Four-compartmental kinetic model of the simultaneous processes occurring throughout biodegradation of polychlorinated biphenyls in the active bacterial cell suspension

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Abstract

Kinetics of distribution of polychlorinated biphenyls (PCBs) in an active bacterial suspension of *Pseudomonas stutzeri* was studied by monitoring the evaporated amounts and the concentration remaining in the liquid medium containing the biomass of the degrader bacterium. A model considering biosorption, evaporation, and primary biodegradation of individual PCB congeners was constructed using the methods of subcellular toxicokinetics. The time hierarchy of the underlying processes (uptake/release of the congeners to/from biomass is much faster than evaporation and degradation) allowed for construction of a simplified disposition function, i.e. the time dependence of the intra- and extracellular PCB concentrations. The resulting model was fitted to the experimental data, with the parameters characterising biosorption and evaporation determined in separate experiments. In this way, the biodegradation rate constants were determined for individual congeners of the technical PCB mixture DELOR 103. Biodegradability decreases with increasing number of chlorines in the molecule, especially if they are located in the *ortho-* and *para-* positions. On the other hand, the increasing number of free 2,3-positions or their equivalents on the biphenyl skeleton leads to an increased biodegradability. A quantitative structure-degradability relationship with these variables was formulated for 2- and 3-chlorinated congeners.

Keywords: biosorption, biodegradation, evaporation, polychlorinated biphenyls

Introduction

Polychlorinated biphenyls (PCBs) are highly persistent pollutants that have been largely produced in the past decades. The production was stopped after they had been proven to be toxic to humans (Langer et al. 2006). Once in the environment, they tend to accumulate in different stages of the food chain and besides their toxicity, they are also carcinogenic (McLachlan 1996). Environmental and economic reasons have urged the development of bioremediation technologies for the PCB removal from contaminated sites. A crucial step is the isolation or genetic construction of microbial strains with biodegradation potential. Several aerobic microorganisms are able to biodegrade some, usually less chlorinated, PCB congeners (Furukawa et al. 1978; Sylvestre et al. 1982; Bedard et al. 1987; Kohler et al. 1988).

Although the complete PCB degradation is the ultimate goal, often only partial solutions of this complex problem can be reached resulting in elimination of the most harmful properties (Pitter and Chudoba 1990). For PCBs, the top priority is to lower their bioaccumulation potential or, equivalently, lipophilicity. In the aerobic biodegradation process, dioxygenases introduce hydroxyl groups into the PCB molecule, lower its lipophilicity and make further cleavage easier (Sondossi et al. 1991). The initial oxidation and following ring cleavage seem to be the rate-limiting steps of the process.

Screening of the metabolic activity of microorganisms was often based on monitoring of the PCB concentration remaining in the medium and the obtained data suggested rapid elimination of PCBs in the aquatic environment (Sugiura 1992). The biodegradation rate was considered to depend only on the aqueous solubility of congeners, being not affected by the substitution pattern of the congener. These false positive results were a consequence of neglecting volatility of PCBs in the aqueous environment in open systems, including cultivation vessels closed with cotton wool stoppers or aerated reactors without the air-stripping monitoring. In a similar way, the degraded amount of a hydrophobic pollutant in continuous bioreactor systems is often calculated as the difference between its effluent and influent concentrations, without quantitative consideration of evaporation and biosorption (Tsezos and Seto 1986).

The biodegradation rate constants are important parameters determining how long it takes to eliminate a PCB congener from the contaminated site. However, there are only few published kinetic studies on the aerobic PCB biodegradation in defined conditions to date.

In our previous works, a simple apparatus for effective monitoring of the PCB evaporation kinetics in batch biodegradation experiments was described (Vrana et al. 1995; Vrana et al. 1996; Dercová et al. 1996), together with a simplified mathematical model (Dercová et al. 1999) and improvement of biodegradation in the presence of terpenes serving as inductors of biphenyldioxygenase metabolic pathways (Tandlich et al., 2001).

The goals of the present work were: (1) to derive a PCB distribution model describing the concurrent processes of evaporation, biosorption, and biodegradation in a suspension of a PCB-degrading bacterium; (2) to use the model for the determination of the primary biodegradation rate constants of individual PCB congeners present in a commercial PCB mixture DELOR 103, in a *Pseudomonas stutzeri* suspension; (3) to conduct an empirical structure-degradability analysis of the obtained data.

Materials and Methods

Chemicals

A commercial mixture of PCB, DELOR 103 containing 40 - 42 % (w/v) of bound chlorine (ex-product of Chemko Strážske, Slovakia), biphenyl (Merck, Germany), n-hexane; pesticide grade (Merck, Germany), acetone p. a. (Mikrochem Bratislava, Slovakia), chemicals for mineral media (Lachema Brno, Czech Republic), octadecylsilica (C18) resin SILIPOR (55-105 μm) (Lachema Brno, Czech Republic), and 3-chlorobenzoic acid (Merck, Germany).

Microorganisms

A PCB degrading bacterium *Pseudomonas stutzeri*, obtained from a long-term contaminated soil by enrichment in synthetic mineral DMA medium with biphenyl as the sole carbon source (Dercová et al., 1995), was used for experiments.

Biomass dry weight determination

For determination of the biomass dry weight a concentrated cell suspension was diluted with appropriate amount of the DMA medium, to give an optical density ranging from 0.1 to 1.0, when measured at 620 nm. A portion (10 ml) of each dilution was filtered through a labelled, dried, and pre-weighed membrane filter (SYNPOR, pore diameter 0.4 μm). All filters were then dried to a constant weight at 105 °C and placed into a desiccator to cool down. The biomass dry weight was calculated, as the difference of the filter weight before and after the procedure. At the end, the calibration curve was constructed as the dependence of the optical

density on the biomass dry weight concentration. The concentrated biomass (100 g d.w.l^{-1}) was diluted in DMA medium to the final concentration 4.0 g d.w.l^{-1}).

