Journal of Industrial Microbiology (1996) 16, 325–329

Biodegradation and evaporation of polychlorinated biphenyls (PCBs) in liquid media

K Dercová, B Vrana, Š Baláž and A Šándorová

Department of Biochemical Technology, Faculty of Chemical Technology, Slovak Technical University, Radlinského 9, 812 37 Bratislava, Slovakia

During microbial degradation of PCBs in a liquid medium, two processes influence the PCB concentration in the medium simultaneously: biodegradation and evaporation. The physical loss of PCB due to evaporation frequently causes false positive results in biodegradation experiments. Therefore, if only PCBs are monitored, the determination of the PCB concentration in both liquid and gaseous phases is necessary for a correct appraisal of biodegradation. The kinetics of PCB evaporation and biodegradation were monitored and described by a simple mathematical model. The evaporation and biodegradation rate constants for individual PCB congeners were determined for PCB degradation in liquid medium by *Pseudomonas stutzeri* and *Alcaligenes xylosoxidans*, both isolated from a long-term PCB-contaminated soil.

Keywords: biodegradation; evaporation; polychlorinated biphenyls; PCB; *Alcaligenes xylosoxidans*; *Pseudomonas stutzeri*

Introduction

Volatility, lipophilicity, and insolubility of PCBs pose particular difficulties in aqueous assays of biodegradation and require special attention. Loss of PCBs due to evaporation or adsorption to the inoculation vessel and the bacterial cells has often been mistakenly attributed to biodegradation. To minimise problems caused by evaporation, Fava *et al* [2] proposed the use of a sorbent for the capture of evaporated PCBs. This approach has been modified with the aim of improving the aeration conditions by selection of a suitable sorbent and analysing the distribution of PCB congeners between the liquid and gaseous phases during incubation in liquid media [7]. The kinetics of PCB evaporation showed [8], that low chlorinated biphenyls especially evaporate rapidly.

The aims of this study were to: (1) monitor evaporation and degradation during incubation of two PCB-degrading micoorganisms: *Pseudomonas stutzeri* and *Alcaligenes xylosoxidans* isolated from a long-term PCB-contaminated soil in a PCB-containing medium; (2) describe the two simultaneous processes by a simple mathematical model; and (3) determine the rate constants of evaporation and degradation for individual PCB congeners.

Materials and methods

Chemicals

A commercial PCB mixture, Delor 103 (equivalent to Aroclor 1242, ex-product of Chemko Strážke, Slovakia) containing 40–42% (w/v) of bound chlorine, Delor 106 (equivalent to Aroclor 1260, ex-product of Chemko Strážke, Slovakia) containing 60% (w/v) of bound chlorine, biphenyl (Lachema Brno, Czech Republic), *n*-hexane UV (Pestiscan, Labscan Ltd, Ireland), acetone (Microchem Bratislava, Slovakia) and chemicals for mineral media (Lachema Brno, Czech Republic) with purity grade were used.

Cultivation conditions

The synthetic DMA medium for bacteria as described by Pirt [6] was used. This is composed of five parts, A to E. Part A is K_2HPO_4, 84.5 g; part B is $MgSO_4 \cdot 7H_2O$, 20 g; part C is $CaCl_2$, 1.0 g; part D consists of $FeSO_4 \cdot 7H_2O$, 5.0 g; $ZnSO_4 \cdot 7H_2O$, 5.0 g; $MnSO_4 \cdot 5H_2O$, 5.0 g; $CuSO_4 \cdot 5H_2O$, 1.0 g; $CoCl_2 \cdot 6H_2O$, 1.0 g; $Na_2B_4O_7$, 1.0 g; $NaMoO_2 \cdot H_2O$, 1.0 g; and part E is NH_4Cl, 20 g. Parts A, B, C and E were each dissolved in 500 ml of distilled water and autoclaved for 20 min at 120 kPa. For part D, the first three ingredients were each dissolved in 100 ml distilled water, and the other ingredients of part D in 1 L each, autoclaved separately and mixed in the volume ratio 10 : 1 : 1 : 10 : 10 : 10 : 10. This solution (52 ml) was mixed with distilled water (448 ml) to give the final solution D. The solutions of parts A to E were then mixed in the volume ratio 10 : 1 : 1 : 1 :10. To 124 ml of this solution, 876 ml of distilled water was added.

