



Project Summary

Effects of Temperature and Redox Conditions on Degradation of Chlorinated Phenols in Freshwater Sediments

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The physical and chemical properties of the sediment environment can vary in time as well as in location. Sediment temperatures change over the seasons, and sediments directly exposed to the sun in summer can reach temperatures in excess of 50°C. Freshwater sediments are primarily methanogenic, whereas marine sediments are dominated by sulfate reducing conditions. These fluctuations and differences can influence chemical as well as microbiological processes involved in the transformation or degradation of hazardous organic chemicals. Therefore, during the development of mathematical models to predict the fate of toxic organic compounds in the environment, it is important to include parameters that describe the influence of temperature and chemical characteristics on anaerobic biodegradation.

The effect of temperature and redox conditions on the anaerobic degradation of 2,4-dichlorophenol (2,4-DCP) was investigated in anaerobic sediment slurries prepared from local freshwater pond sediments. Under methanogenic conditions, 2,4-DCP dechlorination occurred in the temperature range between 5 and 50°C. Although dechlorination was not observed

above 50°C, anaerobic bacterial activity was indicated by methane formation up to 60°C. In sediment samples from two sites and at all temperatures from 5 to 50°C, 2,4-DCP was transformed to 4-chlorophenol (4-CP). The 4-CP was further degraded after several weeks. Adaptation periods decreased between 5 and 25°C, were essentially constant between 25 and 35°C, and increased between 35 and 40°C. Degradation rates increased exponentially between 15 and 30°C, had a second peak at 35°C and decreased to about 5% of the peak activity by 40°C. In one sediment sample, an increase in degradation rates was observed following the minimum at 40°C, suggesting that at least two different organisms were involved in the 2,4-DCP dechlorination.

The addition of sulfate at a level of 0.25% resulted in a marked reduction in the rate of methane formation, a marked reduction in the rate of reductive dechlorination, and an increase in the length of the adaptation period before reductive dechlorination could be detected. The addition of nitrate at a level of 0.15% resulted in the complete inhibition of the reductive dechlorination reaction for at least 127 days.

Storage of the original sediment slurries for 2 months at 12°C resulted in increased adaptation times, but did not affect the degradation rates.

This Project Summary was developed by EPA's Environmental Research Laboratory, Athens, GA, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The full report explains the effect of temperature and redox conditions on the reductive dechlorination of 2,4-dichlorophenol (2,4-DCP) and 4-chlorophenol (4-CP) sediment slurries. It also shows that, for a restricted temperature (e.g., from 15 to 30°C), dechlorination rates under methanogenic conditions can be described by an Arrhenius function.

Both 2,4-DCP and 4-CP are released to the environment by direct disposal or as break-down products of higher chlorinated compounds such as the extensively used wood preservative pentachlorophenol (PCP) or the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). These compounds are highly toxic and 4-chlorophenol is a suspected carcinogen. Thus, there is a clear need for modeling the fate of these compounds in order to evaluate their potential risk to man and his environment.

The reductive dechlorination of chlorophenols has been reported in a variety of anaerobic sediments such as sewage sludge, sediment, and subsurface aquifer environments, all of which were methanogenic. Only recently has the reductive dechlorination of 2,4-DCP been reported to occur under sulfate reducing conditions and, to our knowledge, reductive dechlorination under denitrifying conditions has not been reported. The proposed pathway for the anaerobic degradation of 2,4-DCP is the progressive replacement of the chlorines by hydrogen (reductive dechlorination) leading to phenol, which is then further degraded to methane and carbon dioxide. Similar reductive pathways have been reported for chlorinated benzoates and other chlorinated phenols.

The addition of sulfate to methanogenic aquifer materials has resulted in the inhibition of the dechlorination reaction. Interestingly, however, the degradation intermediate phenol was readily degraded under sulfate reducing conditions.

Adding sulfate to methanogenic aquifer material has also led to increased degradation of cresols. Unfortunately, little else is known about the effects of environmental factors on these reactions. An extensive study of the effects of redox couples or temperature has not been reported. Our work was undertaken to learn more of these effects to provide a better base for the development of environmental fate and effects models.

