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High-Rate Biodegradation of Pentachlorophenol by Biofilm Developed in the Immobilized Soil Bioreactor

DIMITRE G. KARAMANEV* AND RÉJEAN SAMSON

NSERC Industrial Chair on Site Bioremediation, BIOPRO Research Center, Department of Chemical Engineering, Ecole Polytechnique de Montreal, P.O. Box 6079, Station Centre-ville, Montreal, Canada H3C 3A7

A novel type of bioreactor, the immobilized soil biofilm reactor, was used for the biodegradation of pentachlorophenol (PCP) in aqueous solutions. An extremely high volumetric PCP degradation rate was obtained—up to 950 mg of PCP L^{-1} h^{-1}. The study of the biofilm growth kinetics showed that biofilm mass reached 4 g dry weight/L of reactor volume, which is much higher than values generally reported in the literature. The corresponding yield coefficient was found to be 0.054 g of biomass/g of PCP. The biofilm thickness during the PCP degradation constant rate period was below 100 μm. A mathematical model based on a diffusion−reaction mechanism in a cylindrical shell biofilm showed that under these conditions the process is kinetically controlled. Substrate utilization kinetic data showed that the process can be described by a Monod-type model with $\mu_m = 0.016$ h^{-1} and $K_s = 5.74$ mg of PCP/L. It was shown that essentially all PCP is degraded within the biofilm, with negligible liquid-phase biodegradation. The effect of different physicochemical parameters of the liquid phase on PCP biodegradation rate was also studied. The optimal temperature range was between 20 and 35 °C, while a 60% rate decrease was observed at 15 °C. The process was inhibited at pH values above 7.7, while the main water-soluble reaction product (chloride ions) affected negatively the process only at concentrations higher than 15.8 g/L. These results show that aerobic biodegradation of PCP is much less affected by variations in the different physicochemical factors when carried out in a biofilm as compared to a free suspended culture. These considerations become the bases for modeling and designing of immobilized soil bioreactors for PCP degradation in aqueous phase (such as groundwater and process water of soil washing).

Introduction

Among the biggest challenges in biological treatment of PCP in aqueous phase (e.g., groundwater and process water after soil washing) is finding microorganisms that can efficiently degrade the target pollutant and developing bioprocess systems that can provide conditions for peak performance of these microorganisms. Unfortunately, PCP biodegradation rates reported in the literature are quite low—the time required for degradation of aqueous solutions containing up to 200 mg/L PCP is of the order of days or hours (*1*). Efficiency of PCP biodegradation can be improved significantly in two ways: development of an active microbial consortium and bioreactor design.

The use of pure microbial cultures is also problematic because of their low adaptability to natural conditions (*2*) and the requirement for continuous inoculation of the system. To overcome this limitation, it has been proposed to use soil particles collected from a polluted site containing the target pollutant. In many cases these particles show intrinsic biodegradation activity due to the natural selection of appropriate microorganisms (*3*). It has already been shown that a soil sample, once polluted with a certain contaminant, begins degrading it after an acclimation period which can be as short as 1 h or as long as many months, depending of both the pollutant and the nature of the soil (*4*). However, the microflora associated with the soil usually shows a very low activity due to the lack of nutrients, oxygen, and humidity under natural conditions. It was shown that this microflora could be activated by putting the soil particles into a bioreactor with a liquid phase containing nutrients, oxygen, and a carbon source (additional pollutant and/or cosubstrate) (*3*). Among the most important requirements for soil activation are maintaining low hydrodynamic shear rates around particles (this provides appropriate conditions for biofilm growth on the soil particle surface) and retaining smaller soil particles from leaving the reactor with the liquid. The above requirements can be met if the soil particles are fixed into the pores of an inert immobile porous support. The resulting structure was named "immobilized soil" (*5*). The concept of soil immobilization was carried out in the immobilized soil bioreactor (ISBR).

