◇ Over View ◇

# BIODEGRADATION PATHWAYS OF TRICHLOROETHYLENE (TCE) AND METHYL BROMIDE (MeBr)

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## Abstract

트리클로로에틸렌 (trichloroethylene, TCE)는 오랜 시간동안 자연환경에서 잔류할 뿐만 아니라 TCE보다 더욱 더 독성이 강한 중간 생성물들을 만들기 때문에 미국과 대부분의 전세계 국가들로부터 주요 1차 환경오염물질로 분류되었다. 그러한 독성물질들은 혐기성 상태에서는 다이클로로 에틸랜(dichloroethylene, DCE)과 바이닐 클로라이드 (vinyl chloride, VC)와 같은 독성물질들이 생성되고 호기성 상태에서는 TCE epoxide계통의 물질들이 생성된다. 또한 훈증제인 메틸 브로마이드 (methyl bromide)는 대기의 오존층을 파괴하는 것으로 알려져 있고, 2001년경에 미국환경보호청 (USEPA)에 의해 사용이 금지될 것이다. TCE는 혐기성 조건하에서 연속적으로 탈염소화되고, 이어서 호기성 조건하에서 완전 산화될 수 있다. 그리하여 연속적인 혐기성 및 호기성 조건하에서 궁극적으로 TCE의 완전분해를 이루게된다. 메틸브로마이드는 화학적으로 가수분해되어 메틸 알콜 (methyl alcohol)로 되거나 유기물에 강하게 결합 (bound)된다. 또한 그것은 생물학적으로 포름알데하이드 (formaldehyde)로 산화되거나 메틸알콜로 가수분해된다. 수많은 연구자들에 의해 행해진 연구들은 TCE와 MeBr은 메탄 혹은 암모니아 산화 세균에 의한 공동대사과정 (cometabolism)을 통해 분해가 증진될 수 있다는 것을 보여주었다. 두 부류의 세균들이 두 화합물들을 분해시킬 수 있는 monooxygenase를 생산한다는 것은 잘 알려져 있다. 이 연구 논문에서 TCE와 MeBr의 생분해와 관련된 가장 최근의 연구논문들로부터 나온 핵심 연구결과들이 요약 검토된다. TCE와 MeBr로 오염된 현장을 정화하기 위해 이러한 기초연구결과들을 토대로 더욱 더 많은 연구가 필요 할 것으로 사료된다.

## INTRODUCTION

Halogenated hydrocarbons are an important class of chemicals used for many industrial applications.[1] They are used as pesticides, plastics, solvents, and degreasers. Despite their industrially beneficial uses, some have caused serious environmental damage including contamination of air, soil, and groundwater, killing of or injuries to nontarget organisms, depletion of the stratospheric ozone layer, and long-range transport. Many are suspected carcinogens to humans, and persistent in soils and waters.[2] Consequently, the public is concerned about possible adverse effects on the quality of life due to these compounds.

Most halogenated compounds fall into two main groups. The first group of these chemicals

includes short-chain halogenated aliphatic hydrocarbons such as trichloroethylene (TCE), ethylene dibromide(EDB), methyl bromide(MeBr), chloroform, and 1,3-dichloropropene(1,3-D). The second group includes halogenated aromatic compounds such as DDT, polychlorinated biphenyls(PCBs), chlorophenols, chlorobenzenes, chlorobenzoates, chlorotoluenes, and chlorophenoxyacetates.

## MECHANISMS OF DEHALOGENATION

The carbon-halogen bond is strong or recalcitrant to breakdown, due to the increased electronegativity of the substituents, and halogenated compounds with one or at most a few substituents are thought to be more readily degradable than the corresponding polyhalogenated compounds.[3] Of the possible carbon-halogen bonds, the C-F bond is the strongest and results in more recalcitrance, comparable to a carbon-hydrogen bond, and high dissociation energy. On the other hand, the C-I bond is the weakest carbon-halogen bond and is much less recalcitrant. Carbon-halogen bonds can be broken either by biological (enzymatic) or chemical dehalogenation to form unstable intermediates, with biological mechanisms generally being faster than chemical mechanisms under the same condition. Many dehalogenating enzymes are involved in catalyzing the removal of halogen elements.

