CAN BACTERIA SWEEP AWAY OUR MESS?
NEW PERSPECTIVES FOR CLEANING OF PCB-CONTAMINATED ENVIRONMENTS

I. Petrić

OKOLIŠ

Laboratory for analytical chemistry and biogeochemistry of organic compounds / Division for marine and environmental research

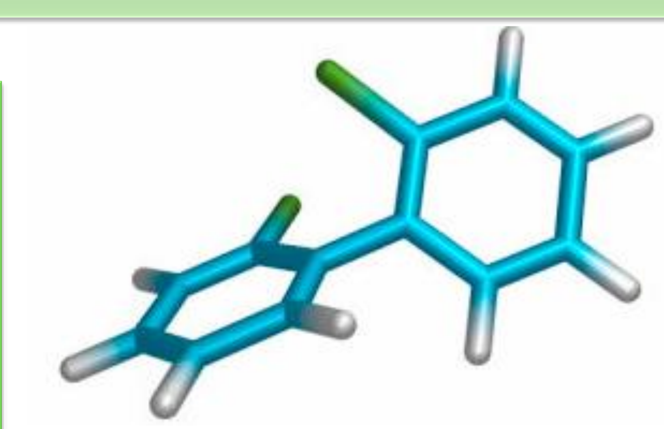
Acknowledgments

Presented work was done under supervision of dr. **D. Hršak** (RBI). Financial support was provided by the MZOŠ and partially by the APOPSBAL contract within European Commission (Fifth Framework Programme). Work was done in collaboration with the Institute for Medical Research and Occupational Health (Zagreb) and with INRA-CMSE, Université de Bourgogne (Dijon, France). Funding for scientific visits and postdoctoral research were provided by the French embassy, FEMS and ADEME (Conseil Régional de Bourgogne, France).

- Over the past century humans have introduced a large number of **synthetic organic xenobiotics** into the environment, among them *halogenated solvents, chlorofluorocarbons, polychlorinated biphenyls, synthetic detergents, pesticides, fungicides* etc. Many of these pollutants were found to produce **harmful impacts to the ecosystems** and were therefore either banned or greatly restricted in their application. However, xenobiotics are sometimes very recalcitrant and persist in soils and sediments, groundwater or surface waters for a long period of time.
- **Microorganisms**, especially bacteria, were found to have capacity to adapt to xenobiotic compounds as novel growth and energy substrate. Microbial diversity and versatility for adaptation to different substrates makes them the best candidates to convey xenobiotics into natural biogeochemical cycles. Adaptation of microorganisms to xenobiotic substrate proceeds via various genetic mechanisms that subsequently determined the evolution of functional degradative pathways. Many factors can negatively control biodegradability of the contaminant: its concentration and bioavailability, pH, temperature, oxygen, availability of water, nutrients, presence of toxic and inhibitory substances, competing substrates, interactions among microorganisms...
- Increasing pollution of the environment has provoked the need for understanding the impact of toxic compounds on microbial populations, the catabolic degradation pathway of xenobiotics and upgrade in bioremediation processes.
- **Bioremediation**, a cost-effective and ecologically friendly method, was proposed as a suitable alternative to physico-chemical remediation strategies. Technology can be applied to contaminated wastewater, ground or surface waters, soils, sediments and air. However, for bioremediation to be successful it is crucial to gain as much information about the biodegradability of the xenobiotic compound, metabolic pathways used by microorganisms in their transformation, catabolic genes coding for specialized enzymes in their degradation, impact of the applied bioremediation on the soil bacterial community etc.

POLYCHLORINATED BIPHENYLS (PCBs)

- ✓ Highly desirable features including chemical and thermal stability, non-flammability, high electrical insulating properties, high resistance to acids, bases... → **extensive use from 1930-1970** (electrical capacitors and transformers, hydraulic fluids and pump, oils and adhesives, pigments, dyes, copy paper, pesticides...)
- ✓ **Widespread adverse effects recognized in aquatic life, birds, animals and humans** (effect on immune, reproductive, nervous, endocrine systems, teratogenic, cause chromosome damage, tumorigenic effects etc.) → use was **banned in 1970s**
- ✓ As of 1975, 4.5 million kg lost to environment through vaporization, leaks, spills, and landfill
- ✓ **Still represent ecological and health issues worldwide** → found in the air, water, soil, sediments, food, animals, plants and humans (bioaccumulation)



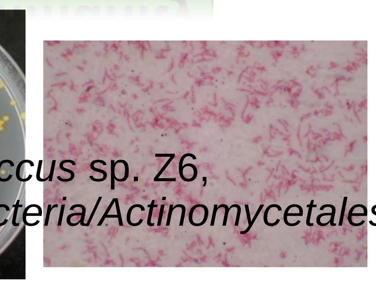
Biphenyl backbone carrying 1-10 Cl
→ **209 possible PCB molecules**

