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The overall objective of the project was to characterize the activity and interactions of diverse anaerobic microbial communities involved in dehalogenation in marine sediments. Understanding the role of anaerobic respiratory processes and different microbial communities for dehalogenation in marine sediments is essential for developing *in-situ* remediation technologies, exploiting intrinsic processes and developing the science base for natural attenuation. Our approach has been to examine the role of anaerobic microbial processes, such as sulfidogenesis and iron-reduction, on the activity of dehalogenating microorganisms. Halogenated aromatic compounds were shown to be biodegradable under a variety of redox conditions central to carbon flow in anoxic sediments and soils, and their complete oxidation to CO_2 can be coupled to processes such as sulfate reduction, Fe(III)-reduction, denitrification and methanogenesis. Reductive dehalogenation is usually the initial step in metabolism under methanogenic, sulfidogenic and iron-reducing conditions. Microorganisms with the capacity for dehalogenation appear to be widely distributed in anoxic marine environments. Complementary biomolecular tools (16S rRNA and phospholipid fatty acid analysis) were used to examine the community structure and dynamics of the anaerobic dehalogenating consortia and to gain more detailed information about the dehalogenating bacteria.

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FINAL REPORT

GRANT #: N00014-94-1-0434

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INSTITUTION: Rutgers, the State University of New Jersey

GRANT TITLE: Diversity of Anaerobic Dehalogenation in Estuarine and Marine Sediments

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OBJECTIVE: The overall objective of the project was to characterize the activity and interactions of diverse anaerobic microbial communities involved in dehalogenation in marine sediments. The aim is to expand our understanding of the biodegradative activity of diverse anaerobic bacteria and how they can be stimulated for remediation of contaminated marine and estuarine sediments.

APPROACH: Our approach has been to examine the influence of alternative electron acceptors (sulfate, Fe(III) and carbonate) on dehalogenation and degradation of halogenated aromatic compounds. The role of anaerobic microbial processes, such as sulfidogenesis and iron-reduction, on the activity of dehalogenating microorganisms were determined. Anaerobic consortia enriched from different estuarine and marine sediments that degrade brominated and chlorinated aromatic compounds under sulfidogenic and iron-reducing conditions were characterized. Specifically, the effect of alternate electron acceptors, such as sulfate and Fe(III), on the activity of dehalogenating microorganisms was examined. Biomolecular tools (16S rRNA and fatty acid analysis) were used to examine the community structure and dynamics of the anaerobic dehalogenating consortia.

ACCOMPLISHMENTS: We demonstrated that halogenated aromatic compounds are biodegradable under a variety of electron accepting conditions (sulfidogenic, iron-reducing and methanogenic) central to carbon flow in marine sediments. Bromophenols were reductively debrominated to phenol with stoichiometric release of bromide, and degradation of phenol to carbon dioxide was coupled to sulfate- and iron-reduction, respectively. Dehalogenation rates were in general slower under sulfidogenic and iron-reducing conditions suggesting that dehalogenation was affected by the alternate electron acceptor. The different substrate specificities observed for the chlorinated and brominated aromatic compounds suggests that distinct dehalogenating microbial populations were enriched under the different redox conditions. Very similar degradation activities were observed at different sites, indicating that the capacity for dehalogenation is widely distributed in marine sediments. While brominated and chlorinated phenols and benzoic acids were readily degraded in sediments from a variety of marine sites, fluorinated aromatic compounds appear to be more recalcitrant. We have observed no degradation of monofluorinated phenols under either sulfidogenic, iron-reducing methanogenic or denitrifying conditions. Fluorobenzoates were utilized only under denitrifying conditions.

To examine the anaerobic metabolism of halogenated phenols in more detail a sulfidogenic consortium was enriched from estuarine sediment and maintained with 4-chlorophenol as the sole source of carbon and energy. This consortium was shown to mineralize 4-halophenols (4-chloro-, 4-bromo-, and 4-iodophenol) to CO_2 with release of halide under sulfidogenic conditions. Further metabolic characterization indicated that 4-chlorophenol was reductively dechlorinated to phenol by the sulfate-reducing culture and mineralization of the phenol ring was coupled to sulfate reduction. Reductive dechlorination as the initial step in chlorophenol degradation by the sulfate-reducing consortium was confirmed with the use of chloro-fluorophenols. Interestingly, this dehalogenation activity appeared to be dependent on sulfidogenesis. Reductive dechlorination was inhibited by molybdate and did not occur in the absence of sulfate. These results indicate that 4-chlorophenol is reductively dechlorinated to phenol under sulfate-reducing conditions and mineralization of the phenol ring to CO_2 is coupled to sulfate reduction. The sulfate-dependency and inhibition by molybdate suggests that sulfate-reducing bacteria may be directly responsible for 4-chlorophenol degradation.