Contamination of liquid medium

A stock PCB solution was prepared from the commercial mixtures of Delor 103 and Delor 106 (2 : 1, w/w) dissolved in 100 ml acetone with a final concentration of 4 mg ml^{-1}. For contamination of the liquid medium 50 μl of the PCB stock solution was added to 20 ml of DMA medium in Erlenmeyer flasks (the final concentration 10 $\mu g\ ml^{-1}$).

Sorbent

To determine the loss caused by evaporation of PCBs during incubation, the flasks were fitted with a column (height 110 mm, diameter 25 mm) filled with the sorbent SILIPOR C18 (diameter of particles 120–160 μm, 0.57 g ml^{-1}, Lach-

Correspondence: K Dercová, Department of Biochemical Technology, Faculty of Chemical Technology, Slovak Technical University, Radlinského 9, 812 37 Bratislava, Slovakia
Received 6 November 1995; accepted 13 March 1996

ema Brno, Czech Republic). For each column 0.5 g of the sorbent was used, resulting in a 1-mm thick sorbent layer.

Microorganisms and culture conditions

Two bacterial isolates were used. Each was obtained from a long-term contaminated soil by enrichment in DMA medium with biphenyl as the sole carbon source [1]. The bacteria were identified as *Pseudomonas stutzeri* and *Alcaligenes xylosoxidans* and are maintained in the Czech Collection of Microorganisms, Masaryk University, Brno. Inocula were prepared by incubating *P. stutzeri* or *A. xylosoxidans* for 7 days in DMA medium (pH 6.7) containing 2.5 g L^{-1} biphenyl at 28°C. The biomass obtained was 1.5 g d.w. L^{-1}.

Biodegradation of PCBs by pure bacterial strains was carried out in 100-ml Erlenmeyer flasks containing 20 ml DMA medium without a carbon source, closed with the column filled with sorbent. The apparatus for biodegradation experiments was described in detail by Vrana *et al* [7] (Figure 1). The PCB stock solution (final concentration 10 μg ml^{-1}) and the bacterial inoculum (final concentration 0.5 g d.w. L^{-1}) were added. To maintain a sterile environment and allow for gas diffusion, the top of the glass column was closed with a cotton wool stopper. The flasks were incubated for 15 days on a rotary shaker (180 rpm) at 28°C in the dark. A 15-day incubation period was used in view of the fact that evaporation of more-chlorinated PCB congeners becomes significant after this time. Whole flasks were taken periodically every 3 days for PCB analysis. The amounts of PCBs in the liquid medium and on the sorbent were determined.

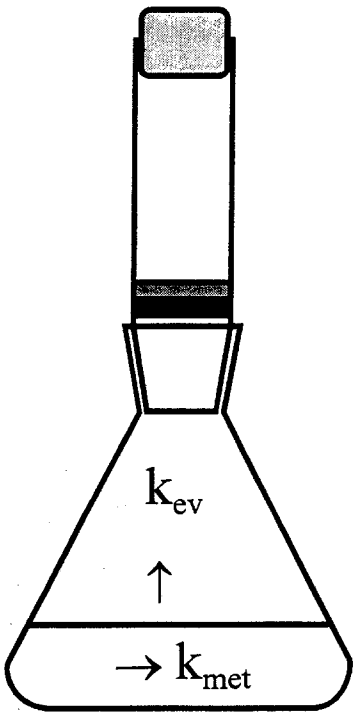

Figure 1 Apparatus for monitoring evaporation and degradation of PCBs during biodegradation experiments: Erlenmeyer flask with a sorbent-filled column closed by a cotton wool stopper.

Extraction of PCBs

After incubation the flask was filled with a mixture of *n*-hexane : acetone (9 : 1, 10 ml) and put into an ultrasonic bath for 15 min. The whole volume of the vessel was then transferred to a separatory funnel and shaken intensively for 1 min. The hexane layer was collected in a 25-ml volumetric flask. The aqueous layer was returned to the original vessel and the procedure was repeated. The hexane layer was added to the volumetric flask which was then filled with *n*-hexane to 25 ml.

