

## ENHANCEMENT OF AEROBIC MICROBIAL DEGRADATION OF POLYCHLORINATED BIPHENYL IN SOIL MICROCOSMS

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(Received 2 April 2002; Accepted 21 October 2002)

**Abstract**—This article reports the results of various biodegradation experiments on polychlorinated biphenyl (PCB)-contaminated sandy soil employing a mixed culture of acclimatized bacteria. Following the optimization of different variables without chemical pretreatment, the elimination rate achieved of Aroclor® 1242 in slurry-phase reactors was 61% after four months of treatment, with the presence of biphenyl as cosubstrate being the most important factor affecting PCB biodegradation. The biodegradation occurred as a first-order process, and it proved most effective in respect to dichlorinated biphenyls (100% removal), followed by trichlorinated (92%) and tetrachlorinated biphenyls (24%). The results also showed that the degradability of PCBs in soil may be enhanced by an advanced oxidation pretreatment (Fenton reaction), producing almost 100% elimination of PCBs at the end of the integrated chemical–biological process and 72% mineralization of the intermediates generated during the chemical pretreatment.

**Keywords**—Polychlorinated biphenyl Sandy soil Biodegradation Chemical pretreatment Kinetic

## INTRODUCTION

Awareness of the toxicity of polychlorinated biphenyls (PCBs) has led to increased research into the development of PCB waste treatment technology. Although incineration is currently the most frequently used method of dealing with waste containing a high concentration of PCBs, waste products containing a large proportion of inert material such as soils and sediments require other alternatives [1].

One of the options for this type of waste is biological treatments, which are made attractive by their low cost and operative simplicity. However, the mechanisms involved in the biodegradation of PCBs sorbed on particles are not yet fully established. This is due to the difficulty in correlating the results of the different researchers in the field and to the fact that most work has been carried out in the aqueous phase. Also, most researchers have used pure cultures and have been concerned primarily with determining the effectiveness of one strain in particular in PCB biodegradation. On the other hand, a significant number of researchers have shown that the degree of biodegradation is improved by using mixed cultures [2].

The primary objective of this research is to evaluate various enhancements that have the potential to make the PCBs more bioavailable, thus achieving lower total PCB concentrations than can be achieved by biodegradation alone. Therefore, the following three enhancements to bioremediation are proposed: supplemental organics, biostimulation, and bioaugmentation; surfactant addition; and chemical treatment. Supplemental organics and biostimulation and bioaugmentation serve to increase the microbial biomass. This can enhance both the rate and extent of biodegradation of the relatively insoluble PCB congeners. Surfactant addition can increase the rate and extent of desorption of PCB from soil, resulting in the congener being

more soluble and thus more bioavailable. Chemical oxidation, via Fentons reagent, could serve to partially oxidize sorbed PCB congeners, with the oxidation products being less toxic, more desorbable, and more bioavailable than the parent congeners.

## MATERIALS AND METHODS

The mixed culture of PCB-degrading bacteria was acquired from New York State Center for Hazardous Wastes Management (Buffalo, NY, USA). The mixed culture of microorganisms was enriched by biphenyl enrichment from PCB-contaminated sediment. To enrich the mixed culture, 5 g of sediment and 0.1 g of biphenyl were placed in 100 ml of a phosphate-buffered mineral nutrient solution [3]. This culture consisted mainly of Gram-negative strains exhibiting Type 2 organism PCB biodegradation patterns. Type 2 organisms are *Pseudomonas* that biodegrade congener profiles in a way similar to the *Pseudomonas* strain LB400 (*Burkholderia*). The mixed culture was grown on biphenyl, aqueous PCB solutions, and a phosphate-buffered mineral nutrient solution.

To obtain aqueous solutions of PCBs needed for the inoculation reactor feed and for biodegradation experiments in an aqueous medium, bidistilled water (0.035 ml/cm<sup>2</sup>/min) was passed through a column ( $\phi = 2.5$  cm,  $L = 20$  cm, Pyrex® glass; Cosela, Sevilla, Spain) filled with glass beads ( $\phi = 0.15$ – $0.212$  mm, G 9018; Supelco, Bellefonte, PA, USA) impregnated with Aroclor 1242 (Supelco) [4].

