Microbial Degradation of Chlorinated Phenols

Edward Topp

Land Resource Research Centre, Agriculture Canada, Research Branch,

Central Experiment Farm, Ottawa, Ontario K1A 0C6

Introduction

Chlorinated phenols can be introduced into the environment as a result of the activities of the wood preservation and pulp and paper industries. Chlorophenol pesticide formulations have been used for a variety of applications for over 50 years. As a result of heavy and widespread use, pentachlorophenol (PCP) is apparently ubiquitous in the environment being found in soil, water, and human urine (6). High levels of PCP contamination can sometimes be found at wood preservation facilities and lumber storage areas (13). There are several hundred PCP-polluted sites that have been identified in North America. These can comprise both soil and groundwater contamination. Currently, about 2000 Tonnes of pentachlorophenol (PCP) are used per year in Canada. Pentachlorophenol is an effective wood preserving agent because it is very toxic to microorganisms and survives a long time in the treated material. Unfortunately, it is this toxicity and environmental stability which make PCP a problematic pollutant.

The objective of this paper is to review the degradation of chlorinated phenols, particularly PCP, by microorganisms. Aspects to be discussed include *in situ* biodegradation, biodegradation by microbial isolates, and strategies being investigated to utilize microorganisms for decontaminating chlorophenol-polluted soil and water. A number of published reviews will be of value to the reader who wishes to investigate the literature in more depth (4,6,12,23).

<u>Fate of chlorinated phenols in the environment</u>

Once chlorophenols enter the environment they are subject to photolysis, sorption to particulates, covalent coupling to large or small molecules, volatilization, and microbial decomposition (biodegradation). It is biodegradation which is generally the predominant mechanism for the breakdown of these compounds. Convincing evidence for biodegradation comes from experiments where radiolabelled chlorophenols are converted to radio-labelled CO_2. This mineralization does not take place in soils that have been sterilized indicating that the activity is of biological origin.

The half-life of PCP in aquatic and terrestrial systems can range from a few hours to many years. Soil type, organic matter content, and moisture are some of the factors that can influence the rate of degradation in terrestrial systems (14). It is important to think of environments in terms of those that are in contact with air (aerobic) and those that are not (anaerobic). The metabolic properties of microorganisms able to function under these two conditions are profoundly different and consequently the reactions, rates, and products of biodegradation are very sensitive to oxygen. In anerobic sewage sludes and flooded soils heavily chlorinated phenols are sequentially dechlorinated to less chlorinated phenols (15,30). On the other hand, when oxygen is available degradation proceeds through chlorinated quinone intermediates. In both anaerobic and aerobic environments chlorophenols, including PCP, can be completely degraded to innocuous products such as carbon dioxide. However, subsidiary pathways may sometimes result in the transformation of a small amount of a chlorophenol to an undesirable product. For example, PCP may be methylated to volatile pentachloanisole. There is some evidence to suggest that microorganisms present in aquifers may be able to degrade chlorophenols. Water samples from a shallow aquifer dehalogenated some chlorophenols (11). Chlorophenol-consuming microorganisms are also present in aquatic systems. The half-life of PCP in a freshwater stream declined in response to an increase in the PCP-degrading microbial population (20).

If, clearly, there are microorganisms in nature which break down chlorinated phenols, why are these chemicals a pollution problem? There are a number of reasons why chlorophenols may not be completely degraded. Microorganisms with the required degradative ability may not be distributed uniformly throughout the environment. Interactions between chlorophenols and other molecules, for example binding of chlorophenols to soil via a number of mechanisms, may immobilize them and make them unavailable for biodegradation. Extremes in physical or chemical conditions such as temperature, pH, and moisture content, may slow down or block biological activity. There may be other pollutants present such as heavy metals, oils, or polyaromatic hydrocarbons (PAHs) which are toxic to chlorophenol-degrading microorganisms. Finally, the concentration of the chlorophenol may be critical. Highly polluted materials will be prohibitively toxic. Very low concentrations may be below the threshold concentration for metabolism. In this case reaction kinetics dictate that the rate of degradation will be very slow.

Degradation by isolated organisms

To date no organisms that metabolize chlorophenols anaerobically have been isolated or described. However, the complete dechlorination and conversion of PCP to carbon dioxide and methane in anaerobic sludges indicates that these microorganisms must exist (15).

A number of aerobic bacteria and fungi have been isolated which degrade PCP and other chlorinated phenols (9). Bacteria which grow on and completely degrade PCP include an *Arthrobacter* species (7), a *Flavobacterium* species (21) and *Rhodococcus chlorophenolicus* (1). The fungus *Geotrichum candidum* excretes a laccase enzyme which converts some chlorophenols to reactive intermediates which become bound to soil and immobilized (22). The white-rot fungus *Phanerochaete chrysosporium* metabolizes PCP, posssibly by means of the ligninase enzyme system (17).

