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Perspectives in biodegradation of alkanes and PCBs

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Abstract: Mixtures of indigenous soil bacteria were applied to remediate local ground waters and soil polluted with petroleum derived substances. Implementation of three months remediation protocols resulted in a decline of the amount of petroleum derived contaminants from an initial concentration of 1-10 $g.kg^{-1}$ soil dry weight to an average of 0.25 $g.kg^{-1}$ soil dry weight. We also studied genetic and biochemical properties of the bacterial strain *Pseudomonas* C12B. It was originally isolated for its ability to utilise alkylsulfates and alkylbenzensulfonates as the sole source of carbon and energy. PCB biodegradation was studied using two biological models, bacterial co-cultures and plant cells cultivated *in vitro*. An industrial mixture of polychlorinated biphenyls (Delor 103) containing about 60 congeners of different degrees of chlorination (an average of three chlorines per biphenyl molecule) was used. Bacterial co-cultures acquired from enrichment protocols were tested in laboratory and semi-pilot experiments. Pilot experiments were performed in a two step process in a ground water decontamination unit (working volume $5m^3$) which was operated semi-continuously. After 45 days of operation the initial PCB concentration had decreased to 20%. In laboratory experiments PCB degradation using plant cells cultivated *in vitro* was also performed. Different cultures of various species differing in their growth parameters and morphology (amorphous, differentiated shoot forming or "hairy root"), transformed or nontransformed by *Agrobacterium*, were used. Differentiated or hairy root cultures exhibited better degradative abilities than undifferentiated amorphous cultures.

Key Words: biodegradation, alkanes, PCBs, bacteria, plant cells.

INTRODUCTION

In recent years a wide spectrum of microorganismshas been found to possess the ability to efficiently degrade numerous xenobiotics. Oil-derived substances, mainly n-alkanes belong to the most easily biodegradable compounds, whereas polychlorinated biphenyls (PCBs) are considered to be the most persistent pollutants open to degradation by a limited number of species. In the Czech Republic aside from other environmental contaminants these above-mentioned compounds represent extremely widespread soil and water pollution. There are many reasons for pollution in the Czech Republic: industrial accidents, traffic accidents, irresponsible behaviour, former bases of Soviet Army primarily polluted by oil and kerosene and imperfect legislation. One example of an industrial accident in 1984 is the leakage of PCBs to the river Skalice from Technological Unit located in Rožmitál pod Radhoštěm. Periodical monitoring of the river sediments during the following eight years proved the extreme persistence of PCBs in an untreated system. In Table 1 there are listed some previous Soviet Army bases heavily polluted mainly by oil derivatives.

Aliphatic hydrocarbons are assimilated by a wide variety of microorganisms, but not all species are capable of utilising aliphatic hydrocarbons as growth substrates. Whilst many details of a microbial hydrocarbon metabolism have already been elucidated (ref 1), including the enzymology (ref 2), regulation and genetics (ref 3), less attention has been paid to the primary interaction of microorganisms and the hydrocarbon involved. The substrate specificity and biochemical properties of bacterial strain *Pseudomonas* C12B, which is used in our laboratory, were described by Košťál *et al.* (ref 4), whilst the genetic characteristics were summarized only recently. Coculture *ASANOL*, (ref 5) was used for practical bioremediation cases.

TABLE 1 : The Main Centres of Soviet Army Bases in the Czech Republic

Place	Area [km²]	Location
Mladá Boleslav	100	Northern Bohemia
Ralsko	29	Northern Bohemia
Milovice	11	Northern Bohemia
Bohosudov	6	Northern Moravia
Olomouc	36	Northern Moravia
Vysoké Mýto	50	Eastern Bohemia
Plzeò	10	Western Bohemia
Libava	36	Northern Bohemia

Also polychlorinated biphenyls (PCBs) are a family of compounds with a wide range of industrial applications in heat transfer fluids, dielectric fluids, hydraulic fluids, flame retardants, plasticizers, solvent extenders and organic diluents. Although the manufacture of PCBs has been forbidden (in the Czech Republic in 1984), they are still an environmental problem because of their presence in electrical transformers and landfills. In the United States and the United Kingdom, complex PCB mixtures were manufactured under the trade name Aroclor. In the Czech Republic the commercial name of the similar mixture was Delor (see Table 2.). All these mixtures consist of a number of congeners which differ in the number and distribution of chlorines on the biphenyl nuclei. Possible congeners (209) were described according to IUPAC nomenclature. About 150 congeners have been reported in the environment. PCBs have entered into soil and sediment environments as a result of improper disposal of industrial PCB wastes and leakage of PCBs from electric transformers. Their thermal and chemical stability, resistance to chemical corrosion, and general inertness have contributed to their persistance in the environment. Chemically stable lipophilic PCBs are easily transported through the food chain. The concentration sometimes reaches thousands of ng of PCB l^{-1} of water, which is also often contaminated by petroleum products or chlorohydrocarbons. Furukawa *et al.* (ref 6) and Bedard *et al.* (ref 7) were among the first who reported the biodegradation of these compounds.