After incubation the evaporated PCBs were eluted from the sorbent directly in the original glass column by 10 ml of *n*-hexane; the eluate volume was made up to 10 ml and the solution was analysed by GC.

PCB analysis

Samples were analysed by GC (HP 5890) with H_2 as a carrier gas (60 kPa, 1.5 ml min^{-1}, split-splitless inlet mode), using an electron capture detector (280°C, make up gas N_2 at 60 ml min^{-1}), and a fused-silica capillary column (50 m × 0.32 mm i.d.) with a non-polar stationary phase HP 1 (thickness 0.17 μm). The injector temperature was 250°C. The column was held at 45°C for 30 s, then increased to 150°C at 20° min^{-1}, followed by an increase to 250°C at 2° min^{-1} and finally held at 250°C for 6 min. Identification of peaks and their calibration was made according to Krupčík *et al* [5]. The reproducibility of the quantitative analysis was controlled using a standard solution containing 20 μg ml^{-1} of Delor 103 and Delor 106 (2:1, w/w). Relative deviations for congeners that did not interfere with the background were around 3%. Individual congeners with the corresponding peak number, IUPAC number and the chlorine substitution pattern are given in Haluška *et al* [4].

Results and discussion

During incubation two processes take place simultaneously: degradation and evaporation of PCBs. For a description of PCB degradation and evaporation kinetics it is assumed that both degradation and evaporation of PCBs can be described by first-order kinetics.

The temporal decrease of the concentration of each PCB congener in the medium (c_l) caused by evaporation and degradation (characterised by the rate constants k with the subscripts *ev* and *met*, respectively) can be described as:

$$-\frac{dc_l}{dt} = c_l\,(k_{ev} + k_{met}) \qquad (1)$$

After integration of Equation (1) the time course of c_l is:

$$c_l(t) = c_0\, e^{-(k_{ev} + k_{met})t} \qquad (2)$$

where c_0 is the initial concentration.

If PCB are adsorbed on the sorbent immediately after evaporation from the aqueous phase, their concentration in the gaseous phase above the surface of the medium is negligibly low. Then the increase of the amount of each PCB congener on the sorbent (m_s) is proportional to the congener concentration in the medium:

$$\frac{dm_s}{dt} = k_{ev}\, V_l\, c_l(t) = k_{ev}\, V_l\, c_0\, e^{-(k_{ev}+k_{met})t} \quad (3)$$

The proportionality constant is represented by the evaporation rate constant k_{met} and the volume of the aqueous medium V_l.

Integration of Equation (3) provides for the kinetics of sorption:

$$m_s = \frac{k_{ev}\, V_l\, c_0}{k_{ev} + k_{met}} (1 - e^{-(k_{ev}+k_{met})t}) \quad (4)$$

For the concentration evaporated from the medium (c_{ev}) it can be written analogously:

$$c_{ev} = \frac{m_s}{V_l} = \frac{k_{ev}\, c_0}{k_{ev} + k_{met}} (1 - e^{-(k_{ev}+k_{met})t}) \quad (5)$$

Equations (2) and (5) in the form $c_l = a_1 \exp(-b_1 t)$ and $c_{ev} = a_2\,[1 - \exp(-b_2 t)]$, respectively, can be fitted by nonlinear regression analysis to the experimentally determined time courses of the concentration of each PCB congener in the aqueous phase (c_l) and on the sorbent (c_{ev}).

The values of evaporation and metabolism rate constants for individual PCB congeners can be obtained using the regression parameters a_1, a_2, b_1, b_2:

$$k_{ev} = b_1\, a_2/a_1 \quad (6)$$

and

$$k_{met} = b_1 - k_{ev} \quad (7)$$

A typical decrease of the PCB congener concentration in the medium (c_l) and its increase on the sorbent (c_{ev}) during incubation with *P. stutzeri* is illustrated in Figure 2. The values of evaporation and degradation rate constants for both bacterial strains were calculated from Eqns 6 and 7. Their values for the individual PCB congeners are given in Table 1. However, some of the chromatographic peaks include more than one PCB congener [4]. The rate constants determined from these peaks represent the weighted average of the rate constants of the co-eluting congeners. Their values, together with the evaporation and degradation rate constants for individual PCB congeners, are shown in Figures 3 and 4 for *P. stutzeri* and in Figures 5 and 6 for *A. xylosoxidans*.