Biodegradation assay

The bacterial culture was prepared by incubating *P. stutzeri* for 7 days in the DMA medium (pH 7.2) (Dercová et al., 1996) containing 2.5 g l^{-1} biphenyl at 28 °C to the resulting concentration 1.5 g d.w.l^{-1}. Biomass was filtered through a paper filter on a Büchner funnel to remove the remaining biphenyl crystals. The filtrate was then centrifuged for 20 min at 3500 rpm. After removing the supernatant, the biomass was resuspended in equal volume of DMA medium (pH 7.2). The centrifugation was repeated under the same conditions as in the first step and after removing the supernatant the biomass was resuspended in the DMA medium, to give one hundredth of the initial medium volume.

Incubation was carried out in Erlenmeyer flasks (100 ml) closed with a glass column filled with the C18 sorbent. The apparatus for experiment was detailed described in Vrana et al. (1995). The stock solution of PCBs was prepared from the commercial mixture of PCBs DELOR 103 dissolved in acetone (100 ml) with the final concentration 4 mg.ml^{-1}. The biomass suspension (20 ml) with the final concentration 4.0 g d.w.l^{-1} was mixed with 50 µl of the PCB stock solution (the final concentration 10 µg.ml^{-1}). The flasks were equipped with the sorbent columns that were closed with a cotton wool stopper, to maintain a sterile environment and allow for gas diffusion. Flasks were incubated for 25 days on a rotary shaker (180 rpm) at 28 °C in the dark. Whole flasks were taken periodically every day for PCB analysis. The amount of PCBs in the liquid medium and on the sorbent was analysed.

Extraction of PCBs

After incubation, the flask was filled with a mixture of n-hexane and acetone (9:1, 10 ml) and PCBs were extracted in an ultrasonic bath for 15 min. The whole volume of the vessel was then transferred to a separatory funnel and intensively shaken for 1 min. The hexane layer was collected into a 25 ml volumetric flask. The aqueous layer was returned to the original vessel and the whole procedure was repeated. The two hexane layers were dried over anhydrous Na2SO$_4$, collected in the volumetric flask, filled up with n-hexane to 25 ml, and analyzed by GC-ECD.

After incubation, the evaporated and trapped PCBs were eluted from the sorbent in the glass column by 10 ml of n-hexane and the solution was analysed by GC.

PCB analysis

PCB extracts were analysed by GC (HP 5890) with H2 as a carrier gas (60 kPa, 1.5 ml.min^{-1}, split-splitless inlet mode), equipped with an electron capture detector (ECD; 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). Temperature conditions: injector 250 °C, column 45 °C (0.5 min - 20 °C/min - 150 °C - 2 °C/min - 250 °C (6 min). Identification of peaks and their calibration was made according to Krupčík et al. (1992). The reproducibility of the quantitative analysis was controlled using the standard solution of DELOR 103 (c = 20 μg ml^{-1}).

Cell counting

DMA agar was prepared in similar way as the synthetic DMA medium, except that the medium components were mixed in the warm (50 °C) agar solution (20 g of agar dissolved in 1 l of distilled water, autoclaved for 15 min at 120 kPa), and transferred into Petri dishes. After proper dilution, the biomass suspension from the incubation vessel (0.1 ml) was applied onto the solidified agar layer. The incubation was carried out in the upside-down position, with a few biphenyl crystals spread over the empty bottom lid. Viable cells were counted as colony forming units per ml (CFU.ml^{-1}) in three series after 48-h incubation at 37 °C. The viable cells were counted at the inoculation time and then once a week during the PCB degradation assay.

Model of PCB distribution

The fate of PCB in the degrading bacterial suspension was described using an extension of the model of the PCB distribution in the non-degrading bacterial biomass suspension (B. Vrana, non-published). This model demonstrated satisfactory agreement with the experimental data and was used to determine the PCB biosorption equilibria. The aim of this work was to incorporate the intracellular metabolism of PCBs into the model with the intention to determine metabolism rate parameters of individual congeners in a PCB mixture DELOR 103.

Model Construction

Diffusion of organic molecules in the bulks of the model compartments is assumed to be fast due to either sufficient mixing (the aqueous medium and the gaseous phase over it) or to small dimensions (cells of degrading bacterium). This fact warrants the replacement of the second order partial differential equations based on the Fick second law by a set of the first order linear differential equations. This approach is common in the methods of subcellular toxicokinetics (Baláž et al. 1992).

Enzymatic reactions are frequently described by the Michaelis-Menten kinetics:

$$-\frac{dm_m}{dt} = -\frac{v_m V_m c_m}{K_m + c_m} \cong \frac{v_m V_m}{K_m} c_m = \kappa_d c_m \qquad (1)$$

where v_m is the maximum (saturation) rate of the reaction, c_m is the concentration of the chemical in the surroundings of the enzyme, V_m is the volume of the system (of the biomass in our case), and K_m is the Michaelis constant. When the initial chemical concentration is well below the value of the Michaelis constant ($c << K_m$), the Michaelis-Menten equation can be reduced to the expression given by the second equality in Eq. 1. Consequently, biodegradation was treated as the first-order process, in addition to evaporation and biosorption that were shown to follow the first-order kinetics in previous studies (Vrana et al. 1996). A scheme of the processes governing the fate of PCBs is outlined in Fig. 1. Each congener in the mixture is assumed to behave independently. At the beginning of the experiment, the PCB mixture is spiked into the aqueous medium, each congener in the amount m_0. After biomass addition, each congener can be (1) partitioned into and (2) released from the biomass, (3) metabolised inside the cells, and (4) evaporated. Previous experiments (Vrana et al. 1995) showed that, after evaporation, each congener is immediately and irreversibly bound to the sorbent. This means the gas-phase PCB concentration is close to zero at each moment and evaporation is irreversible. The uptake into and release from the cells are treated as fully-reversible processes as confirmed in our previous study (Dercová et al. 1999).