TABLE 2: Assignment of Congeners to Peaks of Delor 103 Analyzed on Gas Chromatograph-electron Capture Detector

Peak No.	IUPAC No.	Substitution		
1	5 + 8	2,3	+ 2,4′	
2	15 + 18	4,4′	+ 2,2′,5	
3	17	2,2′,4		
4	16 + 32	2,2′,3	+ 2,4′,6	
5	26	2,3′,5		
6	31	2,4′,5		
7	28	2,4,4′		
8	20 + 33 + 53	2,3,3′	+ 2′,3,4	+ 2,2′,5,6′
9	45	2,2′,3,6		
10	52	2,2′,5,5′		
11	49	2,2′,4,5′		
12	47 + 75	2,2′,4,4′	+ 2,4,4′,6	
13	48	2,2′,4,5		
14	44	2,2′,3,5′		
15	37 + 42 + 59	3,4,4′	+ 2,2′,3,4′	+ 2,3,3′,6
16	41 + 64	2,2′,3,4	+ 2,3,4′,6	
17	96	2,2′,3,6,6′		
18	74	2,4,4′,5		
19	70	2,3′,4′,5		
20	66 + 88 + 95	2,3,4,4′	+ 2,2′3,4.6	+2,2′,3,5′,6
21	101	2, 2′,4,5,5′		
22	77 + 110	3,3′,4,4′	+ 2,3,3′,4′,6	

Indigenous microorganisms capable of effective PCBs biodegradation were isolated from the various sites of the Czech Republic and described by Bokvajová *et al.* (ref 8) . Kaštánek *et al.* (ref 9) published the results from combined microbial treatment and sorption of PCBs from polluted ground water.

Current research efforts focus on the use of plants for biodegradation of various xenobiotic compounds. This technology - phytoremediation, is still in development. Different

projects that target organic contaminants in the water phase (e.g. trinitrotoluene or trichloroethylene) look promising, however, more research on less mobile contaminants, e.g. PCBs, is needed before undergoing pilot and large-scale field testing (ref 10). Metabolic transformation and the biochemical pathways leading to the degradation, solubilization and for example in the case of chlorinated compounds also to dechlorination are not totaly understood, but preliminary results are quite promising. Qualitative differences between plant and bacterial bioremediation showed that this action could be of great environmental importance due to the tendency of plant cultures to transform xenobiotic compounds (PCB etc.) into water soluble products (ref 11).

The scope of this paper is to present the practical approaches to bioremediation used in the Czech Republic, and to show some of the generally accepted conclusions found in the course of experiments.

MATERIALS AND METHODS

Substrates

Alkanes

For screening of the ability of isolated strains to degrade n-alkanes an industrial mixture of alkanes C_{10} - C_{16} was used. Pure alkanes used as growth substrates were purchased from Fluka, Switzerland.

Polychlorinated biphenyls

Commercial mixture of polychlorinated congeners produced as Delor 103 (see Table 2) was used throughout. PCBs producer in the former Czechoslovak Republic was Chemko-Strážné. In field experiments degradation of two types of Delor was followed: Delor 103 (D 103) with maximum of five chlorine atoms per biphenyl molecule, and Delor 106 (D 106), that was highly chlorinated. Usage of PCBs
was forbidden in the Czech Republic in the beginning of the 1980s, but prevailing environmental contamination is still caused by these products.

Organisms Used

Microorganisms

Alkane degrading bacteria: coculture *ASANOL* which was developed in our department using an enrichment technique, comprised the following genera: *Acinetobacter, Klebsiella, Pseudomonas.* Strain *Pseudomonas* C12B (NCIMB 11753) was used throughout for physiologic and genetic studies under laboratory conditions.

PCBs degrading bacteria: several cocultures were developed in our department using soil from the polluted sites of the Czech Republic by an enrichment technique (319A, 319B, Z1, Z2, Z3) and the individual strains were isolated from these mixed cultures. According to preliminary identifications all isolated strains belong to genus *Pseudomonas.*

Plant Cells

To study the ability of plants to degrade PCBs various plant cell, tissue and organ cultures from the Collection of the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, have been used. From some species both amorphous undifferentiated callus cultures and shoot forming terratoma cultures were used as starting material, while in other species callus cultures were compared with strains obtained by genetical transformation using *Agrobacterium tumefaciens* and *A. rhizogenes* (see Table 3). These cultures included undifferentiated ones, shooty terratomas and hairy root clones. Some of the stock cultures were already kept in liquid media as suspensions of small aggregates, some as submerged root cultures, others were transferred from agar to liquid media just before the incubation with PCBs.

Media

Microorganisms

All types of microbial cultures were cultivated on simple mineral media described earlier (Refs 4,8) and supplemented with suitable carbon source. Alkane degrading bacteria were cultivated with a mixture of n-alkanes or with individual aliphatic hydrocarbons (C_6 - C_{16}). PCBs degrading bacteria were cultivated on biphenyl, which was also present in all Delor 103 degrading experiments at the laboratory stage.