Complementary biomolecular tools (16S rRNA and phospholipid fatty acid analysis) were used to examine the community structure and dynamics of the anaerobic dehalogenating consortia and to gain more detailed information about the dehalogenating bacteria. Dehalogenating microbial consortia were enriched under sulfidogenic conditions using an estuarine sediment inoculum with 2-bromophenol, or phenol as the sole the carbon source. Stable consortia were maintained with repeated feeding and serial dilution into fresh medium. 2-Bromophenol was initially dehalogenated to phenol as the first step in degradation. From a sulfidogenic 2-bromophenol-utilizing consortium we identified four phylotypes which based upon their 16S rRNA sequences were clustered into 3 major groups. One sequence was related to the ε subgroup of the Proteobacteria, two clones clustered within the sulfate-reducering bacteria (δ subgroup of Proteobacteria), the fourth phylotype was divergent from previously described bacteria and was most closely related to the genus *Planctomycetes*. In contrast, a sulfidogenic phenol-degrading consortium initiated concurrently with the same sediment inocula yielded only two clonal types. One was placed within the ε sub-division of the Proteobacteria, with *Thiomicrospira denitrificans* as its closest neighbor. The other clone was closest to the genus *Cytophaga* with *Anaeroflexus maritimus* as its closest neighbor. Terminal restriction fragment length polymorphism (T-RFLP) of all individual clones and both microbial consortia indicated that all 16S rRNA types present in both consortia had been cloned and characterized. The dynamics of the microbial communities were also monitored by phospholipid fatty acid analysis (PFLA). Principal component analysis of the phospholipid fatty acids demonstrated that distinct populations were enriched with each substrate and under each electron accepting condition. The combination of PFLA and phylogenetic analysis will help discern the organisms responsible for dehalogenation and degradation of halogenated aromatic compounds under different reducing conditions. These two methods complement each other to allow a more complete analysis of the total consortium.

CONCLUSIONS: Halogenated aromatic compounds are biodegradable under a variety of redox conditions central to carbon flow in anoxic sediments and soils, and their complete oxidation to CO_2 can be coupled to processes such as sulfate reduction, Fe(III)-reduction, denitrification and methanogenesis. Reductive dehalogenation is usually the initial step in metabolism under methanogenic, sulfidogenic and iron-reducing conditions. Similar degradation activities were observed with inocula from different sites indicating that the microorganisms with the capacity for dehalogenation are widely distributed in anoxic marine environments. In addition, the different substrate specificities and activities observed for the halogenated aromatic compounds suggests that distinct dehalogenating microbial populations are enriched under the different reducing conditions, and this is also suggested by molecular characterization of the anaerobic consortia. The presence or absence of suitable electron acceptors will affect the activity of different microbial populations and thus the biodegradability of organohalides in anaerobic environments. A detailed characterization of these anaerobic microbial communities will help to fully understand the role that they play in the degradation of haloaromatics in anoxic environments and for harnessing their activities for treatment of contaminated sediments.

SIGNIFICANCE: Contamination of marine and estuarine sediments by anthropogenic compounds, such as halogenated aromatic compounds, including phenolics, PCBs and dioxins, has become a major problem with far-reaching economic consequences. A fundamental understanding of the processes that control the fate and effects of these pollutants in estuarine and near-shore environments is needed. Enhancing microbial dehalogenation of these compounds has the potential of reducing the toxicity of sediments and thus providing a viable treatment technology for contaminated dredge spoils and sediments. Understanding the role of anaerobic respiratory processes and different microbial communities for dehalogenation in marine sediments is essential for developing *in-situ* remediation technologies, exploiting intrinsic processes and developing the science base for natural attenuation.

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