

Biodegradation Kinetics of Highly Chlorinated Biphenyls by *Alcaligenes* sp. JB1 in an Aerobic Continuous Culture System

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A mixture of nine polychlorinated biphenyl (PCB) congeners with four to six chlorine substituents was cometabolized in an aerobic chemostat culture of *Alcaligenes* sp. JB1, grown on 3-methylbenzoate. Pseudo-first-order biodegradation rate constants (k_b) were calculated using a steady-state kinetic model. These rate constants varied from 7.8 to 0.44 h⁻¹. Comparable results for exposure to high and low influent concentrations confirmed the assumption of pseudo-first-order kinetics for most of the congeners studied. *Alcaligenes* sp. JB1 was found to degrade 2,3-dichlorophenyl rings very rapidly and 2,5-dichlorophenyl rings relatively slowly. Certain penta- and hexachlorobiphenyls were degraded faster than some tetrachlorobiphenyls. For example, 2,2',3,3',6,6'-hexachlorobiphenyl was degraded faster than 2,2',6,6'-tetrachlorobiphenyl. On the other hand, 2,2',3,3',4,4'-HCB was degraded much more slowly than 2,2',3,3'-TCB. A control experiment with *Pseudomonas fluorescens* NCTC10038, a bacterium incapable of PCB (co)metabolism, showed that losses caused by physical or chemical phenomena were negligible.

Introduction

Despite their apparent recalcitrance in the environment, the lower chlorinated (Cl < 5) biphenyls have been mineralized aerobically by several microbial consortia in laboratory studies (1-3). Biphenyl and monochlorobiphenyls can serve as growth substrates for several microorganisms, including the organism dealt with in this study, *Alcaligenes* sp. JB1. The other chlorinated biphenyls can only be degraded by cometabolism, i.e., degradation of PCBs during growth on another substrate. Highly chlorinated

PCBs (Cl > 4) are presumed to be persistent under aerobic conditions. However, exceptions to this presumption are demonstrated by the reported degradation of penta- (PnCB) and hexachlorobiphenyls (HCB) by *Alcaligenes* Y42 (4), *Pseudomonas* sp. LB400 (5), and *Alcaligenes eutrophus* H850 (6).

The anaerobic reductive dehalogenation of highly chlorinated biphenyls to lower chlorinated analogues has been shown to be an important initial step in their destruction (3, 7). It has been recently shown that complete reductive dehalogenation to biphenyl is possible (8). However, to our knowledge, anaerobic carbon metabolism of (chloro)biphenyls has never been detected. Thus, oxidative degradation may be essential for the complete mineralization of PCBs to CO₂, H₂O, biomass, and chloride.

Little is known about the kinetics of PCB degradation. At the moment, only overall anaerobic dehalogenation rates are available, measured in poorly defined mixed cultures and in most cases in the presence of sediment. Different anaerobic enrichments show great variation in dehalogenation rates. In contrast, aerobic biodegradation rates can be determined for pure cultures grown under well-defined conditions. The advantage of using a pure culture instead of a mixed culture is that only one enzyme system is involved in the degradation, the action of which may be described by Michaelis-Menten kinetics. This gives rate constants characteristic of the congener and the enzyme system studied. However, such rate constants are scarce in the literature.

In this study, the aerobic biodegradation kinetics of tetra-, penta-, and hexachlorinated biphenyls were studied in a chemostat culture. The advantages of using such a system for kinetic studies are that the conditions under which degradation takes place are controlled and constant and that the continuous limitation of growth provides a constant biomass in the exponential growth phase. The liquid culture is homogeneously mixed, and growth substrate and PCBs are dissolved in the aqueous medium. This simplifies the kinetics compared to batch cultures, in which the PCBs are often added at concentrations far above their water solubilities, or biofilm reactors, in which the biomass concentration is neither homogeneous nor constant.

The bacterium used to study PCB degradation was *Alcaligenes* sp. JB1. This organism, previously identified as *Pseudomonas* sp. JB1, is able to grow on benzoate, 3-methylbenzoate, biphenyl, and monochlorobiphenyls and to cometabolize various chlorinated benzoates and biphenyls during growth on 3-methylbenzoate (9, 10). A control experiment was conducted by exposing strain *Pseudomonas fluorescens* NCTC10038 to PCBs in the chemostat under the same conditions. This latter strain is unable to grow on biphenyl or to cometabolize PCBs. From this experiment, the disappearance rates due to processes other than biodegradation were determined. Differences in biodegradation rate constants of PCB congeners are discussed.

