**PCBs IN FRESHWATER AND MARINE SEDIMENTS: TRANSPORT, TRANSFORMATION AND TREATMENT**

**Organized by**

**D.A. Dzombak and G.V. Lowry**

Symposia Papers Presented Before the Division of Environmental Chemistry
American Chemical Society
Philadelphia, PA August 22-26, 2004

# MICROBIAL COMMUNITIES IN TWO RIVER SEDIMENTS DEMONSTRATE DISTINCT ANAEROBIC PCB DECHLORINATION PATTERNS

Christine Wang[1], Jeanne M. VanBriesen[2,3], William E. Brown[1] and Edwin G. Minkley, Jr.[3]
[1]Department of Biological Sciences
[2]Department of Civil and Environmental Engineering
[3]Department of Biomedical Engineering
Carnegie Mellon University, 5000 Forbes Ave., Pittsburgh, PA 15213
chriswang@cmu.edu

## Introduction

Persistent chemical contaminants that accumulate in the environment and in food webs result in long-term threats to public health and ecosystem stability. For example, polychlorinated biphenyls (PCBs), widely used in industry between 1929 and 1978, are stable, lipophilic and chemically inert which allows them to adhere to organic particles and persist in the environment. These persistent chemicals enter food webs with lipid-rich cell membranes and adipose tissues eventually become a sink for these contaminants in living organisms. PCBs have been associated with neural, behavioral and developmental defects in children and with the disruption in endocrine and reproductive systems in human adults as well as in many vertebrates[1]. The influence of PCB contamination has caused great concern in fish consumption. Consequently, PCB contaminants in lakes, rivers and oceans damage commercial fishing industries. The extended range of PCB distribution leads to a broad effect on the global ecosystem.

Previous research[2-4] revealed microbial activities in the degradation and dechlorination of PCBs despite their stability. These biological activities that occur naturally can be further stimulated in the laboratory[5-6]. These observations lead to the hypothesis that different microbial activities result in different rates and specificity of anaerobic PCB dechlorination. To test this hypothesis, our research focuses on the microbial communities in contaminated, fresh water, river sediments. Our specific aim is to determine the roles that different populations play in PCB dechlorination by characterizing the microbial communities – identifying the type of organisms, the population size and their activities in PCB dechlorination.

## Materials and Methods

Contaminated river sediments spiked with selected PCB congener

Experiments were set up using sediments from two PCB-contaminated fresh water systems, the Grasse River (NY) and the Hudson River (NY). The addition of PCBs in the sediments under anaerobic condition is designed to stimulate the growth of organisms capable of reductive declorination of PCBs and therefore enrich the population of these organisms in the samples. The experiment presented here used enrichment samples spiked with 40ppm 2,4,5-trichlorobiphenyl (BZ-29). The anaerobic, PCB-spiked sediments were periodically sampled for PCB composition analysis and molecular biological analysis to monitor microbial activities in PCB dechlorination as well as the changes in sediment microbial populations.

PCB analysis

PCB analysis is a quantitative measurement of specific congeners in the sample. Total PCBs were extracted using 1:1 (v/v) acetone:hexane, cleaned with florisil and anhydrous sodium sulfate and subjected to gas chromatography (The extraction and cleanup methods were modified from Quensen *et al*.[7] and EPA Method 3620B[8] respectively.) PCB extracts were applied to a DB-XLB column (J&W) using a thermal profile designed to separate and quantify the amount of the spiked congener (BZ-29) and its dechlorinated products 2,5-dichlorobiphenyl (BZ-9), 2,4-dichlorobiphenyl (BZ-7) and 2-chlorobiphenyl (BZ-1). The results of the PCB analyses indicated the amount, the identity and thus the preferred pathway(s) or congener(s) of dechlorination.

Molecular biology analysis

The molecular biological analysis provides a profile of the microbial communities in the sample. Total DNA was isolated from sediments using two commercially available kits (Mo-Bio UltraClean Soil DNA Kit and QIAGEN QIAamp DNA Stool Mini Kit). The 16S ribosomal RNA gene (16S rDNA) is a genetic marker that was used to categorize and identify specific microbial groups. After PCR (polymerase chain reaction)-amplification, denaturing gradient gel electrophoresis (DGGE) was used to resolve the specific 16S rDNA fragments[9]. The resolved 16S rDNA fragments provide an estimation of the composition of the microbial community at a molecular level. This analysis allows us to visualize any changes in the microbial populations.