Immobilized Soil Bioreactor. This bioreactor is based on a modification of the classic airlift reactor in which the solid separating wall between the riser and downcomer is replaced with a highly porous material, in our case, geotextile (*6*). The aeration in the riser causes a liquid flow pattern in the reactor in two directions: vertically (upward in the riser and downward in the downcomer) and horizontally through the draft tube wall from the downcomer to the riser (Figure 1). Both horizontal and vertical components of liquid velocity were a function of the reactor height (*7*). The driving force for both liquid flow directions is the hydrostatic pressure difference between the riser and the downcomer (*6*). When soil is added to the liquid in the reactor, flow of the resulting slurry through the geotextile causes an entrapment of soil particles into its pores. Since geotextile has a very wide pore size distribution, between 10 and 1000 μm, particles with a similar size distribution are getting entrapped into these pores. Since the structure of soil particles entrapped into a geotextile is analogous to that of enzymes or microbial cells immobilized on an inert solid support, we named this structure immobilized soil. Since soil particles are immobilized, liquid flows around them in a laminar fashion (with minimal mechanical friction) and supplies the soil microorganisms with a carbon source (usually the target contaminant), nutrient salts, and oxygen. Therefore, hydrodynamic conditions in the reactor are favorable for the selective development of a microbial consortium capable of degrading the target pollutant. This consortium then grows and develops as a biofilm, initially on the surface of the soil particles and eventually on the surface of the geotextile fibers.

* Corresponding author present address: Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario, Canada N6A 5B9. Telephone: (519) 679 2111 (ext. 8230); fax: (519) 661 3498; e-mail: dkaramanev@eng-ntadmin.engga.uwo.ca.

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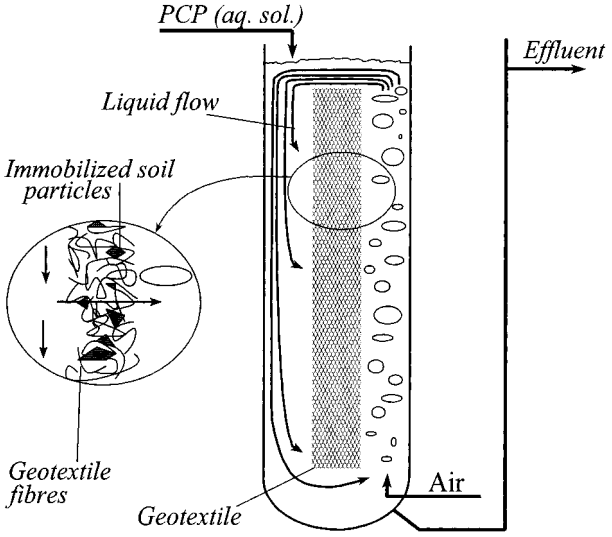

FIGURE 1. Immobilized soil bioreactor (ISBR).

The main aim of this work was to study the kinetic aspects of PCP biodegradation within a biofilm of mixed microbial culture in the immobilized soil bioreactor.

Experimental Section

Soil. The soil used in this work was obtained from a site polluted with PCP during the decade-long operation of a wood-treatment plant. It was a mixture of clawed sand and uniform sand from a site close to the condenser tanks. A full description of the soil was presented by Otte et al. (*3*). Three grams of soil was used for immobilization in the bioreactor.

Immobilized Soil Bioreactor. The reactor was a split-cylinder airlift column with an internal diameter of 5 cm, a total height of 34 cm, and a working volume of 500 mL. The soil immobilization medium was a rectangular piece (5 × 24 cm) of nonwoven polyethylene textile (Matador Co., Montreal, Canada), with a thickness of 30 mm and a porosity of 0.995. The air was distributed in the bioreactor by a perforated stainless steel tube, and its flow rate was controlled by a rotameter at 15 L/h unless otherwise indicated. The liquid solution was fed to the reactor by a Masterflex (Cole-Parmer Co.) peristaltic pump. It contained PCP with concentrations between 10 and 74 mg/L of nutrient salts (*8*) and NaOH, which was added stoichiometrically to neutralize the HCl produced by PCP mineralization.