PCB-polluted site

Sampling of the TS 110/35kV PCB-contaminated soil
(31.4 ± 4 μg PCB per g of soil)

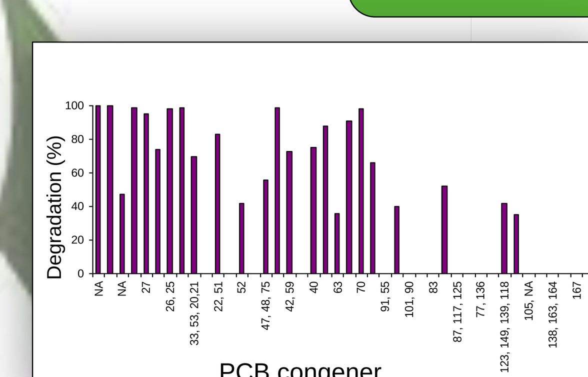
1) Isolation of the potential PCB-degrading bacterial community

Incubation: rotary shaker set on 200 rpm and 28°C

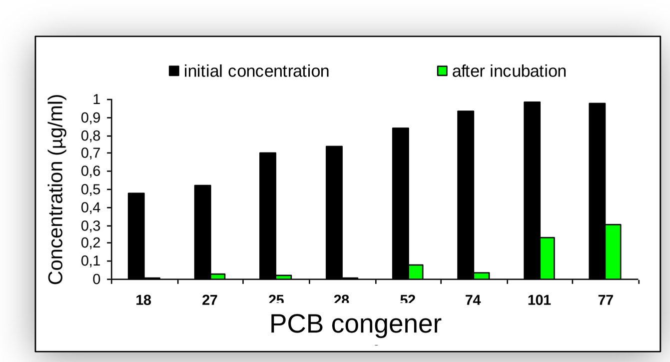


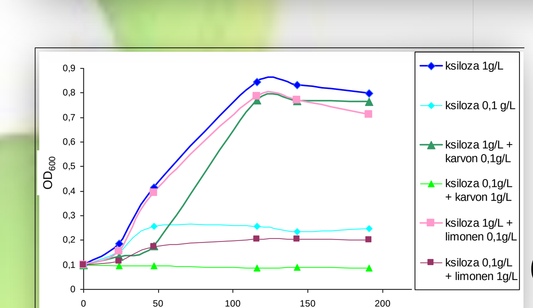
Isolation (mineral medium with biphenyl) and identification (16S rRNA sequence) of catabolically active PCB-degrading members of the community

2) Detailed characterization of the bacterial community as catalyst in PCB-degradation

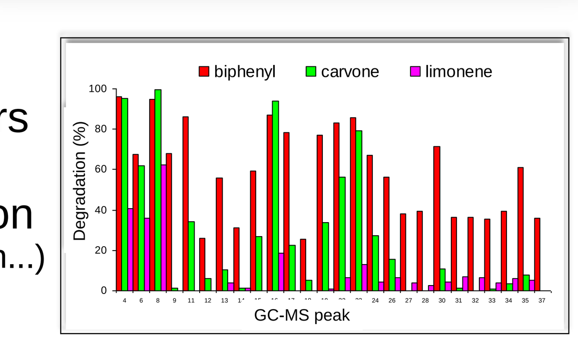


Degradation of different PCBs in liquid medium in the presence of inducer biphenyl (GC/MS)

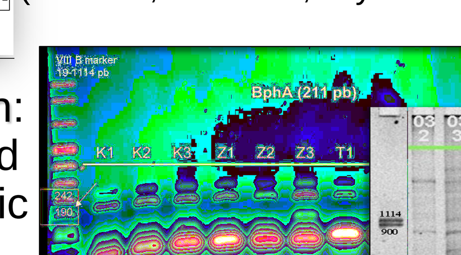


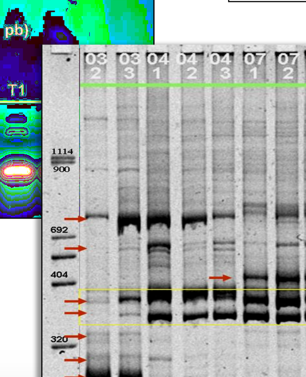


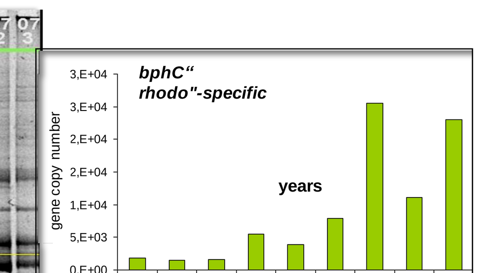
Testing potential inducers and surfactants for enhancing biodegradation (carvone, limonene, soya lecithin...)