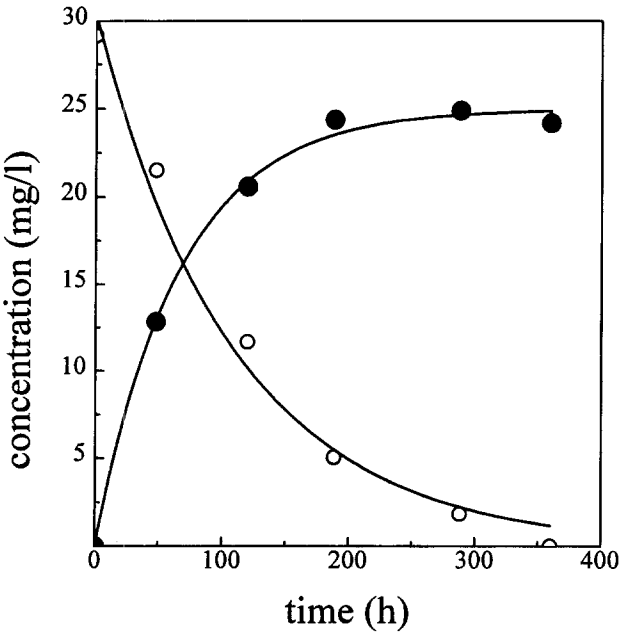

Figure 2 The time course of the concentration of 2,3′-dichlorobiphenyl (peak No. 3) in DMA medium (○) and on the sorbent (●) during incubation with *Pseudomonas stutzeri*. Conditions: DMA medium (pH 6.7), initial concentration of PCB 10 μg ml^{-1}, aerobic incubation for 15 days on a rotary shaker (180 rpm), 28°C, initial concentration of inoculum 0.5 g d.w. L^{-1}.

Chlorine substituents make the biphenyl skeleton less volatile and less degradable. The magnitude of k_{ev} decreased, in the presence of *P. stutzeri*, from 7.47×10^{-3} (h^{-1}) for dichlorobiphenyls to 1.0×10^{-4} (h^{-1}) for pentachlorobiphenyls and in the presence of *A. xylosoxidans* from 1.21×10^{-2} (h^{-1}) to 1.30×10^{-4} (h^{-1}) (Table 1). The differences between values of the rate constants are large. The highest rate of degradation was observed for congeners included in peak No. 2 (2,4- and 2,5-dichlorobiphenyl) in the experiments with both bacteria (Figures 4 and 6), but predominantly for *A. xylosoxidans* ($k_{met} = 0.126$ h^{-1}).

On the other hand, peak No. 1, containing two congeners with two chlorine atoms in the *ortho* position (2,2′-dichlorobiphenyl with chlorines on both rings and 2,6-dichlorobiphenyl on a single ring), showed a lower degradation rate in the experiment with *P. stutzeri* and striking resistance to degradation by *A. xylosoxidans*, as illustrated in Figures 4 and 6. These observations agree with those of Furukawa's [3]. The degradation rate of congeners included in peaks No. 3 (2,3′-dichlorobiphenyl), No. 4 (2,3- and 2,4′-dichlorobiphenyl) and No. 7 (4,4′-dichlorobiphenyl and 2,2′,4-trichlorobiphenyl) are high in comparison to other congeners. It can be seen from Figures 4 and 6, as well as from Table 1, that degradation of di- and trichlorobiphenyls strongly depends on the congener structure. From a comparison of evaporation and degradation rate constants obtained for both bacterial strains it can be concluded that: (1) the degradation rate of dichlorinated congeners is comparable with the evaporation rate; (2) the evaporation rate of trichlorinated congeners is higher by one order of magnitude than the degradation; (3) the rates of evaporation and degradation of tetra- and pentachlorinated congeners are comparable.