Analytical Procedures. The concentration of pentachlorophenol in the aqueous solution was determined by HPLC (Thermo Separation Products Ltd.) equipped with a column type ODS-1, 250 × 4.6 mm. The mobile phase was a 70% aqueous solution of methanol corrected to pH 2.0 by concentrated H_3PO_4. A spectrophotometric detector was used at 210 nm. The sample was filtered by a 0.45 μm Millex-HV filter (Millipore Co.). For more routine measurements, PCP concentration was measured by a visual-UV spectrophotometer (Sequoia-Turner 390) at a wavelength of 320 nm. The Cl^- concentration was measured also by a HPLC (Dionex Co.) using a 250 × 40 IONPAC AS4A-SC column and an aqueous bicarbonate buffer as an eluent.

Measurement of the Biofilm Mass. To measure the total biofilm inventory, the bioreactor operation was stopped, and the biofilm was mechanically detached from the geotextile support. The biofilm was very fragile, and it was easily detached by squeezing the geotextile and washing it with distilled water. This procedure was repeated three times. The quantity of biofilm detached during the third cycle was negligible. The biofilm, separated from the wash water by centrifugation at 10 000 rpm (8000*g* relative centrifugal force) was dried at 103 °C and weighed.

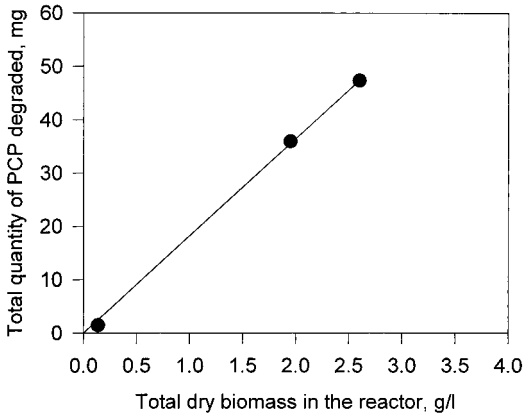

FIGURE 2. Relationship between biofilm mass in the reactor and the total quantity of PCP degraded.

Results and Discussion

It was found that the process of PCP degradation in ISBR can be divided into three separate phases: activation, constant degradation activity, and decline. The process of activation of the microbial consortium was described in details elsewhere (*5*). After the inoculation of the bioreactor with soil, it was operated in fed-batch regime. New substrate was added after 90% of PCP dissolved in liquid was degraded. The bioreaction rate increased in time, and after reaching a volumetric efficiency of 0.4 mg of PCP L^{-1} h^{-1}, the reactor was switched to a continuous operation. The entire activation period lasted between 15 and 30 days, after which time the PCP degradation activity became constant. No hysteresis of the reaction rate was observed after a temporary increase or decrease of the input parameters such as dilution rate and inlet PCP concentration. This period lasted usually about 45 days and was followed by a sharp decrease in the PCP degradation rate due to the blocking of the geotextile pores by biofilm. The results reported in this work were obtained during the period of constant degradation activity. The PCP biodegradation rate in the period of constant degradation activity varied within 10% for different reactor runs.

Biofilm Study. The quantity of biofilm was measured in a bioreactor identical to the one used for kinetic experiments but without soil. This bioreactor was inoculated with a microbial consortium from the ISBR. The biodegradation rate of PCP in this reactor was measured to be the same as that in the bioreactor with soil. Three different biofilm measurements were made (Figure 2): one at the initial period of biofilm growth and two at steady-state conditions. The total dry weight of the biofilm measured during the period of constant activity was over 2 g. This value would represent an average biomass concentration, *X*, based on a pseudo-homogeneous liquid-phase bioreactor of just over 4 g/L, which is much higher than those reported in the literature. The biomass concentration in a free suspended cells culture was reported to be between 0.0002 and 0.06 g/L (*1*, *9*) while in a polyurethane-immobilized culture it reached 0.4 g/L (*10*). The biomass weight in Figure 2 is given as a function of the total quantity of PCP degraded before the biofilm sample was taken. It can be seen that these parameters are proportional, and therefore (1) the yield coefficient is constant and (2) there is no detachment of biofilm. The biofilm detachment rate was estimated by measuring the UV light absorption of liquid leaving the reactor. No measurable difference between light absorption of the influent and effluent liquid was detected. The lack of biofilm fragments