The presented model can be described by a set of the first-order linear differential equations with constant coefficients. Only the subsystem of reversibly connected compartments (aqueous phase and biomass) needs to be included in the first step. The PCB

distribution in the remaining compartments can be calculated using the solutions for the aqueous phase and biomass. In the aqueous phase, the free PCB concentration is of interest because only free molecules can be evaporated or transported from the aqueous phase. The relationship between the total (subscript *a*) and free (*af*) concentration can be expressed from the definition of the association constant K_b

$$c_a = c_{af} (1 + K_b b)$$ (2)

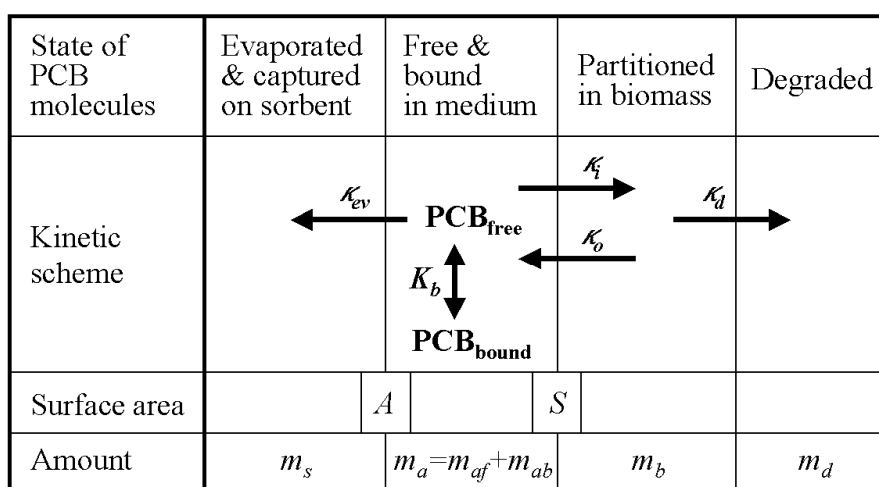| State of PCB molecules	Evaporated & captured on sorbent	Free & bound in medium	Partitioned in biomass	Degraded
Kinetic scheme	κ_{ev}	PCB_{free} K_b $\text{PCB}_{\text{bound}}$	κ_i κ_o	κ_d
Surface area		A	S	
Amount	m_s	$m_a = m_{af} + m_{ab}$	m_b	m_d

Fig. 1. A kinetic scheme of the fate of a PCB congeners in the degrading bacterial biomass suspension. The rate constants κ characterise four concurrent processes: evaporation (subscript *ev*) through the air/water interface *A*, uptake into the biomass (*i*), release from the biomass (*o*), both through the membrane/water interface *S*, and intracellular degradation (*d*). Binding to the extracellular colloidal particles is much faster than the above processes and is described by the association constant K_b. Distribution is expressed in the terms of amounts *m*. The evaporated molecules bind immediately and irreversibly to the sorbent (subscript *s*). In the extracellular aqueous phase (*a*), the congener molecules are present as free (*af*) and bound (*ab*). After partitioning in the biomass (*m*), the congener molecules can be degraded (*d*).

The concentration of the binding sites on the colloidal material (*b*) is assumed to be much higher than the concentration of the congener and, therefore, not influenced by the PCB binding. The loss of the congener from the aqueous phase is given by the rate of participating processes

$$-\frac{dm_a}{dt} = \left(\kappa_i S + \kappa_{ev} A\right)c_{af} - \kappa_o S\, c_m \tag{3}$$

The amounts will be used as variables in the set of differential equations because the fate of the congeners is monitored as evaporated amount captured on the sorbent and the amount remaining in the liquid medium and biomass. The free PCB concentration in the aqueous phase will be first substituted by the total aqueous concentration using Eq. 2. After these conversions, Eq. 3 reads

$$-\frac{dm_a}{dt} = \left(k_i + k_{ev}\right)m_a - k_o c_m \tag{4}$$

In a similar way, the mass balance for the biomass can be written as

$$-\frac{dm_m}{dt} = -k_i m_a + \left(k_o + k_d\right)m_m \tag{4}$$

The amount-based rate parameters k includes the volumes of individual compartments and their interfacial areas:

$$k_i = \frac{\kappa_i S}{V_a(1 + K_b b)} \tag{5}$$

$$k_o = \frac{\kappa_o S}{V_m} \tag{6}$$

$$k_d = \frac{\kappa_d}{V_m} = \frac{v_m}{K_m} \tag{7}$$

$$k_{ev} = \frac{\kappa_{ev} A}{V_a(1 + K_b b)} \tag{8}$$

The system of linear differential equations (Eqs. 3 and 4) can be solved using the Laplace transform methods for the boundary condition that at the beginning of the experiment the congener is only present in the aqueous phase in the amount m_0 as

$$m_a(t) = \frac{m_0}{\lambda_1 - \lambda_2}\left[\left(\lambda_1 - k_o - k_d\right)e^{-\lambda_1 t} - \left(\lambda_2 - k_o - k_d\right)e^{-\lambda_2 t}\right] \tag{9}$$

$$m_m(t) = \frac{m_0 k_i}{\lambda_1 - \lambda_2}(e^{-\lambda_2 t} - e^{-\lambda_1 t}) \tag{10}$$

where

$$\lambda_1 = \frac{1}{2}\left(k_i + k_o + k_{ev} + k_d + \sqrt{D}\right) \tag{11}$$

$$\lambda_2 = \frac{1}{2}\left(k_i + k_o + k_{ev} + k_d - \sqrt{D}\right) \tag{12}$$

$$\sqrt{D} = (k_i + k_o + k_{ev} + k_d)\sqrt{1 - \frac{4(k_o k_{ev} + k_i k_d + k_{ev} k_d)}{(k_i + k_o + k_{ev} + k_d)^2}} \tag{13}$$