Fetzner and Lingens[2] suggested that seven mechanisms can be involved in biological dehalogenation:

1. Reductive dehalogenation. Reductive dehalogenase can catalyze replacement of the halogen substituent on aliphatic or aromatic ring carbons by hydrogen atoms.

2. Oxygenolytic dehalogenation. Monooxygenase or dioxygenase can be involved in incorporating one or two atoms of molecular oxygen into the substrate, concurrently removing halogen atoms.

3. Hydrolytic dehalogenation. Hydrolytic dehalogenase can catalyze the replacement of halogen atoms by the hydroxyl(OH) groups of water.

4. Thiolytic dehalogenation. A dehalogenating glutathione S-transferase, which is produced by dichloromethane-utilizing bacteria, can catalyze the formation of a S-chloromethyl glutathione conjugate, with concomitant release of chlorine atoms.

5. Intramolecular substitution. Intramolecular nucleophilic displacement accelerates the formation of epoxides, which are involved in the dehalogenation of vicinal haloalcohols.

6. Dehydrohalogenation. The concurrent elimination of HCl from halogen compounds can result in the formation of a double bond.

7. Hydration. Hydratase enzyme can catalyze the step of adding a water molecule to form unstable intermediates during the breakdown of halogenated compounds.

Because of the widespread use of halogenated hydrocarbons in many industries and their persistence in conjunction with inadequate past techniques for handling, storage, and disposal of these chemicals, halogenated hydrocarbons have become ubiquitous environmental pollutants. In fact, these compounds are the most frequent groundwater contaminants in the United States.[4] These chemicals are toxic to humans, and many of them are suspected carcinogens. Consequently, the public is concerned over the contamination of these chemicals in drinking water.[5, 6]

Trichloroethylene(TCE) and methyl bromide (MeBr) each have structural similarity(i.e., short-chain halogenated aliphatic hydrocarbons) and are each degraded by cometabolism.

# TRICHLOROETHYLENE

Trichloroethylene belongs to a family of synthetic chlorinated aliphatic hydrocarbons manufactured as industrial solvents having a greatly reduced potential for fire or explosion.[7] It is widely used for degreasing and cleaning metals and electronic components. Because of frequent detection in drinking water aquifers,[8] TCE has been classified as a priority pollutant in the U.S.A. In addition, TCE is the most frequently reported contaminant at hazardous waste sites on the National Priority List of the U.S. Environmental Protection Agency(USEPA).[9] Major metabolites of TCE(TCE-epoxide, dichloroethylene, and vinyl chloride) and perchloroethylene(PCE) also cause serious groundwater contamination problems.[10] These chemicals are suspected carcinogens and generally tend to resist biodegradation in the environment.[8, 11]

Microorganisms capable of utilizing TCE as a sole source of carbon and energy for growth have not been reported. However, TCE can be degraded by bacterial cometabolism under anaerobic(Fig. 1) and aerobic(Fig. 2) conditions. Under anaerobic conditions, TCE can be transformed as a non-growth-supporting elec-

tron acceptor. As a result, TCE can be sequentially dechlorinated, producing less-chlorinated metabolites.[12] However, under anaerobic conditions, dechlorination is very slow, leading to accumulation of a more toxic metabolite, vinyl chloride. Under aerobic conditions, TCE can be biologically oxidized by broad specific monooxygenases and dioxygenases, which are normally produced to initiate the oxidation of growth-supporting substrates such as methane, ammonia, phenol and toluene.[7, 13] Although all oxygenases have the capacity to oxidize their primary substrates, only a handful of oxygenases have the capacity to oxidize TCE or other short-chain, halogenated, aliphatic hydrocarbons.

Methanotrophic bacteria are aerobic microorganisms and are ubiquitously present in oxic soil and aquatic environments. These bacteria possess unique methane monooxygenase (MMO) enzyme system which enables them to utilize methane as a sole carbon and energy source.[14] There are two types of MMO, soluble methane monooxygenases(sMMO) and particulated(membrane-associated) MMO(pMMO), produced by some methanotrophs that have the capacity to degrade TCE, with sMMO being the major
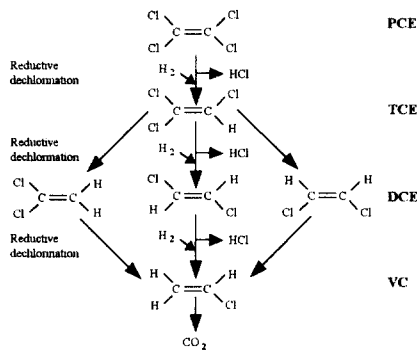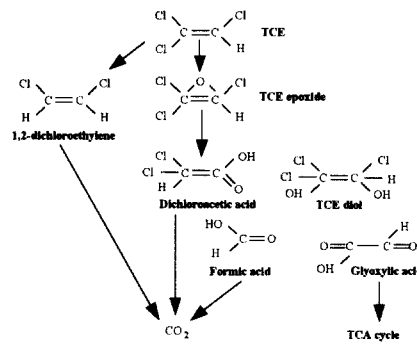