The type of soil employed was sandy quartz (X-Ray Diffraction PW 1830; Phillips Ibérica, Madrid, Spain) from Guadalete River (Southwest of the Iberian Peninsula) containing very low levels of organic material (<0.05% weight). Sand was selected because the surface soils—the region most affected by accidental spills—are often predominantly made up of sand [5]. Furthermore, due to its low natural organic composition, this sand was expected to have low interaction with PCB, thus resulting in better interpretation of the experimental data. Using Aroclor 1242 and following the method described

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Presented at the Organic Soil Contaminants Meeting, SETAC Europe, Copenhagen, Denmark, September 2–5, 2001.

by Barriault and Sylvestre [6], the soil was contaminated to a concentration of 100 mg/kg (dry wt).

Before performing biodegradation experiments on soils, the effectiveness of the inoculum was tested in aqueous phase. Batch testing was conducted in 125-ml amber-colored glass bottles with Teflon® septums that were sacrificed for sampling. The aqueous PCB solution (100 ml) used for this experiment was obtained from the glass-bead generator column, and the aqueous concentration was approximately 71 µg/L for the sum of eight congeners. The medium contained the phosphate-buffered mineral nutrient solution [3], and biphenyl was supplied at an initial concentration of 100 mg/L as a cosubstrate directly to the sample bottles. The acclimated mixed culture was supplied at approximately  $2 \times 10^5$  active cells/ml. Oxygen was supplied by starting the experiment with a 20% volume/volume (v/v) oxygen headspace in each bottle. After their setup, sample bottles were placed on an orbital shaker (Unimax 2010; Heidolph Instruments, Schwabach, Germany) and rotated at 200 rpm in the upright position at room temperature ( $25 \pm 2^\circ\text{C}$ ).

After confirming aerobic biodegradation of soluble-phase PCB, a study was conducted to assess the effect of different variables in the biodegradation of PCB-contaminated soil. The experiments were conducted in 15-ml Pyrex glass test tubes with screw tops and polytetrafluoroethylene septa, which were sacrificed for sampling. Each contained 1 g of PCB-contaminated soil, 10 ml (except in those cases where the purpose of the experiment was to determine the optimum value of volume of the cell suspension) of the phosphate-buffered mineral salts medium with the inoculum ( $\sim 10^6$  active cells/ml), and the oxygen-saturated headspace (33% v/v, maintained by flushing with oxygen weekly). Biphenyl was supplied at an initial concentration of 1,000 mg/L (except in those cases where the purpose of the experiment was to determine the doses of the cosubstrate) as a cosubstrate directly to the sample test tubes. After their setup, test tubes were placed on an orbital shaker (Unimax 2010, Heidolph Instruments) and rotated at 260 rpm in a horizontal position at room temperature ( $25 \pm 2^\circ\text{C}$ ) for four months.

The experiments were performed in stirred tank reactors (STR), using the previously optimized conditions, in 2.5-L Pyrex glass vessels covered with aluminum foil that were stirred with steel agitators at 200 rpm and kept at a temperature of  $23 \pm 3^\circ\text{C}$ . At the same time, experiments were performed to test the PCB-degradation capabilities of another inoculum, Microbe-Lift, of proven efficiency in the treatment of municipal wastewater (Ecological Laboratories, Forepart, NY, USA).

All experiments in bottles, test tubes, and STR were performed in duplicate (in addition, two samples were taken from the STR), with controls to evaluate abiotic losses (400 ppm of  $\text{HgCl}_2$  to ensure cessation of biological activity). These loss percentages were nonsignificant ( $\alpha = 0.95$ ) in the case of the bottle and test-tube assays (including those flushed with oxygen) due to the application of the extraction procedure in the same reaction vessels. Loss percentages in STR were dependant on the group of homologs (4.6% for dichlorinated biphenyls [DiCBs], 5.7% for trichlorinated biphenyls [TriCBs], 9% for tetrachlorinated biphenyls [TetraCBs], and 24.7% for pentachlorinated biphenyls [PentaCBs]) due to wall sorption (determined by solvent extraction). In the STR experiments, these control reactors contained activated carbon traps to determine loss due to evaporation. Analysis of the activated car-

bon in the atmospheric emissions trap revealed that no appreciable losses occurred.

Evaluation of the active and total number of aerobic microorganisms was determined with an Epifluorescence Microscope (AFX-DX; Nikon Instrument Europe, Badhoevedorp, The Netherlands) using as the reagents the fluorochromes [7] 5-cyano-2,3-ditoyl-tetrazolium chloride and 4,6-diamido-2-phenylindole, respectively.