The rate of PCB evaporation from the medium and practically immediate binding on the sorbent is described by first-order kinetics and can be obtained as

$$m_s(t) = \int_0^t m_a(t)dt \tag{14}$$

The time course of the congener amount on the sorbent, $m_s(t)$, can be described by the solution of Eq. 14 as

$$m_s(t) = \frac{k_{ev} m_0}{\sqrt{D}}\left[\frac{\lambda_1 - k_o - k_d}{\lambda_1}(1 - e^{-\lambda_1 t}) - \frac{\lambda_2 - k_o - k_d}{\lambda_2}(1 - e^{-\lambda_2 t})\right] \tag{15}$$

The solutions of the differential equations, describing PCB distribution in the experimental system (Eqs. 9, 10 and 15), represent the so-called disposition functions of subcellular toxicokinetics (Baláž et al. 1992).

Model Simplification

Transport of PCBs in the biomass-medium system, their evaporation, and elimination proceed on different time scales. The widely different rates of these processes in the experimental system can be use to simplify the disposition functions to the forms complying with the information comprised in experimental data.

Transport of non-amphiphilic chemicals in cellular suspensions is usually a fast process finished within seconds or minutes (Kubinyi 1978). The transport rate parameters for entering (κ_i) and leaving the membrane (κ_o) depend on the membrane/water partition coefficient $P_m = \kappa_i / \kappa_o$ as

$$\kappa_i = \frac{aP_m}{bP_m + 1} \cong \frac{a}{b} \tag{16}$$

$$\kappa_o = \frac{a}{bP_m + 1} \cong \frac{a}{bP_m} \tag{17}$$

The parameters a and b are specific for the experimental set up (the apparatus geometry, viscosity of the non-polar phase, agitation rate), but do not depend on structure of the transported molecules. The second equalities in Eqs. 16 and 17 are valid for compounds with high values of the equilibrium parameter P that are typical for extremely hydrophobic chemicals like PCB. Eq. 18 indicates that κ_o for individual congeners are several orders of magnitude lower than their κ_i values.

Evaporation and degradation are much slower processes, the time scale is days even for less chlorinated congeners. The time hierarchy of the processes governing the fate of PCBs in the degrading bacterial suspension can be written as $\kappa_i + \kappa_o \gg \kappa_{ev} + \kappa_d$ or $k_i + k_o \gg k_{ev} + k_d$. The second expression, in terms of the amount-based rate parameters k, is warranted by identity of the denominators in the definitions of the pairs k_i and k_{ev} (Eqs. 5 and 8) as well as k_o and k_d (Eqs. 6 and 7). The expression for D (Eq. 13) can be rewritten as

$$\sqrt{D} \cong (k_i + k_o + k_{ev} + k_d)[1 - \frac{2(k_o k_{ev} + k_i k_d + k_{ev} k_d)}{(k_i + k_o + k_{ev} + k_d)^2}] \tag{18}$$

The second equality is based on the substitution of the square root expression $\sqrt{1-x}$ by the first two terms of its Taylor expansion ($\sqrt{1-x} \cong 1 - x/2$) that is sufficiently precise for $x <$ 0.1. For individually standing $\lambda_1 - \lambda_2 = \sqrt{D}$ we get

$$\sqrt{D} \cong k_i + k_o \tag{19}$$

Using the time hierarchy ($k_i + k_o >> k_{ev} + k_d$), Eq. 11 as combined with Eq. 19 provides

$$\lambda_1 \cong k_i + k_o \tag{20}$$

Eq. 12, with D expressed by Eq. 19, results in

$$\lambda_1 = k_a + k_d \tag{21}$$

The second near-equality originated from neglecting some terms due to application of the time hierarchy ($k_i + k_o >> k_{ev} + k_d$): in the numerator, $k_{ev}k_d$ and in the denominator, k_{ev} and k_d. The equilibrium constant is $K = k_i/k_o$.

Since $\lambda_2 \approx \dfrac{k_{ev}K}{K+1}$, $e^{-\lambda_2 t}$ will determine the time course of the PCB amounts in various states because the exponential $e^{-\lambda_1 t}$ will vanish after a much shorter time than $e^{-\lambda_2 t}$. For a substantial portion of the duration of the experiment but initial moments, the term $e^{-\lambda_1 t}$ can be neglected in Eqs. 9, 10 and 15. The time courses of the PCB amounts were experimentally monitored in whole liquid medium (with biomass) and on the sorbent. The expression for the whole medium, the sum of Eqs. 9 and 10, will become, after application of the time hierarchy ($k_i + k_o >> k_{ev} + k_d$)

$$m_l(t) = m_a(t) + m_m(t) \cong \frac{m_0}{\lambda_1 - \lambda_2}(k_i + k_o + k_d - \lambda_2)e^{-\lambda_2 t} \cong m_0 e^{-\frac{k_{ev} + Kk_d}{K+1}t} \tag{22}$$

Eq. 15 describing the kinetics of PCB deposition on the sorbent will be changed, after application of the time hierarchy ($k_i + k_o >> k_{ev} + k_d$), to

$$m_s(t) \cong \frac{m_0 k_{ev}}{k_i + k_o}[1 - \frac{\lambda_2 - k_o - k_d}{\lambda_2}(1 - e^{-\lambda_2 t})] \cong \frac{m_0 k_{ev}}{Kk_d + k_{ev}}(1 - e^{-\frac{k_{ev} + Kk_d}{K+1}t}) \tag{23}$$

The total amount of a congener in the system, $m_t(t)$, decreases during the incubation as a result of its proceeding biodegradation. This amount is equal to the sum of $m_l(t)$ and $m_s(t)$ given by Eqs. 22 and 23:

$$m_t(t) = m_l(t) + m_s(t) = m_0 \left(\frac{k_{ev}}{Kk_d + k_{ev}} + \frac{Kk_d}{Kk_d + k_{ev}} e^{-\frac{k_{ev} + Kk_d}{K+1}t} \right) \tag{24}$$

Eqs. 22 - 24 are simplified disposition functions suitable for fitting the experimentally determined time courses of the individual congeners concentration in individual compartments of the system using non-linear regression analysis.