Metabolism-related compound addition

In addition to a specific PCB congener, metabolism-related compound(s), including (1) nitrogen (as ammonium chloride), (2) phosphorus (as potassium phosphates) or (3) fatty acids (single chain of carbon atom[s] with a carboxyl group at one end, *i.e.*, formic and acetic acids) (as sodium formate and acetate) were added either as a single addition component or as different mixtures of the above three compounds. By supplying various metabolism-related compounds (nutrients) to the sediments under controlled conditions, the changes in microbial populations can be correlated to different dechlorination patterns that would suggest the preferred substrate or conditions for those groups of organisms.

## Results and Discussion

Hudson River sediment microcosms showed rapid dechlorination of BZ-29 with a minimal gas production, while the Grasse River microcosms showed a longer lag period, accompanied by extensive methane and $CO_2$ production. The two possible pathways for the degradation of BZ29 include: BZ29 to BZ9 to BZ1 and BZ29 to BZ7 to BZ1. Currently we have completed PCB and molecular microbial analyses of the BZ-29-spiked, nutrient-added Hudson samples after 10 weeks of anaerobic incubation at room temperature. Figure 1 shows the effects of various nutrient additions on the distribution of PCBs in the 10-week Hudson samples. The addition of nitrogen alone seems to suppress the production of BZ-9, resulting in the accumulation of BZ-7. The presence of phosphorus, either as a single component addition or as a mixture with other compounds led to little or no BZ-7 production and yielded BZ-9 and BZ-1 as the dechlorination products. The addition of fatty acids (formate and acetate) appears to drive the reaction to BZ1 compared to the combination of acetate and formate and nitrogen that appears to inhibit this effect.

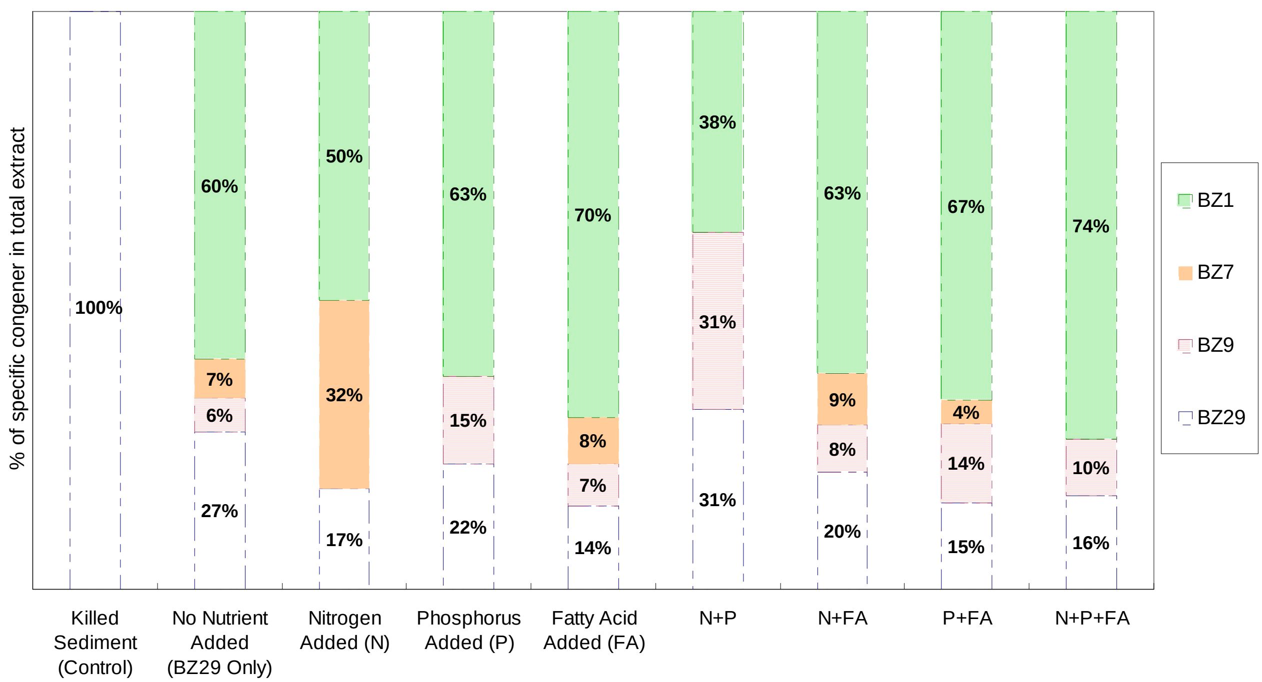


**Figure 1.** The influence of different nutrient additions on BZ29 dechlorination in BZ29-spiked, 10-week Hudson River sediment samples**.**

The 16S rDNA derived PCR fragments resolved in the denaturing gradient gel (Figure 2) showed distinct banding patterns for each of the varied BZ-29-spiked, nutrient-added samples. Sequencing data and BLAST results of DNA recovered from some of the bands on the gel suggest that DNA fragments with similar mobility are very likely to be phylogeneticly related (*i.e.*, these 16S rDNA fragments belong to the same organisms, even though they were amplified from different samples). The results demonstrated the power and the feasibility of using the 16S rDNA marker and denaturing gradient gel electrophoresis to profile the microbial communities in our samples. Work is in progress to complete the comparison to the Grasse River sediments.