Although long-term biodegradation experiments in soils seem not to be influenced by evaporative losses of PCBs as adsorption to the soil matrix prevents evaporative losses, the influence of evaporation on degradation in liquid media cannot be neglected. Therefore it is necessary to evaluate degradation and evaporation simultaneously. When a particular contribution of evaporation to the loss of PCBs from the medium is known, it is possible to quantify the contribution of degradation, if the total loss of PCBs from the liquid medium during the degradation is determined.

Acknowledgements

Financial support from the Slovak Grant Agency (Grant No. 1/990969/93) and NATO International Scientific Exchange Programs (Linkage Grant Envir.LG.940637) is gratefully acknowledged. This work was also carried out

Table 1 The rate constants of degradation and evaporation for individual PCB congeners in the presence of *Pseudomonas stutzeri* or *Alcaligenes xylosoxidans* (0.5 g d.w. L^{-1}). Incubation was carried out in DMA medium pH 6.7 with an initial concentration of 10 mg L^{-1} PCB on a rotary shaker in the dark at 28°C for 15 days

			Microorganism			
			Pseudomonas stutzeri		*Alcaligenes xylosoxidans*	
No. chlorines	Substitution	Peak number	$k_{ev}\cdot10^4$ (h^{-1})	$k_{met}\cdot10^4$ (h^{-1})	$k_{ev}\cdot10^4$ (h^{-1})	$k_{met}\cdot10^4$ (h^{-1})
2	2,3′	3	74.7	16.1	121.1	68.7
3	2,2′,6	5	57.8	12.1	–	–
	2,2′,5	6	–	–	60.8	10.9
	2,3′,5	11	39.6	3.44	27.7	11.8
	2,3′,4	12	42.5	0.57	29.4	14.5
	2,4′,5	13	31.7	3.87	24.1	12.8
	2,4,4′	14	26.4	2.88	21.9	8.4
	3,4,4′	24	5.81	6.57	–	–
4	2,2′,3,6	17	26.9	5.84	20.1	11.2
	2,2′,3,6′	18	26.3	5.47	17.3	11.5
	2,2′,5,5′	19	13.4	7.86	9.9	8.7
	2,2′,4,5′	20	14.5	7.82	10.0	9.1
	2,2′,4,5	21	14.2	5.45	10.0	9.8
	2,2′,3,5′	23	11.0	7.88	7.6	9.4
	2,2′,3,4′	25	10.3	7.36	6.8	8.5
	2,2′,3,3′	27	7.82	8.72	5.8	10.4
	2,3′,4,4′	32	–	–	3.9	16.0
5	2,2′,3,4,6′	36	–	–	2.6	10.3
	2,2′,3,4,5	40	1.28	7.21	1.7	9.0
	2,3,3′,5,5′	41	1.22	7.37	1.7	9.9
	2,3,3′,4′,6	43	1.00	6.24	1.3	7.6
	2,2′,3,3′,4	44	4.86	3.22	–	–

