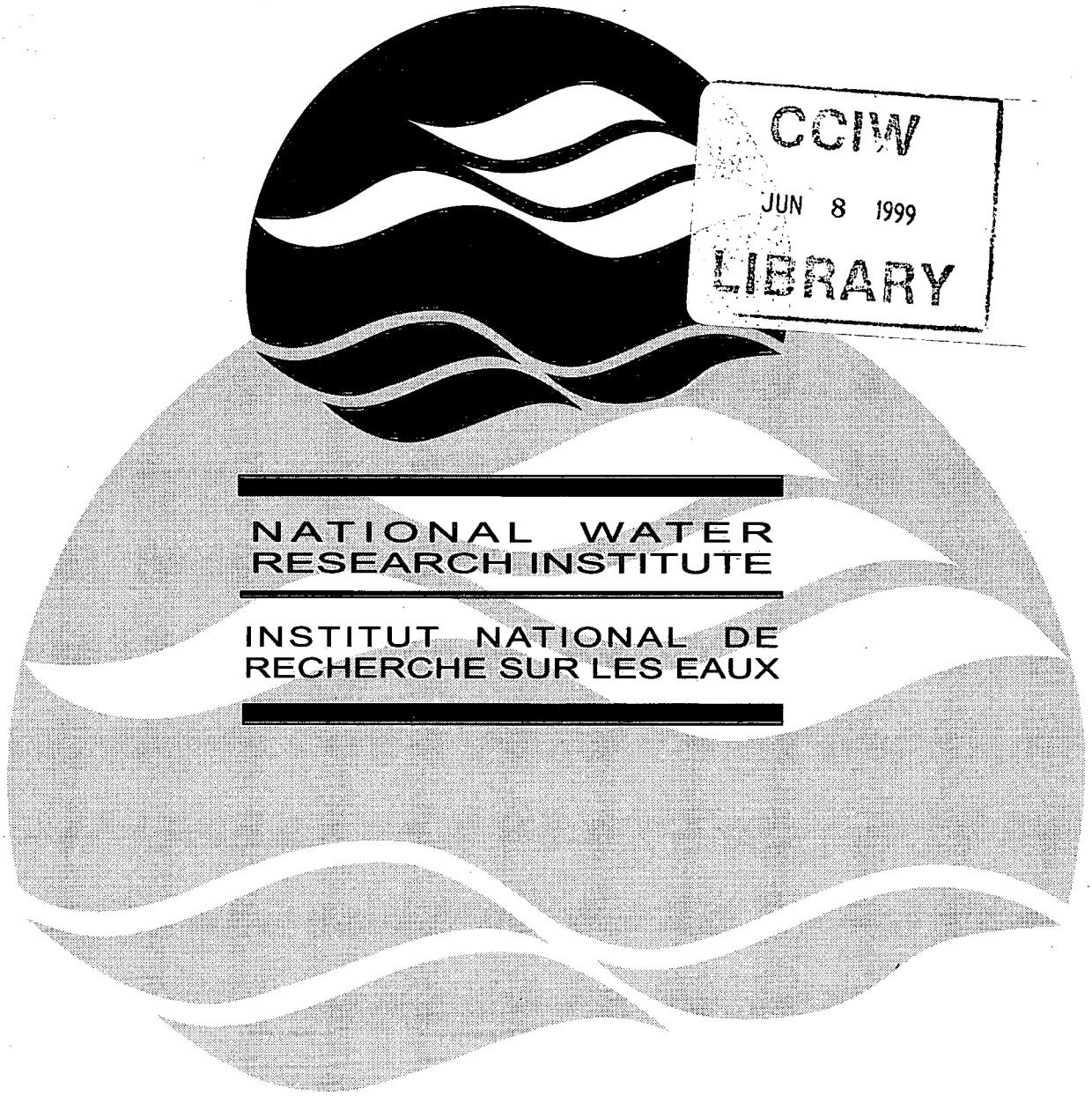

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THE BACTERIAL DEGRADATION OF PCB IN SYDNEY HARBOUR SEDIMENT

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Bacterial Degradation of PCB in Sydney Harbour Sediment

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Management Perspective

The degradation of polychlorinated biphenyls (PCBs) by the biphenyl-utilizing bacteria *Alkaligenes xylosoxidans* and *Pseudomonas stutzeri* was studied in different sediment samples. The aim of the present study was to evaluate the capability of these bacterial strains to degrade PCB in sediment microcosms, and to identify some of the factors likely to favor the degradative performance of seeded bacteria. The most important factor affecting PCB degradation in the test systems was the necessity to have biphenyl as a co-substrate. Because biphenyl was rapidly depleted in sediment, repeated addition of small amounts of biphenyl to maintain a constant level of the co-substrate allowed the degradation of lower chlorinated biphenyl congeners. In addition to the congener specificity, significant differences were observed between the two bacterial strains in the degradation of PCBs in the different sediment samples. The efficiency of degradation was generally low for higher chlorinated congeners. These results indicate that the degradation of PCBs is probably related not only to the bacterial strain employed and the high concentration of PAHs in the sediment, but also to sediment sorption of the PCB congeners. The degradation is faster in sediments containing an intermediate amount of organic carbon with a high proportion of total aromatic carbon in the humic acid fraction.