Materials and Methods

Bacterial Strains. *Alcaligenes* sp. JB1 (previously tentatively identified as *Pseudomonas* strain JB1) was isolated as described by Parsons et al. (10). Identification by the API-20NE test system (API systems S.A., Montalieu, France)

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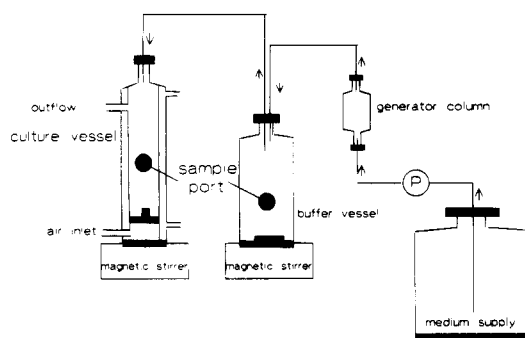


FIGURE 1. Schematic design of the chemostat culture system used in this study.

revealed that it was an *Alcaligenes denitrificans* strain (91.5% certainty). However, as this organism is not able to reduce nitrite to nitrogen, we describe it as *Alcaligenes* sp. JB1. *Alcaligenes* was maintained on agar slopes with 0.25 mM 3-methylbenzoate. *P. fluorescens* NCTC10038 was a gift from the Department of Microbiology, University of Amsterdam. This organism is able to grow on benzoate, but not on 3-methylbenzoate or biphenyl. We assumed this organism to be unable to cometabolize PCBs. The purity of both strains was controlled by frequent plating on nutrient agar and API-20NE tests.

Culture Conditions. Precultures were grown in batch at 25 °C on 8.3 mM benzoate in an Evans mineral medium with the following composition: $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ (1.0 mM), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.01 mM), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.625 mM), NH_4Cl (50 mM), KH_2PO_4 (30 mM), $\text{N}(\text{CH}_2\text{COOH})_3$ (1 mM), ZnO (12.7 μM), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (50 μM), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (25.3 μM), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (2.54 μM), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (5 μM), HBO_3 (2.58 μM), and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.046 μM). The pH was adjusted to 7.0 ± 0.1 .

Chemostat. The design of the chemostat system is shown in Figure 1. The culture was supplied continuously with Evans medium containing 7.5 mM 3-methylbenzoate as the growth substrate for *Alcaligenes* sp. JB1 cultures or 8.3 mM benzoate for *P. fluorescens* NCTC10038. The medium flow rate was 20 mL/h, resulting in a dilution rate of $0.040 \pm 0.004 \text{ h}^{-1}$ or a hydraulic residence time of approximately 1 day. Under these conditions, the concentration of the carbon source limits the growth, resulting in a constant biomass concentration during steady state. The dry weight, measured by the procedure of Herbert et al. (11), was approximately $0.6 \pm 0.06 \text{ g dryweight/L}$ (g dw L^{-1}) in all cases. The aeration rate was $\pm 1200 \text{ mL/h}$, the stirring speed was 300 rpm, and the temperature was kept constant at 25 °C.

This study consisted of three experiments. Two experiments were carried out with *Alcaligenes* sp. JB1 (one with relatively high influent concentrations and another with relatively low influent concentrations) and the control experiment with *P. fluorescens* NCTC10038. The chemostat cultures were exposed to a mixture of the following PCB congeners: 2,2',3,3',-2,2',5,5',- and 2,2',6,6'-tetrachlorobiphenyls (TCBs); 2,2',4,4',6-, 2,2',4,5,5',-, 2,3',4,4',6-, and 2,3',4,5',6-pentachlorobiphenyls (PnCBs); and 2,2',3,3',4,4'- and 2,2',3,3',6,6'-hexachlorobiphenyls (HCBs). All PCB congeners were obtained from C. N. Schmidt B.V., Amsterdam, The Netherlands. This mixture was dissolved in 50 mL of pentane containing 8–12 g of Chromosorb GAW 45–60 Mesh (Chrompack, Middelburg, The Netherlands). The pentane layer was evaporated under a gentle nitrogen

stream. The Chromosorb coated with PCBs was transferred to a glass column, in which it was held in position by a glass filter on top and glass wool on the bottom. The column was closed with silicon rubber-lined screwcaps. The column was sterilized by circulating a formaldehyde/ H_2O (1/1 v/v) mixture for 24 h. Three liters of sterile medium was then pumped through the column to remove the formaldehyde. A separate column was prepared for each experiment.