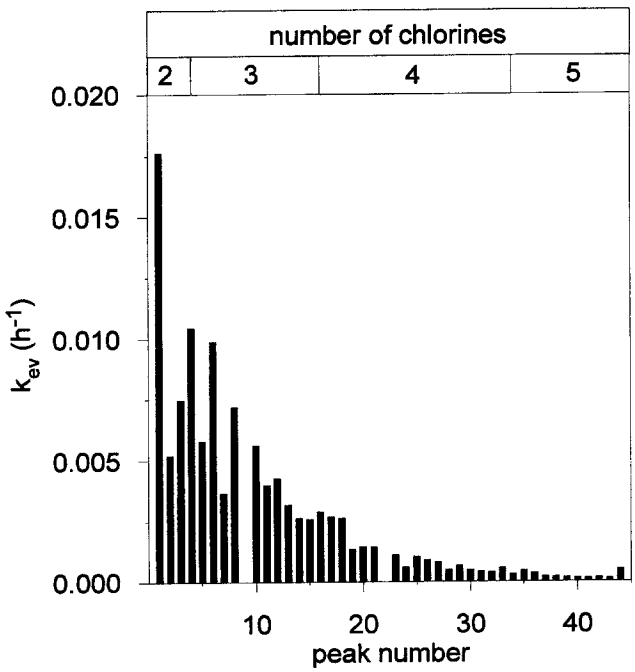

Figure 3 PCB evaporation rate constants (k_{ev}) during degradation by *Pseudomonas stutzeri* for individual peaks. DMA medium, initial concentration of PCB 10 μg ml^{-1}, 15 days, aeration in dark at 28°C, rotary shaker (180 rpm), inoculum (0.5 g d.w. L^{-1}). The composition of individual peaks, IUPAC number and the chlorine substitution pattern are given in [4].

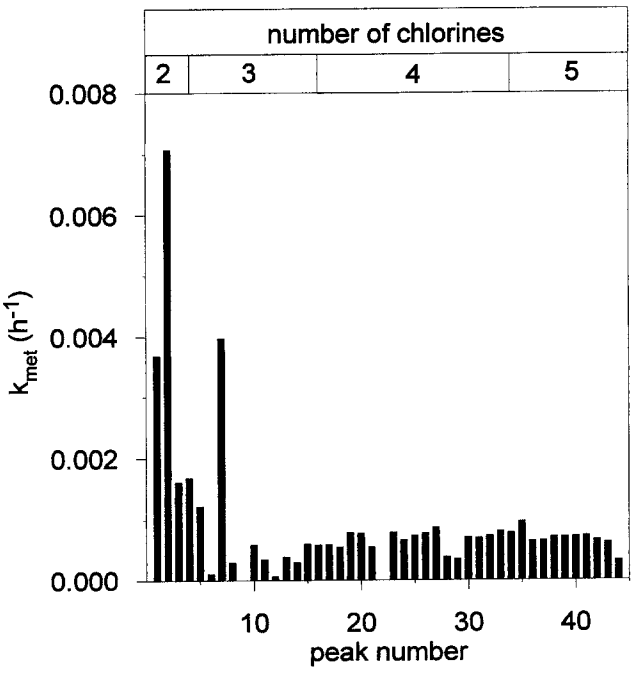

Figure 4 PCB degradation rate constants (k_{met}) determined for *Pseudomonas stutzeri* for individual peaks. DMA medium, initial concentration of PCB 10 μg ml^{-1}, 15 days, aeration in dark at 28°C, rotary shaker (180 rpm), inoculum (0.5 g d.w. L^{-1}) adapted on biphenyl (7 days, 2.5 g L^{-1}).

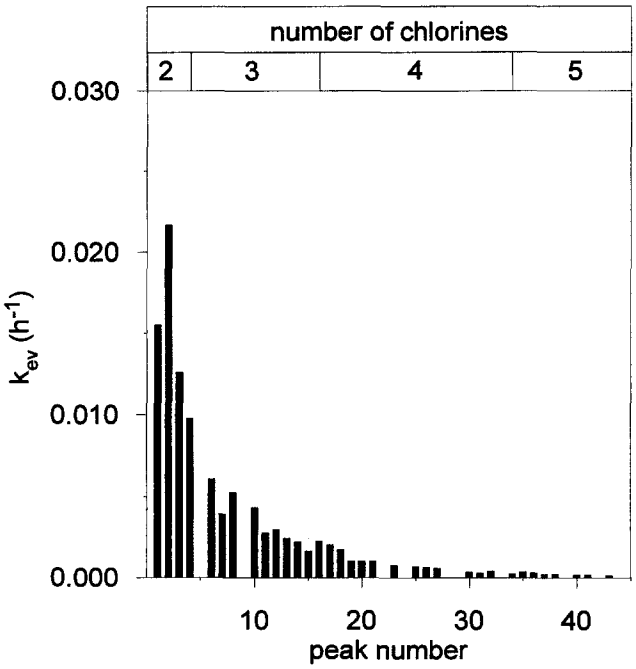

Figure 5 PCB evaporation rate constants (k_{ev}) determined for *Alcaligenes xylosoxidans*. Incubation conditions as in Figure 3.