Model Behavior

Simulation of the PCB distribution using the full disposition functions (Eqs. 9, 10, and 15) is shown if Fig. 2. The gradual increase of PCB in the biomass can be seen because unrealistically low values of the used transport rate parameters k_i and k_o have been used. In reality, the maximum on the curve m, representing the relative PCB amount in biomass will occur at a much shorter time after the start of the experiment. Therefore, the simplified disposition functions (Eqs. 22 - 24) are suitable description of the real situation (Fig. 3).
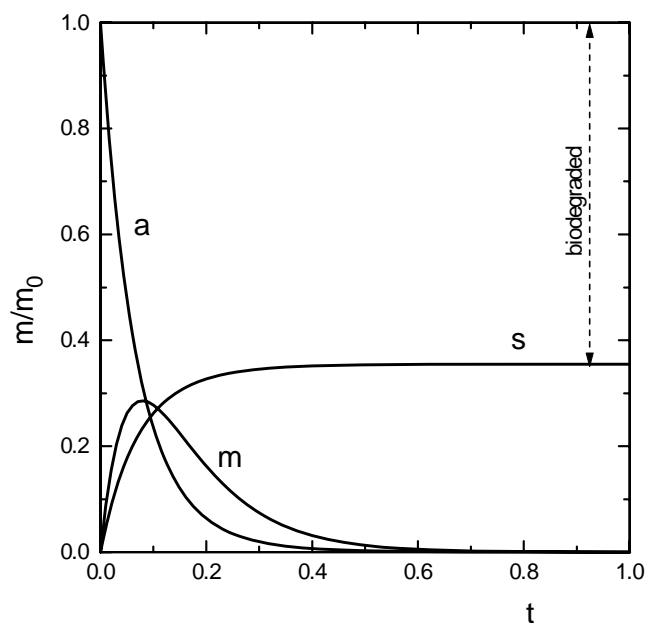

Fig. 2. Simulation of PCB distribution in an active bacterial suspension as outlined in Fig. 1. Four simultaneous processes are going on: evaporation ($k_{ev} = 5$), uptake into the biomass ($k_i = 10$) and release from the biomass ($k_o = 1$) and intracellular metabolism ($k_d = 10$). After evaporation, the congener is irreversibly bound to the sorbent (curve s). The relative amounts in the aqueous phase and in the biomass are represented by the curves a and m, respectively. Lines correspond to the full disposition functions (Eqs. 9, 10 and 15).
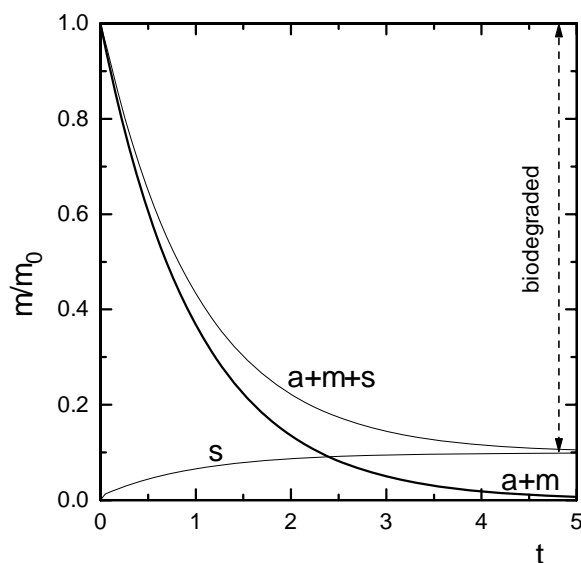
Fig. 3. Simulation of a PCB congener distribution in an active bacterial suspension. Three simultaneous processes are going on: evaporation ($k_{ev} = 1$), fast and reversible partitioning in the biomass (characterised by equilibrium constant $K = k_i/k_o = 10$) and intracellular metabolism ($k_d = 1$). After evaporation, the congener is irreversibly bound to the sorbent (curve *s*). The relative amounts in the whole liquid medium (in the aqueous phase and in the biomass) are described by the curve a+m. The lines correspond to simplified disposition functions (Eqs. 22 - 24).

Statistical Analyses

The experimental data were fitted to model functions by non-linear regression analysis (Origin, version 4.1). In order to utilise the available experimental information to the maximum possible extent, the time courses of the PCB amounts both in the whole liquid medium (including biomass) and captured on the sorbent after evaporation were fitted simultaneously to the respective Eqs. 22 and 23 in the form

$$m(t) = m_0 \left(\frac{k_{ev}}{Kk_d + k_{ev}} \right)^{\frac{z}{2}} \left[\frac{z}{2} - (z-1)e^{-\frac{k_{ev}+Kk_d}{K+1}t} \right] \tag{25}$$

The indicator variable z takes the value $z = 0$ for the amount *m* in the whole medium including biomass and $z = 2$ for the amount on the sorbent. The optimised parameters were the degradation rate constants k_d and the equilibrium constants K characterizing the partitioning of PCB congeners in the biomass. The values of the evaporation rate parameters k_{ev} were determined previously according to Vrana et. al. (1996) in the same experimental

system and were not optimised now. Quality of the fit was characterised by the standard errors of the optimised parameters, as well as by the correlation coefficient (r), the standard deviation (SD), and the Fisher criterion (F) of the relationship between calculated and experimental values.