Plant Cells

Plant cell cultures were cultivated using media prepared according to Linsmeier and Skoog (ref 12), 2% of the sucrose was used as carbon source (ref 13). The biodegradation experiments were performed in liquid medium of the same composition, but without any growth regulators added.

Cultivation conditions - microorganisms

Alkanes

Cultivation on agar plates in gaseous vapours of alkanes: The cell suspension (0.1 ml) was inoculated on agar plates with basal salts liquid medium and the cells were cultivated in alkane vapours at 28 °C.

Cultivation in liquid media: The organisms were grown in batch culture made up of a basal salts liquid medium. For growth experiments 500 ml Erlenmayer flasks containing 100 ml of medium were inoculated with tested strains and shaken at 28 °C. Concentrations of alkanes used and details about different cultivation conditions and modes are given by Košťál *et al.* (ref 4).

Microorganisms for the decontamination process were grown in a three step procedure: flask-bioreactor of total volume 7 l - bioreactor of total volume 70 l (ref 5).

TABLE 3: Plant Cell Cultures Used for PCB Degradation and Their Morphological Characteristics

Plant cell cultures	*Agrobacterium* transformation	Type of culture
Solanum aviculare		
KK1N	no	callus, nondifferentiated
AVI-3	no	callus, nondifferentiated
AVR-1	yes	differentiated
Armoracia rusticana		
K62K	no	callus, nondifferentiated
K50	no	callus, nondifferentiated
K54	no	differentiated
Solanum nigrum		
SNC90	yes	hairy root
SNT-2	yes	differentiated
SNC-9L	yes	callus, nondifferentiated
Atropa bella-donna		
R1BC	no	callus, nondifferentiated
ATR-1	yes	callus, nondifferentiated
Angelica sylvestris		
ANG-X	no	callus, nondifferentiated
Cucurbita melo		
Mel	no	callus, nondifferentiated

Polychlorinated biphenyls

Cultivation on agar plates in gaseous vapours of PCBs: Cell suspension (0.1 ml) was inoculated on agar plates with basal salts liquid medium and the cells were cultivated in PCB vapours at 28 °C.

Cultivation in liquid media: The microorganisms were grown in batch culture made up of a basal salts liquid medium (ref 8). For growth experiments 500 ml Erlenmayer flasks containing 100 ml of medium were inoculated with tested strains and shaken at 28 °C under conditions described by Bokvajová *et al.* (ref 8). For biodegradation experiments the Delor103 concentration was 50 $mg.l^{-1}$.

Microorganisms for the decontamination process were grown in a three step procedure: flask - bioreactor total volume 7 l - bioreactor total volume 70 l (ref 9) .

Cultivation conditions - plant cell cultures

4g of inoculum cultivated on the solid medium were aseptically transferred to 100 ml of the liquid medium in 300ml Erlenmayer flasks. 10 mg per 100 ml of Delor 103 (methanol solution) was added to each flask. Cultures were incubated with PCBs 14 days on a rotary shaker at 26°C. The process was finished by heating in a water bath (80-100°C) for 15 minutes. Further treatment was the same as with microbial cells.

Analytical Procedures

Alkane analysis

Residual alkanes were estimated by gas chromatography on GS Labio GC-94 equipped with a fused silica capillary column coated with polyethyleneglycol. The flame ionization detector (FID) was operated at 230°C.

