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Paper from: *Brownfield Sites*, CA Brebbia, D Almorza & H Klapperich (Editors).
ISBN 1-85312-918-6

Biological degradation of PCBs in soil. A kinetic study

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Abstract

In this paper a kinetic study is made of the biodegradability of Aroclor 1242 in sandy soil employing a mixed culture of acclimatized bacteria. The assays were done in stirred tank reactors, and the biodegradation process was monitored by High Resolution Gas Chromatography (HRGC) with Electron Capture Detector. These results are supported by other indirect measurements and indicators of the existence of microbial degradation process, as well as the parameters for the control of the process.
The biodegradation occurred as a first order process and it proved most effective in respect of dichlorinated (100% removal), followed by trichlorinated (92%) and tetrachlorinated biphenyls (24%).

1 Introduction

Awareness of the toxicity of 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 are biological treatments, made attractive by their low cost and operative simplicity. The aim of this research has been to study the kinetic of the aerobic biodegradation of PCBs adsorbed to soil particles by employing a mixed culture of acclimatized bacteria.

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2 Materials and methods

The mixed culture of PCB degrading bacteria was acquired from New York State Centre for Hazardous Wastes Management (Buffalo, N.Y.). The mixed culture of microorganisms was isolated by biphenyl enrichment from PCB contaminated sediment. This culture consisted mainly of gram negative strains exhibing Type II organism PCB biodegradation patterns. Type II organisms are Pseudomonas that biodegrade similar congener profiles as the Pseudomonas strain LB400. The mixed culture was grown on biphenyl, aqueous PCB solutions, and a phosphate-buffered mineral nutrient solution.

The type of soil employed was sandy quartz (X-Ray Diffraction, Phillips PW 1830) from Guadalete river (South-West 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 an accidental spill- is often predominantly made up of sand [2]. Futhermore, due its low natural organic composition, this sand was expected to have low interaction with PCB and thus results in better interpretation of the experimental data. Using Aroclor 1242 (SUPELCO), and following the method described by Barriault and Sylvestre [3], the soil was contaminated to a concentration of 100 mg/Kg (dry weight).

The experiments in stirred tank reactors (STR) were performed in 2.5L Pyrex glass vessels, covered with aluminum foil and stirred with steel agitators at 200 rpm, at a temperature of 23±3°C. The experiments were performed in duplicate with controls to evaluate abiotic losses (400 ppm of $HgCl_2$ to ensure cessation of biological activity).

Evaluation of the active and total number of aerobic microorganisms was determined with the Epifluorescence Microscope (Nikon AFX-DX) using the following reagents as fluorochromes [4]: 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC) and 4,6-diamido-2-phenylindole, respectively (DAPI).

The PCB were analyzed by capillary gas chromatography using an electron capture detector (ECD). All samples were run on a Perkin Elmer Autosystem HRGC equipped with a 30 x 0.32 mm I.D. fused silica colum (SPB-5, Supelco Inc., Bellefonte, Pa). The chromatographic protocol employed was that of Ofjord et al. [5] employing hexachlorocyclohexane as an internal standard. PCB extraction was performed using the method developed by Quensen et al. [6].

And finally, to determine pH, temperature, and dissolved oxygen concentration, selective electrodes were used in accordance with standard methods [7].

3 Results and discussion

In STR assays, the m/V ratio was 1/10 g/mL and the cosubstrate (biphenyl) was added every 2 days with 100 mg/L. Fig. 1 shows the evolution of residual Aroclor 1242 in the experiments.

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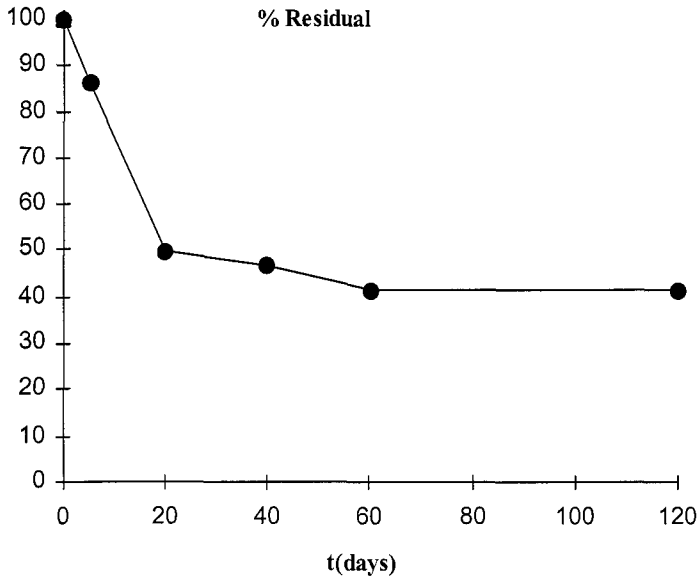

Figure 1: Evolution of residual Aroclor 1242 in soils in the experiments using mixed culture of PCB degrading inoculum.