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Sommaire à l'intention de la direction

La dégradation des polychlorobiphényles (PCB) par des bactéries utilisant le biphényle, *Alkaligenes xylosoxidans* et *Pseudomonas stutzeri*, a été étudiée pour différents échantillons de sédiments. Le but de cette étude était d'évaluer la capacité de dégradation des PCB de ces souches bactériennes dans des microcosmes sédimentaires, et d'identifier quelques facteurs facilitant la performance de dégradation des bactéries ensemencées. Le principal facteur affectant la dégradation des PCB dans les systèmes expérimentaux était la nécessité d'utiliser le biphényle comme co-substrat. En raison de l'élimination rapide du biphényle dans les sédiments, l'addition répétée de petites doses de biphényle, pour maintenir un niveau constant de co-substrat, a permis la dégradation de congénères de chlorobiphényles inférieurs. En plus de la spécificité selon les congénères, des différences importantes ont été observées entre les deux souches bactériennes dans la dégradation des PCB selon les échantillons de sédiments. L'efficacité de la dégradation était généralement faible pour les congénères chlorés supérieurs. Ces résultats indiquent que la dégradation des PCB est probablement reliée non seulement aux souches bactériennes utilisées et à la forte concentration d'HAP dans le sédiment, mais aussi à la sorption des congénères de PCB dans les sédiments. La dégradation est plus rapide dans les sédiments contenant une quantité intermédiaire de carbone organique avec une forte proportion de carbone aromatique total dans la fraction de l'acide humique.

Ce rapport a été rédigé pour l'Organisation du Traité de l'Atlantique Nord afin de remplir les conditions reliées à la subvention n° SA 12.2-02 (Envir.LG 940637) (95) LVdC et son supplément, n° SA 12.5-02 (CN.Suppl.950 464) 453/JPN/065.

Abstract

The degradation of PCBs by two biphenyl-utilizing bacterial strains, *Alkaligenes xylosoxidans* and *Pseudomonas stutzeri*, was studied in sediment from Sydney Harbour, Nova Scotia which was contaminated by PCBs and coal tar. In addition to the congener specificity, significant differences were observed between the two bacterial strains in the degradation of PCBs in different sediment samples. The efficiency of degradation was generally low for higher chlorinated congeners. These results indicate that the degradation of PCBs is probably related not only to the bacterial strain employed and the high concentration of PAHs in the sediment, but also to sediment sorption of the PCB congeners. The degradation is faster in sediments containing an intermediate amount of organic carbon with a high proportion of total aromatic carbon in the humic acid fraction. This study was supported by the North Atlantic Treaty Organization.

Résumé

La dégradation des PCB par deux souches bactériennes utilisant le biphényle, *Alkaligenes xylosoxidans* et *Pseudomonas stutzeri*, a été étudiée dans des sédiments du port de Sydney, en Nouvelle-Écosse, qui ont été contaminés par des PCB et du goudron de houille. En plus de la spécificité selon les congénères, des différences importantes ont été observées entre les deux souches bactériennes dans la dégradation des PCB selon les échantillons de sédiments. L'efficacité de la dégradation était généralement faible pour les congénères chlorés supérieurs. Ces résultats indiquent que la dégradation des PCB est probablement reliée non seulement aux souches bactériennes utilisées et à la forte concentration d'HAP dans les sédiments, mais aussi à la sorption des congénères de PCB dans les sédiments. La dégradation est plus rapide dans les sédiments contenant une quantité intermédiaire de carbone organique avec une forte proportion de carbone aromatique total dans la fraction de l'acide humique. Cette étude a été subventionnée par l'Organisation du Traité de l'Atlantique Nord.

Introduction

Polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) in bottom sediments have been associated with tumor-forming effects in bottom feeding fish. Brown *et al.* (1985) have discussed an increased prevalence of hepatic neoplasms and other hepatic lesions in English sole *(Parophys vetulus)* containing toxic chemicals in sediments and biota from a creosote-polluted harbor. The harbour at Sydney, Nova Scotia (Fig. 1) has been heavily polluted by industrial contaminants, primarily as a result of coal tar discharges from a nearby steel mill over a period of more than a half of century. These discharges are concentrated in a waste disposal area known as the Sydney Tar Pond (Kiely *et al.*, 1988). Analyses of sediment samples from the Tar Pond have indicated total PAH concentrations in the range 0.13-2800 μg/g dry weight (Matheson *et al.*, 1983). The total PCB content is also very high.

The purpose of this study was to determine factors affecting the degradation of PCBs by two biphenyl-utilizing bacterial strains, *Alkaligenes xylosoxidans* and *Pseudomonas stutzeri*, in sediment from the Tar Pond area. This study reports a modified method to optimize aeration conditions, and the use of a sorbent trap to determine the distribution of PCB congeners between the gaseous phase and the aqueous incubation medium.

Experimental Methods

Chemicals

A commercial mixture of PCB congeners, Aroclor 1260 containing 60% (w/v) of bound chlorine, biphenyl, *n*-hexane (UV-grade purity), acetone (p.a.) and GTK agar were used.

Sediment

Sediment from the Tar Pond area (see Fig. 1) was kindly provided by Dr. T. Murphy of the National Water Research Institute. The water content of the Sydney sediment was 11.1± 1.5%, determined by drying to constant weight at 65 °C. The pH value of the sediment was 6.7, was determined in 0.1 mol.L^{-1} KCl solution. The sediment contained about 100 mg/kg dry weight of PCBs, mainly highly chlorinated congeners (the source of contamination was probably Aroclor 1260). In addition, it contained high concentrations of polynuclear aromatic hydrocarbons and other hydrocarbons. Details have been previously reported by Marvin *et al.* (1993, 1994).