Fig. 1. Proposed anaerobic degradation pathways of TCE.[12]



Fig. 2. Proposed aerobic degradation pathways of TCE.[13]

factor.[15] Additionally, some sMMO can degrade a wide range of carbon substrates[16] which are economically and environmentally important organic compounds.[17]

Wilson and Wilson[18] were the first to demonstrate that TCE could be mineralized to $CO_2$ and $H_2O$ in methanotrophic soil. Fogel et al.[19] reported that TCE was also mineralized to $CO_2$, $H_2O$, and $Cl^-$ by methane-oxidizing bacteria in a liquid medium. Arciero et al.[20] reported that the ammonia-oxidizing bacterium Nitrosomonas europaea cometabolically degraded TCE in the presence of ammonia. Ammoniaoxidizing bacteria oxidize ammonia to nitrite for growth. They produce ammonia monooxygenases (AMO), membrane-bound enzymes, that catalyze the oxidation of ammonia to hydroxylamine.[21]

Ammonia monooxygenases are also capable of cooxidizing a variety of organic compounds including methane,[21] methanol,[22] ethylene,[23] propylene,[24] benzene and phenol,[25] cyclohexane,[24] bromocarbons,[23] and carbon monoxide.[26]

## METHYL BROMIDE

Methyl bromide(MeBr) is presently the most important fumigant commercially available.[27] At 4 ℃, MeBr is a gas.[28] This chemical is extensively used for the control of nematodes, soil-borne fungi, and weeds.[29] This chemical is also used as a space fumigant for commodities, for structural pest control, and for quarantine and regulatory purposes.[28]

Despite its beneficial uses in agriculture, use of MeBr will be suspended in the year 2001.[27] It is a potent depleter of the stratospheric ozone layer,[30] due to its release of bromine atoms into the atmosphere. Bromide ion is as much as 100-fold more efficient at scavenging ozone than Cl ion.[31] It is currently believed that anthropogenic MeBr sources, such as from agricultural
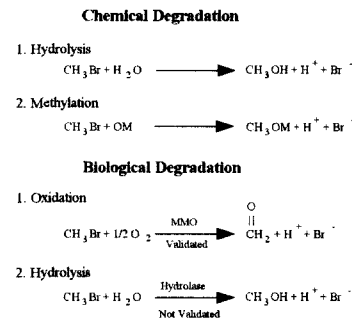


Fig. 3. Proposed degradation pathways of MeBr in soils.[28]

soil fumigation, account for about one-third of the annual flux of $200 \times 10^6$ kg, with the rest derived from natural sources such as the oceans and the burning of forests.[32, 33]

In water, MeBr is chemically hydrolyzed to methanol, and may exchange with other halides.[34] In soil, MeBr undergoes both chemical and biological degradation, which include chemical hydrolysis,[35] methylation to soil organic matter[36] and microbial oxidation.[37, 28]

Similar to TCE, MMOs are involved in the oxidation of MeBr to formaldehyde and a bromide ion. Some ammonia-oxidizing bacteria and methane-oxidizing bacteria in the presence of their primary substrates, ammonia and methane, have been shown to have the capacity to oxidize MeBr.[37, 39] Ou et al.[28] reported that the degradation of MeBr in soil can be stimulated by increase in the activity of soil nitrifiers through the application of ammonium fertilizers. In this case, the major degradation pathway of MeBr is the oxidation of MeBr to formaldehyde(Fig. 3).

## CONCLUSIONS

1. TCE can be sequentially less dechlorinated to DCE and VC under anaerobic condition,

resulting in more toxic metabolites than the parent chemical.