Research on PCP-degrading bacteria has recently focused most intensively upon the *Flavobacterium* and *R. chlorophenolicus*. These bacteria can readily be grown and studied in the laboratory. Considerable information on the biochemistry of chlorophenol degradation is available (3,24). A variety of chlorophenols are degraded by each of the organisms (2,25). Various parameters including growth conditions (27) and nutrient supplements (26) can be manipulated to enhance PCP degradation by the *Flavobacterium*. The *Flavobacterium* can tolerate and degrade PCP concentrations greater than 100 mg/l. *R. chlorophenolicus* can tolerate about 10 mg/1 PCP. It can metabolize a variety of polychlorinated phenols as well as chlorinated guaicols. It will also methylate these compounds.

Exploiting biodegradation for environmental remediation

Several approaches to enhance the biodegradation of chlorophenols in contaminated soil are currently being investigated. These include the addition of microorganisms to the soil, composting, and soil washing followed by biological treatment of the leachate. Decontamination of polluted groundwater has focussed on pump and treat methods.

A number of pure cultures have been examined for their ability to enhance biodegradation when inoculated into polluted soils. Addition of 10^6 PCP-degrading Arthrobacter cells per gram soil reduced the half life of PCP from 2 weeks to less than a day (7). Flavobacterium cells added to soil or soil slurries will degrade PCP concentrations up to the 100 ppm range within a few days or weeks (5,28). Residual PCP concentrations will be in the ppb range. Parameters which influence the degradation rate and should be optimized include pH, temperature, water content, PCP concentration and how many Flavobacterium cells are added. High concentrations of copper/chromate/arsenate or creosote inhibit the Flavobacterium. Salkinoja-Salonen and colleagues were able to decontaminate soil polluted with 5000 ppm PCP by a combination of composting and inoculation with R. chlorophenolicus.

Addition of PCP-acclimated anerobic sewage sludge to soil enhanced PCP degradation (16).

Reactors containing PCP-degrading bacteria can be used to treat groundwater, soil leachate or process water. The reactor may contain a biofilm growing on an inert packing (10,19) or cells immobilized in a matrix such as alginate or polyurethane (18,29). Contaminated groundwater is pumped through the reactor where the microorganisms degrade the PCP. Other contaminants such as PAHs can be degraded or adsorbed (10). Parameters that can be manipulated to optimize the activity include temperature, pH, and organic and inorganic nutrients.

Conclusion

Degradation of hazardous materials by microorganisms has been the subject of considerable research in recent years. Results to date indicate that biodegradation may be a feasible technology for environmental remediation. Research to isolate and understand microorganisms with desirable degradative properties should continue. The continued input of several scientific and engineering disciplines will be required to design systems which exploit biodegradation at the field scale.

References

1. Apajalahti, J.H.A., and M.S. Salkinoja-Salonen. 1986. Degradation of polychlorinated phenols by Rhodoccus chlorophenolicus. Appl. Microbiol. Biotechnol. 25:62-67.

2, Apajalahti, J.H.A., and M.S. Salkinoja-Salonen. 1987a. Dechlorination and para-hydroxylation of polychlorinated phenols by Rhodococcus chlorophenolicus. J. Bacteriol. 169:675-681.

3. Apajalahti, J.H.A., and M.S. Salkinoja-Salonen. 1987b. Complete dechlorination of tetrachlorohydroquinone by cell extracts of pentachlorophenol-induced Rhodocoddus chlorophenolicus. J. Bacteriol. 169:5125-5130.

4. Boyd, S.A., M.D. Mikesell, and J.-F. Lee. 1989. Chlorophenols in Soils. p. 209-228 in: Reactions and Movement of Organic Chemicals in Soils. Special publication no. 22. Soil Science Society of America. Madison WI.

5. Crawford, R.L., and W.W. Mohn. 1985. Microbiological removal of pentachlorophenol from soil using a Flavobacterium. Enzyme Microb. Technol. 7:617-620.

6. Crosby, D.G. 1981. Environmental chemistry of pentachlorophenol. Pure Appl. Chem. 53:1051-1080.

7. Edgehill, R.V. and R.K. Finn. 1982. Isolation, characterization and growth kinetics of bacteria metabolizing pentachlorophenol. Eur. J. Appl. Microbiol. Biotechnol. 16:179-184.

8. Edgehill, R.V. and R.K. Finn. 1983. Microbial treatment of soil to remove pentachlorophenol. Appl. Environ. Microbiol. 45:1122-1125.

9. Engelhardt, G., P.R. Wallnofer, W. Mucke, and G. Renner. 1986. Transformations of pentachlorophenol. Part II: Transformations under environmental conditions. Toxicol. Environ. Chem. 11:233-252.