Results and Discussion

Survival of Pseudomonas stutzeri

Table 1 shows the ability of *Pseudomonas stutzeri* to survive during the PCB degradation assay. The means of viable cell concentrations determined after proper incubation periods were compared with the initial concentration using one-way ANOVA test (Origin version 4.1) and found not to differ significantly at the 0.05 level during the whole PCB degradation assay, except after 12 days of incubation. The number of metabolically active cells and, consequently, activity of the PCB-degrading enzyme were treated as constant during the degradation assay in the model.

Table1. Survival of *Pseudomonas stutzeri* during the PCB degradation assay.

Incubation time (days)	CFU.ml^{-1}
0	$(7.90 \pm 2.63) \times 10^8$
5	$(5.30 \pm 0.66) \times 10^8$
12	$(2.80 \pm 0.56) \times 10^8$
19	$(5.15 \pm 3.15) \times 10^8$
25	$(6.40 \pm 2.40) \times 10^8$

Degradation Rate Constants

A typical decrease of the PCB congener amount in the medium $m_l(t)$ and its increase on the sorbent $m_s(t)$ during incubation with *Pseudomonas stutzeri* is illustrated in Fig. 4.

For all 31 peaks identified in the DELOR 103 mixture, the time courses of the amounts captured, after evaporation, on the sorbent (m_s) and remaining in the whole medium including biomass were fitted using Eq. 25. The evaporation rate parameters k_{ev} were determined in independent experiments (Vrana et al., 1996; Dercová et al. 1996). The optimised parameters were the degradation rate constant k_d and the equilibrium constants K characterizing the partitioning of the congeners in the biomass.
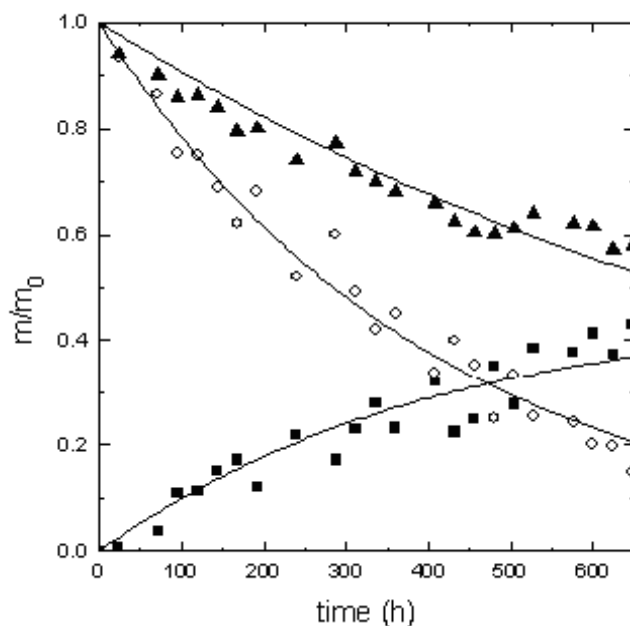
Fig. 4. The time course of the relative amount of 2,2′,5-trichlorobiphenyl (PCB 18) in the biomass suspension (o), on the sorbent (■) and their sum (▲) during incubation with *Pseudomonas stutzeri*. The lines correspond to Eqs. 22 - 24 with the optimised values of adjustable parameters given in Table 2. Incubation conditions: DMA medium, initial concentration of PCB 10 μg ml⁻¹, aerobic incubation for 25 days on a rotary shaker (180 rpm), 28 °C, inoculum (4 g d.w.l⁻¹) adapted on biphenyl (7 days, 2.5 g l⁻¹).

The sorption coefficients K_p are related to K as

$$K = K_p X \tag{26}$$

where X is the biomass concentration. The parameters $K_X = K_p + K_b \beta$ (K_b is the association constant for binding of PCB congeners to extracellular colloidal material containing β binding sites per gram of dry weight) were measured in the similar way as in the biodegradation assay, except the addition of 3-chlorobenzoic acid (5 mmol l⁻¹) inhibiting the PCB degradation (Dercová et al., 1995). The values of the constants K were also optimized because the K_p values could not be extracted from the values of K_X. The results are summarized in Table 2. In most cases, the fits were based on 24 time measurements and their quality was satisfactory at the 0.05 significance level. The correlation coefficient varies from 0.963 to 0.998, the highest standard deviation of the fit is S.D. = 90.924 and the Fisher test criterion is equal to or higher than 287 in all cases. The degradation rate constant k_d and the partitioning equilibrium constant K were significant in all the cases at the 0.05 significance level.

The sorption coefficients K_p were determined according to Eq. 26, as the values of the equilibrium constant K divided by the biomass concentration (4 g l^{-1}). The K_X values are in most cases slightly higher than the K_p values, indicating that the binding of PCB congeners to the extracellular colloidal material is possible.

Some chromatographic peaks include more than one congener (Krupčík et al. 1992) (peaks No. 1, 2, 4, 7, 8, 9, 13, 14, 22, 26, 27, 29, 30 and 31). In these cases, the time course of the cumulative amount of all congeners eluting in the given peak is monitored. The corresponding description consists of the sum of expressions given by Eq. 22 or 23, written for each congener. If the constants k_{ev}, k_d, and K were substantially different for the co-eluting congeners, the time courses of the amounts in the medium and on the sorbent would not be mono-exponential. This was not the case, all the time courses for co-eluting peaks were fitted using Eq. 25, in the same way as the data for the peaks containing separate congeners. The fits were of good quality and no systematic deviations indicating the multi-exponential behaviour were detected. This fact suggests that the constants characterising the fate of PCB (k_{ev}, k_d, and K) are not substantially different for the co-eluting congeners, at least within the experimental error of the used analytical techniques. In some peaks, one dominant congener can be found (Ballschmiter and Zell 1980): 2,2′,4-trichlorobiphenyl is probably dominant in peak No. 7, 2′,3,4-trichlorobiphenyl in peak No. 13, and 2,3,4′-trichlorobiphenyl in peak No. 14.

