Microbial monooxygenases

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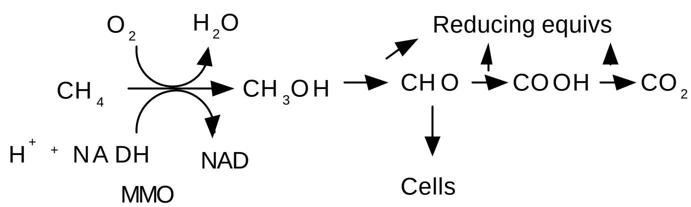
Dechlorination of chlorinated aliphatic hydrocarbons by methane-oxidizers, methanotrophs

Methanotrophs are obligate methane oxidizers, obligate aerobes, important part of carbon cycle, completing oxidation of methane to CO_2.

Widely distributed, particularly at the interface between aerobic and anaerobic environments from which methane diffuses to the atmosphere.

Methane monooxygenase first step in methane oxidation to CO_2



Requires oxygen and energy (NADH). NADH is produced by the oxidation of methanol, formaldehyde and formate.

Methane monooxygenase comes in two varieties: soluble and membrane bound. The soluble MMO (sMMO) is very nonspecific and is present primarily in Type II methanotrophs, where it is induced by low copper conditions enzyme (<50 µg/L). The membrane bound or particulate MMO (pMMO) is constitutive, but limited by the availability of copper. It is more specific for methane and oxidizes fewer xenobiotics

Methanotrophs are classified by the pathway used to assimilate carbon from formaldehyde, their internal membrane structure, G+C content of DNA, and metabolism:

Type	Representative species	C assimilative pathway	G+C%	Metabolism	Internal membranes	Type of MMO
I	*Methylomonas albus* BG8	hexulose monophosphate pathway	50-54	incomplete TCA, unable to fix N_2	bundles or stacks	Mostly pMMO
II	*Methylosinus trichosporium* OB3b	serine pathway	58-66	complete TCA, able to fix N_2	paired peripheral membranes	Both pMMO & inducible sMMO

The soluble sMMO attacks trichloroethylene; 1,1,1-trichloroethane; mono & dichloroethane, methane and ethene; chloroform; others:

Environmentally Significant Compounds

Oxidized by Methanotrophs

Compound	Product (pure culture)
Chloromethane	Formaldehyde
Dichloromethane	Carbon monoxide
Trichloromethane(Chloroform)	Carbon dioxide
Bromomethane	Formaldehyde
Benzene	Phenol & Hydroquinone
Toluene	*p* -Cresol, Benzyl alcohol, Benzoate
Styrene	Styrene oxide & Hydroxystyrene
m-Cresol	Hydroxybenzaldehydes
o-Cresol	5-Methyl-1,3-benzenediol
m-Chlorotoluene	Benzyl alcohols
Naphthalene	Naphthols
1- and 2- Methylnaphthalene	ND
Vinyl chloride	ND
1,2 & 1,1-dichloroethylene	ND
Trichloroethylene	Dichloroacetic acid & TCE Diol
1,1- & 1,2-dichloroethane	ND
1,1,1- & 1,1,2-trichloroethane	ND

The oxidation of these compounds provides no benefit for the methanotrophs and drains the organism of energy (NADH); thus it is **cometabolism**

There are 3 components to the sMMO: the hydroxylase, the reductase, and a component B. The reactions involving methane occur at the hydroxylase. The active site has a unique hydoxo-bridged dinuclear iron center. The diiron is first reduced to ferrous, which then reacts with O_2 to yield water and a di-Fe[IV]=O species, which pulls an H from CH_4 to form a CH_3* radical and diiron cluster hydroxyl radical. Recombination forms methanol. Comp B and the reductase feed NADH electron transfer and regulate the reaction by forming complexes that alter MMO activities. (Lipscomb, J. D. (1994). "Biochemistry of the soluble methane monooxygenase." Annual Review of Microbiology **48**(1994): 371-399)

Four types of inhibitory effects on the methanotrophs:

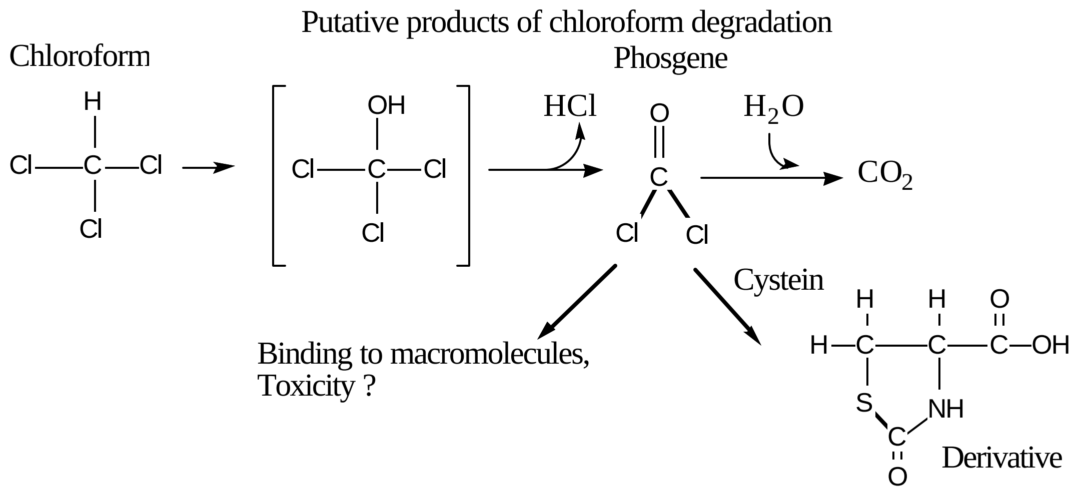
1. Depletion of energy (NADH)
2. Competition with methane for the MMO site, competitive inhibition with the following kinetics:

$$\frac{dS_t}{dt} = r_{tm} \frac{S_t / K_t}{1 + S_s / K_s + S_t / K_t}$$

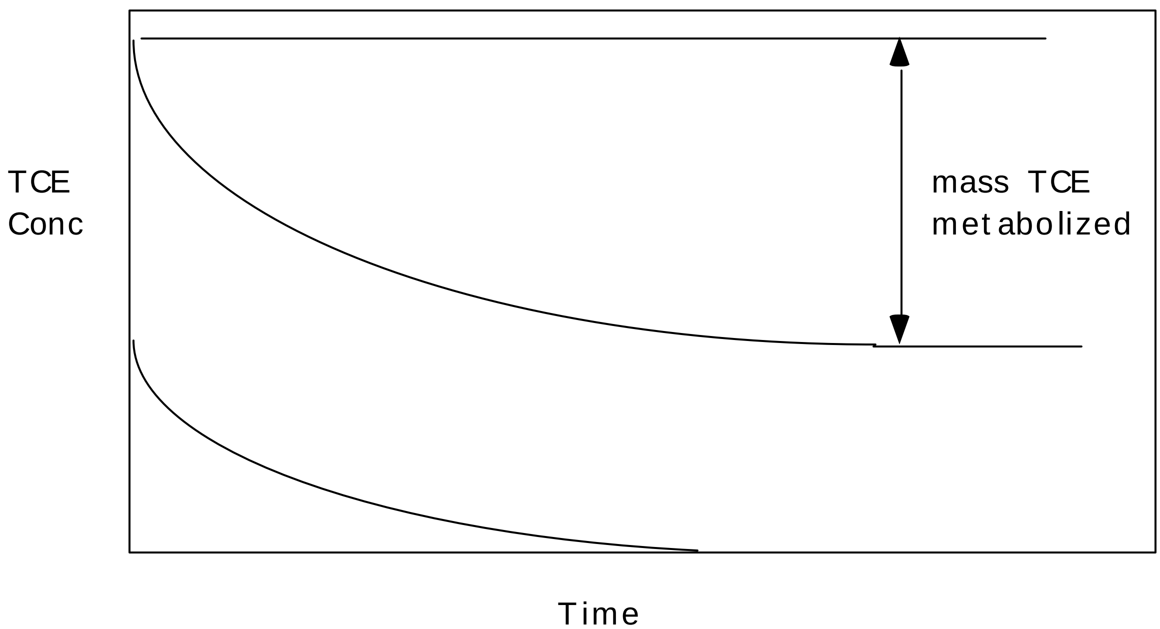
S_t is the concentration of the toxic compound, K_t is the affinity constant for the toxic compound, S_s is the concentration of the growth substrate, methane, K_s is the affinity constant for methane. This expression degenerates to the Michaelis-Menten rate expression if S_s is zero

3. Toxic effects of the chlorinated solvents on membranes and metabolism
4. Toxic effects of the metabolites, Particularly in the case of TCE and Chloroform. These metabolites are the same as those that cause damage in the mammalian transformations by cytochrome P450

Toxic metabolites:



Metabolite toxicity effects detectable by course of CF transformation.