Bacteria from a contaminated Slovak soil

Two bacterial strains were studied, *Alcaligenes xylosoxidans* and *Pseudomonas stutzeri*. Both were isolated from a long-term contaminated Slovak soil by enrichment in defined mineral medium with biphenyl as the sole carbon and energy source (Tickett *et al.*, 1991; Dercova *et al.*, 1995). The enrichment was done by adding 10 g of the sediment to a 1000 L flask containing 500 mL of a cultivation medium (Furukawa and Matsumura, 1976) containing 0.5 g/L of glucose, 0.5 g/L of peptone, and 0.5 g/L KH_2PO_4 (pH 7.0), and incubating for 3 days on a rotary shaker (180 rpm) at 30 °C. Isolation was carried out in a 500 mL flask containing 200 mL of a defined

mineral medium (DMA) (Pirt, 1967) with 1 g/L of biphenyl as the sole carbon source. An inoculum of the enrichment culture (4 mL) was transferred to this flask and incubated for 7 days on a rotary shaker. To isolate the organisms, an inoculum of the above liquid cultures was streaked on the surface of the agarized DMA medium with excess biphenyl (5 g/L) in Petri dishes. After incubation, a portion of each single colony was picked up and sub-cultured aerobically in liquid DMA medium with biphenyl and streaked on the solid medium again. The inoculum of *Alcaligenes xylosoxidans* was cultivated to 0.25 g dry weight per litre in the synthetic DMA medium (pH 6.7) with biphenyl as a sole carbon source (2.5 g/L) for 7 days at 28 °C on a rotary shaker (180 rpm) in the dark.

The strains were later provided in lyophilized form by the Slovak Technical University, Bratislava, Slovak Republic. The lyophilized bacteria in ampoules were reconstituted in 0.3 mL of broth medium, transferred to liquid broth medium, and incubated for at least 48 hours at 37 °C on a rotary shaker. Used ampoules were soaked in a disinfection solution or autoclaved.

The synthetic medium for bacteria is composed of five parts, A-E. Part A is KH_2PO_4 (84.5 g); B is $MgSO_4.7H_2O$ (20 g); C is $CaCl_2$ (1.0 g); D is a mixture of $FeSO_4.7H_2O$, (5.0 g), $ZnSO_4.7H_20$, (5.0 g), $MnS0_4.5H_20$ (5.0 g), $CuSO_4.5H_20$ (1.0 g), $CoCl_2.6H_20$ (1.0 g), $Na_2B_2O_7$ (1.0 g) and $Na_2MoO_4.2H_20$ (1.0 g); and E is NaCl (20 g). Parts A, B, C and E were each dissolved in 500 mL of distilled water and autoclaved for 20 minutes at 120 kPa. The first three ingredients of part D were each dissolved in 100 mL of distilled water, and the last 4 ingredients of part D were each dissolved in 1 L of distilled water. These solutions were autoclaved separately (20 minutes at 120 kPa), mixed 10:1:1:10:10:10:10 (by volume) and then 52 mL of that mixture was mixed with 448 mL distilled water to give the final D solution. The solutions of parts A-E were then mixed 10:1:1:1:10 by volume, and 120 mL of the final mixture was combined with 880 mL of distilled water. The pH was adjusted to 7.2 with a 5% solution of NaOH. The volumes of samples and carbon source are specified in the description of each experiment in the Tables (see below).

For the preparation of solid medium, 20 g of agar in 880 mL of distilled water were autoclaved for 20 minutes at 120 kPa. To the hot (60 ^{0}C) agar solution, 120 ml of the A-E mixture (10:1:1:1:10) were added and mixed well.

Isolation of PCB degrading microorganisms from Sydney Tar Pond sediments

Microorganisms with the capacity to degrade PCBs were isolated by preparing bacterial cultures from different sediment samples contaminated with PCBs from Sydney Harbour.

For the isolation, 1 g of sediment sample was diluted in defined mineral medium to an optical density A = 0.1 at 625 nm (UV/VIS spectrometer) against pure mineral medium. To 100 mL of sediment suspension in a 250 mL Erlenmeyer flask, 2.5 g of biphenyl were added as a sole carbon source. The flasks were incubated at 30 ^{0}C on a rotary shaker (150 rpm). Optical density readings were taken periodically. After 7 days, samples with increased optical density (A = 1.0 or higher) were collected for further isolation work.

An inoculum of the enrichment culture was diluted on the surface of broth medium agar. After 24 hours incubation at 30 °C a portion of each single colony was picked up and transferred to a Petri dish with broth medium agar. After a further 24 hours incubation at 30 °C a portion of the pure culture on the agar surface was transferred to a Petri dish with mineral medium agar without any carbon source. These Petri dishes were incubated in the upside down position with a few biphenyl crystals on the bottom lid of the Petri dish. This allowed the microorganisms to grow using biphenyl vapour as a sole carbon and energy source. The cultures with positive growth were transferred to test tubes with "oblique" broth medium agar and after 24 hours of cultivation at 30 ^{0}C they were stored in a refrigerator at 5 ^{0} C for further use.

Bacteria able to grow on biphenyl as a sole carbon and energy source in aerobic conditions were isolated only from two samples of Hamilton Harbour sediments, samples labeled as "Bunker oil 2" and Bunker oil 3". No significant increase of turbidity during the incubation of the enrichment culture (A_{625}>1.0) was observed.

The isolated pure cultures were stored in test tubes with "oblique" agar marked B2/1 to B2/7 for isolates from "Bunker oil 2" and B3/1 to B3/7 for isolates from "Bunker oil 3" in a refrigerator at 5 ^{0}C. For longer storage, they should be re-inoculated to a fresh agar medium every three weeks or the cotton wool stoppers should be soaked in molten paraffin (50 ^{0}C) to avoid the rapid evaporation of water. After this treatment the cultures can survive on the agar surface in the test tubes for 3 months. If the PCB degradation results with the isolates are positive, the isolates can be identified and lyophilized for long-term storage.