leaving the reactor can be explained by the filtration nature of the ISBR. If a piece of biofilm detaches from a geotextile fiber, it is instantly reattached to another fiber within the geotextile according to the mechanism of soil immobilization described earlier. Therefore, mainly single cells and small aggregates are expected to leave the reactor since the time of attachment of micron-size particles is significantly larger than the retention time of liquid in the reactor (*11*). This assumption was confirmed by the experiment on liquid-phase kinetics, described below. The above results prove to be very useful for estimating the lifetime of biofilm in the reactor since both biofilm thickness and porosity of geotextile with biofilm can be estimated from the cumulative quantity of PCP degraded.

The yield coefficient of the process was calculated on the basis of the total quantity of PCP degraded in the reactor (Figure 2). The yield coefficient was found to be 0.054 g of biomass dry wt/g of PCP, determined from the slope of the curve in Figure 2. Assuming a biomass carbon content of 50% (*1, 12*), the yield coefficient can be represented as 0.107 g of biomass carbon/g of PCP carbon. This value is very close to the yield coefficient ($Y = 0.11$) determined using [^{14}C]PCP in a suspended culture of the same microorganisms (*5*). Similar values of yield coefficient for PCP biodegradation were reported in the literature (*1, 10, 13, 14*).

Since biofilm thickness is one of the most important parameters in the kinetic study of biofilm processes, it will now be considered. Unfortunately, the direct measurement of biofilm thickness was very difficult due to the very fragile nature of the biofilm, and it was estimated indirectly. According to the review of Characklis and Marshall (*15*), the density ρ_{bf} (dry mass per unit wet volume) of different biofilms varies between 0.02 and 0.13 g/cm^3. It varies not only from one microbial culture to another, but even as a function of biofilm thickness. A value of 0.05 kg/m^3 was chosen as the most representative for this system (it is the mean value of published data). At the beginning of steady-state conditions, the biomass dry weight was $m = 1.95$ g (Figure 2), and therefore the total biofilm volume in the reactor was determined as $V_{bf} = m_{bf}/\rho_{bf} = 39$ cm^3. It was observed visually that during the period of constant PCP degradation rate the shape of biofilm covering geotextile fibers remained close to cylindrical. Since geotextile fibers have a cylindrical shape, the biofilm thickness can be calculated by the following equation derived on the basis of cylindrical geometry of the support−biofilm structure:

$$\delta = \sqrt{\frac{V_{bf}\rho_{gt}r_{gt}^2}{m_{gt}} + r_{gt}^2} - r_{gt} \qquad (1)$$

Taking into account that the geotextile in the reactor weighed 2.0 g, the mean fiber radius was 25 μm, and the polyethylene density is 0.93 g/cm^3, the biofilm thickness calculated using eq 1 was found to be 84 μm.

Kinetics of PCP Biodegradation. The first set of kinetic experiments was conducted in batch regime. During continuous operation of the bioreactor, the input flow was stopped and the reactor was spiked with PCP. The results are shown in Figure 3. It can be seen that PCP degradation rates are very high. At initial PCP concentrations above 18 mg/L, the maximal biodegradation rates (determined from the slope of the respective curves) were about 950 mg L^{-1} h^{-1}. To calculate reaction rates from the data in Figure 3, the curves were smoothed by a polynomial regression. The experimental points at time = 0 were discarded in order to avoid the effect of a possible lag phase. These rates will be used later to determine the kinetic parameters of the process. The residual PCP concentrations at the end of each batch reached between 1 and 5 ppb.