Sample Preparation, Cleanup, and Analysis. Medium samples (50 mL) were collected from the buffer vessel, and culture samples (50 mL) were collected from the culture vessel by means of a syringe through the sample ports. The cleanup procedure and analysis were adapted from the method described by Parsons et al. (9). Hexachlorobenzene (25 ng) was added to these samples as an internal standard. Culture samples were acidified with 4 M H_2SO_4 to pH = 1 and extracted with 50 mL of pentane to which 1.5 mL of 2,2,4-trimethylpentane (TMP) was added. After centrifugation (500g) and removal of the pentane layer, the extracts were concentrated to ca. 1 mL under a gentle nitrogen stream or by Kuderna–Danish distillation (in the case of the *P. fluorescens* experiment). The medium samples were extracted with 1.5 mL of TMP by shaking for 2 min. Both extracts were eluted with 10 mL of TMP through a column (cross-section 5 mm) containing 1 g of 100–120 mesh silica + 40% w/w H_2SO_4 above 1 g of 100–120 mesh silica + 33% w/w 1 M NaOH to remove polar compounds. The samples were then concentrated to a final volume of 1 mL under a gentle nitrogen stream. Analysis was GC with cold-on-column injection (Carlo Erba 6000 Vega Series 2 or Hewlett Packard 5890, 30 m \times 0.32 mm DB5 columns). The detection occurred with a ^{63}Ni electron capture detector (ECD). For the PCBs used in these experiments, this detector was least sensitive for detection of 2,2',6,6'-TeCB, of which the detection limit amounted to 0.02 pg (corresponding with a concentration of 0.0004 $\mu\text{g/L}$ in the experiments).

Calculation. Triplicate 50-mL samples of the medium and culture were taken at each time point. A period of at least 4 days was allowed for the re-establishment of the steady state between sampling. The biodegradation experiment with *Alcaligenes* sp. JB1 was sampled four times at steady state for both high and low influent (medium) PCB concentrations, whereas the control experiment with *P. fluorescens* NCTC10038 was sampled five times. Average medium (C_m) and culture concentration (C_c) were calculated for each time point.

At steady state in a chemostat, the rate at which a chemical enters the chemostat is equal to the rate at which it is lost by dilution, degradation, and abiotic processes such as adsorption and volatilization. The mass balance is then be described by

$$DC_m = DC_c + \nu \quad (1)$$

in which D is the dilution rate of the culture (h^{-1}), C_m is the concentration of PCB in medium influent ($\mu\text{g L}^{-1}$), C_c is the concentration of PCB in the culture ($\mu\text{g L}^{-1}$), and ν is the disappearance rate. Assuming the disappearance is caused by biodegradation, the disappearance rate can be expressed by

$$\nu = qX \quad (2)$$

in which q is the specific substrate consumption rate (μg

TABLE 1

Mean Concentrations of PCBs in the Medium (C_m) and Culture (C_c) for Two Experiments with *Alcaligenes* Strain JB1 and One with *Pseudomonas fluorescens* NCTC10038

PCB congener	<i>Alcaligenes</i> sp. JB1 high influent concentration ($n = 4$)		<i>Alcaligenes</i> sp. JB1 low influent concentration ($n = 4$)		<i>P. fluorescens</i> NCTC10038 control ($n = 5$)	
	concn in medium C_m ($\mu\text{g L}^{-1}$) \pm SD	concn in culture C_c ($\mu\text{g L}^{-1}$) \pm SD	concn in medium C_m ($\mu\text{g L}^{-1}$) \pm SD	concn in culture C_c ($\mu\text{g L}^{-1}$) \pm SD	concn in medium C_m ($\mu\text{g L}^{-1}$) \pm SD	concn in culture C_c ($\mu\text{g L}^{-1}$) \pm SD
2,2',3,3'-TCB	10.574 \pm 1.011	0.055 \pm 0.003	1.164 \pm 0.045	0.006 \pm 0.002	11.975 \pm 1.511	7.301 \pm 1.168
2,2',5,5'-TCB	25.309 \pm 4.333	2.033 \pm 0.491	0.663 \pm 0.024	0.058 \pm 0.012	4.094 \pm 0.612	2.948 \pm 0.248
2,2',6,6'-TCB	8.911 \pm 1.272	0.770 \pm 0.247	0.464 \pm 0.016	0.030 \pm 0.009	7.517 \pm 1.294	2.874 \pm 0.368
2,2',4,4',6,6'-PnCB	3.712 \pm 0.899	0.133 \pm 0.028	1.145 \pm 0.067	0.026 \pm 0.004	3.564 \pm 0.722	2.476 \pm 0.581
2,2',4,5,5'-PnCB	3.520 \pm 0.544	0.235 \pm 0.025	1.185 \pm 0.071	0.079 \pm 0.006	4.250 \pm 0.799	3.679 \pm 0.754
2,3',4,4',6,6'-PnCB	3.163 \pm 0.396	0.109 \pm 0.013	1.155 \pm 0.070	0.039 \pm 0.004	5.106 \pm 1.100	4.358 \pm 1.186
2,3',4,5',6-PnCB	3.729 \pm 0.300	0.159 \pm 0.032	1.251 \pm 0.074	0.043 \pm 0.005	5.924 \pm 1.189	5.128 \pm 1.261
2,2',3,3',4,4'-HCB	0.534 \pm 0.224	0.023 \pm 0.010	0.401 \pm 0.029	0.018 \pm 0.001	1.632 \pm 0.409	2.247 \pm 0.708
2,2',3,3',6,6'-HCB	1.862 \pm 0.160	0.050 \pm 0.006	0.405 \pm 0.035	0.007 \pm 0.001	3.770 \pm 0.678	3.669 \pm 0.949