The PCBs were analyzed by capillary gas chromatography using an electron-capture detector. All samples were run on a Perkin-Elmer Autosystem HRGC (Norwalk, CT, USA) equipped with a  $30 \times 0.32$  mm i.d. fused silica column (SPB-5, Supelco). The chromatographic protocol employed was that of Ofjord et al. [8] employing hexachlorocyclohexane as an internal standard. Multiple injections were performed from a single sample stock solution to assess the reproducibility of the gas chromatography PCB analysis (relative standard deviation, 1.2%). The PCB extraction was performed using the method developed by Quensen et al. [9]. Extraction efficiency for Aroclor 1242 in the sediment was  $89 \pm 3\%$  and more than 95% for liquid samples.

A total organic carbon analyzer (Shimadzu TOC-5050A; Izasa SA, Barcelona, Spain), operating in accordance with the standard method of infrared combustion, was used to establish the dissolved organic carbon in liquid samples [10]. The dichromate oxidation method developed by El Rayis was used to measure organic carbon in the contaminated soil [11]. In determining the nitrates, nitrites, chlorides, sulfates, and phosphates, a Capillary Electrophoresis (Waters Quanta 4000 CIA System; Bedford, MA, USA) was employed, in accordance with Waters method N601 and in compliance with American Society for Testing and Materials D1066 regulations (Philadelphia, PA, USA). And finally, to determine pH, temperature, and dissolved oxygen concentration, selective electrodes were used in accordance with standard methods [10].

## RESULTS AND DISCUSSION

### *Aqueous PCB solution prepared by glass-bead generator columns*

Typically, the use of cosolvents and dissolution from neat PCBs have been used to generate aqueous PCB solutions because of their low solubility. In the first method, the large amount of cosolvent present in aqueous solution along with the PCB made such solutions inappropriate for degradation tests to be conducted. Using the second method, in which a drop of neat PCB is positioned in the bottom of a large glass vessel, the dissolution rate was found to be very slow.

Using a glass-bead generation column, it was possible to produce aqueous PCB solution suitable for PCB degradation experiments in reasonably short times. The PCB congener concentrations were below their aqueous solubilities (the average overall concentration of PCB in the composite sample was 261 µg/L) and different from the congener profile characteristics of Aroclor 1242, suggesting that aqueous-phase PCB congener concentrations resulting from operation of the generator column are influenced by PCB congener availability and solubility. Higher fractions of lower chlorinated PCB congeners were found in the effluent during the initial phases of solution generation from a fresh generator column compared with the later phases of a generator column operation (Fig. 1). Collection of the total effluent from a generator column for a long duration more closely approximated the congener distribution in the original Aroclor mixture. The composite sample from

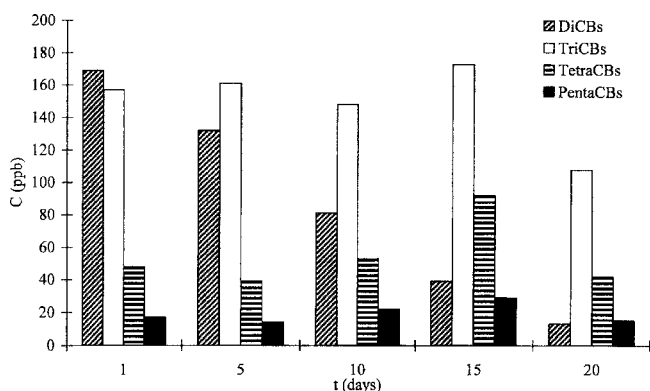


Fig. 1. Polychlorinated biphenyl homolog distribution in generator column effluent with time. Refer to Table 1 for explanation of acronyms.

the column effluent contained more DiCBs (21%) and TriCBs (44%) than Aroclor 1242 (14% DiCBs, 34% TriCBs) and less of the higher chlorinated congeners (23% TetraCBs, 8% PentaCBs) than Aroclor 1242 (34% TetraCBs, 15% PentaCBs).

#### Acclimation reactor

During acclimation reactor maintenance, measurements were taken to quantify reactor conditions and assess biomass growth. Acclimation reactor pH, dissolved oxygen, and temperature were measured prior to each feeding. The room temperature also was recorded at this time. Typically, the pH and dissolved oxygen concentration would decrease during the feed cycle, reflecting the occurrence of biodegradation. The reactor temperature also remained fairly constant at 21°C ( $\pm 2^\circ\text{C}$ ) and consistently fluctuated with the room temperature. A fluorescent yellow-green color was observed one time in the reactor 1 h after feeding. The phenomenon was postulated to result from biphenyl intermediate accumulation, possibly catechol, which served as a good indicator of aggressive biphenyl biodegradation. The acclimation reactor biomass concentration was occasionally measured with the epifluorescence microscope using as fluorochrome the 5-cyano-2,3-ditolyl-tetrazolium chloride reagent.