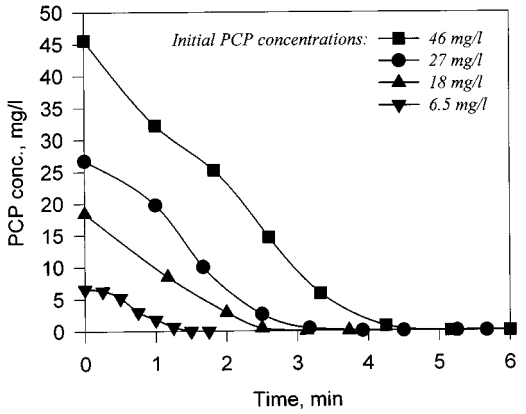

FIGURE 3. Dynamics of PCP biodegradation in batch regime.

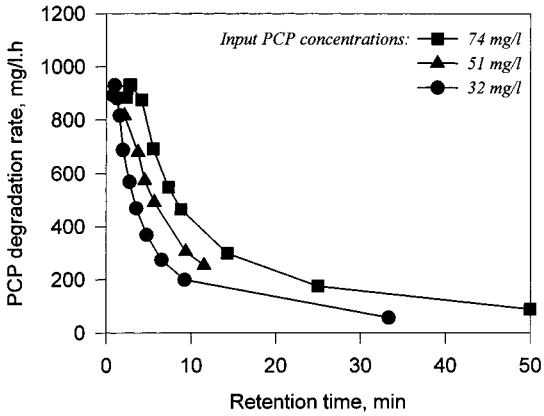

FIGURE 4. Degradation of PCP in continuous regime.

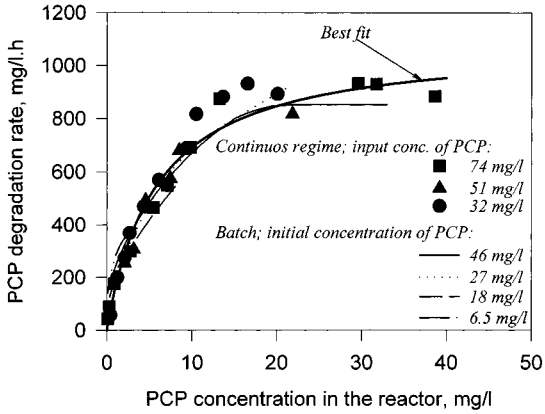

FIGURE 5. Relationship between PCP concentration in the reactor and its biodegradation rate.

The results of the kinetic experiments in continuous regime are presented in Figure 4. These experiments were conducted at the beginning of the constant degradation activity period. Three different initial concentrations were used: 74, 51, and 32 mg of PCP/L. The experiments were conducted by a stepwise increase of the liquid flow rate, followed by a stepwise decrease. It can be seen overall that PCP degradation rate was higher at higher input concentration. This result was expected since the substrate (PCP) concentration in the reactor is higher at higher input concentration, which leads to higher degradation rates. The highest degradation rates are observed at short retention times (the shortest time being 47 s), reaching 950 mg L^{-1} h^{-1}. This rate is orders of magnitude higher than any PCP degradation rate reported in the literature (*1, 10, 13, 16*). However, the highest reaction rates were obtained at low substrate conversions (around 50%). Figure 5 shows PCP

degradation rates calculated from the data in Figure 4 as a function of substrate (PCP) concentration. The reaction rates calculated from the batch experiments are also presented in this figure. It can be seen that the relationships between reaction rate and substrate concentration for both continuous and batch regimes for all initial and input concentrations used in this work can be represented by a single curve. The shape of the curve is also typical of Monod-type kinetics with no substrate inhibition.