Inocula of isolates for the PCB degradation study were prepared by incubating isolates for 3 days in defined mineral medium containing 2.5 g/L biphenyl at 30 ^{0}C. After incubation, cell counting in the inoculum was done. For the PCB degradation study only isolates B2/l and B3/1 were taken.

Cell counting

Broth agar (broth medium with agar) (5.0 g of enzymatic casein hydrolysate, 2.5 g of yeast extract, 1.0 g of glucose and 15 g of agar suspended in 1000 mL of distilled water) (pH 7.2) was autoclaved for 15 minutes at 120 kPa, and transferred into Petri dishes. After proper dilution in a sterile 0.90% NaCl solution, the diluted bacterial inoculum and sediment extract (0.1 mL) was pipetted onto the agar source in the Petri dishes. The colonies were counted in three series after 48 h incubation at 30 ^{0}C. Growth was monitored over time by measuring absorbance at 600 nm.

PCB degradation experiments

Incubations were carried out in 150 mL Erlenmeyer flasks fitted with column traps (height 150 mm, diameter 25 mm) filled with 0.3 g of C-18 resin (diameter of particles 55-105 μm) (see Fig. 2). The traps were used to determine the loss of PCBs caused by evaporation during incubation.

Three g of sediment was added to a 150 mL Erlenmeyer flask. The sediment was spiked

with a toluene solution of decachlorobiphenyl (50 µg of decachlorobiphenyl in 44.2 µL). Then 0.1 g of biphenyl and 25 mL of defined mineral medium (pH=7.2) was added. Five ml of bacterial inoculum (1.3 10^{10} CFU/mL of *Pseudomonas stutzeri* or 2.1.10^{9} CFU/ml of *Alcaligenes xylosoxidans* or 2.5.10^{9} CFU/mL B2/1 or 4.2.10^{9} CFU/mL of B3/1) was added. The flasks were closed with the sorbent column described above. All experiments were done in triplicate. Controls without added microorganisms were also made in triplicate. The experimental protocol is described in Table 1. Flasks were incubated at 30 °C on a rotary shaker (150 rpm) without illumination for 30 and 60 days, respectively. Flasks were weighed, and water lost was replaced on a weekly basis. At the end of the incubation time, the concentrations of PCBs were determined in the water, the sediment (after centrifugation) and the trap. Appropriate control experiments were conducted without added microorganisms. The degradation of Aroclor 1260 (without sediment) in this test system was also studied.

For the chemical analysis, the aqueous portion of the medium was separated from the sediment and extracted with methylene chloride (3 x 10 mL). The C-18 resin trap was washed with methylene chloride and this extract was combined with the organic aqueous extract. Extraction of sediment samples was accomplished using a Soxhlet extractor for 8-14 hours (typically such conditions exhibit coefficients of variation of 8-18 % for PCB homologue concentrations). The sediment sample (3 g) was extracted with 350 mL hexane/acetone (1/1, v/v) after spiking with 50 µg of decachlorobiphenyl as an internal standard. The extract was dried by passage through a Na_2SO_4 column. Sample extracts were combined and then cleaned up on a Florisil column and by deactivated silica chromatography (3% water). Sulfur was removed using mercury. One hundred µg of PCB 204 (nonachloro-derivative) was used as a surrogate standard and the samples were made up to volume for quantitative analyses.

Chemical analyses

(a) gas chromatography (HRGC-ECD)

Determination of the individual PCB congeners was done with a Hewlett-Packard 5890 GC with H_2 as carrier gas (60 kPa, 1.5 mL/min split-splitless inlet mode), using an electron capture detector (ECD) (350 °C, make up gas N_2 at 60 mL/min), and a 50 m x 0.32 mm I.D. fused-silica capillary column with the nonpolar stationary phase HP 1 (thickness 0.17 µm). Temperature conditions: injector 250 °C, column 45 °C for 0.5 minute, then programmed at 20 °C/min to 150 °C, then 2 °C/min to 250 °C, with a 6 minute final hold. Reproducibility of the quantitative analysis was controlled using a standard solution of Aroclor 1260 (7.5 µg/mL). Relative deviations for congeners which had no interference from the background were around 3%. (see Fig.3).

(b) high resolution gas chromatography - mass spectrometry (HRGC-MS)

Identification and quantitation of PCBs was carried out with a Hewlett-Packard 5890 Series II gas chromatograph coupled to a Hewlett-Packard Engine 5989A mass spectrometer. The column was a 30 m x 0.25 mm i.d. DB-5MS capillary column connected to a deactivated 1 m x 0.32 mm i.d. fused silica pre-column. The injector temperature was 250 °C and the transfer line

temperature was 200 °C. The MS source temperature was set at 200 °C and the quadrupole temperature at 100 °C. The carrier gas was helium at 190 kPa in constant flow mode. Sample volumes of 1 μL were injected using split/splitless injection mode (valve opened after 30 s) employing the following oven temperature program: 80 °C hold for 1 minute, then programmed to 150 °C at 15 °C/min then to 270 °C at 6 °C/min, with a 10 minute final hold (Onuska *et al.*, 1983). Because of the many interferences in the samples, selected ion monitoring mode (SIM) was used, rather than full scan mode. Table 2 shows ions monitored for identification and quantitation purposes. Quantitation was performed relative to decachlorobiphenyl as internal standard and PCB 204 was used as a surrogate standard. HRGC-ECD was initially used in Bratislava but later HRGC-MS was used at NWRI.

