LABORATORY METHODS FOR THE AEROBIC DESTRUCTION OF PCBS: A VIABLE APPROACH TOWARDS ENVIRONMENTAL CLEANUP ?

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ABSTRACT

The mineralization of Aroclors 1242 in soil, measured as $^{14}CO_2$ evolution, was greatly enhanced upon inoculation with *Acinetobacter* sp. strain P6 (a PCB cometabolizer) and *Pseudomonas aeruginosa* strain JB2 (a commensal which grows on chlorobenzoates), when amended with biphenyl. Alternatively, an aerobic continuous reactor system with pure axenic cultures of stain P6 and *Acinetobacter* sp. strain 4-CB1 was able to mineralize selected PCB congeners. Both systems appear to be promising approaches towards application.

INTRODUCTION

The potential of aerobic degradation of PCBs has been investigated [1-5]. For these processes to be applicable in *in situ* (no displacement of the soil matrix) and on site (with displacement) bioremediation methods, extensive information is required on the degradation kinetics of PCBs (both in pure culture and at the concentrations and environmental conditions present in soil), on the nature of the degradative processes and the interactions between the microorganisms, on the fate of the PCB co-metabolites (chlorobenzoates, chlorocatechols) involved, and lastly on the influence of the physico-chemical parameters (i.e. aqueous solubility, vapor pressure and sorption reactions) of PCBs.

EXPERIMENTAL METHODS

Resting cell incubations with [^{14}C] Aroclor 1254 have been described in Kohler et al. [1], while the soil incubation procedures with [^{14}C] Aroclor 1242 have been described previously [5,6]. The continuous aerobic reactor set-up is described in Adriaens and Focht [3]. The following water soluble amounts of PCB congeners were supplied to the microbial biofilm (per day): 4,4'-dichlorobiphenyl (3.0 mg), 3,4-dichlorobiphenyl (1.3 mg), and 3,3'4,4'-tetrachlorobiphenyl (0.3 mg) Additionally, on day 17, a shock load of 4,4'-di- (100 mg l^{-1}) and 3,3',4,4'-tetrachlorobiphenyls (10 mg l^{-1}) was applied to the biofilm.

RESULTS AND DISCUSSION

Microbial substrate kinetics in soil indicated that microorganisms behave similar to resting cells (high maintenance requirements) or growing cells (lower maintenance), depending on the type of substrate added [7]. The results from Table I show that growing cells are physiologically more stable than resting cells with respect to metabolism of biphenyl and transformation of PCBs. Moreover, it was noted that growing cells outperformed resting cells in their ability to transform Aroclor 1254 in two ways: (i) growing cells transformed congeners to a greater extent than resting cells did, and (ii) growing cells transformed congeners that were not attacked by resting cells (Figure 1, from ref. 1).

Table I : Transformation of [14C] Aroclor 1254 by resting and growing cells of Acinetobacter sp. strain P6 and Arthrobacter sp. strain B1B. (From ref. 1).

Strain and cell type	% ^{14}C in hexane after acidic extraction[a]	% ^{14}C in hexane after basic reextraction[a]
Acinetobacter sp. strain P6 resting cells		
Control[c]	99	97
OD 4.5[d]	91	81
OD 0.9	93	80
Acinetobacter sp. strain P6 growing cells		
Control	99	101
Sample[e]	69	69
Arthrobacter sp. strain B1B resting cells		
Control	101	103
OD 6.0	91	95
OD 1.5	92	95
Arthrobacter sp. strain B1B growing cells		
Control	99	99
Sample	80	77

[a] The incubation was stopped by addition of concentrated H_2SO_4 (10%). After addition of Triton X-100 and Na_2SO_4, the incubation mixture was extracted with 4 volumes of hexane for 1 h. The hexane phase was reextracted with 2 ml of 0.1 N NaOH to separate acidic and polar metabolites.
[b] Percentages based on 100% being 79,000 and 9,500 dpm/ml for resting and growing cells, respectively.
[c] Controls were killed with $AgNO_3$ (10 mM).
[d] OD, Optical density
[e] Grown on 500 mg of biphenyl liter^{-1}; optical density = 1.0 at the end of the incubation.