PCB analysis

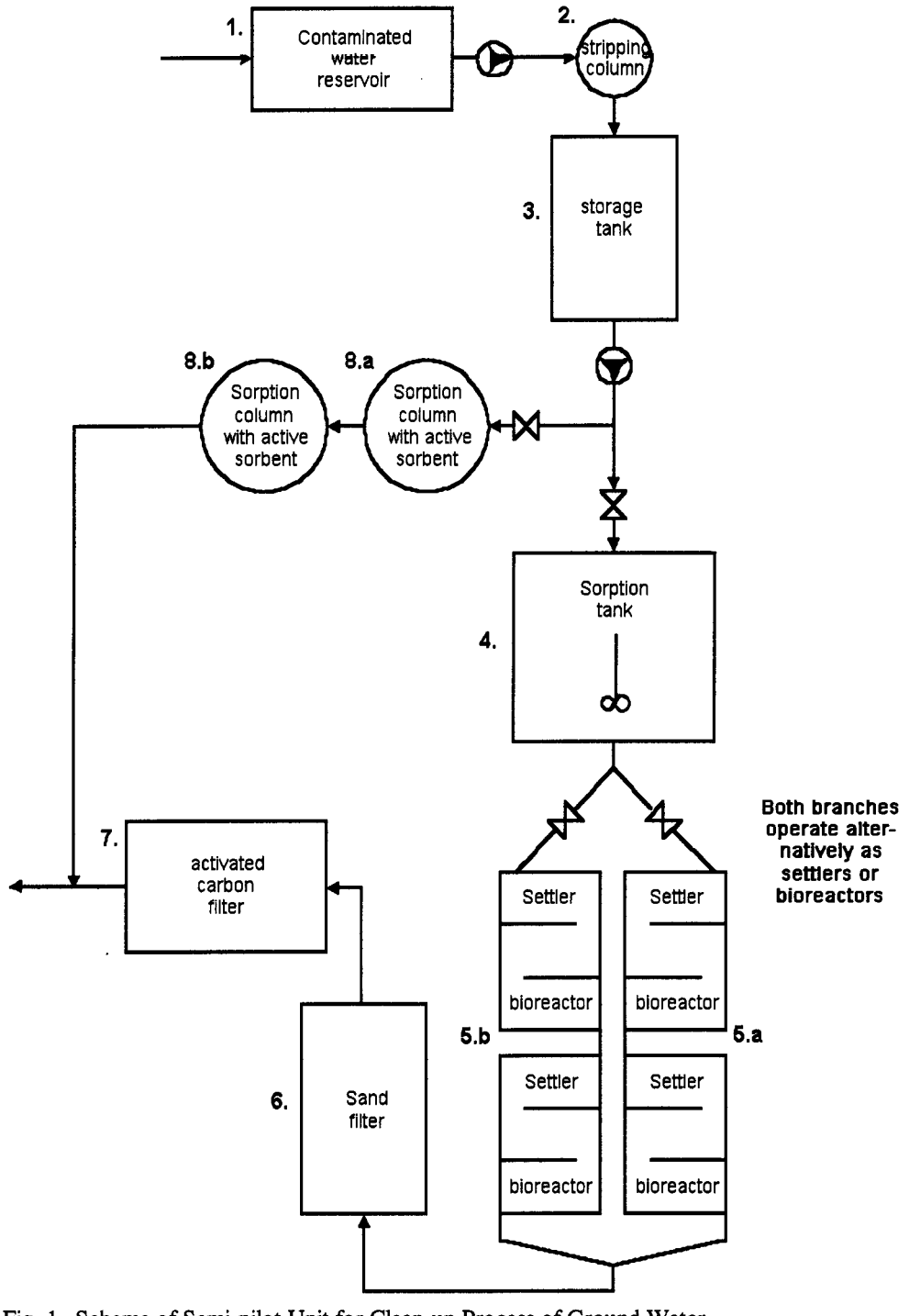
PCB concentration in material used (water media treated by microbial or plant tissue cultures, slurry, sediment and sorbent) was analysed by gas chromatography. Remaining PCBs content was extracted with hexane at ambient temperature and according to the procedure described earlier (ref 4). A Hewlett-Packard 5890 gas chromatograph with an electron capture detector and a fused silica capillary column coated with an immobilized nonpolar phase was employed.

Bioreactor used for decontamination of ground water contaminated with alkanes

The bioreactor was an open vessel with a capacity of $2m^3$ equipped by built-in polyamide netting with 5mm meshes forming a vertical surface in distance of 100mm. The suspension of bacterial cells was immobilized on the netting by adsorption. The content of bioreactor was aerated continuously by a compressor using perforated distribution tubes. Contaminated water was pumped into the bioreactor from drill holes through a tempered vessel, into which solid phase nutrients (ammonium phosphate) were periodically added. After passing through the bioreactor water returned to the infiltration drill holes. The water remained in the reactor for 20-30 hours. During this process the content of nutrients, dissolved oxygen and the input and outlet values of the hydrocarbons were monitored. The temperature in the bioreactor was dependent on the weather conditions and was kept lower than the optimum used for bacteria.

Two step technology for removing PCBs from ground water

Decontamination for semi-pilot plants was arranged as shown in Fig. 1. Water was pumped from the well to the reservoir (1) with a capacity of 3000 l and further to the top of the stripping column (2) where chloroethylenes were removed. Water was stored in the tank (3). Sorption of PCBs on bentonite activated by ferric sulphate took place in the sorption tank (4). The sorbent sedimented in the two sedimentation basins (5). The retention time of water during sedimentation was 130-170 min. After several sorption cycles, the sufficient amount of the slurry with entrapped PCBs in the parallel couple of basins (5) was used in a further step as a bioreactor with following parameters: volume: 1700 l, content of mud with sorbed PCBs: 5%, used bacteria: co-culture of indigenous strains, inoculum: 2%, nutrients: 0.7% of commercial fertilizer, aeration: periodical, time of decontamination: 40 days.



Fig, 1 Scheme of Semi-pilot Unit for Clean-up Process of Ground Water

Plasmid curing

The method of Rheinwald *et al.* (ref 14) was followed. 10^3 to 10^4 cells from an overnight culture of *Pseudomonas* C12B were inoculated into 2 ml of nutrient broth containing mitomycin C at concen-trations from 5 to 25 $\mu g.ml^{-1}$ and shaken at 28°C until growth occured (48 hours). Aliquot of the cell suspension

with sublethal concentration of mitomycin C (5 $\mu g.ml^{-1}$) was 5x diluted and 0.1 ml portions were spread on nutrient agar plates. Colonies were then tested for growth on n-decanoic acid, n-decane and laurylsulfate.