Mathematical Model of Diffusion and Reaction in a Biofilm. The process of PCP biodegradation in a biofilm can be described by the following model. The coupled diffusion−reaction process in a biofilm covering a cylinder-shaped support can be described by the following differential equation (*17*):

$$\frac{d^2S}{dr^2} + \frac{1}{r}\frac{dS}{dr} = \frac{R_s}{D_{eff}} \tag{2}$$

with boundary conditions

$$\frac{dS}{dr} = 0 \qquad \text{at } r = r_{gt} \tag{3}$$

$$S = S_b \qquad \text{at } r = r_{gt} + \delta \tag{4}$$

These boundary conditions were based on the following assumptions: no reaction or sorption at the geotextile fiber surface (eq 3) and no external mass-transfer limitation (eq 4). Substrate sorption had no effect on the reactor operation at steady-state regime. The rate of biodegradation (R_s) in eq 2 can be described by the Monod equation:

$$R_s = \frac{\mu_m X}{Y}\frac{S}{(K_s + S)} \tag{5}$$

Since the substrate (PCP) concentration in the bioreactor is expected to be usually below 2 mg/L, the Monod equation can be transformed to a first-order kinetic model (according to Figure 5). Therefore, eq 2 becomes

$$\frac{d^2S}{dr^2} + \frac{1}{r}\frac{dS}{dr} = \frac{\mu_m X}{YD_{eff}K_s}S \tag{6}$$

This is a Bessel equation that has the following solution

$$S = AJ_0\left(r\sqrt{\frac{\mu_m X}{YD_{eff}K_s}}\right) + BY_0\left(r\sqrt{\frac{\mu_m X}{YD_{eff}K_s}}\right) \tag{7}$$

where J_0 is Bessel function of zero-order and Y_0 is Weber function of zero-order. The effectiveness factor of PCP degradation in the biofilm shows the effect of diffusion on the rate of the process. It is defined as the ratio between the actual reaction rate and the one without a diffusional resistance.

After solving the above mathematical model, it was found that the effectiveness factor at 84 μm thick biofilm is above 0.70. The effectiveness factor increased when the reaction order decreased (at PCP concentrations over 2 mg/L). On the basis of these results, it can be assumed as a first approximation that the PCP biodegradation in the ISBR is kinetically controlled. The experimental data in Figure 5 were then used to estimate the Monod kinetic constants (eq 5) by nonlinear regression. The kinetic parameters obtained were K_s = 5.74 mg/L and ($\mu_m X/Y$) = 1090 mg L^{-1} h^{-1}.

Using the values previously obtained for X and Y, μ_m was calculated to be 0.016 h^{-1} from the term ($\mu_m X/Y$) obtained from the regression. Compared to the published data on PCP biodegradation, this coefficient is relatively low; μ_m of

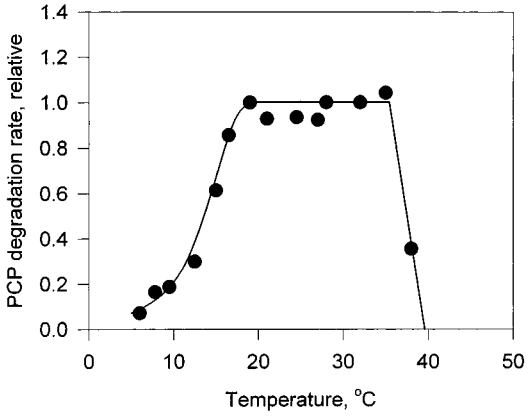

FIGURE 6. Influence of temperature on PCP biodegradation rate.