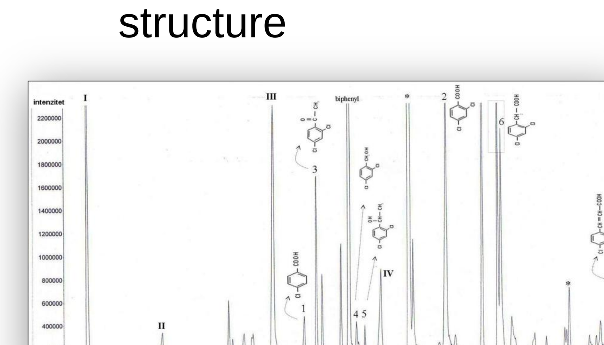


Molecular characterization: Determining presence and abundance of key catabolic *bph* genes
Analysis of the community structure

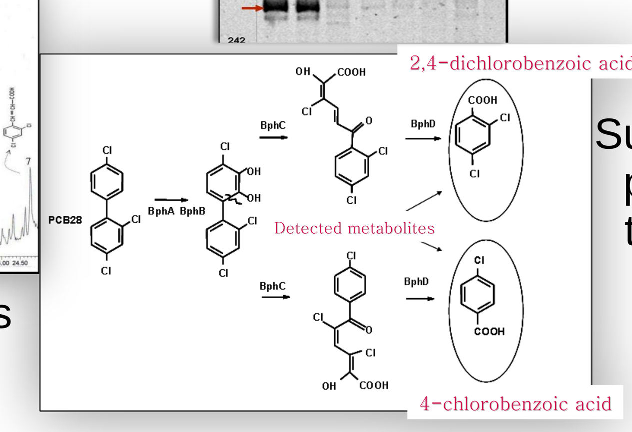






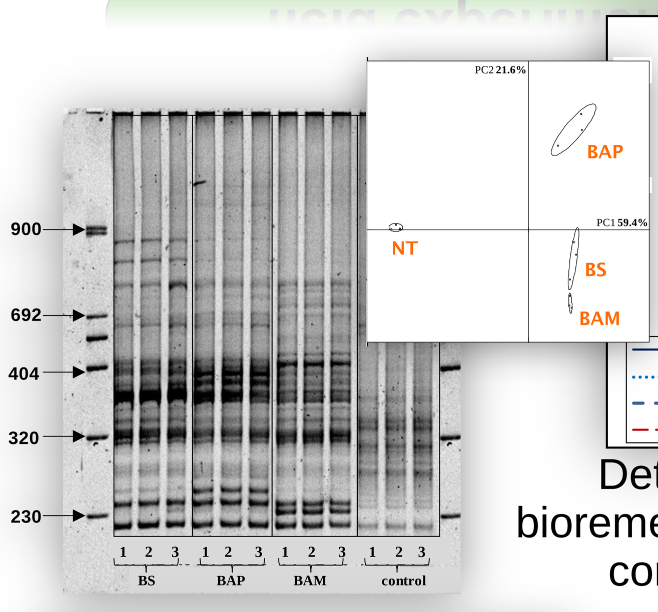


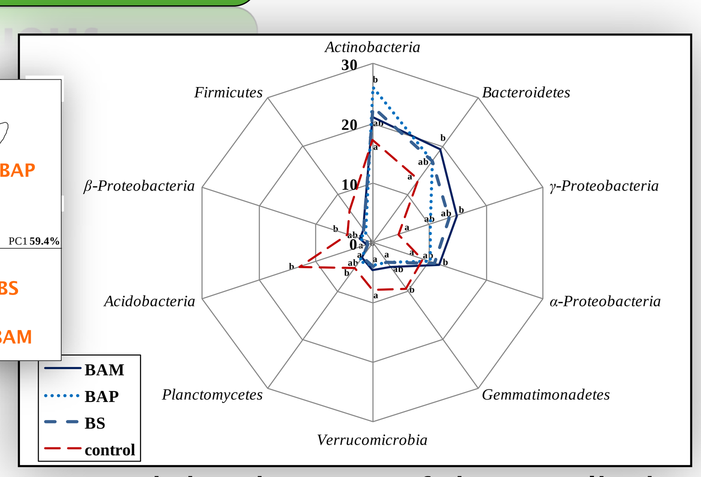
Identification of metabolites produced during PCB degradation (GC-MS)