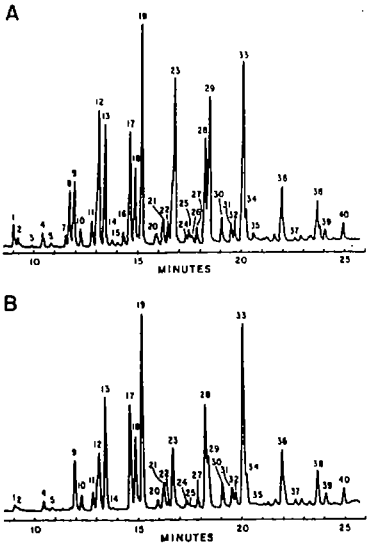

Figure 1 : GC comparisons of Aroclor 1254 transformation by resting cells (A) and growing cells (B) of *Acinetobacter* sp. strain P6. Note the differences in peaks 1, 2, 4, 7, 8, 12, 16, 22, 23, 25, 26, 29, and 35. Chromatograms were normalized with respect to peak 36.

Metabolism and mineralization of [^{14}C] Aroclor 1242 in soils were greatly enhanced by the addition of biphenyl (in concert with the indigenous microflora), compared to the addition of PCB cometabolizing bacteria alone. The mineralization of PCBs, measured as $^{14}CO_2$ evolution, was

delayed until after the mineralization of BP, and accounted for 48 % of the amount of PCB added (Table II).

Table III : Residual Aroclor 1242 and ^{14}C recovery from soils incubated for 49 days. From Focht and Brunner [6].

Treatment[a]	% PCBs[b]	% ^{14}C partitioning[c]						% ^{14}C recovered
		Hexane-acetone	Fulvic acid	Humic acid	Soil residue	HCO_3^- + CO_3^-	CO_2	
9B	25.2 ± 0.9	10.4	5.8 ± 0.1	2.7 ± 0.2	12.4 ± 0.1	17.2 ± 1.6	31.4 ± 1.0	79.9
5B	34.9 ± 0.5	12.7	4.4 ± 0.2	2.1 ± 0.2	12.4 ± 0.1	15.3 ± 1.9	32.9 ± 0.2	79.8
UB	35.0 ± 0.8	16.9	4.2 ± 0.01	2.0 ± 0.2	13.5 ± 1.9	11.2 ± 1.0	38.2 ± 0.1	86.0
Control	91.6 ± 2.1	71.6	4.2 ± 0.06	2.7 ± 0.05	16.8 ± 2.1	0	1.8 ± 0.1	95.9
Control + $AgNO_3$	93.1 ± 2.2	71.6	4.2 ± 0.06	2.7 ± 0.05	16.8 ± 2.0	0	0	94.1

[a] 9B, 5B, and UB treatments contained 0.33 g of BP per g of soil.
[b] Percentage of original Aroclor 1242 remaining after extraction with hexane-acetone (1:1, vol/vol) and determined by gas chromatography. Values are reported as the mean ± standard deviation and were corrected on the basis of an 84 ± 4% extraction efficiency.
[c] Values are reported as the mean ± standard deviation. When no standard deviation is given, only one determination was made.

Based on the $^{14}CO_2$ evolution data, it was concluded that the $^{14}CO_2$ production of PCBs could not be based on the metabolic activity of one species, the inoculated BP oxidizer, but is rather the result of metabolism of the cometabolic products from PCBs by indigenous commensals. Additionally, it was observed that both [^{14}C] Aroclor and [^{14}C] residues were incorporated in soil biomass and stabilized in humus (Table II). Soil incubations with Aroclor 1242, and inoculated both with *Acinetobacter* sp. strain P6 and *Psudomonas aeruginosa* JB2 (a versatile chlorobenzoate commensal) indicated that the rate of $^{14}CO_2$ liberation was dependent on the chlorobenzoate metabolizing strain.