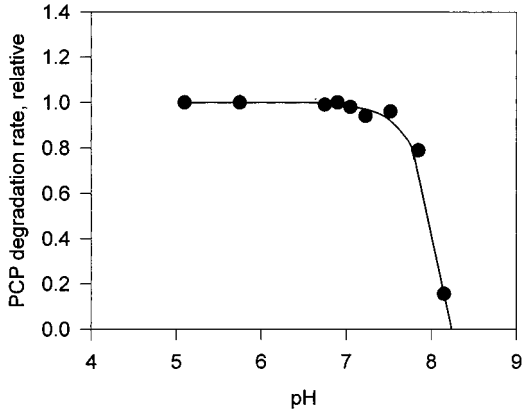

FIGURE 7. Effect of pH of the liquid phase on PCP biodegradation rate.

both suspended and immobilized microorganisms was reported to be in the range 0.02−0.3 h^{-1} (*1, 10, 18, 19*). The value of K_s was within the limits reported by other authors.

Effect of the Main Physicochemical Parameters on Biodgradation Rate. The following experiments were performed in the immobilized soil bioreactor under steady-state conditions. The liquid retention time was kept constant at 7.3 min while the input PCP concentration was 32 mg/L. The effect of temperature on the PCP biodegradation rate was also studied in the range between 6 and 40 °C. The results are shown in Figure 6. It can be seen that the degradation rate remains nearly constant for temperatures between 20 and 35 °C. Outside this range, the rate of degradation decreases sharply. No PCP degradation was observed at temperatures over 40 °C. These results were obtained without any previous adaptation period of microorganisms to temperatures higher and lower than 25 °C—the temperature at which the bioreactor was operated. Recent results of a separate study indicated that microorganisms can be adapted to work at lower temperatures, typical of groundwater conditions: the reaction rate at 9° reached 600 mg L^{-1} h^{-1}.

The effect of the liquid-phase pH in the bioreactor was also studied. pH was varied by adding either 10 N NaOH or 10 N H_2SO_4 to the inlet solution. It was found (Figure 7) that pH variation does not affect the rate of PCP biodegradation in the range between 5.1 and 7.7. For pH values over 7.7, the degradation rate is strongly suppressed, and no degradation was detected at pH higher than 8.3. It was impossible to study the effect of pH below 5.1 because solubility of PCP was very low under these conditions. No adaptation period to the variations of pH was performed in this work. The results in Figure 7 were obtained after a hydraulic steady-state condition was obtained. The steady-state conditions

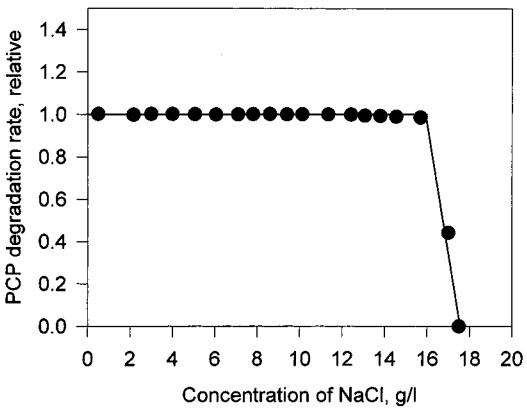

FIGURE 8. Effect of chloride ions on the PCP biodegradation rate.

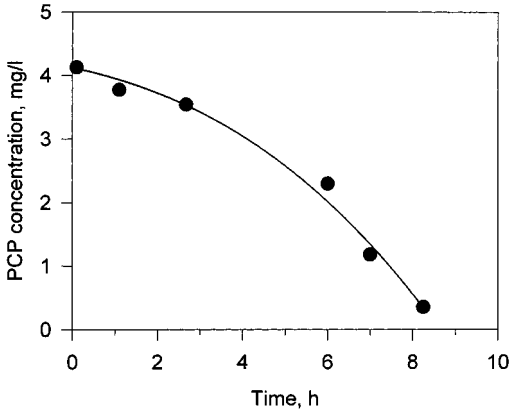

FIGURE 9. Biodegradation of PCP by the liquid phase in the bioreactor.

were obtained after a period equal to between three and five liquid retention times.