#### Aerobic biodegradation of PCBs in aqueous phase

To evaluate the effectiveness of the mixed culture in the biodegradation process, aqueous phase experiments were performed. Table 1 shows the overall extent of biodegradation and with respect to each homolog. The results reveal that, after 3 d, only 20% of the original PCBs remained unamenable to degradation, and after 7 d, only 5%. It is also clear that the degree of chlorination is an influencing factor given that a high

chlorine content has the effect of reducing the extent of biodegradation.

These results reflect those obtained by Bedard et al. [12] and are higher than those obtained by Barriault and Sylvestre [6] due probably to the fact that, in our experiments, a mixed culture was used, which generally leads to greater levels of elimination [2].

#### Influence of variables in the bioremediation of PCB-contaminated soils

Experiments were performed with PCB-contaminated soils in order to evaluate the influence of biostimulation (10% increase per month of the inorganic nutrients added at the beginning of the assay) and bioaugmentation (addition of  $10^8$  active cells/g soil/month), ratio of soil mass/volume of solution (m/V), addition of a nonionic surfactant (nonylphenol polyethoxylated, Empilan NP8; Campi e Jové, Barcelona, Spain) at different concentrations, and addition of a cosubstrate (biphenyl) at different concentrations and frequencies.

Figure 2 shows residual Aroclor 1242 in the different assays. The soil mass/volume of solution ratio (m/V) was 1/10 g/ml and the amount of cosubstrate added at the start of the assay was 1,000 ppm except in those cases where the purpose of the experiment was to determine the optimum value of the m/V ratio and that of the doses of the substrate.

The results show that the concentration of biphenyl is the variable that exerts the greatest influence on the process (Fig. 2D) and that optimal conditions are obtained by the addition of small but frequent amounts of biphenyl, 100 ppm every 2 d, producing 60% biodegradation in two months. This method avoids the competition of substrates caused by high biphenyl/PCB ratios and the possible toxic effects of biphenyl. The worst results were obtained from experiments in which no biphenyl was added, although during the first month, the lightly chlorinated biphenyls (more soluble compounds) underwent significant degradation (70% DiCBs, 30% TriCBs) due to the absence of any other carbon source in the medium.

With respect to the influence of biostimulation and bioaugmentation (Fig. 2A), the results show that there are no significant differences between either of the experiments, which would imply that the amount of nutrients added initially is sufficient and that it is not necessary to reinoculate with fresh bacteria. On the other hand, Figure 2B shows that, while there is no significant difference between the levels of biodegradation achieved with m/V ratios of 1/2 and 1/5 g/ml, there is a slight change when the m/V ratio is 1/10 g/ml. This is probably due to the fact that PCB solubilization is enhanced at this level and results in an additional 12 and 9% degradation of

Table 1. Polychlorinated biphenyl biodegradation in aqueous phase using a mixed culture of acclimatized bacteria (temperature =  $25 \pm 2^\circ\text{C}$ ,  $\text{pH}_i = 7.05$ ,  $\text{pH}_f = 6.71$ ,  $2 \times 10^5$  cells/ml)<sup>a</sup>

| Homolog group                         | Percent biodegradation <sup>b</sup> (3 d) | Percent biodegradation <sup>b</sup> (7 d) |
|---------------------------------------|-------------------------------------------|-------------------------------------------|
| Dichlorinated biphenyls (DiCBs)       | 100                                       | 100                                       |
| Trichlorinated biphenyls (TriCBs)     | 79                                        | 95                                        |
| Tetrachlorinated biphenyls (TetraCBs) | 75                                        | 90                                        |
| Pentachlorinated biphenyls (PentaCBs) | 55                                        | 81                                        |
| Total polychlorinated biphenyl        | 80                                        | 95                                        |

<sup>a</sup>  $\text{pH}_i$  = initial pH;  $\text{pH}_f$  = final pH.

<sup>b</sup> Values are given as the mean of two replicates. Percent biodegradation was calculated considering all quantifiable peaks in Aroclor 1242.