Results and Discussion

The success of bioremediation of PCBs in Sydney Harbour sediment involved introduction of a laboratory bacterial strain into a sediment. This process depends on the survival of a specific bacterial strain and on its biological activity in the sediment. As shown in Fig. 3, *A. xylosoxidans* can degrade several lower chlorinated congeners of Aroclor 1260 mixture under defined conditions over a 14 day period. Only di-, tri- and tetra-chlorinated congeners were degraded. Removal of higher chlorinated PCB congeners was less than 5% in all experiments. Therefore, the results for congeners with more than four chlorine substituents are not reported.

Among the important factors affecting PCB degradation in sediment, the principal factor was maintaining a constant level of biphenyl. The use of biphenyl as a growth substrate is known to enhance PCB degradation in liquid media (Kohler *et al.*, 1988). The initial degradation rates of PCBs in sediment by the added bacterial strains *A. xylosoxidans* and *P. stuzeri* were independent of the biphenyl concentration at the time of inoculation. However, repeated additions of a small amount of biphenyl to maintain a constant level of the co-substrate in sediment led to more degradation of the most highly chlorinated components of PCBs than a single addition of biphenyl at the time of inoculation.

When considered individually, the degradation of di- and trichlorinated biphenyls was very fast in sediment inoculated with both strains (Table 3). In the presence of biphenyl, there was a sharp decrease in the concentrations of several Aroclor 1260 congeners during the first few days, then the concentration more slowly decreased thereafter (Table 4). It is likely that the more readily available congeners (the more water soluble ones) are degraded first, then the less water-soluble congeners are released slowly from sediment and degraded at a lower rate. In this respect, Sugiura (1992) showed a direct correlation between the rate of degradation of various PCB congeners and their water solubility. However, solubilization of PCB congeners in water did not appear to be the only factor responsible for the lowering of PCB degradation measured in the older culture. Among the factors that are likely to decrease the rate of PCB degradation, one is certainly the accumulation of toxic metabolites from biphenyl and chlorinated biphenyls that interact with the enzyme activities involved in PCB transformation. Interestingly there was no direct correlation between survival of *Alcaligenes xylosoxidans* and its degradation ability in the individual sediment types. The degradation of PCBs is probably related not solely to the capabilities of the strain employed, but also to the bioavailability of the substrate.

It is noteworthy from the data illustrated in Fig. 4 for a single isomer 2,2',4,4', 5,5'-hexachloro biphenyl (IUPAC # 153) that degradation resumed immediately after viable *P. stutzeri* were massively re-introduced in the sediment. Although the rate of degradation in reintroduced microcosms was lower than immediately following the first inoculation, this rate was maintained for the remainder of the experiment.

Comparison of Isolates with Adapted Strains

Table 1 summarizes the results of biodegradation tests of isolates in relationship with the adapted collection strains *Pseudomonas stutzeri* and *Alcaligenes xylosoxidans* after 14 days cultivation. Degradation in the studied bacterial strains ranged from 5.2 to 6.3 % degradation. The best single strain *Alcaligenes xylosoxidans* exhibited good biodegradative ability and the best growth on biphenyl as the sole carbon source was selected for the following experiments.

Biphenyl utilization test under anaerobic conditions

For the biphenyl utilization test under anaerobic conditions with nitrate as electron acceptor, 1.805 g of KNO_3 was added to 250 mL of deionized water in a 500 mL Erlenmeyer flask. This solution was autoclaved for 20 minutes at 120 kPa. To this solution, 31 mL of A-E solution (10:1:1:1:10) of defined mineral medium (see above) were added and mixed well. The pH was adjusted to 7.2 and 0.625 g of biphenyl was added. The flask was purged with nitrogen and inoculated with 5 mL of the diluted bacterial inoculum (A= 0.1 at 625 nm). Samples were incubated at 30 ^{0}C on a rotary shaker (150 rpm) in argon atmosphere. The optical density increase of the samples was checked after 7 days. No bacterial growth was observed in the samples after this time.

In conclusion, it appears that actively growing cells on biphenyl can degrade PCBs very rapidly, immediately after they are implanted into sediment, either because of a residual metabolism of biphenyl or after absorption into cells. The results show that (1) the survival of the inoculated strain does not correlate with the degradation of PCB congeners, (2) there are greater differences between degradation of PCB in different sediment types, and (3) efficiency of *Alcaligenes xylosoxidans* is much higher in liquid media than in soils, indicating that the degradation of PCB is probably related not solely to the capabilities of the strain used and to nature and amount of competitive species inhabiting the sediment, but also some other factors are at play.

PCB are hydrophobic compounds (octanol/water partition coefficients K_{ow}, a measure of hydrophobicity, for 58 PCB congeners range from the average log K_{ow} of 4.5 for monochlorinated compounds to the average log K_{ow} of 8. 1 for congeners with seven chlorines (Rapaport and Eisenreich, 1984) and therefore sorption of PCB on soil particles may play an important role in bioavailability of the compounds. The soil sorption of organic compounds can influence microbial degradation, implying that microorganisms are unable to access the sorbed materials (Mihelcic and Luthy, 1988). Commonly it is believed that only the dissolved portion of a chemical is directly available for uptake by microorganisms and increased sorption reduces biodegradation

rates (Peter and Weber, 1985a, 1985b; Basham et al., 1987; Sims et al., 1991). Barriault and Sylvestre (1993) observed stimulation of the PCB degradation by the strain *Pseudomonas testosteroni* B-356, caused probably by the decrease in the sorption of PCB due to the production of surfactants by added *Alcaligenes faecalis* B-556. The soil organic matter has a strong influence on the PCB adsorption. Paya-Perez et al. (1991) found linear correlation between the soil sorption coefficients of six chlorobenzenes and twenty PCB congeners and the organic carbon content of the tested soils. Other factors tested (pH content of silt and clay) were not significant. Sorption was favored by a high degree of chlorination and the absence of ortho-substitution in the biphenyl.