(g dw) $^{-1}$ h $^{-1}$) and X is the biomass concentration (g dw L $^{-1}$). A combination of eqs 1 and 2 yields

$$qX = D(C_m - C_c) \quad (3)$$

The kinetics of bacterial degradation may be dependent on the kinetics of the enzymes involved, which can be described by the Michaelis–Menten equation

$$qX = q_{\max} X \left(\frac{C_c}{K_m + C_c} \right) \quad (4)$$

in which q_{\max} is the maximum specific substrate consumption rate ($\mu\text{g (g dw)}^{-1}$ h $^{-1}$) and K_m is the affinity constant of enzymes for the substrate ($\mu\text{g L}^{-1}$).

Little is known of the affinity constants of the enzymes involved in (chloro)biphenyl degradation. Bergeron and Bopp determined an apparent K_m of ca. 8 μM for the degradation of 2,4'-dichlorobiphenyl by *Pseudomonas putida* LB400 (12). *Acinetobacter* sp. strain P6 showed K_m values of 3.2–12.2 μM for growth on biphenyl and between 45 and 97 μM for growth on 4-chlorobiphenyl (13). Due to their low solubilities, the PCB concentrations in the experiments reported here were very low (less than 50 nM). So it seems reasonable to assume that the affinity of these enzymes for PCBs is very low and $C_c \ll K_m$. In this case, the Michaelis–Menten equation simplifies to

$$qX = \left(\frac{q_{\max} X}{K_m} \right) C_c \quad (5)$$

Assuming a constant biomass concentration X , the pseudo-first-order biodegradation rate constant k_b (h $^{-1}$) is defined as

$$k_b = \frac{q_{\max} X}{K_m} \quad (6)$$

Equations 3, 5, and 6 can be combined to give

$$k_b = D \left(\frac{C_m - C_c}{C_c} \right) \quad (7)$$

The possible losses of PCBs due to abiotic processes such as volatilization will also follow first-order kinetics, described by a rate constant k_a :

$$\nu = k_a C_c \quad (8)$$

A combination of eqs 1 and 8 yields

$$k_a = D \left(\frac{C_m - C_c}{C_c} \right) \quad (9)$$

Recovery Experiments. The analytical recoveries of the PCBs were determined by analyzing 50 mL of sterile medium and acid-killed culture samples containing known concentrations of PCBs, comparable to the values of C_m and C_c in the degradation experiments. The ratios of the amounts measured by GC-ECD and those added were used to calculate the recoveries of the analytical procedure. The recoveries were essentially quantitative (90–110% for medium samples and 80–120% for culture samples).

Results

Continuous Cometabolism of Highly Chlorinated PCBs by *Alcaligenes* sp. JB1. Chemostat cultures of *Alcaligenes* sp. JB1, grown on 3-methylbenzoate, were exposed to a mixture of PCB congeners with four to six chlorine substituents. The steady-state concentrations of the PCBs in the culture and the medium influent to which the cultures were exposed are listed in Table 1, as are the results of the control experiment. The concentrations of two representative PCB congeners (2,2',3,3'-TCB and 2,2',3,3',6,6'-HCB) in the three experiments are also shown in Figure 2a–f. The concentration of 2,2',3,3'-TCB in the cultures was 200 times lower than in the medium, indicating a 99.5% removal of this compound. The lowest removal was found for 2,2',6,6'-TCB (92%); others were intermediate. These congeners were degraded by this organism at specific consumption rates (q) ranging from $\approx 1.5 \mu\text{g (g dw)}^{-1}$ h $^{-1}$ for 2,2',3,3'-TCB to $\approx 26 \text{ ng (g dw)}^{-1}$ h $^{-1}$ for 2,2',3,3',4,4'-HCB. The pseudo-first-order biodegradation rate constants (k_b) of the PCBs were all significantly different from zero (Table 2). The order of biodegradation rate constants is as follows: 2,2',3,3'-TCB \gg 2,2',3,3',6,6'-HCB $>$ 2,2',4,4',6-PnCB \geq 2,3',4,4',6-PnCB \geq 2,3',4,5',6-PnCB \geq 2,2',3,3',4,4'-HCB \geq 2,2',4,5,5'-PnCB \geq 2,2',6,6'-TCB \geq 2,2',5,5'-TCB. Attempts to resolve the k_b values into q_{\max} and K_m in a Lineweaver–Burk plot of the Michaelis–Menten equation were unsuccessful due to the restricted range in concentrations used in this experiment (data not shown).