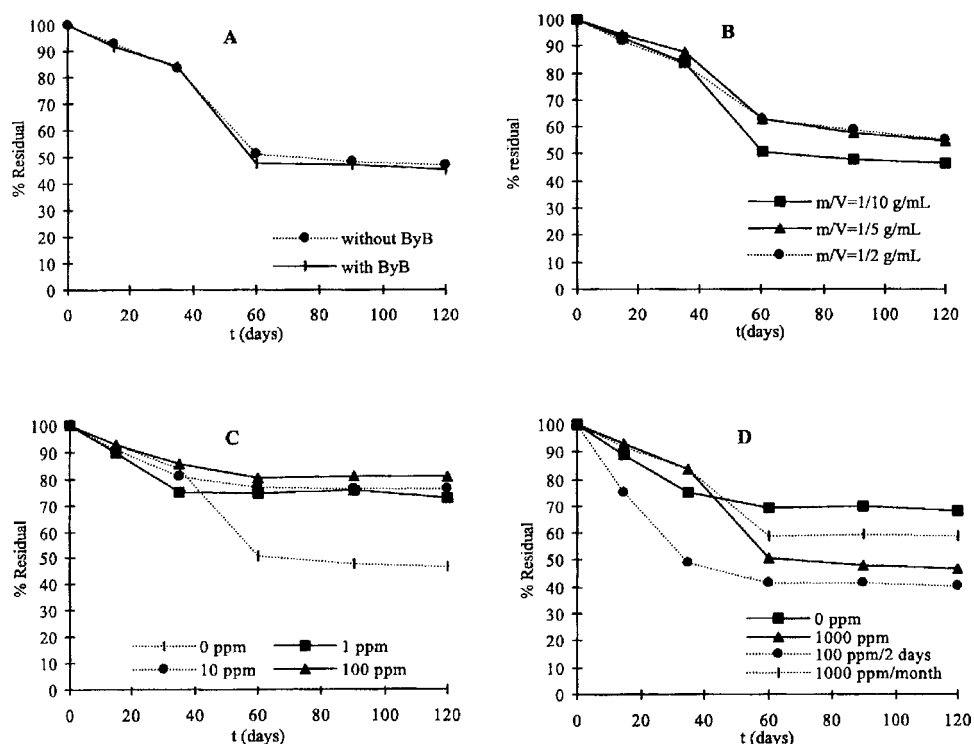


Fig. 2. Residual Aroclor 1242 in the different assays. (A) Biostimulation and bioaugmentation (ByB), (B) ratio of soil mass/volume of solution, (C) addition of nonionic surfactant, and (D) addition of biphenyl as cosubstrate. Values are given as the mean of two replicates, and percentage biodegradation was calculated considering all quantifiable peaks in Aroclor 1242.

Aroclor 1242 at two and four months, respectively, compared with the other ratios.

In the experiments performed with the addition of the surfactant Empilan NP8 (Marchon, London, UK) (Fig. 2C), the results recorded during the first month show that the highest rates of PCB elimination were obtained in the experiments with 1 and 10 ppm of the surfactant (25 and 20%, respectively), followed by the surfactant-free experiment (17%) and, finally, by the experiment using 100 ppm of the aforementioned surfactant (14% biodegradation). This sequence of results seems to be due to the fact that, with small concentrations of surfactant (1 and 10 ppm), PCB bioavailability is increased and, consequently, PCB biodegradability, whereas higher concentrations (100 ppm) inhibit biodegradation. From the end of the first month onward, the tendency is reversed, with the surfactant-free experiments achieving the highest rates of elimination. This may be due to the high toxicity of metabolites generated during nonylphenol polyethoxylated biodegradation [13].

The results of these experiments, together with the measurement of other parameters such as pH, inorganic nutrients, and viable microorganisms, allowed us to establish the optimal working conditions for subsequent experiments.

#### Bioremediation experiments in stirring tank reactors

Two different inocula were used to compare the degradation capabilities of each. The experiment with bacteria acclimatized to PCB degradation was surfactant free and bioaugmentation and biostimulation techniques were not applied. The m/V ratio was 1/10 g/ml, and the cosubstrate was added every 2 d with 100 mg/L of biphenyl. The experiment employing the Microbe-Lift culture (Ecological Laboratories) was performed under the same conditions except that no cosubstrate was used

and the inoculum was added once a week ( $10^6$  active cells/ml), in accordance with the supplier's instructions.