Detection of plasmid DNA

We used the method of Wheatcroft *et al.* (ref 15) which is a modification of that of Eckhardt (ref 16). The method is based on the cell lysis directly in the agarose gel prior to electrophoresis. To suit particular properties of *Pseudomonas* C12B, we had to further modify the method as follows: because the cells of *Pseudomonas* C12B lysed readily when exposed to cold, the entire procedure was performed at room temperature. To further slow down cell lysis, the concentration of n-lauroylsarcosine solution used for washing the cells had to be decreased to 0.06% (w/v). The electrophoresis (distance of electrodes 150 mm) was run at 100 V backwards for 15 minutes and 20 V forwards for 1 hour followed by 100 V for another hour.

Growth tests

Resistance to mercury was determined on nutrient agar plates with 100 μg $HgCl_2$ $.ml^{-1}$. To test resistance against various antibiotics, the antibiotic disks supplied by Lachema-Chemapol (Neratovice, Czech Republic) were placed on freshly inoculated Mueller Hinton Agar plates (Diagnostics Pasteur, France). Growth was examined after 1 day incubation at 28°C.

RESULTS

Alkanes

Our work was focused on resolving two main problems; (a) - environmental-technological application and (b) - physiological and genetic characteristics of microorganisms involved.

a) environmental-technological application.
The principle of the method was the use of microorganisms coming directly from the contaminated locality, after their selection in the laboratory and their production to the necessary quantity. From the samples of soil which were taken from several localities, around 300 strains were isolated after cultivation on the mineral medium with kerosene as the only carbon source (ref 16). Their ability to degrade oil pollution was tested. The isolated strains were identified and species selected were not pathogenic or conditionally pathogenic microorganisms. For the locality Vysoké Mýto, a coculture consisting of three strains was suggested: *Acinetobacter* DBM 155, *Acinetobacter* DBM 163 and *Micrococcus* DBM 153.

On the basis of detailed monitoring of the contents of the oil substances in the soil and ground water in this locality, the following biodegradation technologies were used; (i) - surface decontamination of the soil *in situ*, (ii) - decontamination of the soil at the decontamination area and (iii) - sanitary pumping of contaminated water and the use of the bioreactor.

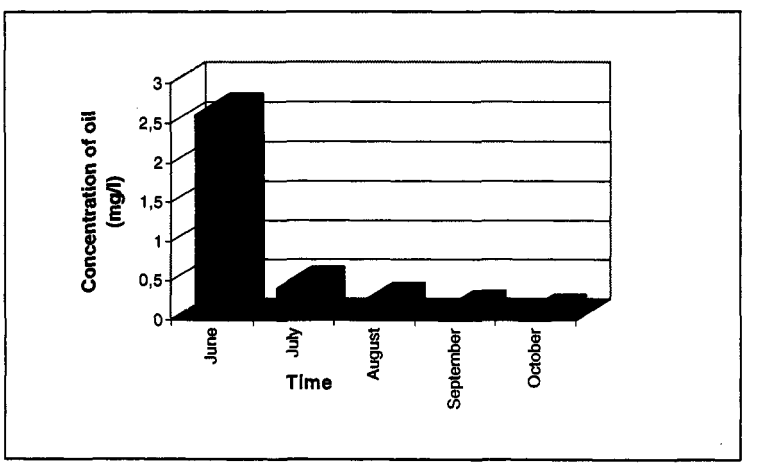


Fig. 2 Decontamination Course in Ground Water

In all cases the coculture of the above named microorganims was used for biodegradation of oil substances (details see Kaštánek *et al.* 1992) (ref 16).

The decontamination course of ground water in bioreactor is shown on Fig.2. It can be seen that water was cleansed practically to the purity prescribed by

Czechoslovak standard for drinking water, i.e. less than 0.05 mg.l^{-1} oil. For decontamination of soil special decontamintion area was used.

TABLE 4: Comparison of n-alkane Utilising Systems in *Pseudomonas* C12B and *Pseudomonas putida* (CAM-OCT plasmid).

Pseudomonas C12B	OCT plasmid alk. system
· n-alkanes C9 - C12	· n-alkanes C6 - C10
· no rubredoxin detected	· contains rubredoxin
· plasmid encoded	· plasmid encoded
· NOT IncP2 group	· IncP2 incomp. group
· very specific inductors	· less specific inductors

b) Studying the physiological and genetic characteristics of the microorganisms involved.

Although most studies to date (ref 17) suggest that there is not too much diversity in alkane oxidation systems among the gram negative bacteria, we have found important new features (see Table 4) after closer examination in *Pseudomonas* C12B of a similar system. The system is plasmid encoded (the only one plasmid known to code the alkane biodegradation was the OCT plasmid, belonging to the IncP2 incompatibility group and encoding resistance to mercury and the ability to grow on n-alkanes from n-hexane through n-decane). The plasmid in PC12B does not belong to the IncP2 incompatibility group. It confers oxidation of n-alkanes from n-octane through n-tridecane and of n-alkenes of similar length. If *Pseudomonas* C12B is cured of the plasmid, the resulting strain still grows on longer n-alkanes and alkenes and on the same range of aliphatic alcohols as the original strain. The plasmid does not confer resistance to either the tested antibiotics (ampicillin, penicillin, ko-trimoxazol, chloramphenicol, tetracyclin, gentamycin, linkomycin, neomycin, streptomycin, oxacillin, cefalotin, erythromycin, sulfothioxazol, kanamycin), or to mercury.