Validation of Pseudo-First-Order Kinetics. We found in previous studies with chemostat cultures that at a flow rate of 20 mL/h PCBs dissolved in the medium at concentrations comparable to their reported aqueous solubilities (9). However, in these experiments the concentrations

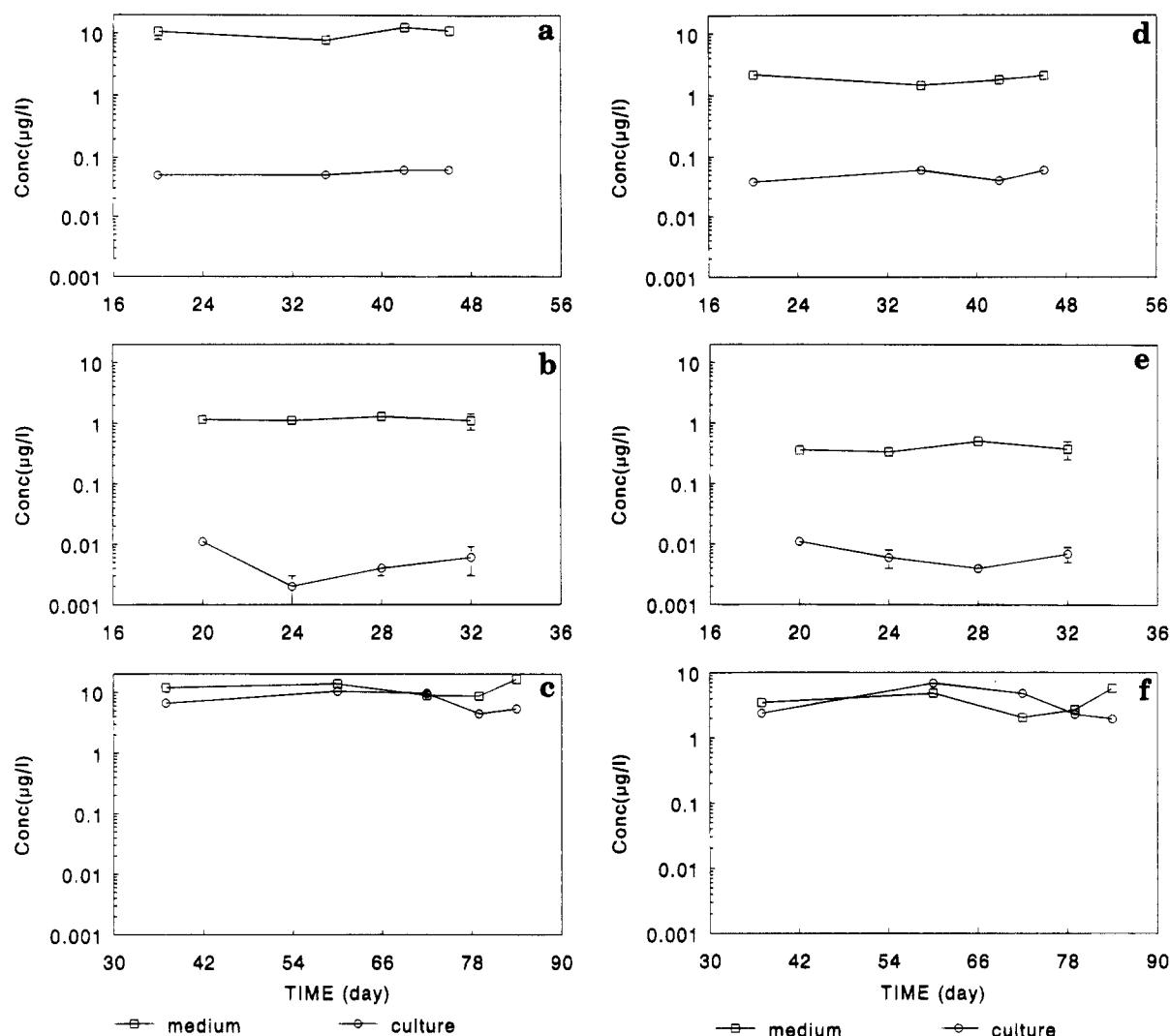


FIGURE 2. Concentrations of PCBs against time, measured in the medium influent (C_m) and culture (C_c) for two cultures of *Alcaligenes* strain JB1 (with high and low influent concentrations) and a culture of *P. fluorescens* NCTC10038. 2,2',3,3'-Tetrachlorobiphenyl: (a) high influent concentration, (b) low influent concentration, (c) *P. fluorescens*. 2,2',3,3',6,6'-Hexachlorobiphenyl: (d) high influent concentration, (e) low influent concentration, (f) *P. fluorescens*.

TABLE 2

Biodegradation Rate Constants k_b , Calculated for High and Low Influent Concentrations and Abiotic Disappearance Rate Constants k_a

PCB congener	<i>Alcaligenes</i> sp. JB1		<i>P. fluorescens</i> NCTC10038 k_a (h^{-1}) \pm SD	<i>Alcaligenes</i> sp. JB1 av biodegradation rate constant corrected for abiotic disappearance rate constant $k_b - k_a$ (h^{-1}) \pm SD
	high influent concn k_b (h^{-1}) \pm SD	low influent concn k_b (h^{-1}) \pm SD		
2,2',3,3'-TCB	7.6 \pm 1.1	8.0 \pm 2.2	0.03 \pm 0.02	7.8 \pm 1.2
2,2',5,5'-TCB	0.46 \pm 0.13	0.42 \pm 0.08	0.02 \pm 0.01	0.42 \pm 0.08
2,2',6,6'-TCB	0.42 \pm 0.13	0.57 \pm 0.15	0.07 \pm 0.02 ^b	0.43 \pm 0.10
2,2',4,4',6-PnCB	1.1 \pm 0.3 ^a	1.7 \pm 0.3 ^a	0.04 \pm 0.03	1.4 \pm 0.2
2,2',4,5,5'-PnCB	0.56 \pm 0.12	0.56 \pm 0.08	0.02 \pm 0.02	0.54 \pm 0.07
2,3',4,4',6-PnCB	1.1 \pm 0.2	1.2 \pm 0.2	0.02 \pm 0.02	1.1 \pm 0.1
2,3',4,5',6-PnCB	0.9 \pm 0.19	1.1 \pm 0.2	0.02 \pm 0.02	1.0 \pm 0.1
2,2',3,3',4,4'-HCB	0.9 \pm 0.5	0.85 \pm 0.12	-0.01 \pm 0.01	0.88 \pm 0.25
2,2',3,3',6,6'-HCB	1.5 \pm 0.2 ^a	2.3 \pm 0.5 ^a	0.01 \pm 0.02	1.9 \pm 0.3

^a Statistically significant difference (t -test, α 5%, β 5%) between k_b calculated from high and low influent concentrations. ^b Statistically significant difference (t -test, α 5%) between k_a and zero.

