BIODEGRADATION OF CHLORINATED DIBENZOFURANS BY AN *ALCALIGENES* STRAIN

J.R. PARSONS*, C. RATSAK AND C. SIEKERMAN

Department of Environmental and Toxicological Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands.

Abstract

We report the biodegradation of chlorinated dibenzofurans by *Alcaligenes denitrificans* strain JB1. Rates of biodegradation of these compounds decrease with increasing number of chlorine substituents. 5-Chlorosalicylic acid is tentatively identified as a metabolite of 2-chlorodibenzofuran.

Introduction

Despite all the interest in the toxicity and environmental distribution of polychlorinated dibenzofurans (PCDFs), surprisingly little is known about their metabolism by microorganisms, or biodegradation. In 1979, Cerniglia et al. reported the hydroxylation of dibenzofuran by a fungus and a bacterial strain[1]. Recently, Foght and Westlake described a *Pseudomonas* strain that degrades dibenzofuran and other polycyclic hydrocarbons[2]. Other authors have also recently reported the isolation of dibenzofuran-utilizing *Pseudomonas* strains by selective enrichment from a variety of environmental sources[3,4]. These bacteria degrade dibenzofuran by a mechanism that leads to the formation of salicylic acid as intermediate. However, to date there have been no reports of the biodegradation of chlorinated dibenzofurans.

As we have reported previously, the biphenyl-utilizing *Alcaligenes* strain JB1 is capable of the cometabolism (degradation, but not growth on) of chlorinated biphenyls[5] and dibenzo-*p*-dioxins[6]. We here report a study to investigate whether chlorinated dibenzofurans are also degraded by this strain.

Materials and methods

Strain JB1 was isolated from soil by selective enrichment on biphenyl. Although this strain was initially characterized as a *Pseudomonas* strain[5], it since been identified as an *Alcaligenes denitrificans* strain by the API-20NE test system.

* To whom correspondence should be addressed.

Degradation of chlorinated dibenzofurans was studied in benzoate- or 3-methylbenzoate-grown batch and chemostat cultures as previously described for PCDDs[6]. Samples were extracted with hexane after addition of an internal standard (pentachlorobenzene). Clean-up of the samples was as described[6]. PCDFs were analysed by GC-ECD (Hewlett-Packard 5890; 30 m x 0.33 mm DB5 column). Concentrations given are the means of those for duplicate or triplicate samples. Filtered culture samples were analysed by reversed-phase HPLC (UV detection at 235 nm, 15 cm x 6 mm Rosil-phenyl column, 55/45 or 25/75 MeOH/10 mM H_3PO_4 eluent).

Results and discussion

Strain JB1 is able to grow on biphenyl and monochlorinated biphenyls. As we have reported previously, benzoate- or 3-methylbenzoate-grown cultures of this strain degrade chlorinated biphenyls, benzoates and dibenzo-*p*-dioxins[5,6]. Initial experiments to test this strain's ability to degrade PCDFs were performed by adding a mixture of 2-chloro-, 2,8-dichloro- and 1,3,7,8-tetrachlorodibenzofuran (2-CDF, 2,8-DCDF and 1,3,7,8-TCDF, resp.) to batch cultures grown on benzoate. The results (after 52 h incubation) indicated ready degradation of 2-CDF, but little or no degradation of the more highly chlorinated congeners (Table 1).

TABLE 1
DEGRADATION OF PCDFs IN BATCH CULTURES OF STRAIN JB1

PCDF	Initial conc. (µg/l)	Final concentration (µg/l)	
		Culture	Control
2-CDF	408	60.7	275
2,8-DCDF	47.5	29.8	33.9
1,3,7,8-TCDF	3.93	2.73	2.48

Continuous culture systems such as chemostats have a number of advantages for the study of biodegradation compared to the simpler batch cultures. These include the possibility of continuously exposing a growing population of bacteria to chemicals and thus favouring adaptation of the bacteria. Further advantages of chemostat cultures include the fact that in such cultures bacteria grow under well-defined conditions and the fact that their constant biomass concentrations simplify the kinetics of biodegradation.

Chemostat cultures of strain JB1 were grown on medium containing 1 g/l 3-methylbenzoate as carbon source. These cultures were exposed continuously to mixtures of PCDFs, which were dissolved in the medium supplied by means of a generator column system as described previously[6]. The results for two such experiments are given in Table 2. Mean concentrations of each congener in the medium (C_m) and in the culture (C_c) during the experiments (15 and 49 days long, respectively) are given in the table, together with the pseudo first-order biodegradation rate constants calculated from these concentrations from the steady state mass balance equation:

$$D\ C_m = k_b'\ C_c + D\ C_c$$

where D is the dilution rate constant of the culture and k_b' the pseudo first-order biodegradation rate constant. k_b' can be calculated as:

$$k_b' = \frac{D\ (\ C_m - C_c\ )}{C_c}$$

TABLE 2
DEGRADATION OF PCDFs IN CHEMOSTAT CULTURES OF STRAIN JB1*

PCDF	C_m (μg/l)	C_c (μg/l)	k_b' (h^{-1})
Experiment 1			
2-CDF	235 ±23	7.17 ±0.11	1.29 ±0.11
2,8-DCDF	5.39 ±0.41	0.33 ±0.06	0.565 ±0.025
1,3,7,8-TCDF	0.15 ±0.04	0.09 ±0.03	0.039 ±0.039
Experiment 2			
2,8-DCDF	5.95 ±0.27	0.425 ±0.091	0.545 ±0.106
1,3,7,8-TCDF	0.204 ±0.022	0.141 ±0.036	0.017 ±0.010
Control experiment			
2,8-DCDF	5.81 ±0.37	4.36 ±0.20	
1,3,7,8-TCDF	0.125 ±0.012	0.178 ±0.021	

*C_m: concentration in medium; C_c: concentration in culture; k_b': pseudo first-order biodegradation rate constant.

The results given in Table 2 show that in these cultures significant degradation of 2-CDF and 2,8-DCDF took place. Although the concentrations of 1,3,7,8-TCDF were always lower in the cultures than in the medium, the difference was not significant ($p>0.05$). The results of a control experiment in which a bacteria suspension sterilized with 10 mM sodium azide was exposed to 2,8-DCDF and 1,3,7,8-TCDF are also given in Table 2. Although the sodium azide was not completely effective, as was seen when samples of the culture showed some growth on nutrient agar plates, these results demonstrate that the removal of PCDFs in the cultures was in fact due to biodegradation.

The biodegradation of dibenzofuran is reported to proceed by a pathway which includes the intermediate formation of salicylate[3,4]. If if stain JB1 degrades chlorinated dibenzofurans by the same pathway, chlorinated salicylates should be formed as metabolites. For example, 5-chlorosalicylate should be formed as a metabolite of 2,8-DCDF. This was investigated by growing a batch culture of strain JB1 on 3-methylbenzoate in the presence of a large excess of 2-CDF (ca. 50 mg/l). The culture was sampled daily and analysed by reversed-phase HPLC. After 3 days a new peak appeared in the chromatograms with a retention time identical to that of an authentic sample of 5-chlorosalicylate. This peak corresponded to a concentration of ca. 0.4 mg/l. After a further 4 days incubation the concentration of 5-chlorosalicylate had decreased to ca. 0.3 mg/l. Thus, it appears that 5-chlorosalicylate is formed as a metabolite of 2-CDF by strain JB1.

In conclusion, cultures of Alcaligenes strain JB1, grown on benzoate or 3-methylbenzoate, are able to degrade 2-CDF and 2,8-DCDF. Biodegradation rates appear to decrease with increased chlorination. At the moment there is no evidence for significant degradation of 1,3,7,8-TCDF: more data are needed before firm conclusions can be drawn in this regard. We have evidence that 5-chlorosalicylate is formed as a metabolite of 2-CDF. This result obviously requires confirmation, e.g. by GC-MS analysis.

Further work in our group on the biodegradation of PCDFs (and PCDDs) will concentrate on the following aspects:

1. The relationship between structure and biodegradation rates of PCDFs, including those with 4 or more chlorine substituents.
2. Elucidation of the mechanism and pathway of biodegradation of PCDFs.
3. Effect of sorption of PCDFs and PCDDs on particulates on their biodegradation rates.
4. Attempted isolation of bacteria from PCDF- and PCDD-contaminated environments able to degrade highly chlorinated PCDFs and PCDDs under aerobic or anaerobic conditions.

References

1. C.E. Cerniglia, J.C. Morgan and D.T. Gibson, Bacterial and fungal oxidation of dibenzofuran, Biochem. J., 180(1979)175-185.

2. J.M. Foght and D.W.S. Westlake, Degradation of polycyclic aromatic hydrocarbons and aromatic heterocycles by a *Pseudomonas* species, Can. J. Microbiol., 34(1988)1135-1141.

3. P. Fortnagel, H. Harms, R.-M. Wittich, W. Franke, S. Krohn, H. Meyer, V. Sinnwell, H. Wilkes and W. Francke, Metabolism of dibenzofuran by *Pseudomonas* sp. strain HH69 and the mixed culture HH27, Appl. Environ. Microbiol. 56(1990)1148-1156.

4. V. Strubel, H.G. Rast, W. Fietz, H.-J. Knackmuss and K.H. Engesser, Enrichment of dibenzofuran utilizing bacteria with high co-metabolic potential towards towards dibenzodioxin and other anellated aromatics, FEMS Microbiol. Lett., 58(1989)233-238.

5. J.R. Parsons, D.T.H.M. Sijm, A. van Laar and O. Hutzinger, Biodegradation of chlorinated biphenyls and benzoic acids by a *Pseudomonas* strain, Appl. Microbiol. Biotechnol., 29(1988)81-84.

6. J.R. Parsons and M.C.M. Storms, Biodegradation of chlorinated dibenzo-p-dioxins in batch and continuous cultures of strain JB1, Chemosphere, 19(1989)1297-1308.