An important factor from the viewpoint of sorption seems to be, in addition to concentration, the nature of the organic matter, especially one of the most important components, humic acids. Humic acids are characterized by a high biological stability and hydrophobicity and thus they are the most suitable sediment components for sorption of hydrophobic chemicals. The importance of quality of the organic matter, mainly the amount and structure of humic; acids (elementary structure, amount of aliphatic and aromatic carbon) on sorption of non-ionic hydrophobic xenobiotics have been stressed (Gautier et al., 1987; Grathwog, 1990). It is difficult to judge the influence of single factors on degradation in such complex system as sediments, where many other factors are at play. Further experiments with sediment samples should be carried out. The selected sediments should not be so heavily contaminated with PAHs as the presently used ones. After biodegradation solid particles of the tar remained undisturbed and the inoculum did not penetrate them. For this reason it is assumed that the contact between inoculum and the PCB contamination was poorly defined (Table 5). Similarly, the presence of at least 10 fold greater concentration of PAHs probably resulted in competition between PAHs and PCBs during biodegradation, or the inhibitory effect of these co-pollutants was dominating during the process.

From this point of view it would be reasonable in future studies to vary the sediment characteristics individually in a model sediment system. However, the results from this study seem to confirm the importance of environmental factors, which significantly influence the degradation process. The understanding of the physico-chemical, as well as biological, factors controlling rates of degradation of PCB in soils is critical to the development of successful *in situ* bioremediation methods and may provide insight into the traits important for survival and activity of introduced microorganisms in sediment.

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Table 1. Protocol for the PCB degradation study with bacterial strains from Slovakia

Sample #	Microorganism	Sediment weight, g	Weight of flask with medium and sediment without column, g
1	none	2.910	129.9
2	none	2.919	128.5
3	none	2.946	128.1
4	*A. xylosoxidans*	2.977	132.8
5	*A. xylosoxidans*	2.906	131.4
6	*A. xylosoxidans*	2.971	129.4
7	*P. stutzeri*	2.940	127.8
8	*P. stutzeri*	2.941	129.5
9	*P. stutzeri*	3.037	128.7
10	none	2.827	129.4
11	none	2.979	130.4
12	none	3.035	131.3
13	*A. xylosoxidans*	2.806	126.7
14	*A. xylosoxidans*	2.976	128.2
15	*A. xylosoxidans*	2.965	133.7
16	*P. stutzeri*	2.998	131.3
17	*P. stutzeri*	3.043	129.0
18	*P. stutzeri*	2.919	131.3

Table 2. Selected ions for monitoring PCB congeners

Window	Mass, m/z	Dwell time, ms	Start, min.	Finish, min.
1	222,220,152,256,258,188,292, 290	60	10.5	16.0
2	326,328,254,360,362,290	60	16.0	20.2
3	360,362,290,394,396,324	60	20.2	21.8
4	394,396,324,430,428,358	60	21.8	25.8
5	464,462,466,498,500,428	60	25.8	30.0

Table 3. Degradation of PCB (initial concentration 9.5 μg/mL) by adapted strains of microorganisms after 14 days' cultivation, aeration, 30 °C, synthetic medium DMA, pH 6.7

Microorganism	**Degradation (%)**
P. stutzeri	5.2
A. xylosoxidans	6.3

Table 4. Biodegradation of Aroclor 1260*

No.	Date	Inoculum	Molar Percentages			
			Pentachloro	Hexachloro	Heptachloro	Octachloro
PCB Std.	8/29/96-9/27/96	none	12.7	57.2	26.9	3.2
1	8/29/96-9/27/96	none	13.5	54.1	28.3	4.1
4	8/29/96-9/27/96	*A. xylosoxidans*	13.0	53.3	28.9	4.1
7	8/29/96-9/27/96	*P. stutzeri*	12.8	55.0	27.4	4.8
10	8/29/96-10/28/96	none	13.3	54.6	27.9	4.2
13	8/29/96-10/28/96	*A. xylosoxidans*	13.8	57.5	27.6	1.4
16	8/29/96-10/28/96	*P. stutzeri*	12.7	54.7	30.7	1.9
1	9/19/96-10/28/96	none	11.9	48.7	33.3	6.1
4	9/19/96-10/28/96	B2/1	11.1	51.1	34.8	2.9
7	9/19/96-10/28/96	B3/1	11.0	54.3	31.1	3.6
10	9/19/96-11/19/96	none	10.7	50.7	33.2	5.4
13	9/19/96-11/19/96	B2/1	11.1	51.7	31.8	5.4
16	9/19/96-11/19/96	B3/1	11.8	51.0	31.7	5.5

*Analyses were performed in triplicate by HRGC-MS-SIM.