in the medium could not be kept constant. Insertion of a 1-L buffer vessel in the experimental setup (Figure 1) reduced these fluctuations considerably, and the concentration of PCBs entering the culture was almost constant (Figure 2a-f). Table 1 clearly shows that the influent concentrations varied for the different experiments due to

the preparation of different columns. This enabled the validation of the assumption: $C_c \ll K_m$ and thus of pseudo-first-order kinetics (see Materials and Methods). If the degradation can be described by pseudo-first-order kinetics, experiments with different medium concentrations should give the same values of k_b . This is apparent from the data

in Table 2. The rate constants obtained for high and low medium concentrations were the same (*t*-test, α 5%; β 5%) for all the PCBs apart from 2,2',4,4',6-PnCB and 2,2',3,3',6,6'-HCB for which the differences are small.

Control Experiment. Caution has to be taken in quantifying data from biodegradation assays, as many factors other than biodegradation can cause loss of PCBs. Although PCBs have low vapor pressures and high boiling points, volatilization, adsorption to glass or plastic, and differential extraction and clean-up could be potential sources of serious analytical error. Losses due to the analytical procedure were not found (see Materials and Methods). To investigate the losses other than biodegradation in the chemostat, an experiment was done with an organism not able to grow on biphenyl, *P. fluorescens* NCTC10038. This is a Gram-negative, aerobic rod, capable of metabolizing benzoate aerobically, similar to *Alcaligenes* strain JB1. The results of this experiment were used to calculate abiotic disappearance rate constants k_a . All these disappearance rate constants, except one, were not significantly (*t*-test, α = 5%) different from zero (Table 2). A very low value of k_a was found for 2,2',6,6'-TCB; however, this was much lower than its k_b value.