10. Frick, T.D., R.L. Crawford, M. Martinson, T. Chresand, and G. Bateman. 1988. Microbiological cleanup of groundwater contaminated by pentachlorophenol, p.173-191 in: Environmental Biotechnology. Reducing risks from environmental chemicals through biotechnology. G.S. Omenn (Ed.).

11. Gibson, S.A., and J.M. Suflita. 1986. Extrapolation of biodegradation results to groundwater aquifers: reductive dehalogenation of aromatic compounds. Appl. Environ. Microbiol. 52:681-688.

12. Jones, P.A. 1984. Chlorophenols and their impurities in the Canadian environment: 1983 supplement. Environment Canada Report EPS 3-EP-84-3.

13. Kitunen, V.H., R.J. Valo, and M.S. Salkinoja-Salonen. 1987. Analysis of chlorinated phenols, phenoxyphenols and dibenzofurans around wood preserving facilities. Int. J. Environ. Anal. Chem. 20:13-28.

14. Kuwatsuka, S. and M. Igarashi. 1975. Degradation of PCP in soils. II. The relationship between the degradation of PCP and the properties of soils, and the identification of the degradation products of PCP. Soil Sci. Plant Nutr. 21:405-414.

15. Mikesell, M.D., and S.A. Boyd. 1986. Complete reductive dechlorination and mineralization of pentachlorophenol by anaerobic microorganisms. Appl. Environ. Microbiol. 52:861-865.

16. Mikesell, M.D., and S.A. Boyd. 1988. Enhancement of pentachlorophenol degradation in soil through induced anaerobiosis and bioaugmentation with anaerobic sewage sludge. Env. Sci. Technol. 22:1411-1414.

17. Mileski, G.J., J.A. Bumpus, M.A. Jurek, and S.A. Aust. 1988. Biodegradation of pentachlorophenol by the white rot fungus _Phanerochate chrysosporium_. Appl. Environ. Microbiol. 54:2885-2889. 1988.

18. O'Reilly, K.T., and R.L. Crawford. 1989. Degradation of pentachlorophenol by polyurethane-immobilized _Flavobacterium_ cells. Appl. Environ. Microbiol. 55:2113-2118.

19. O'Reilly, K.T., R. Kadakia, R.A. Korus, and R.L. Crawford. 1988. Utilization of immobilized bacteria to degrade aromatic compounds common to wood-treatment wastewaters. Wat. Sci. Tehc. 20:95-100.

20. Pignatello, J.J., M.M. Martinson, J.G. Steiert, R.E. Carlson, and R.L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. Appl. Environ. Microbiol. 46:1024-1031.

21. Saber, D.L., and R.L. Crawford. 1985. Isolation and characterization of _Flavobacterium_ strains that degraded pentachlorophenol. Appl. Environ. Microbiol. 50:1512-1518.

22. Shannon, M.J.R. and R. Bartha. 1988. Immobilization of leachable toxic soil pollutants by using oxidative enzymes. Appl. Environ. Microbio. 54:1719-1723.

23. Steiert, J.G., and R.L. Crawford. 1985. Microbial degradation of chlorinated phenols. Trends Biotechnol. 3:300-305.

24. Steiert, J.G., and R.L. Crawford. 1986. Catabolism of pentachlorophenol by a _Flavobacterium_ sp. Biochem. Biophys. Res. Comm. 141:825-830.

25. Steiert, J.G., J.J. Pignatello, and R.L. Crawford. 1987. Degradation of chlorinated phenols by a pentachlorphenol-degrading bacterium. Appl. Environ. Microbiol. 53:907-910.

26. Topp, E., R.L. Crawford, and R.S. Hanson. 1990. Influence of readily metabolizable carbon on pentachlorophenol metabolism by a pentachlorophenol-degrading Flavobacterium sp. Appl. Environ. Microbiol. 54:2452-2459.

27. Topp, E., and R.S. Hanson. 1990a. Degradation of pentachlorophenol by a Flavobacterium species grown in continuous culture under various nutrient limitations. Appl. Environ. Microbiol. 56:541-544.

28. Topp, E., and R.S. Hanson. 1990b. Factors influencing the survival and activity of a pentachlorophenol-degrading Flavobacterium in soil slurries. Can. J. Soil Sci. 70:83-91.

29. Valvo, R.J., M.M. Haggblom, and M.S. Salkinoja-Salonen. 1990. Bioremediation of chlorophenol containing simulated ground water by immobilized bacteria. Wat. Res. 24:253-258.

30. Weiss, U.M., I. Scheunnert, W. Klein, and F. Korte. 1982. Fate of pentachlorophenol-^{14}C in soil under controlled conditions. J. Agric. Food Chem. 30:1191-1194.