Table 5. Normalized results in mol % for biodegradation of Aroclor 1260 homologs in Tar Pond sediment.*

Treatment	Total, mg/kg	Pentachloro	Hexachloro	Heptachloro	Octachloro
Aroclor 1260 std.	50	11.26	56.10	28.90	3.74
none	161	11.98	52.87	30.39	4.79
A. xylosoxidans	175	11.50	52.15	30.99	5.36
P. stutzeri	146	11.31	53.75	29.35	5.59
none	139	11.76	53.41	29.92	3.31
A. xylosoxidans	165	11.86	56.54	29.69	1.64
P. stutzeri	197	11.24	53.58	32.96	2.22
none	121	10.42	47.18	35.36	7.00
B2/1	167	9.77	50.06	37.13	3.37
B3/1	121	9.69	52.90	33.22	4.18
none	137	9.37	49.13	35.26	6.24
B2/1	166	9.74	50.18	33.83	6.24
B3/1	175	10.36	49.53	33.85	6.37

*N.B. Normalized total concentration for all samples is 100 mg/kg for wet sediment. % RSD for individual homologue groups is 11.12%.

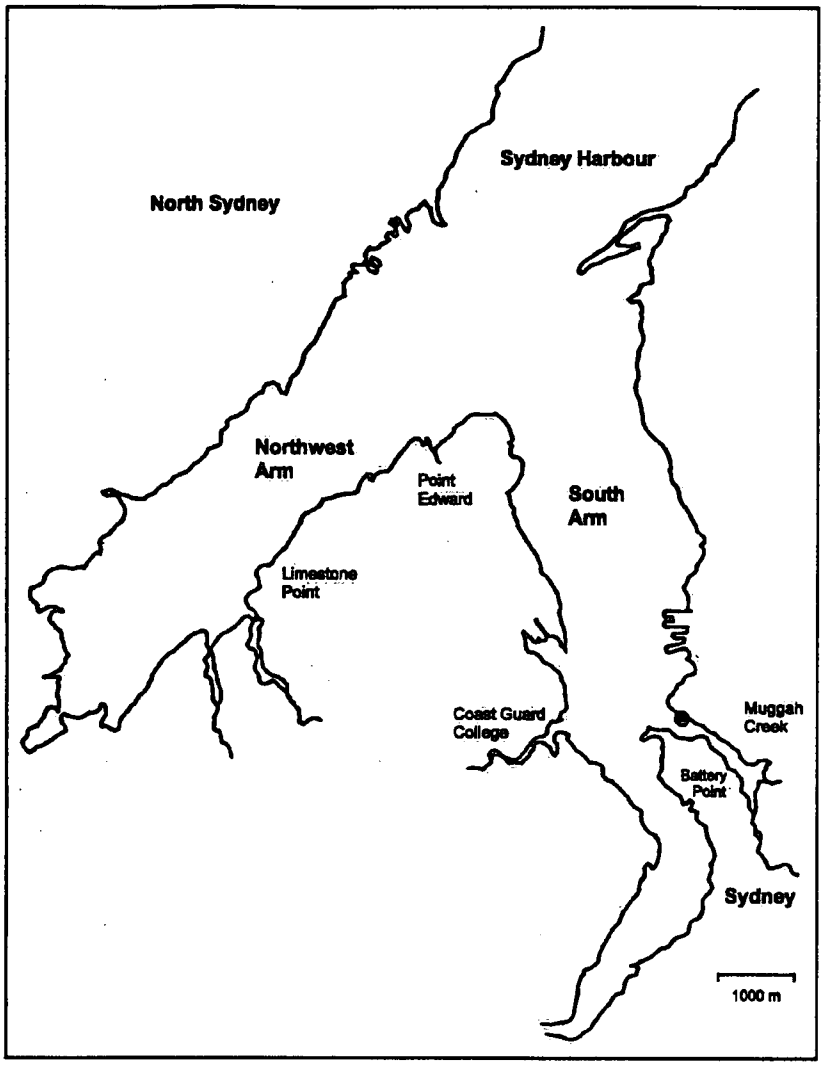


Figure 1. Map of Sydney Harbour, Nova Scotia showing the sampling site in Muggah Creek.

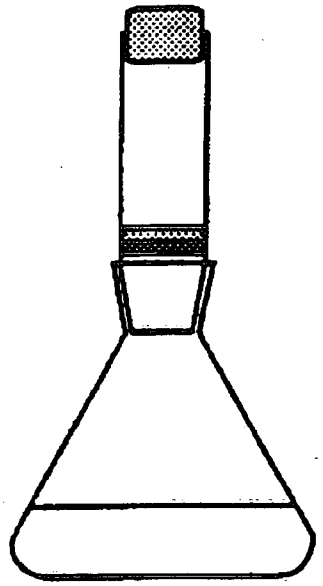


Figure 2. Erlenmeyer flask for PCB degradation experiments, fitted with a trap containing a sorbent (and cotton wool plug) in order to monitor the volatilization of PCBs.