Figure 3 shows the amount of residual Aroclor 1242 in the experiments using each of the inocula. The results show that Aroclor levels in the slurry decrease rapidly in the experiment with acclimatized bacteria (14% at 5 d and 50% over the first 20 d of the experiment), while in the Microbe-Lift culture, no biodegradation occurred in the course of the first 20 d. After 120 d, the biodegradation rates achieved in the experiments with acclimatized bacteria and with Microbe-Lift were 60 and 4%, respectively.

The biodegradation extents obtained for Aroclor 1242 with the acclimatized culture are higher than those obtained by Brunner et al. [14] (13% removal with *Ancinetobacter* P6 over

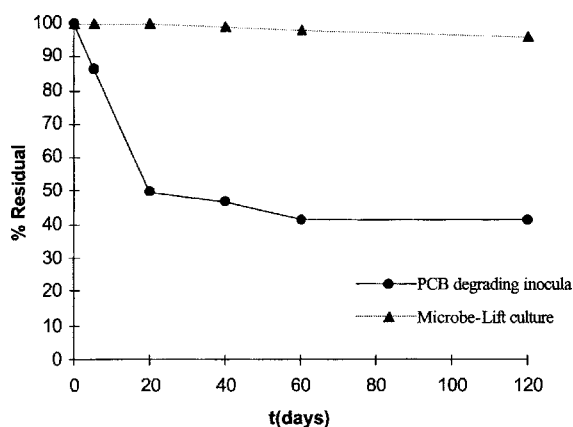


Fig. 3. Residual Aroclor 1242 in soils in the experiments using a mixed culture of polychlorinated biphenyl (PCB)-degrading inocula and the Microbe-Lift culture.

Table 2. Parameters adjusted to the Middleton kinetic model<sup>a</sup>

|                        | Dichlorinated biphenyls | Trichlorinated biphenyls | Tetrachlorinated biphenyls | Total polychlorinated biphenyl |
|------------------------|-------------------------|--------------------------|----------------------------|--------------------------------|
| $C_r$ (mg/kg)          | 0.12 (0.09)             | 2.62 (2.7)               | 25.41 (25.9)               | 40.7 (39.8)                    |
| $k$ (d <sup>-1</sup> ) | 0.0823                  | 0.0654                   | 0.044                      | 0.074                          |
| $r^2$                  | 0.982                   | 0.967                    | 0.966                      | 0.987                          |

<sup>a</sup> The data in parentheses are the values derived from the experimental results.

210 d) and by Barriault and Sylvestre [6] (4.5% removal with *Pseudomona Testoteroni* B-356 over 90 d). On the other hand, the results obtained in these experiments are somewhat lower than those recorded by Focht and Brunner [15], who noted 75% biodegradation after 49 d with *Acinetobacter* P6.

As before, an increase in the level of chlorination resulted in a lower extent of biological degradation (100% DiCBs, 91% TriCBs, 29% TetraCBs, and nonappreciable degradation for PentaCBs after 60 d). The Microbe-Lift culture was able to degrade only 10 and 4% of the DiCBs and TriCBs, respectively.

With regard to the measurement of PCB concentration in the aqueous phase of the experiments performed with acclimatized bacteria and in the abiotic assays (400 ppm HgCl<sub>2</sub>), the results show that the PCB concentration was less in the microorganism assays (12 ± 5 ppb) than in the abiotic assays (23 ± 4 ppb). However, PCBs were not entirely eliminated in the aqueous phase, and consequently, the reduction in the potential, through biodegradation, of soil-bound organic contaminant to leach and migrate through groundwater or volatilize into the air and biostabilization of the soil did not occur.

Our results reflect those of other researchers [3,12] who revealed a series of tendencies within each of the groups of homologs. For example, congeners with two chlorine atoms in an ortho position in one ring or on both rings proved more resistant to biodegradation. Thus, after 60 d, congener 22'6 had still not begun to decompose while congener 244' had achieved 62% degradation. On the other hand, congeners having all the chlorine atoms in just one ring are more readily degradable than those with the same overall number of chlorines spread over two rings. For example, after 40 d of the experiment, the biodegradation rates of congeners 24 and 24' were 100 and 31%, respectively.

