

**BIOREMEDIATION OF ARYL HALIDE-CONTAMINATED ENVIRONMENTS:
LEARNING FROM NATURAL ATTENUATION PROCESSES****Peter Adriaens**

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1. Introduction

The ultimate environmental sink for most industrial and domestic target pollutants, whether released to the atmosphere or directly discharged in waterways, are the Earth's soils and sediments. Chlorinated aromatic hydrocarbons (CAH) are particularly prone to be particle-associated, due to their low water solubilities and tendency to partition in or complex with soil organic matter and dissolved organic carbon. Until recently, it was believed that CAH were truly anthropogenic in nature, and that the soil microbiota had not been exposed to these chemicals until the advent of Industrial Age. Whereas it is now generally accepted that CAH are produced naturally and have always been present, albeit in trace concentrations, it has become well established that autochthonous (i.e. naturally occurring) microorganisms of many genera are able to adapt to and evolve metabolic capabilities to detoxify a range of CAH in time spans ranging from weeks to decades ¹).

However, even though our knowledge on the abilities of aerobic and anaerobic microorganisms to either transform or completely biodegrade CAH has steadily expanded over the last two decades, successful field-scale applications of bioremediation processes for CAH-contaminated environments are scarce ²). The purpose of this abstract is to address some of the most important bioengineering issues facing the bioremediation industry regarding CAH contamination, based on information which can be derived from extensive environmental analysis for on-going natural attenuation processes in historically-contaminated soils and sediments.

2. Natural Bio-attenuation Processes at Historically CAH-Contaminated Sites

Natural bio-attenuation of CAH is the result of metabolic activities exhibited by natural communities of microorganisms present in the contaminated environment. The physiological versatility of these communities allows them to adapt to environmental contamination and, in effect, establish a 'niche' (i.e. function) in the presence of the contaminant ³). Thus, natural attenuation of environmental

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pollutants in general, and of CAH in particular is likely the result from combined metabolic activities mediated by several interacting populations ^{4,5}).

Direct proof of natural attenuation of CAH is obtained from extensive site characterization, not only for the suspected contaminant(s), but also for structurally related and unrelated compounds which may have resulted from microbial action. A careful integration of environmental microbiology and environmental (analytical) chemistry is thus central to interpreting the 'snapshot-in-time' since the original contamination took place. Some examples of products observed from natural attenuation are given in Table 1.

Table 1. Products Observed in Historically-Contaminated Environments Resulting from Natural CAH Attenuation¹

Compound	Environmental Conditions	Product(s) Identified
Aroclors	Anaerobic (methanogenic)	Enrichment in lesser chlorinated (often <i>o</i> - and <i>o/p</i> -) congeners
Aroclors	Aerobic/Anaerobic	Chlorobenzoates, Hydroxylated PCBs
2,4-D	Aerobic	2,4-Dichlorophenol
PCP	Anaerobic	Chlorinated quinones and anisoles, Nonextractables
Chlorobenzenes	Anaerobic	Monochlorobenzene, Alicyclic compounds

¹ Established to be due to microbial metabolic activities.

The next steps are (i) to demonstrate that the transformation which has occurred was due to microbial action, (ii) to convince legislators and the public that the site is being naturally remediated, or (iii) to propose alternative cleanup strategies, including methods to stimulate the natural processes. For example, the U.S. EPA proposed in 1992 that the appearance of chlorobenzoates has to be demonstrated as proof for aerobic PCB remediation. 'Nonextractable' fractions, due to complexation and condensation reactions, often account for a significant portion of the contaminant lost, based on laboratory experiments. Whether incorporation of the CAH into soil organic matter constitutes an acceptable risk associated with bioremediation processes should be up for debate.

3. Bioengineering Issues Related to CAH Degradation

The limited evidence available to date on natural attenuation of CAH is in part due to the lack of extensive environmental analysis performed on historically-contaminated sites, in particular for transformation products. However, many CAH are persistent in the environment due to structural features of the molecules of concern, physiological limitations of the autochthonous microbial

populations, or properties of the environment in which the compounds are found ^{6,7}). Thus, if no appreciable natural attenuation has occurred, the success of alternative bioremediation strategies to stimulate or enhance either *in situ* or *ex situ* (i.e. bioreactor applications) CAH degradation will depend on the mechanism of recalcitrance (Table 2).