2. TCE can be oxidized to TCE epoxide and further degraded to inorganic compounds.

3. Sequential anaerobic and aerobic conditions make the persistent TCE completely degraded into innocuous inorganic compounds.

4. MeBr can be chemically hydrolyzed to methanol or methylated to organic matter.

5. MeBr can be biologically hydrolyzed to methanol or oxidized to formaldehyde.

6. TCE and MeBr have a common short-chain halogenated hydrocarbon compounds.

7. Microorganisms capable of utilizing TCE and MeBr as a sole source of carbon and energy for growth have not been reported.

8. Degradation of TCE and MeBr can be enhanced by ammonia oxidizing bacteria and methane oxidizing bacteria through a cometabolic process.

## REFERENCES

1. Chaudhry, G.R., and Chapalamadugu, S. : Biodegradation of halogenated organic compounds. Microbiol. Rev. 55, 59-79, 1991.

2. Fetzner, S., and Lingens, F. Bacterial dehalogenase : Biochemistry, genetics, and biotechnological applications. Microbiol. Rev. 58, 641-685, 1994.

3. Reineke, W. : Microbial degradation of halogenated aromatic compounds. pp. 319-360. In Microbial Degradation of Organic Compounds(ed. D.T. Gibson). Marcel Dekker, Inc., New York, NY. 1984.

4. Baker, K.H., and Herson, D.S. : Bioremediation. McGraw-Hill, Inc., New York, NY. 1994.

5. Geiger, W., and Molner-Kubica, E. : Tetra-chloroethylene in ground and drinking waters. Bull. Environ. Contam. Toxicol. 19,

475-580, 1977.

6. Petura, J.C. : Trichloroethylene and methyl-chloroform in groundwater: A problem assessment. J. Am. Water Works Assoc. 73, 200-205, 1981

7. Ensley, B.D. : Biochemical diversity of trichloroethylene metabolism. Ann. Rev. Microbiol. 45, 283-299, 1991.

8. Love, O.T. Jr., and Eilers, R.G. : Treatment of drinking water containing trichloroethylene and related industrial solvents. J. Am. Water Works Assoc. 74, 413-425, 1982.

9. U.S. Environmental Protection Agency. : Substances found at proposed and final NPL sites through update number three. Document NPL-U3-6-3. U.S. Environmental Protection Agency, Washington, D.C. 1985.

10. Westrick, J.J., Mello, J.W. and Thomas, R. F. : The groundwater supply survey. J. Am. Water Works Assoc. 76, 52-59, 1984.

11. Infante, P.F., and Tsongas, T.A. : Mutagenic and oncogenic effects of chloromethanes, chloroethanes, and halogenated analogs of vinyl chloride. Environ. Sci. Res. 25, 301-327, 1982.

12. Vogel, T.M., and McCarty, P.L. : Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49, 1080-1083, 1985.

13. Little, C.D., Palumbo, A.V., Herbes, S.E., Lidstrom, M.E. Tyndall, R.L., and Gilmer, P.J. : Trichloroethylene biodegradation by a methaneoxidizing acterium. Appl. Environ. Microbiol. 54, 951-956, 1988.

14. Dalton, H., Prior, S.D., Leak, D.J., and Stanley, S.H. : Regulation and control of methane monooxygenase. pp.75-82. In Microbial Growth on C1 Compounds(eds. R.L. Crawford and R.S. Hanson). Proceedings

of the 4th International Symposium. American Society for Microbiology, Washington, D.C. 1984.

15. DiSpirito, A.A., Gulledge, J., Shiemke, A.K., Murrell, J.C., Lidstrom, M.E. and Krema, C.L. : Trichloroethylene oxidation by the membraneassociated methane monooxygenase in type I, type II and type X methanotrophs. Biodegradation 2, 151-164, 1992.

16. Dalton, H. Methane oxidation by methanotrophs : Physiological and mechanistic implications. pp. 85-113. In Methane and Methanol Utilizers(eds. J.C. Murrell and H. Dalton). 1992.

17. Higgins, I.J., Best, D.J. and Hammond, R.C. : New findings in methane-utilizig bacteria highlight their importance in the biosphere and their commercial potential. Nature 286, 561-564, 1980.