In the tetra- and pentachlorobiphenyl homolog group, the results showed that congeners with two chlorine atoms at position 2,3 on the ring were more easily degradable than others. The congeners most susceptible to biodegradation in all our experiments were 2,2',3,6- and 2,2',3,3'-CB, at 35 and 55%, respectively, compared with a maximum elimination rate of 25% for the remainder of the tetrachlorinated congeners.

With regard to the amount of total and viable microorganisms, the results from the experiment performed with PCB-degrading inoculum show that, initially, the bacterial population was small (3.5 × 10<sup>7</sup> total cells/ml and 10<sup>6</sup> active cells/ml). It increased with each application of biphenyl and stabilized after 20 d at values for total and active cells ranging between 4 and 5 × 10<sup>8</sup> and 1.5 and 3 × 10<sup>7</sup> cells/ml, respectively (5% viable). The pH, temperature, and inorganic anion values all remained within an optimum range for bacterial development.

#### Kinetic modeling of the bioremediation process of PCB-contaminated soils

Middleton et al. [16] postulated an empirical model to evaluate the levels of polycyclic aromatic hydrocarbons (PAH) in soils via bioremediation. The model, also known as the general bioremediation model, is based on soil/sludge bioremediation data from laboratory tests and pilot and full-scale field studies. The proposed equation is

$$C_t = C_r + (C_0 - C_r)e^{-kt}$$

where

- $C_t$  = concentration of the organic compound at time  $t$  (in mg/kg),
- $C_0$  = initial concentration of the organic compound (in mg/kg),
- $C_r$  = concentration of the organic compound that is resistant to biodegradation or has no bioavailability (in mg/kg), and
- $k$  = the first-order rate constant (in units/time).

The model, which has been used to predict biodegradability in matrices with a high degree of adsorption, is based on two important premises, i.e., that a residual concentration of substrate exists that is resistant to biodegradation due to its non-bioavailability and that biodegradation occurs as a first-order process.

This model has been applied to the results obtained in this research. In Table 2, e.g., the results deriving from the adjusted model are given for the experiments relating to the aerobic biodegradation of PCB-contaminated soils in the STR, with optimized conditions and using acclimatized bacteria.

The model was applied successfully, as is evident from the correlation coefficients obtained. Figure 4 shows the evolution of total and homolog PCB concentrations, including those obtained by experiment (represented by bullets) and those predicted by the model (represented by a continuous line). Their similarity is evidence of the validity of the model.

The results also show how an increase in the number of congener chlorines is accompanied by an increase in relative residual amounts and a decrease in the kinetic constants of the degradation rate.

#### Integrated chemical-biological treatment in stirring-tank reactors

Figure 5 charts the Aroclor 1242 removal rate in the Fenton oxidation experiments. This treatment was effected in slurry tank reactors with previously optimized operating conditions [17]. The concentrations of oxygenated water and iron (III), applied in the form of iron sulfate, were 5% and 100 ppm, respectively. The mixture of soil and oxidant solution was prepared in a proportion of 1/3 kg/L. The experiments were

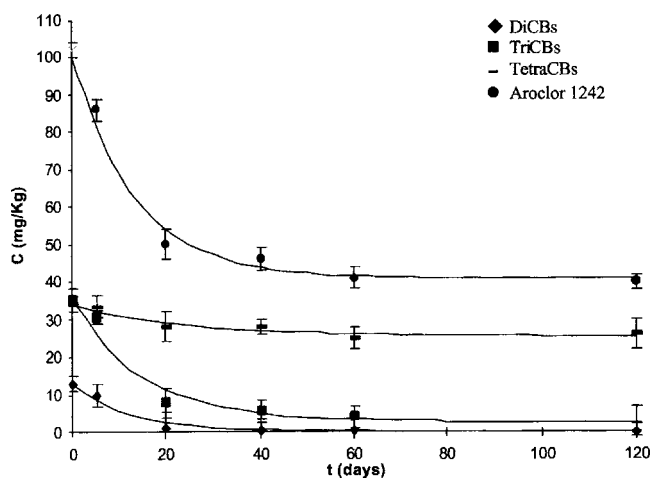


Fig. 4. Overall and homolog polychlorinated biphenyl concentration. Bullets represent experimental values and continuous lines represent those predicted by the model. Refer to Table 1 for explanation of acronyms.

carried out at room temperature ( $22 \pm 1^\circ\text{C}$ ), and the pH value was adjusted to maintain acidity at 2.65 to 2.85.