Table 2. Reasons for Persistence and Engineered In Situ Bioremediation Strategies

Reasons for Persistence	Strategy	Applications (successes)
Lack of Metabolic Capabilities	Bioaugmentation	Aerobic PCB degradation Chlorinated pesticides Pentachlorophenol
Lack of Enzyme Induction	Chemical Amendments	PCB dechlorination/cometabolism PCDD/PCDF dechlorination
Bioavailability	Physical Amendments Co-solvents, Surfactants	?
Environmental Factors	Nutrient Amendments 'Niche Adjustment'	Pesticides

Lack of Metabolic Capabilities: Even though active microbial communities may exist at the CAH-contaminated site, none of the sub-populations has evolved the required metabolic capabilities to metabolize or transform the contaminant(s). This extreme case may present unique opportunities for soil inoculation with previously isolated microorganisms from other sites or with genetic laboratory constructs. For any microbial inoculum to establish successfully, a 'competitive edge' over other sub-populations to degrade a particular pollutant is required, either by outcompeting the naturally-occurring microorganisms, or by establishing a previously unoccupied niche. Successful inoculations for CAH-contaminated soils (on a laboratory scale) include PCP, PCBs, 2,4-D, and 2,4,5-T ^{3,4} .

Induction Issues: Few CAH, in particular those substituted with more than three chlorines, serve as microbial sources of carbon and energy, and are degraded via cometabolic reactions which yield no energy for the microbiota. Thus, if CAH do not serve as the driving force for biodegradation themselves, they can only be degraded in the presence of another (degradable) compound. Under aerobic conditions, this often means that an enzyme-synthesis-inducing compound is required for biodegradation/biotransformation to occur. Under anaerobic conditions, where degradation of CAH is often initiated via reductive dechlorination, the requirements for stimulation of the degradation process are unclear to date.

Bioavailability Issues: Since biodegradation is generally assumed to take place in the aqueous phase, CAH are considered to be bioavailable to a limited extent, due to their low aqueous solubility

and strong tendency to partition into organic matter. Moreover, low aqueous concentrations often do not trigger the requisite enzyme production. Even though several strategies to overcome this problem have been proposed, the issue of bioavailability remains unresolved to date.

4. Natural PCDD/F Attenuation and Potential for Bioremediation

Extensive characterization and chemometric analysis of 2,3,7,8-substituted PCDD/PCDF residues in river, estuary and lake sediments around the world have revealed the occurrence of distinctively different patterns. The variations among the isomer patterns were largely explained by the distributions of the higher chlorinated isomers and tentative source-sink correlations. The lack of clear source identification based on characteristic congener profiles for the process which generates them has been speculated to be due to natural attenuation ('weathering') of the PCDD by biological or abiotic processes.

To elucidate this possibility, and to evaluate whether a bioremediation technology would be feasible, the following three questions need to be answered:

- (i) Can dioxins be dechlorinated at environmentally relevant concentrations (and temperatures) ?
- (ii) What is the distribution of 2,3,7,8-substituted PCDD (including 2,3,7,8-TCDD) during sequential reductive dechlorination ?
- (iii) Are dioxin residues present in historically-contaminated sediments available to transformation reactions ?

Sediments incubated anaerobically with parts-per-billion concentrations of penta- to octaCDD mediated reductive dechlorination, resulting in the nonstoichiometric formation of lesser chlorinated isomers. Isomer-specific analysis revealed the production of 2,3,7,8-substituted hepta- and hexaCDD isomers^{8,9)}. Pattern fingerprints of OCDD dechlorination pathways using sediment-eluted cells, heavy metals and model humic compounds indicated a difference between abiotic and biological dechlorination processes. *Peri*-dechlorination was preferential during biologically-mediated processes, resulting in transient 2,3,7,8-TCDD production. However, whereas this isomer appears to be the bottleneck, it is not the endpoint of microbial dechlorination as mono-, di- and trichlorinated CDDs were produced. In the natural environment, 2,3,7,8-substituted isomers partition preferentially to biological cell membranes. It was shown that 20 ppb of 2,3,7,8-TCDD, which had partitioned to microbial cells derived from historically-contaminated sediments, dechlorinated to monoCDD¹⁰⁾.

These results indicate that 2,3,7,8-residue patterns in sediments are likely the cumulative result of source patterns and abiotic or biological dechlorination fingerprints. Since bioavailability does not appear to be a limiting factor, off-site remediation using the biological and abiotic catalysts present in contaminated soils or sediments has great potential as a treatment strategy.

5. Conclusions

Although natural or enhanced bioremediation of environments contaminated with CAH has great potential, the application of these technologies is still in its infancy, and field trials have only recently emerged. The difficulties being faced relate to process development and such issues as bioavailability, concentration-dependent toxicity, rates of degradation and sequential anaerobic/aerobic processes. Moreover, there is an urgent need for extensive collaboration between environmental analytical chemists and microbiologists, to expand our knowledge about the occurrence, extent, and limitations of natural attenuation processes at historically contaminated sites, such that proper enhancement techniques can be conceived.

The acceptance and importance of bioremediation as an innovative technology is underscored by the U.S. EPA Bioremediation Field Initiative, which addresses the research needs to help foster implementation of this technology at contaminated sites. Currently, over 150 sites, of which 50% are contaminated with chlorinated compounds are being treated using bioremediation. Once the aforementioned hurdles are overcome with future research, both in the laboratory and in the field, the application of microbial processes should prove increasingly successful with respect to CAH contamination

6. References

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