Discussion

The results of the experiments described in this work show that a range of tetra-, penta-, and hexachlorinated biphenyls is degraded in chemostat cultures of *Alcaligenes* sp. strain JB1. The cultures removed between 92 and 99.5% of the PCBs to which they were exposed in the medium influent (Table 1 and Figure 2). The lack of significant degradation of these PCBs (except for a slight reduction in concentration of 2,2',6,6'-TCB) in a culture of *P. fluorescens* NCTC10038 demonstrates that the removal was due to biodegradation and not to physical-chemical losses. The rates of biodegradation may be expressed as specific consumption rates (*q*). However, these rates are a function of the concentrations of the compounds (see eq 3). From these experiments, two different specific consumption rates for the same PCB congener may be calculated. For example, the high influent concentration experiment yields for 2,2',3,3'-TCB $q = 0.7 \mu\text{g (g dw)}^{-1} \text{ h}^{-1}$, whereas the low influent concentration experiment yields $q = 0.08 \mu\text{g (g dw)}^{-1} \text{ h}^{-1}$. Therefore, a better comparison of biodegradation rates would be in terms of Michaelis-Menten parameters or as rate constants. One of the biggest advantages of chemostat cultures for kinetic studies is that their constant biomass concentrations simplifies the kinetics of degradation. In the case of this study, where the concentrations of the PCBs were much lower than their likely K_m values, pseudo-first-order kinetics described the degradation reasonably well. This is indicated by the comparable values of the pseudo-first-order biodegradation rate constants (k_b) in cultures exposed to different concentrations of PCBs (Table 2). The pseudo-first-order biodegradation rate constants were between 7.8 h^{-1} for 2,2',3,3'-TCB and 0.44 h^{-1} for 2,2',5,5'-TCB. These rate constants correspond to half-lives of 0.09 and 1.58 h, respectively. Division of k_b by the biomass concentration ($0.6 \pm 0.06 \text{ g dw/L}$ in these cultures) gives second-order biodegradation rate constants.

The order of the biodegradation rate constants of the PCBs in this study is as follows: 2,2',3,3'-TCB \gg 2,2',3,3',6,6'-HCB $>$ 2,2',4,4',6-PnCB \geq 2,3',4,4',6-PnCB \geq 2,3',4,5',6-PnCB \geq 2,2',3,3',4,4'-HCB \geq 2,2',4,5,5'-PnCB \geq 2,2',6,6'-TCB \geq 2,2',5,5'-TCB. The major aerobic microbial degradation

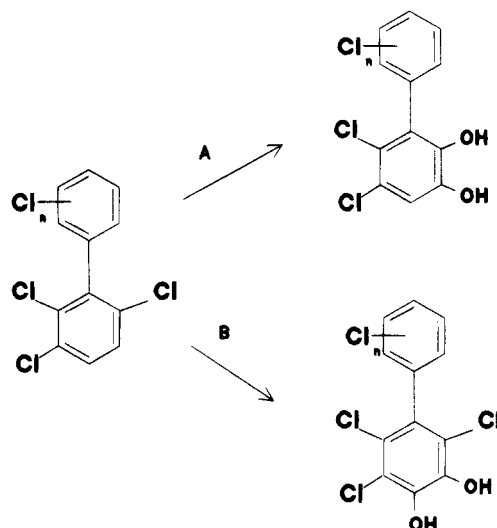


FIGURE 3. Proposed pathways for the first steps in the aerobic biodegradation of chlorobiphenyls. Route A: 2,3-dioxygenase accompanying oxidative dehalogenation. Route B: 3,4-dioxygenase for rings with 2,5-chlorophenyl substitution.

pathway of PCBs starts with 2,3-dioxygenation of the less substituted aromatic ring (Figure 3, route A), followed by ring cleavage and further degradation to (chloro)benzoates (3). The most highly chlorinated phenyl ring is presumed to be the nonreacting ring. The higher biodegradation rates of certain HCBs and PnCBs than those of TCBs may be caused by the influence of the nonreacting ring. However, from these results, it is not clear whether the lowest chlorinated ring is indeed always the reacting ring. The remarkable fact that the degradation rate constant of 2,2',3,3',6,6'-HCB is higher than that of 2,2',6,6'-TCB suggests that in this case an extra chlorine substituent has a stimulating effect on degradation. Bedard et al. also reported faster degradation of 2,2',3,3',6,6'-HCB than 2,2',6,6'-TCB in case of *Alcaligenes eutrophus* H850 (14). Further investigation of the relationships between substitution patterns of PCBs and their biodegradation rates may give more insight into these results. A preliminary investigation of such relationships showed that the biodegradation rate constants measured in this study correlate with both electronic and hydrophobic effects of chlorine substituents. The electronic effects of increasing chlorine substitution reduce biodegradation rate constants. On the other hand, the increase in hydrophobicity as a result of increasing chlorine substitution stimulates biodegradation (15).