Using radiotracer methods, we determined the pathway for n-decane utilisation by this strain. These experiments are not completed yet, but all results so far obtained suggest that n-decane is oxidised via corresponding alcohol and aldehyde to n-decanoic acid, which is further metabolised by either α- or β-oxidation.

Polychlorinated biphenyls

Microbial degradation of PCBs was developed on the laboratory scale for aerobic bacteria. In the beginning, we were focused on the study of physiological characteristics of the involved microorganisms.

TABLE 5: Influence of Delor 103 Concentration on Viability of Strain No.2

Delor 103 (µl/50ml medium)	cfu after 24hrs	cfu after 1 week
0	4.0×10^7	9.8×10^6
4	1.2×10^8	2.8×10^7
40	8.5×10^8	7.2×10^7
400	9.2×10^8	2.0×10^7

cfu = colony forming units (vital count) per ml culture

A number of bacteria found after the biodegradation experiments, using the vital count technique, was in agreement with PCBs reduction (data not shown). In environmental conditions (polluted soil and water) enormous differences exist in PCBs concentration because the PCB congeners are water insoluble. The effect of increased PCBs level in the cultivation medium is summarised in Table 5. It was obvious that higher PCBs concentration did not exhibit any lethal effect. The lowest vital count was found in the control, which was not supplemented by any carbon source, and the cells were starving.

The comparison of growth abilities of the pure culture (strain No. 2) and coculture 319 is presented in Fig. 3. The growth kinetics of these two microbial populations were different. The growth curve of isolate No.

2 was steeper and followed classical growth kinetic patterns belonging to the coculture.

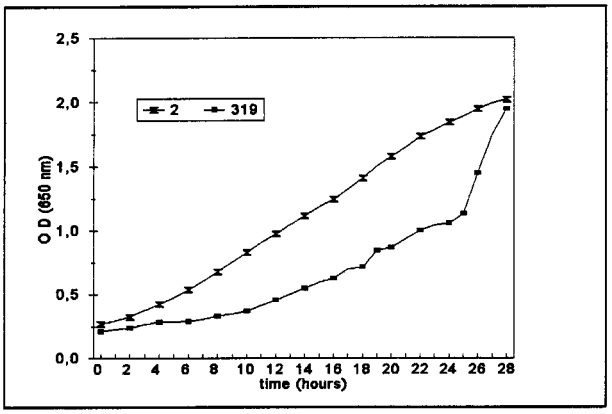


Fig. 3 Growth Curves of Coculture 319 and Isolate No.2 on Biphenyl.
Squares = coculture 319, double triangles = isolate No.2

Finally Fig. 4. represents the biodegradation pattern of coculture 319 and strain No. 2, which was derivatived from the parent coculture. Data from several experiments were collected and the average values are shown on this graph.

Using GC analysis, we measured the decrease of the original content of Delor 103 individual congeners. Results on Fig. 4 are expressed in percents of remaining congeners after the biodegradation. The industrial PCBs mixtures were very complex and the estimation of the individual congeners concentrations presented a difficult analytical task. Therefore, EPA has recommended expressing the total content of PCBs as the sum of chosen "indicator" congeners. For Delor 103 (see Table 2) the following congeners (IUPAC nomenclature) were recommended: 28 (peak No.7), 52 (peak No.10) and 101 (peak No. 21). According to this criterion, the quantity of remaining congeners after biodegradation was found to be 27% for the strain No.2, while coculture 319 left only 10.5% of the original PCBs. The further investigation on the physiology of the strains involved in this study has been planned.

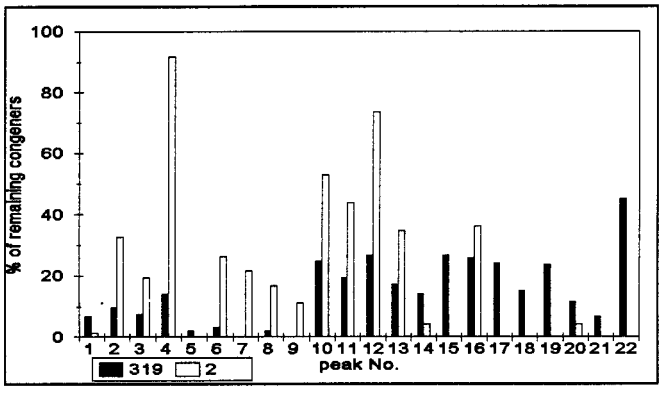


Fig. 4 Efficiency of Delor 103 Degradation by Two Types of Cultures.

The decomposition of PCBs on the semi-pilot scale was developed for the cleaning of ground water in the area of a factory in the Elbe (Labe) river basin. The concentration of PCBs in the water reached thousands of $ng.l^{-1}$, both types of Delor (Delor 103 and Delor 106) and also some chloroethylenes were present. The substantial part of the PCBs was adsorbed on particles of sediment in the amount of 20-30 $\mu g.g^{-1}$ (specific surface area 29 $m^2.g^{-1}$). The process schematically shown on Fig. 1 combines biodegradation by selected microbial cocultures after binding of PCBs on the surface of a bentonite sorbent.