Procedures

Freshwater lake sediments from two different sites near Athens, GA, were used in this investigation. Individual reaction flasks consisted of anaerobic tubes that were filled with 20 ml of sediment slurry and crimp sealed with butyl rubber stoppers. All procedures were conducted under flowing nitrogen that had been purged of oxygen. Methanogenic slurries were prepared by combining sediment with site water to give 0.2 to 0.4 mg dry wt. sediment/ml of slurry, adding sufficient 1 M K_2HPO_4/KH_2PO_4 to yield a buffer concentration of 20 mM, and then adjusting the pH to 6.9 by the addition of small quantities of 2 N NaOH. The slurries were prepared in a blender and were periodically mixed to obtain a homogeneous solution. Sulfate reducing or denitrifying slurries were prepared by adding sulfate (0.25%) or nitrate (0.15%), respectively.

Degradation experiments were initiated by adding 0.7 ml of an aqueous 2,4-DCP stock solution to give a final concentration of about 125 $\mu\text{mol/l}$ (20 ppm). Duplicate samples were incubated in waterbaths at different temperatures. Sediment slurries were removed periodically and combined with an equal volume of acetonitrile to terminate the reaction and to facilitate recovery of compounds bound to the sediment matrix. These mixtures were subsequently analyzed by high performance liquid chromatography (HPLC) for 2,4-DCP, 4-CP, phenol, and benzoate. Abiotic controls were prepared by autoclaving the sediment samples on 3 consecutive days for 45 min each before 2,4-DCP was added.

For methane formation experiments, 10 ml of the slurry without 2,4-DCP were placed in Hungate tubes and the filled tubes were then incubated in a temperature gradient incubator (7 to 80°C) with continuous shaking. Periodically, 100- μl samples were taken from the headspace with a gas lock syringe and the methane concentration determined by gas chromatography.

Conclusions

The authors observed a sequential dechlorination of 2,4-DCP in the temperature range between 5 and 52°C for unacclimated sediment dominated by methanogenic activity. At all temperatures, the first product was 4-CP, and although at least two different groups of microorganisms were involved in 2,4-DCP degradation at the different temperatures, they all used the same reaction to initiate degradation.

The authors also found that, although anaerobic microbial dechlorination of 2,4-DCP occurred from 5 to 52°C, only a small portion of the data (15 to 35°C) could be fit to an Arrhenius equation. Above and below the linear range the temperature dependence varied markedly. Although this range appears limited, it does indicate the temperatures over which microorganisms will significantly influence the fate of chlorinated aromatic compounds in anaerobic environments and does cover a significant fraction of environments where these microorganisms would be active. Furthermore it indicates that laboratory tests could be performed at the faster rates (e.g. at 28°C) and then extrapolated to environmental temperatures (e.g. at 20°C).

Unlike a chemical reaction where temperature may only affect the reaction in a microbiologically mediated process temperature affects both the composition of the active community and the enzyme catalyzed reaction. The effects of population become even more pronounced when more than one organism as in the case of reductive dechlorination may be required for activity and different organisms may catalyze the rate determining step at different temperatures. In this study, the major peaks of activity observed above and below 40°C probably resulted from the contribution of thermophilic and mesophilic organisms, respectively. Interestingly, because thermophilic activity was only observed in half of the samples from one of the sediments, the key thermophilic organisms appeared to be present at significantly lower numbers.

Storing sediment samples before use appeared to affect adaptation more than the rate of dechlorination. For example adaptation periods, determined over the temperature range of 5 to 25°C decreased from a range of 140 to 8 days for the fresh sediments to a range of 27 to 12 days for the sediments stored for months at 12°C. The corresponding 2,4-DCP transformation rates were not significantly affected. Therefore the us

of stored samples could lead to erroneous estimates of the adaptation period.

Under sulfate reducing conditions, the rate of reductive dechlorination activity was observed only between 18 and 40°C after 365 days incubation. More than 30% of the sulfate remained following complete degradation of the 2,4-DCP and methanogenic activity was inhibited for the duration of the experiment. The rates varied from 2 to 19% of the rates observed under methanogenic conditions. No reductive dechlorination was observed under denitrifying conditions after 127 days of incubation.

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The complete report, entitled "Effects of Temperature and Redox Conditions on Degradation of Chlorinated Phenols in Freshwater Sediments," (Order No. PB 89-129 118/AS; Cost: \$13.95, subject to change) will be available only from:

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