The penta- and hexachlorobiphenyl degrading strains *Pseudomonas* sp. LB400 and *Alcaligenes eutrophus* H850, are thought to possess both 3,4- (Figure 3, route B) and 2,3-biphenyl dioxygenase activities (16). 3,4-Dioxygenase activity is thought to be stimulated by PCBs having a 2,5-chlorophenyl ring. The relatively slow degradation of 2,5-chlorophenyl rings by *Alcaligenes* sp. JB1 may be an indication that 3,4-dioxygenase activity is not present in this strain. Assuming 2,3-dioxygenase attack by strain JB1 on the 5,6-position, 2,2',3,3',6,6'-HCB must be oxidatively dehalogenated in the first reaction steps, as shown in Figure 3, route A. The oxidative dehalogenation of PCBs has been reported earlier (17, 18), although the elimination of chlorine is usually thought to be a fortuitous event that occurs in the later metabolic steps.

After studying the degradation spectra of eight PCB-degrading strains, a four-class division of such strains was

suggested by Bedard and Haberl (16). Class I is characterized by a reactivity preference for 3- > 4 > 2-chlorophenyls, no degradation of 2,4- or 2,5-chlorophenyl rings, and a large influence of the nonreacting ring. Class II has the same preferences as class I but combined with negligible effect of the nonreacting ring. Class III has a reactivity sequence of 4- > 2- > 2,4- > 3-chlorophenyls with a strong effect of the nonreacting ring and no attack on 2,5-chlorophenyl rings. Class IV has a sequence of 2- > 2,5- > 3- > 2,4- > 4-chlorophenyls with influence of the nonreacting ring. Strictly speaking, *Alcaligenes* sp. JB1 would not fit into any of these proposed classes. It degrades 2,4- and 2,5-chlorophenyl rings to a significant extent, which leads to class IV, but the order of reaction rates is 2,4- > 2,5-chlorophenyl, which is in contrast to class IV. Furthermore, the sequence of degradation of monochlorinated rings is 4- > 2- > 3-chlorophenyl (10). Thus, with a slight alteration of the conditions from "no evidence of 2,5-chlorophenyl degradation" to "relatively slow degradation rates of 2,5-chlorophenyl rings", this organism fits into class III.

The results of this study clearly show that, under extremely favorable conditions and with special microorganisms, aerobic biodegradation of highly chlorinated biphenyls can be very fast. The persistence of these compounds in the natural environment may have several causes. It is possible that bacteria able to carry out these reactions are rare in the environment and/or that the restricted bioavailability of these extremely hydrophobic, and therefore strongly sorbing, compounds is responsible. It is also possible that other environmental factors, such as the presence or absence of nutrients or the influence of different organic substrates may affect the cometabolism of PCBs and other xenobiotics in the environment. For example, K_m for the degradation of biphenyl and 4-chlorobiphenyl by *Acinetobacter* strain P6 was influenced by the substrate the strain was grown on (13). Since bacteria can be grown under strictly defined conditions in chemostats, these systems are very suitable for studies of the influence of environmental conditions on biodegradation kinetics. More insight into the relationships between environmental factors and biodegradation would aid the extrapolation of the results of laboratory studies to field conditions.

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Nomenclature

PCB	polychlorinated biphenyl
TCB	tetrachlorobiphenyl

PnCB	pentachlorobiphenyl
HCB	hexachlorobiphenyl
D	dilution rate (h^{-1})
C_m	PCB concentration in the medium ($\mu\text{g L}^{-1}$)
C_c	PCB concentration in the culture ($\mu\text{g L}^{-1}$)
q	specific substrate consumption rate ($\mu\text{g (g dw)}^{-1} \text{h}^{-1}$)
X	biomass concentration (g dw L^{-1})
q_{\max}	maximum specific substrate consumption rate ($\mu\text{g (g dw)}^{-1} \text{h}^{-1}$)
K_m	affinity constant of the enzymes for the substrate ($\mu\text{g L}^{-1}$)
k_b	pseudo-first-order biodegradation rate constant (h^{-1})
k_a	first-order abiotic disappearance rate constant (h^{-1})

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