In the first sedimentation basin (see Fig.1) suspension contained 5% of solids and in the second sedimentation basin suspension contained 3% of solids. The deposit was composed of natural sediment, bentonite, ferric and calcium sulphates and flocculant. The deposit in the second sedimentation basin contained a larger amount of very fine particles. The different composition of deposits also correlated with differences in the ratio of higher and lower chlorinated PCBs congeners. While in the first sedimentation basin the ratio of D103:D106 in the slurry was 7 and in the solids 17, the same ratio in the second sedimentation basin was 10 in both the slurry and solids.

Both basins were periodically aerated. The air was injected from inlets located at the bottom and in regular intervals parallel branches (Fig.1, 5a,5b) were supplied. The initial pH was 7.4 in the first sedimentation basin and it was 5.3 after biodegradation. In the second sedimentation basin the pH changed from pH 7.6 to 7.4.

The biodegradation course in the sedimentation basins suspension is shown in Table 6. After 5 weeks of incubation, when PCB concentration did not exhibit further decrease, in the first sedimentation basin (5a), see Fig.1 the level of D 103 was found to be 6 500 ng.l^{-1}. It represented 25% of the initial concentration. In the second basin (5b) after the same elapsed time, the D 103 concentration decreased to 2 000 ng.l^{-1}, which was also 25% of the original content. The concentration of D 106 decreased from 3 700 to 600 ng.l^{-1} in the first basin. In the second basin the content of D 106 changed from 900 to 300 ng.l^{-1}. Content of D 106 decreased in the first sedimentation basin to 15% of the initial value and in the second basin it decreased to 30% of that.

TABLE 6: Decrease of PCB Concentration in Suspension during Biodegradation Process

Days of biodegradation	First sediment basin		Second sediment basin	
	D 103 ng/l	*D 106 ng/l*	*D 103 ng/l*	*D 106 ng/l*
0	26 369	3 743	7 996	872
14	11 860	1 205	5 940	488
22	7 341	495	5 563	451
36	6 563	601	2 073	323

The content of PCBs in the slurry solids from the decontamination basins was followed separately. In the first tank the content of D 103 decreased from 8 000 to 2 000 ng.l^{-1}, which was 25% of the value at the beginning of biodegradation. In the solids from the second basin the concentration of D 103 decreased from 2000 to 600 ng.l^{-1}, which was 30% of the initial value, and the concentration of D 106 decreased from 170 to 120 ng.l^{-1}, which was 70% of the value at the beginning of the experiment.

For comparison, in the slurry treated under laboratory conditions (in shake flasks, temperature 28°C) the D 103 concentration decreased to 6% of its initial value which was 8040 ng.l^{-1} in the solids, and the content of D 106 decreased from 479 ng.l^{-1} to 30% of this value after 22 days of biodegradation. Under laboratory conditions, lower-chlorinated congeners were subjected to more substantial degradation than higher-chlorinated congeners. In the first sedimentation basin, higher-chlorinated congeners were degraded in a similar manner as lower-chlorinated congeners. The different results can be attributed to the difference at the biodegradation time, the temperature and aeration conditions in the laboratory and in the basins. The synergistic effect of natural microorganisms in the basins was also possible.

Plant cells

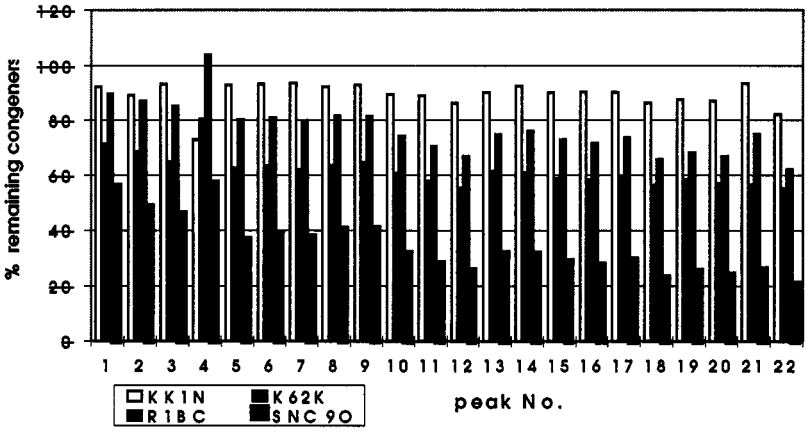


Fig. 5 Efficiency of Delor 103 Degradation by Some Plant Cell Cultures

To establish a reliable plant model system, in the first step the analytical approach was optimized for the use of plant material (ref 18) by changing, inoculum age and size and also biodegradation time period. Furthermore cultures of more species with various levels of differentiation were compared.