The results show that Aroclor levels in the slurry decrease rapidly ,14% at five days and 50% over the first 20 days of the experiment. After 120 days, the duration of the experiment, the biodegradation rates achieved was 60%.
The biodegradation rates obtained for Aroclor 1242 with the acclimatized culture are higher than those obtained by Brunner et al. [8] (13% removal with *Ancinetobacter* P6 over 210 days) and by Barriault and Sylvestre [6] (4.5% removal with *Pseudomona Testoteroni* B-356 over 90 days). On the other hand, the results obtained in these experiments are somewhat lower than those recorded by Fotch and Brunner [9] who noted 75% biodegradation after 49 days with *Acinetobacter* P6.
An increase in the level of chlorination resulted in a lower extent of biological degradation (100% DiCBs, 92% TriCBs, 24% TetraCBs and non appreciable degradation for PentaCBs after 60 days.
With regard to the evolution of total and viable microorganisms, the results from the experiment show that initially the bacterial population was small ($3.5x10^7$ total cells/mL and 10^6 active cells/mL), that it increased with each application of biphenyl, and stabilised after 20 days at values for total and active cells ranging between 4-5 $x10^8$ y 1.5-3 $x10^7$ cells/mL, respectively (5% viable). The pH, temperature and inorganic anion values all remained within an optimum range for bacterial development.
Middelton et al. [10] 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

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bioremediation data from laboratory tests, pilot and full scale field studies. The proposed equation is as follows:

$$C_t = C_r + (C_o - C_r)\, e^{-kt}$$

Where:

C_t = concentration of the organic compound at time t, mg/Kg
C_o = initial concentration of the organic compound, mg/Kg
C_r = concentration of the organic compound which is resistant to biodegradation or has no bioavailability, mg/kg
K = first order rate constant, t^{-1}

The model, which has been used to predict biodegradability in matrices with a high degree of adsorption, is based on two important premiss; (a) that a residual concentration of substrate exists which is resistant to biodegradation due to its non bioavailability and (b) that biodegradation occurs as a first order process.
This model has been applied to the results obtained in this research. In Table 1 the results deriving from the adjusted model are given for the experiments relating to the aerobic biodegradation of PCB-contaminated soils in the STR using acclimatized bacteria.

Table 1: Parameters adjusted to the Middelton kinetic model

	DiCB	TriCB	TetraCB	Total
C_r (mg/Kg)	0.12 (0.09)	2.62 (2.7)	25.41 (25.9)	40.7 (39.8)
k(days^{-1})	0.0823	0.0654	0.044	0.074
r^2	0.982	0.967	0.966	0.987

* The data in brackets are the values derived from the experimental results.

The model was applied successfully, as is evident from the correlation coefficients obtained. 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. Fig. 2 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.

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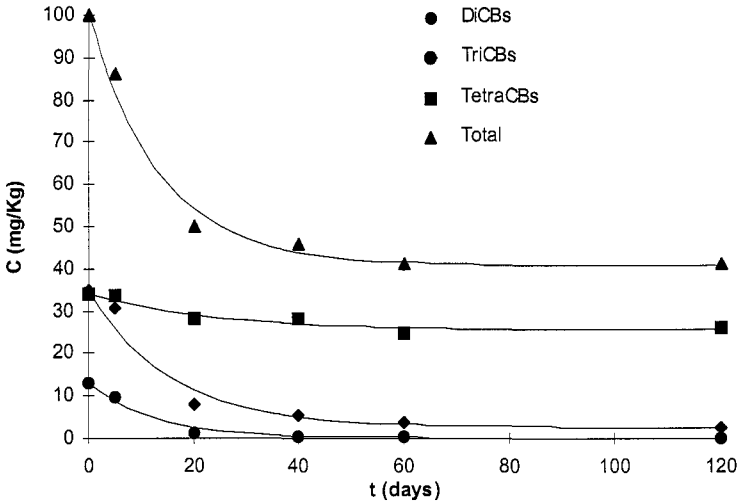

Figure 2: Evolution of overall and homolog PCB concentration.

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