Table 2. Optimised values of the parameters characterising distribution of individual PCB congeners in the active biomass suspension of *Pseudomonas stutzeri*.

Peak	IUPAC #[a]	Chlorine positions	m_0 [μg]	$k_{ev} \times 10^4$ [h⁻¹][b]	$k_d \times 10^4$ [h⁻¹]	K	K_p [L g⁻¹][c]	K_X [L g⁻¹][d]	logP[e]
1	4	2, 2′	419.3	350	89.93±4.4	3.199±0.1	0.80±0.0	1.06±0.	4.97[f]
	10	2, 6							4.98[f]
2	7	2, 4	259.5	248.9	478.7±45.	2.874±0.2	0.72±0.0	1.28±0.	5.21
	9	2, 5							5.21
3	6	2, 3′	88.24	176.6	289.6±13.	3.266±0.1	0.82±0.0	1.29±0.	5.21
4	5	2, 3	88.24	160.3	1001±216	3.771±0.6	0.94±0.1	1.61±0.	5.09
	8	2, 4′							5.21
5	19	2, 2′, 6	88.24	153.1	2.510±0.4	5.465±0.0	1.37±0.0	2.48±0.	5.38
6	18	2, 2′, 5	1596.	64.3	15.70±1.2	4.695±0.2	1.17±0.0	1.70±0.	5.67
7	15	4, 4′	734.2	82	3.330±0.4	5.921±0.1	1.48±0.0	1.83±0.	5.46
	17	2, 2′, 4							6.01
8	24	2, 3, 6	73.56	74	4.170±0.4	7.603±0.2	1.90±0.0	2.29±0.	5.55
	27	2, 3′, 6							5.67
9	16	2, 2′, 3	73.56	73.6	4.980±0.7	11.50±0.6	2.87±0.1	2.44±0.	5.55
	32	2, 4′, 6							5.67
10	26	2, 3′, 5	195.4	43	27.36±3.6	7.482±0.6	1.87±0.1	2.23±0.	5.92
11	25	2,3′,4	--	42.6	--	--	--	--	5.92
12	28	2, 4, 4′	6617.	27.9	21.93±2.0	12.65±0.8	3.16±0.2	2.91±0.	5.92
13	33	2′, 3, 4	1267.	32.6	8.730±0.7	5.281±0.2	1.32±0.0	1.72±0.	5.87[f]
	53	2, 2′, 5, 6′							6.13
14	22	2, 3, 4′	693.3	29.7	55.78±15.	6.587±1.3	1.65±0.3	1.54±0.	5.68
	51	2, 2′, 4, 6′							6.13
15	45	2, 2′, 3, 6	142.8	41.8	3.020±0.4	9.170±0.4	2.29±0.1	2.66±0.	6.01
16	46	2, 2′, 3, 6′	142.8	35.4	3.210±0.4	10.97±0.7	2.74±0.1	2.97±0.	6.01
17	52	2, 2′, 5, 5′	492	22.2	2.680±0.4	9.167±0.7	2.29±0.1	2.62±0.	6.38
18	49	2, 2′, 4, 5′	435.6	22.1	2.670±0.4	9.127±0.7	2.28±0.1	--	6.36[f]
19	48	2, 2′, 4, 5	519.1	21.1	11.59±1.4	6.964±0.5	1.74±0.1	1.66±0.	6.26
20	44	2, 2′, 3, 5′	615.2	19	3.450±0.6	10.36±1.1	2.59±0.2	2.38±0.	6.26
21	42	2, 2′, 3, 4′	--	18.1	--	--	--	--	6.26
22	41	2, 2′, 3, 4	646.3	16.2	4.840±0.9	9.749±1.2	2.44±0.3	2.30±0.	6.14
	64	2, 3, 4′, 6							6.26
	71	2, 3′, 4′, 6							6.26
	72	2, 3′, 5, 5′							6.63
23	40	2, 2′, 3, 3′	123.4	15.6	4.940±1.0	10.19±1.3	2.55±0.3	2.72±0.	6.18[f]
24	67	2, 3′, 4, 5	26.1	14.8	4.650±1.0	11.12±1.5	2.78±0.3	3.48±0.	6.51
25	63	2, 3, 4′, 5	33.4	12.9	5.700±1.5	11.30±2.0	2.83±0.5	3.33±0.	6.51
26	74	2, 4, 4′, 5	506.7	9.4	4.260±1.3	10.22±2.1	2.56±0.5	3.13±0.	6.51
	97	2, 2′, 3, 5, 6′							6.72
27	70	2, 3′, 4′, 5	693.6	9.4	2.170±0.7	9.617±1.8	2.40±0.4	3.87±0.	6.51
	76	2′, 3, 4, 5							6.39
28	66	2, 3′, 4, 4′	1223.	8.7	2.240±0.9	7.725±1.9	1.93±0.4	7.03±1.	6.51
29	55	2, 3, 3′, 4	39.6	9.9	4.290±1.8	17.09±4.9	4.27±1.2	4.52±1.	6.39
	91	2, 2′, 3, 4′, 6							6.72
30	56	2, 3, 3′, 4	661.7	7.4	3.410±0.9	6.196±1.1	1.55±0.2	3.56±0.	6.39
	60	2, 3, 4, 4′							6.39
31	84	2, 2′, 3, 3′, 6	45.99	10.2	1.910±0.7	15.07±3.6	3.77±0.9	6.36±1.	6.6
	92	2, 2′, 3, 5, 5′							6.97

[a]Congeners in boldface type are major components of the peak (Ballschmiter and Zell 1980)
[b]Obtained from independent experiment (Vrana et al. 1996)
[c]Calculated using Eq. 26 for a biomass concentration of 4 g d.w.l⁻¹
[d]Obtained from independent experiment (in preparation)
[e]Calculated by the ClogP software (ClogP for Windows)
[f]Determined by the slow-stirr method (De Bruijn et al. 1989)