A 98% removal of the original PCB concentration was observed in the first 72 h of the experiment, with the highest rates reported in the first half hour (35%). The kinetics observed were of pseudo-first order with a constant of  $0.632/\text{h}^{-1}$  and a correlation coefficient ( $r^2$ ) of 0.96. These results are similar to those reported by other authors investigating the degradability of organic compounds in soil using the Fenton reaction. Watts et al. [1], e.g., obtained a 99.9% pentachlorophenol removal in 24 h; Gates and Siegrist [18] witnessed a 99.8% reduction of trichloroethylene, and Watts and Dilly [19] reported a 99% elimination of diesel fuels.

The elimination percentage rates obtained for homologs were 99.7% of DiCBs, 97.5% of TriCBs, 96.9% of TetraCBs, 96.5% of PentaCBs, and 96.2% of HexaCBs. The degree of chlorination was a slight influencing factor, with the elimination rate decreasing as the number of chlorine atoms increased, in agreement with Dercova et al. [20].

It may be concluded from these results that the subsequent application of an aerobic biological treatment on the already oxidized soil would produce no appreciable result, the said treatment being effective mainly with lightly chlorinated congeners. On the other hand, prior to chemical treatment, the organic carbon concentrations in the solid matrix and in the

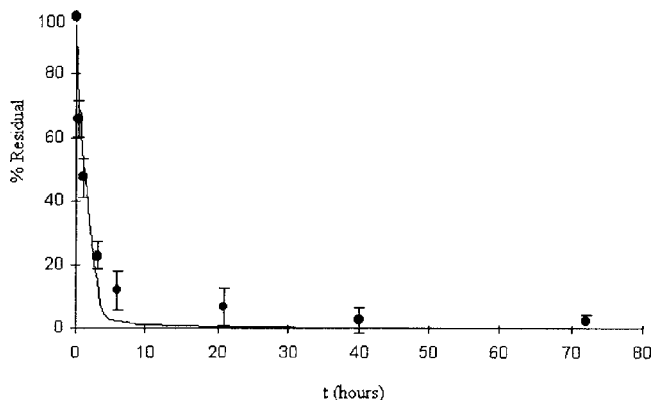


Fig. 5. Rate of elimination of Aroclor 1242 in the Fenton oxidation experiments.

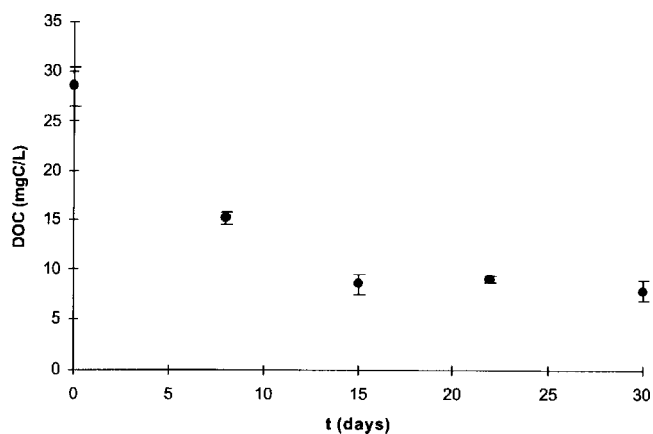


Fig. 6. Dissolved organic carbon during the aerobic biodegradation process (temperature =  $22 \pm 2^\circ\text{C}$ , pH = 7.05).

aqueous solution were 64 mg C/kg and 1.7 mg C/L, respectively. However, once the chemical treatment was complete, after 72 h, the level in the solid matrix had dropped below 5 mg C/kg and was 28.5 mg C/L in the liquid phase. Consequently, it may be deduced that the PCBs were not mineralized during the chemical treatment process and instead generated a series of partially oxidized soluble products, which triggered an aerobic biological process in the aqueous phase.

The aerobic biological treatment of the aqueous phase was performed after neutralization with 1 N NaOH, addition of the phosphate buffer (0.05 M), and dissolution of the mineral nutrient with the inocula ( $1.1 \times 10^6$  active cells/ml). Figure 6 shows the residual dissolved organic carbon in this experiment.

After 30 d of biological treatment, the results show a 72% mineralization of the compounds generated by Fenton oxidation, indicating that the products arising from this chemical pretreatment are readily degradable.

*Acknowledgement*—We thank the Interministerial Commission of Science and Technology (Spanish Ministry of Education and Culture) for its economic support and the New York State Center for Hazardous Waste Management (Buffalo, NY, USA) for its valuable scientific collaboration.

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