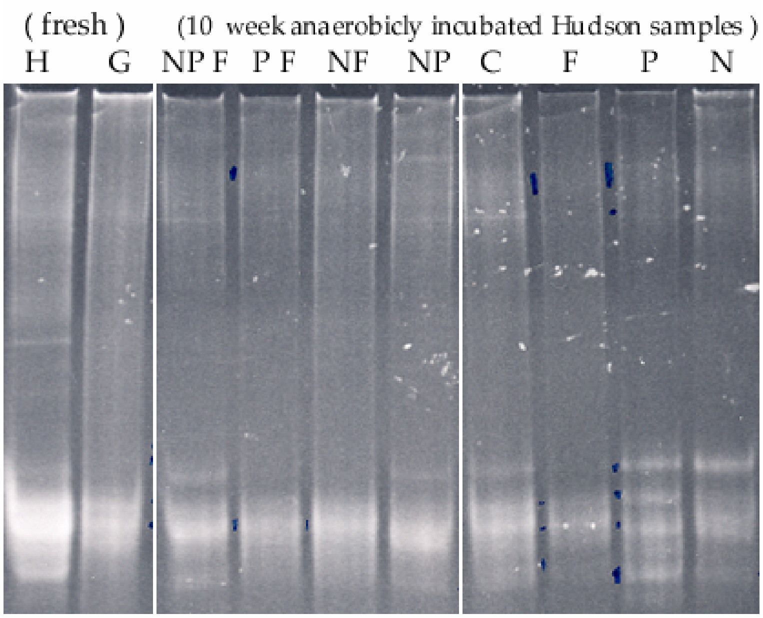


**Figure 2.** Denaturing gradient gel electrophoresis of bacterial 16S rDNA fragments amplified from fresh Grasse (G) and fresh Hudson (H) sediments, 10-week anaerobic incubated, BZ-29-spiked, no nutrient added Hudson (C), and 10-week anaerobic incubated, BZ-29-spiked, nutrient-added (N, P, F, NP, NF, PF and NPF) Hudson samples

**Acknowledgements**
This research is supported by the Interdisciplinary Science Program of the David and Lucile Packard Foundation.

**References**

1. National Research Council (U.S.) (2001) Committee on Remediation of PCB Contaminated Sediments. *A risk-management strategy for PCB-contaminated sediments* (National Academy Press, Washington, DC).
2. Adriaens, P. and T. M. Vogel. (1995) in *Microbial transformation and degradation of toxic organic chemicals* (eds. Young, L. Y. & Cerniglia, C.) 435-486 (Wiley-Liss, New York).
3. Brown, J. F. *et al*. (1984) PCB transformations in upper Hudson sediments. *Northeast Environ. Sci.* **3**, 167-169.
4. Bopp, R. F. *et al*. (1984) The persistence of PCB components in sediments of the lower Hudson. *Northeast Environ. Sci.* **3**, 180-184.
5. Anid, P. J. *et al*. (1993) Effect of hydrogen peroxide on the biodegradation of PCBs in anaerobically dechlorinated river sediments. *Biodegradation* **4**, 241-8.
6. Anid, P. J. *et al*. (1991) in *On-site bioreclamation: Processes for xenobiotic and hydrocaron treatment* (eds. Hinchee, R. E. & Olfenbuttel, R. F.) 428-436 (Butterworth-Heinemann, Boston).

7. Quensen, J. F. *et al*. (1990) Dechlorination of 4 commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Appl Environ Microbiol* **56**, 2360-9.
8. Environmental Protection Agency (U.S.) (1996) Cleanup Method 3620B: Florisil Cleanup. *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. (U.S. Environmental Protection Agency, Washington D.C.)
9. Walia, S. *et al*. (1990) Construction and applications of DNA probes for detection of polychlorinated biphenyl-degrading genotypes in toxic organic-contaminated soil environments. *Appl Environ Microbiol* **56**, 254-9.