18. Wilson, J.T. and Wilson. B.H. : Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49, 242-243, 1985.

19. Fogel, M.M., Taddeo, A.R. and Fogel. S. : Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51, 720-724, 1986.

20. Arciero, D., Vannelli, T., Logan, M. and Hooper, A.B. : Degradation of trichloroethylene by the ammonia-oxidizing bacterium Nitrosomonas europaea. Biochem. Biophys. Res. Commun. 159, 640-643, 1989.

21. Hyman, M.R. and Wood, P.M. : Methane oxidation by Nitrosomonas europaea. Biochem. J. 212, 31-37, 1983.

22. Vosey, P.A. and Wood, P.M. : Methanol and formaldehyde by an autotrophic nitrifying bacterium. J. Gen. Microbiol. 33, 283-290, 1987.

23. Hyman, M.R. and Wood, P.M. : Ethylene oxidation by Nitrosomonas europaea. Arch. Microbiol. 137, 155-158, 1984.

24. Drozd, J.W. : Respiration in the ammonia oxidizing chemotrophic bactia. Vol. 2. pp. 87-111. In Diversity of Bacterial Systems(ed. C.J. Knowles). CRC Press, Inc., Boca Raton, FL. 1980.

25. Hyman, M.R., Sansome-Smith, A.W., Shears, J.H. and Wood, P.M. : A kinetic study of benzene oxidation to phenol by whole cells of Nitrosomonas europaea and evidence for further oxidation of phenol to hydroxyquinone. Arch. Microbiol. 143, 302-306, 1985.

26. Jones, R.D. and Morita, R.Y. : Carbon monoxide oxidation by chemolithotrophic ammonium oxidizers. Can. J. Microbiol. 29, 1545-1551, 1983.

27. Noling, J.W. and Becker, J.O. : The challenge of research and extension to define and implement alternatives to methyl bromide. Suppl. J. Nematol. 26, 573-586, 1984.

28. Ou, L.T., Joy, P.J. Thomas, J.E., and Hornsby, A.G. : Stimulation of microbial degradation of methyl bromide in soil during oxidation of an ammonia fertilizer by nitrifiers. Environ. Sci. Tech. 31, 717-722, 1997.

29. Ferguson, W. and Padula, A. Economic effects of banning methyl bromide for soil fumigation. USDA Economic Research Service, Agricultural Economic Report No. 677. USDA, Beltville, MD. 1994.

30. Watson, R.T., Albritton, D.L., Anderson, S.O. and Lee-Bapty, S. : Methyl bromide : Its atmospheric science, technology, and economics. United Nations Environmental Program, United Nation Headquarters, Nairobi, Kenya. 1992.

31. Prather, M.J. and Watson, R.T. : Stratospheric ozone depletion and future levels of atmospheric chlorine and bromine. Nature(London) 344, 729-734, 1990.

32. Butler, J.H. : The potential role of the ocean

in regulating atmospheric CH₃Br. Geophys. Res. Lett. 21, 185-188, 1994.

33. Khalil, M.A.K., Rasmussen, R.A. and Gunawardena, R. : Atmospheric methyl bromide: Trends and global masss balance. J. Geophys. Res. 98, 2887-2896, 1993.

34. Elliott, S. and Rowland, F.S. : Nucleophilic substitution rates and solubilities for methyl halides in seawater. Res. Lett. 20, 1043-1046, 1993.

35. Gentile, I.A., Ferraris, L., and Crespi, S. The degradation of methyl bromide in some natural fresh waters. Influence of temperature, pH, and light. Pestic. Sci. 25, 261-272, 1989.

36. Gan, J., Yates, S.R. Spencer, W.F., Ernst, F.F. and Yates, M.V. : Effect of soil properties on degradation and sorption of methyl bromide in soil. Chemosphere 29, 2685-2700, 1994.

37. Oremland, R.S., Miller, L.G., Culbertson C.W., Connell, T.L. and Jahnke, L. : Degradation of methyl bromide by methanotrophic bacteria in cell suspension and soils. Appl. Environ. Microbiol. 60, 3640-3646, 1994.

38. Meyers, A.J. : Evaluation of bromoethane as a suitable analogue in methane idation studies. FEMS Microbiol. Lett. 9, 297-300, 1980.

39. Rasche, M.E., Hyman, M.R. and Arp, D.J. : Biodegradation of halogenated hydrocarbon fumigants by nitrifying bacteria. Appl. Environ. Microbiol. 56, 2568-2571, 1990.