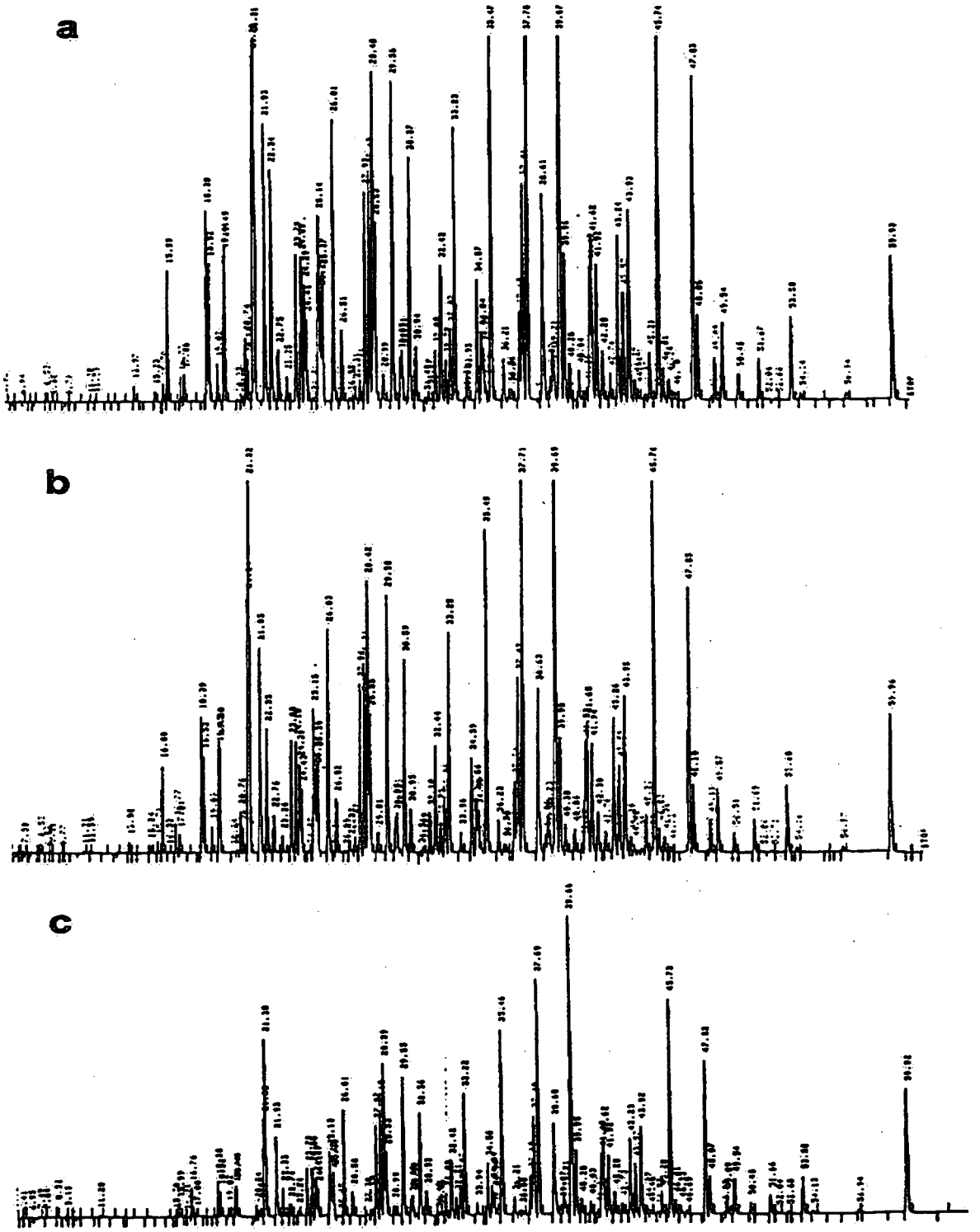


Figure 3. Chromatograms of PCB after 0 (a), 7 (b), and 14 days (c) incubation of *Alcaligenes xylosoxidans* in DMA medium (pH 6.7) with PCB (initial concentration 9.5 μg/mL) as the sole carbon source at 30 ^{0}C.

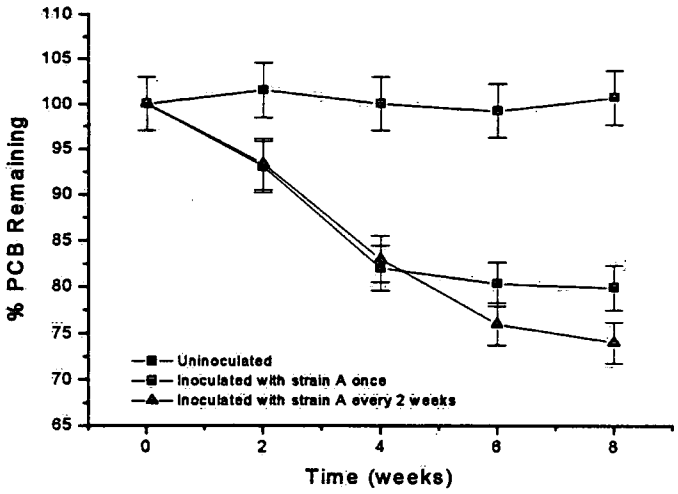
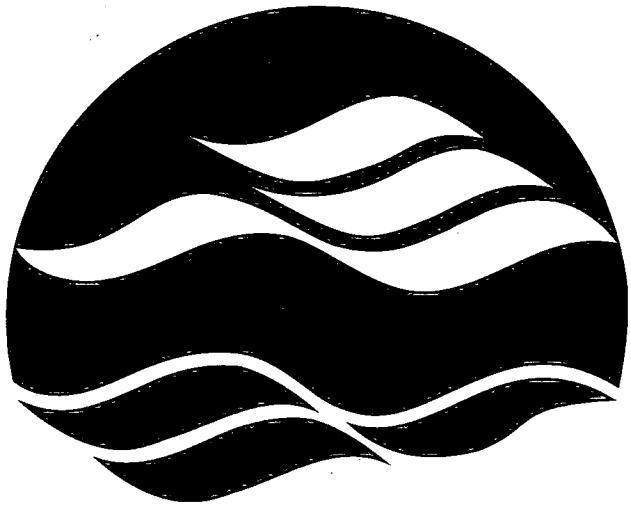


Figure 4. Degradation of PCB 153 in sediment. The error bars represent the percentage from the mean of 3 samples.



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