The overall PCB metabolising capability and also degradation of individual congeners greatly differed from strain to strain. The results obtained for some of the tested cultures are summarized in Table 7. Figure 5 shows an example of the typical degradation course of individual congeners by some plant cultures. The highest ability to metabolize PCB revealed some *Agrobacterium* transformed cultures of *S. nigrum* and *S. aviculare*. These cultures degraded PCBs under the given conditions up to 40 - 50% of the starting content of PCBs (10mg/100ml).

From the results obtained until now we can conclude that in contrast to microorganisms (induction by biphenyl) plant cells are able to biodegrade chlorinated compounds without previous induction and in the presence of other carbon source (sucrose). Among the tested cultures transformed cells were more effective than non-transformed ones, and differentiated cultures were more effective than amorphous non-differentiated ones. Cultures of *Solanum nigrum* degraded PCBs with higher efficiency than other species.

TABLE 7: Ability of Various Plant Cell Cultures to Degrade PCBs

Culture	Morphology	PCB biodegradation
Solanum aviculare		
KK1N	callus, nondifferent	20 %
AVI-3	callus, nondifferent	0 %
AVR-1 (T)*	differentiated	56 %
Armoracia rusticana		
K62K	callus, nondifferent	35 %
K50	callus, nondifferent	35 %
K54	differentiated	44 %
Solanum nigrum		
SNC 90 (T)	hairy root	40 %
SNT-2 (T)	differentiated	53 %
SNC-9L (T)	callus, nondifferent	20 %
Atropa bella-donna		
R1BC	callus, nondifferent	20 %
ATR-1(T)	callus, nondifferent	42 %
Angelica sylvestri		
ANG-X	callus, nondifferent	0 %
Cucurbita melo		
Mel-2	callus, nondifferent.	10 %

* (T) = cultures transformed by *Agrobacterium*

CONCLUSIONS

The feasibility of bioremediation for the cleanup of contaminated sites starts with an assessment of the degradation potential of the contaminants. This assessment includes evaluations of the ease or difficulty of degradation, the ability to achieve total mineralization, as well as the environmental conditions necessary for mineralization. Regulatory officials and citizens demand information on the potential degradation products and their potential hazard.

Microbial transformations of organic compounds are frequently described using the terms detoxification, degradation, and mineralization. Detoxification is the transformation of the compound to some intermediate form that is less toxic. Degradation means that the parent compound no longer exists. Mineralization refers to the complete conversion of the organic structure to inorganic forms e.g. H_2O, CO_2 and Cl^-.

When bioremediating aliphatic hydrocarbons, the following should be considered. Firstly, tThe oxygen is required because biodegradative pathways are aerobic processes. Secondly, many microorganisms are capable

of aliphatic hydrocarbons degradation. Thirdly, soil normally contains an adequate inoculum of natural organisms for bioremediation, nevertheless the application of indigenous strains consortia as *ASANOL* stimulate and speed up the clean-up processes. Short hydrocarbons that have less than nine carbon atoms are toxic for microorganisms and therefore bacterial biodegradation processes are not generally applied for their bioremediation. However, bacterial strains evolved that are capable of dissimilating even these toxic compounds (refs 19 and 20).

There is more than one type of plasmid encoded enzyme system that gram negative bacteria use for dissimilation of short alkanes. We found a biodegradative plasmid that enables the strain *Pseudomonas* C12B to grow on n-alkanes from n-nonane through n-tridecane. Alkenes are oxidized by this system, too. This microorganism uses the common biodegradative pathway that includes the oxidation of the primary carbon atom to form the corresponding primary alcohol, aldehyde and carboxylic acid.

Both aerobic and anaerobic metabolism modes affect some biotransformation of PCBs. However, PCBs chlorinated with four and more chlorine atoms per molecule are rather resistant under aerobic conditions. For degradation of multiple chlorinated PCBs the microbial consortia of selected organisms provide the optimal results. The catabolic pathway of the entire PCB mineralization is encoded by two different sets of genes (chlorobiphenyl and chlorobenzoate degradation) that are normally not found in the same organism. Preparation of hybrid strains bearing the genes of both pathways represents the future of PCBs biodegradation technology.

In addition, these days there is no approved technology in the Czech Republic which would efficiently remove PCBs from the environment. The clean-up of PCB contaminated sites generally presents quite a difficult problem and technologies used are costly and time consuming. Thus, bioremediation through the use of specific biodegrading bacteria seems to be an effective approach towards cleaning of such sites.

Phytoremediation is defined as the use of green plants to remove, accumulate or render harmless environmental contaminants. Plants are the primary source of energy for all other above ground and for all below ground organisms. They have evolved in an environment where they are continually assaulted with a wide array of microbial, plant and, more recently, man-made toxins. In addition they have significant metabolic activities in their roots and the shoots that may be exploited for phytoremediation. Phytoremediation technology is a relatively new concept and although it is still in development it has been already shown to be an emerging technology that promises effective and inexpensive cleanup of certain hazardous waste sites. Also the role of vegetation in the process of enriching *in situ* microbial populations for enhanced degradation of organics by the provision of appropriate beneficial primary substrates (plant exudates - sugars, amino acids, vitamins etc.) supplied by vegetation must also be taken into account.

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