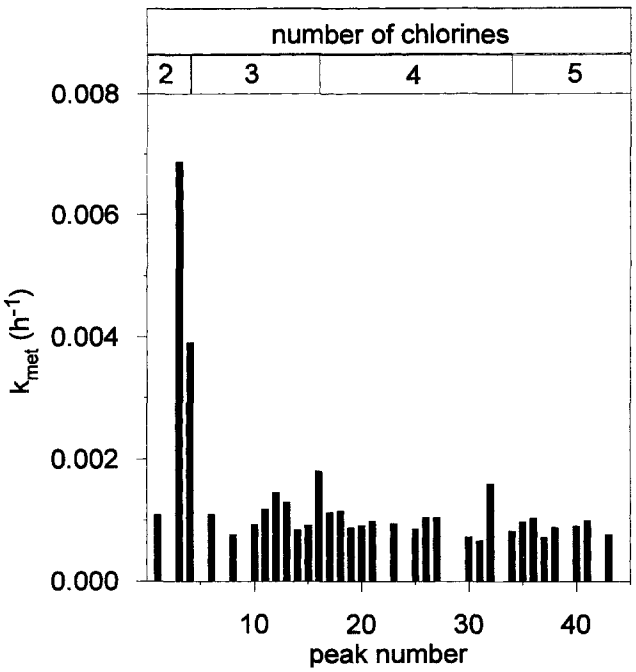

Figure 6 PCB degradation rate constants (k_{met}) determined for *Alcaligenes xylosoxidans*. Incubation conditions as in Figure 4. The value of k_{met} for peak No. 2 is not shown because it was out of range shown. It was 0.126 h^{-1}.

under the framework of the project Quantitative Structure-Activity Relationships for Predicting Fate and Effects of Chemicals in the Environment, financially supported by the Environmental Technologies RTD Programme (DG XII/D-1) of the Commission of the European Communities under contract number EV5V-CT92-0211. Financial support from the European Communities is gratefully acknowledged.

Symbols

a_1,b_1,a_2,b_2	fitting parameters
c_0	initial concentration of PCB congener in liquid medium
c_l	concentration of PCB congener in liquid medium
c_{ev}	concentration of PCB congener in sorbent
k_{ev}	rate constant of PCB congener evaporation
k_{met}	rate constant of PCB congener metabolization
n_s	amount of PCB congener in sorbent
$t_{1/2}$	half-time of evaporation
V_l	volume of liquid medium

References

1 Dercová K, Š Baláž, L' Haluška, V Horňák and V Holecová. 1995. Degradation of PCB by bacteria isolated from long-time contaminated soil. Int J Environ Anal Chem 58: 337–348.
2 Fava F, S Zappoli, L Marchetti and L Morselli. 1991. Biodegradation of chlorinated biphenyls (Fenclor 42) in batch cultures with mixed and pure aerobic cultures. Chemosphere 22: 3–14.
3 Furukawa K. 1982. Microbial degradation of polychlorinated biphenyls. In: Biodegradation and Detoxification of Environmental Pollutants (Chackrabarty AM, ed), pp 34–57, CRC Press, Boca Raton, FL.
4 Haluška L', Š Baláž, K Dercová, E Benická, J Krupčík, P Bielek and G Lindišová. 1995. Anaerobic degradation of PCB in soils. Int J Environ Anal Chem 58: 327–336.
5 Krupčík J, A Kočan, J Petrík, PA Leclerq and K Ballschmiter. 1992. On the use of reference standards for quantitative trace analysis of PCBs by HRGC analyses of technical PCB formulations by HRGC/FID. Chromatographia 33: 514–552.
6 Pirt SJ. 1967. A kinetic study of the mode of growth of surface colonies of bacteria and fungi. J Gen Microbiol 47: 181–197.
7 Vrana B, K Dercová and Š Baláž. 1995. Monitoring evaporation polychlorinated biphenyls (PCB) in long-term degradation experiments. Biotechnol Tech 9: 333–338.
8 Vrana B, K Dercová and Š Baláž. 1996. Evaporation kinetics of polychlorinated biphenyls (PCB) during biodegradation experiments. Biotechnol Tech 10: 37–40.