The effect of the PCP degradation products that remain dissolved in the liquid phase (chloride ions) on the process rate was studied by adding NaCl to the liquid entering the bioreactor. The results are shown in Figure 8. Chloride ion concentrations below 15.8 g/L did not affect the rate of PCP degradation while higher concentrations caused a strong inhibition. The tolerance of the process to chloride ions was found to be much higher than that reported in the literature. For example, Gonzalez and Hu (*20*) reported an inhibition of PCP biodegradation at Cl^- concentration of 3 g/L. This difference could be explained by the increased resistance of biofilms to variations of the physicochemical parameters of the liquid phase as compared to free suspended cultures (*21*).

The kinetics of PCP biodegradation by microorganisms suspended in the liquid phase of the bioreactor was also studied. A sample of 250 mL of liquid leaving the bioreactor was spiked with 4 mg/L of PCP and placed in a rotary shaker at 21 °C. The dynamics of PCP removal is shown in Figure 9. The rate of PCP biodegradation was much lower as compared to the biofilm process. Assuming a Monod-type kinetic model, the variation of the substrate concentration in a free-suspended cell culture with time can be described by the following equation (*22*):

$$\mu_m t(X_o + YS_o) = X_o + Y(S_o + K_s)\ln\frac{X_o + Y(S_o - S)}{X_o} - K_s Y \ln\frac{S}{S_o} \quad (8)$$

The data of Figure 9 were used to calculate the coefficients μ_m, K_s, and Y as well as the initial concentration of biomass X_o by a nonlinear regression. It was found that $Y = 0.045$ g of biomass/g of PCP, which is similar to that of the biofilm process. However, the values obtained for the kinetic constants $\mu_m = 0.21$ and $K_s = 0.22$ were higher as compared to the respective values in biofilm. The initial biomass concentration (equal to that in the liquid phase of the ISBR) was found to be 0.037 mg/L. Since at the time the liquid sample was obtained from the bioreactor, the PCP degradation rate by biofilm was 210 mg L^{-1} h^{-1}, it was calculated that the biomass produced by biofilm if detached should result in a concentration of 11.3 mg/L in the liquid phase. Compared to the estimated value of 0.037 mg/L, a conclusion can be made that the entire biofilm produced by cell growth practically remains in the bioreactor and only a small part of it detaches and leaves with liquid flow. This finding supports the assumption made previously, and thus the yield coefficient was correctly determined from the biofilm study experiments. The maximal rate of PCP degradation in the liquid phase was found to be 1.2 mg L^{-1} h^{-1}, which is 830 times lower than that by biofilm.

The results of the present study will be of great importance for the design of immobilized soil bioreactors for an aboveground treatment of aqueous solutions of PCP as well as for the development of innovative systems for in situ biotreatment of groundwater contaminated with PCP. The main advantages of the process are

(a) very high volumetric removal efficiency;

(b) high-rate degradation at temperatures as low as 15 °C and possibility for adaptation to lower temperatures (9 °C);

(c) efficient PCP degradation over a wide range of pH values;

(d) efficient PCP degradation without limitation at high salt concentration;

(e) Practically total PCP mineralization to CO_2 and Cl^- (*5*).

Acknowledgments

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Nomenclature

A, B	constants (eq 7)
D_{eff}	effective biofilm diffusivity
J_0	Bessel function of zero-order
K_s	Michaelis constant
m_{gt}	mass of geotextile
r	radial coordinate of a cylinder
r_{gt}	radius of a geotextile fiber
r_{tot}	radius of geotextile fiber covered with biofilm ($r_{tot} = r_{gt} + \delta$)
R_s	volumetric rate of PCP biodegradation
S	limiting substrate (PCP) concentration
S_b	substrate concentration in bulk of liquid
S_o	initial concentration of substrate (PCP) in batch regime
t	time
V_{bf}	volume of biofilm
X	biomass concentration
X_o	initial biomass concentration in batch regime

Y biomass/substrate yield coefficient

Y_0 Bessel function of the second kind of zero-order

Greek Letters

δ biofilm thickness

μ_m maximum specific growth rate

ρ_{gt} density of geotextile

ρ_{bf} biofilm density

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