Structure-biodegradability relationship

Biodegradation of DELOR 103 and DELOR 106 by *Pseudomonas stutzeri* (Dercová et al. 1996; Dercová et al. 1995) was studied previously. The results are in accord with other reports on microbial degradation of PCB (Furukawa et al. 1978; Massé et al. 1984; Bedard et al. 1986; Bedard and Haberl 1991). Biodegradability decreases with the increasing number of chlorine atoms in the PCB molecule. If the first step of biodegradation is the biphenyl dioxygenase attack in positions 2 and 3 of one of the rings, then at least one pair of *ortho-* and *meta-* positions must be free in the congener molecule. Most of the congeners, included in the formulation DELOR 103 used, do comply with this condition. There are only three exceptions, and those are: 2,2′,5,5′-tetrachlorobiphenyl (peak No. 17), 2,2′,3,5,5′-pentachlorobiphenyl (peak No. 31) and 2,2′,3′,4,5-pentachlorobiphenyl (peak No. 26), but the rate constants of biodegradation of the congeners included in these peaks are very low (lower than $4.2{\times}10^{-4}$ (h^{-1})). It is generally accepted that the rates of biodegradation are higher for congeners with one non-substituted ring in the molecule. The congeners included in peak No. 2 (2,4- and 2,5-dichlorobiphenyl) are an example. Some other congeners with one non-substituted ring are also included in peak No. 1 (2,6-dichlorobiphenyl) and No. 4 (2,3-dichlorobiphenyl). However, there are two co-eluting congeners in each peak (2,2′- and 2,6-dichlorobiphenyl in peak No. 1; 2,3- and 2,4′-dichlorobiphenyl in peak No. 4) and, obviously, just one of the two congeners has one non-substituted ring. The degradation rate of the congener with non-substituted ring does not differ from the degradation rate of its co-eluting congener, as indicated by absence of the multi-exponential behavior of the time courses of the amounts for co-eluting congeners.

For a simple empirical determination of the influence of the chlorine substitution pattern on biodegradability, the di- and trichlorobiphenyl rate constants of biodegradation were analysed using multiple linear analysis. As the independent variables, the total number of chlorine atoms in the molecule, the number of chlorine atoms in *ortho-* , *meta-* and *para-* positions, and the number of free 2,3- positions on the biphenyl molecule were used. The logarithmic value of the congener degradation rate constant was the dependent variable. The k_d values of congeners co-eluting in one peak were considered identical. If one of the co-eluting congeners was dominant, the other congener in the peak was neglected. The values of the descriptors for individual congeners are summarised in Table 3 and the results of the regression analysis results in Table 4.

Table 3. Descriptors used for structure-degradability analysis representing the number of the given structural features in the congener molecule.

IUPAC No.	$\log k_d$	chlorines total	free 2,3- positions	*ortho -* chlorines	*meta* -chlorines	*para -* chlorines
4	-2.046	2	2	2	0	0
5	-1.000	2	3	1	1	0
6	-1.538	2	2	1	1	0
7	-1.320	2	3	1	0	1
8	-1.000	2	3	1	0	1
9	-1.320	2	2	1	1	0
10	-2.046	2	2	2	0	0
16	-3.303	3	2	2	1	0
17	-3.478	3	2	2	0	1
18	-2.804	3	1	2	1	0
19	-3.600	3	1	3	0	0
22	-2.254	3	3	1	1	1
24	-3.380	3	2	2	1	0
26	-2.563	3	1	1	2	0
27	-3.380	3	1	2	1	0
28	-2.659	3	3	1	0	2
32	-3.303	3	2	2	0	1
33	-3.059	3	2	1	1	1

Table 4. Values of parameters obtained by multiple linear regression analysis of the dependence of degradation rate constants from descriptors of the PCB substitution pattern. Statistical indices: n = 18, r = 0.967, S.D. = 0.272, F = 35.

parameter	optimised value	t-value	$|t_{krit}|$-1 value
const	1.387 ± 0.738	1.879	0.085
chlorines total	-1.093 ± 0.119	-9.159	<0.0001
free 2,3-positions	0.153 ± 0.166	0.925	0.373
ortho - chlorines	-0.713 ± 0.119	-6.004	<0.0001
meta - chlorines	-0.114 ± 0.098	-1.172	0.264
para - chlorines	-0.265 ± 0.110	-2.421	0.032

The analysis was conducted for 18 congeners and the quality of the regression is satisfactory at the 0.05 significance level. The negative influence was established for the total number of chlorine atoms in the molecule and the number of chlorine atoms in *ortho*-positions. A negative influence of lower magnitude was also found for chlorine atoms in *meta-* and *para-* positions. The number of free 2,3-positions on the biphenyl molecule influence the biodegradation rate positively, although the standard deviation of the parameter

is rather high. These results agree well with the present view of structure-degradability relationship of PCBs: the *ortho*-substituted congeners are most resistant and the positions 2 and 3 have to be free in order for successful microbial attack (Massé et al. 1984; Bedard et al. 1986; Bedard and Haberl 1991). The only discrepancy is the behaviour of the *para*-substituted congeners that are degraded by *Comamonas testosteroni* B-356 faster than the *meta*-substituted congeners (Massé et al. 1984).

Conclusion

The biodegradation rate constants are important parameters for the prediction of the time required to eliminate a PCB congener from the contaminated site. However, there are only few kinetic data on the aerobic bacterial PCB-degradation obtained under the defined conditions available to this date. A suggested four-compartmental model that takes into account biosorption, evaporation, and primary biodegradation represents a model system that provides valuable information on the studied process, thus serving both scientific and application purposes. It can be used to determine the kinetic parameters of a biodegradation process including the biodegradation rate constants. Our results also imply that biodegradability, especially of di-and trichlorobiphenyls, is a structure dependent process in comparison with the chemical oxidation of PCBs.

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