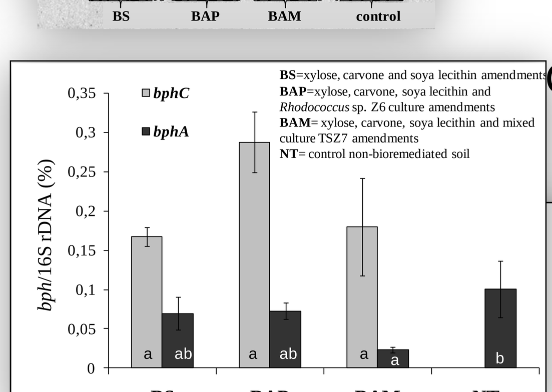
Suggestion of the potential PCB-transformation pathways

Designing of a small-scale field experiment

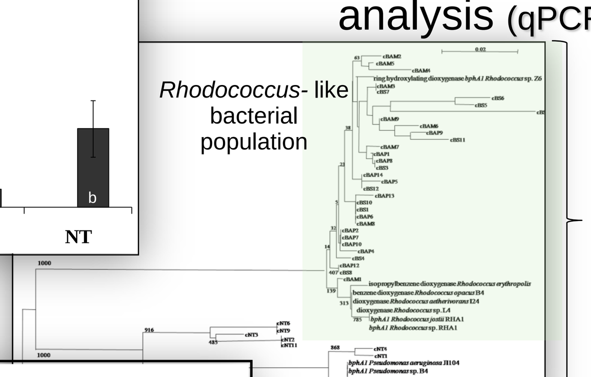




Determining impact of the applied bioremediation treatments on the bacterial community structure (RISA,, qPCR)

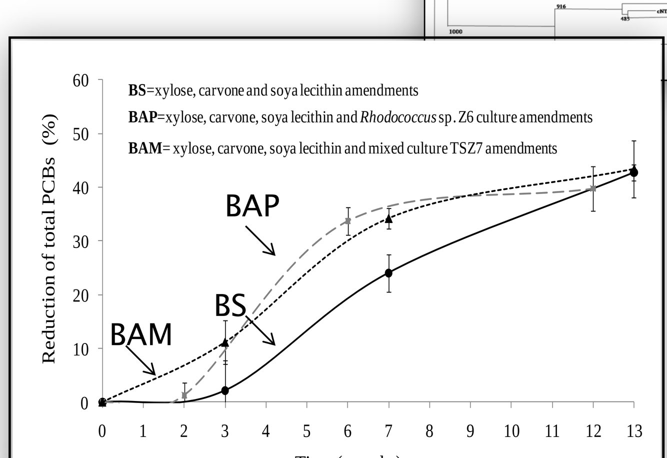


Characterization of functional PCB-degrading community throught *bphA* and *bphC* gene analysis (qPCR, sequencing)



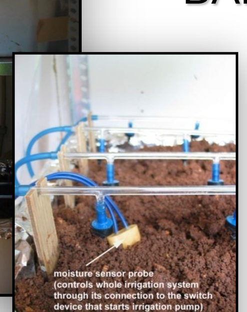
bphA sequencing

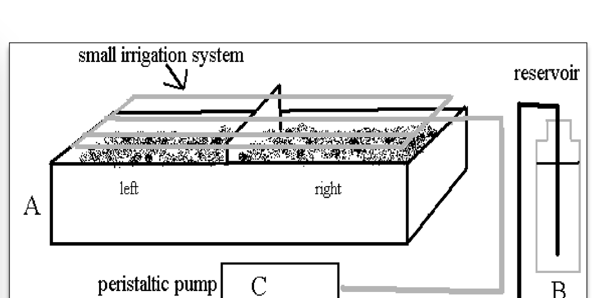
Direct extraction of DNA from soil
(Procedure ISO 11063)



Determining kinetics of PCB removal from soil (GC-MS)

Scale-up experiment





Testing of different bioremediation strategies:
(i) Biostimulation of indigenous bacterial community, BS
(ii) Bioaugmentation with mixed bacterial culture, BAM
(iii) Bioaugmentation with pure strain culture, BAP

Conclusions

1. Enriched bacterial community originally present in the TS contaminated soil was shown to posses ability to transform wide range od different PCBs
2. Applied bioremediation led to removal of approx. 40% PCBs from the soil. Assay gave new insights into behaviour and functioning of the PCB-degrading community during bioremediation and revealed potential impact of the bioremediation on the total soil microbial community

Although bioremediation holds great promise for dealing with intractable environmental problems, it is important to recognize that much of this promise has yet to be realized...