To mimick the soil environment, an aerobic biofilm consisting of *Acinetobacter* sp. strain P6 (a PCB cometabolizer) and *Acinetobacter* sp. strain 4-CB1 (a commensal that grows on chlorobenzoates) was established. Since benzoate was used as the growth substrate, biphenyl vapors were introduced to induce the biphenyl dioxygenase required for PCB cometabolism. Although the PCBs were degraded to an array of intermediates, the degree of mineralization was low : 6.5% and 10% of 4,4'-dichlorobiphenyl and 3,4-dichlorobiphenyl was recovered as inorganic chloride (Table III). The second order rate constants for the growth and cometabolized substrates involved demonstrated that both a low cosubstrate concentration and and a non-selective growth substrate (benzoate) slow down the degradation rate of the chlorinated compounds compared to the rate of utilization of benzoate. Moreover, the interaction of physico-chemical parameters (i.e. solubility and diffusion of the substrates) and biological parameters (i.e. Monod constant K_M, second order rate constant k/K_M, microbial competition for the growth substrate) was responsible for the restconcentrations observed in the effluent and on the foam matrix, and the low degree of mineralization.

Although a succession of microorganisms, whether pure axenic cultures or a combination of an inoculum with the indigenous microflora, was able to mineralize Aroclors and individual PCB

congeners bound to an inert medium or soil matrix, careful attention has to be given to the accumulation of partially metabolized PCBs and low concentration metabolites which may link into humic constituents. Whether this fact constitutes an environmentally acceptable criterion of metabolism to inocuous products bears attention in future studies.

Table III : Degradation of PCB isomers by a coculture of two *Acinetobacter* sp. during a 45 day period (values are given in mM chloride). From Adriaens and Focht [3].

Congener (mM added)	Metabolite	Recovery from Aqueous phase	Solid phase*
		..... mM Chl.	
4,4'-DCBP	4,4'-DCBP	-	1.566
(4.400 mM)	ringfission product	0.718	1.580
	4-chlorobenzoate	0.272	0.029
	inorganic chloride	0.180	-
3,4-DCBP	3,4-DCBP	-	<0.001
(0.468 mM)	ringfission	-	-
	3,4-dichlorobenz.	0.328	0.060
	3-chl-4-hydroxybenz.	0.052	-
	quinone	Not quantified	-
	inorganic chloride	0.052	-
3,3'4,4'-TCBP	3,3'4,4'-TCBP	-	0.118
(0.376 mM)	ringfission	-	-
	3,4-dichlorobenz.	-	0.120
	3-chl-4-hydroxybenz.	-	-
	quinone	-	-
	inorganic chloride	-	-

* Based on total Soxhlet extract of polyurethane foam and biofilm.

REFERENCES

1. H.-P. E. Kohler, D. Kohler-Staub and D. D. Focht, Appl. Environ. Microbiol. 55, 1940 (1988).
2. P. Adriaens, H.-P. E. Kohler, D. Kohler-Staub and D. D. Focht, Appl. Environ. Microbiol. 55, 887 (1989).
3. P. Adriaens and D. D. Focht, Environ. Sci. and Tech., In press.
4. P. Adriaens, C. M. Huang and D. D. Focht, Proc. Meet. Am. Chem Soc. Boston, In press (1990).
5. W. J. Hickey, PhD Dissertation, Univ. of Calif., Riverside (1990).
6. D. D. Focht and W. Brunner, Appl. Environ. Microbiol., 50, 1058 (1985).
7. W. Brunner, F. H. Sutherland and D. D. Focht, J. Environ. Qual., 14, 324 (1985).