation would probably be even more enhanced.

The difference in breakthrough and biodegradation between macropore and matrix flow paths in two cases under quite different conditions indicates that it is necessary to investigate each of these regions to better understand the impact of preferential flow. This investigation was carried out by the comparison of channel and homogeneous columns or by a new procedure, using fiberglass wicks to individually sample the matrix and macropore regions of a macropore column simultaneously and comparing the BTC from the two regions.

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