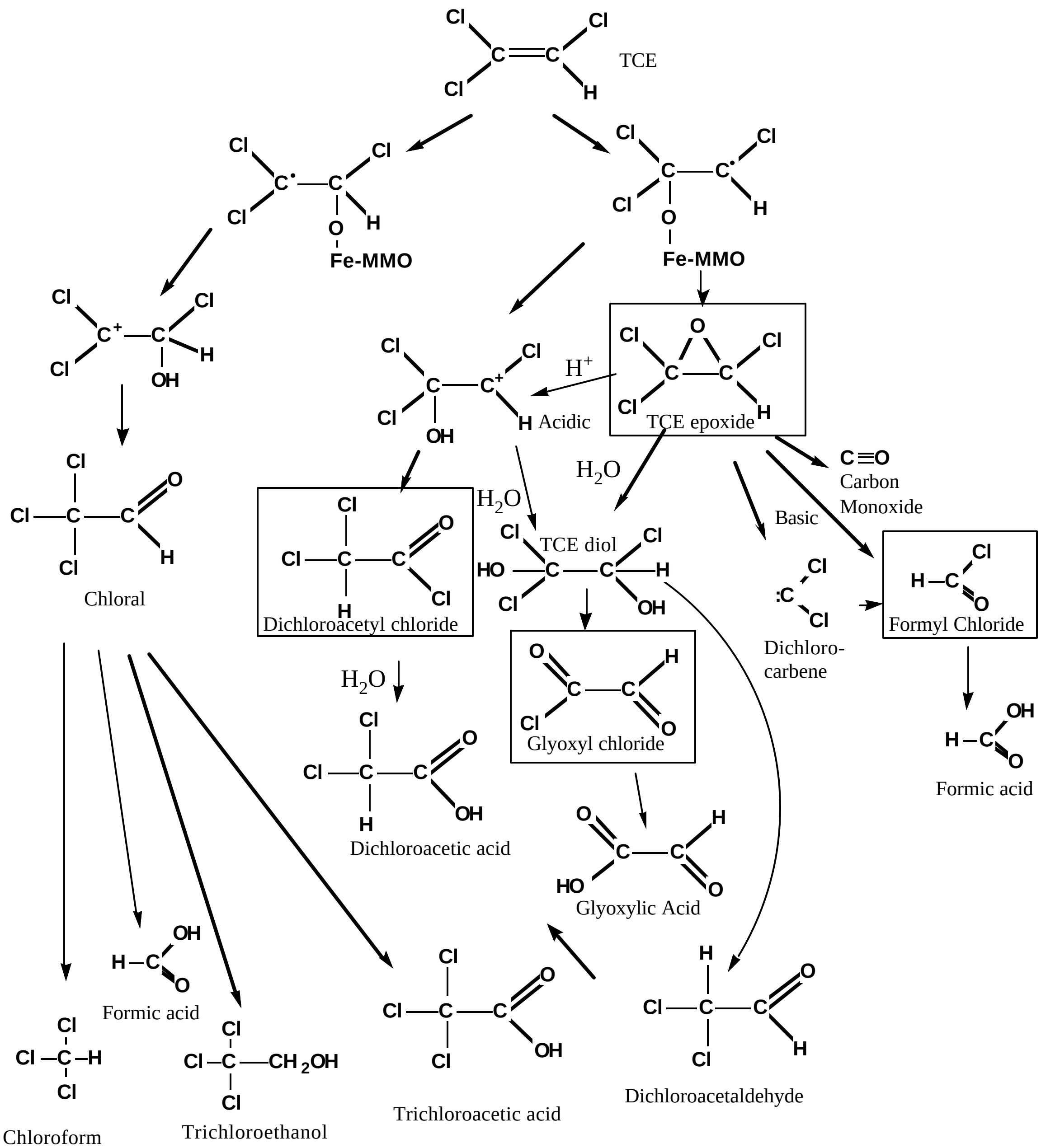
At the end of the time for the higher TCE concentration, the cells will be inactivated, unable to oxidize either TCE or their substrate.

Quantify toxicity effect using transformation capacity:

T_C = (mass TCE metabolized)/(biomass inactivated)

Putative products of TCE degradation

The products most likely to covalently bind enzymes resulting in cell death are shown in boxes.



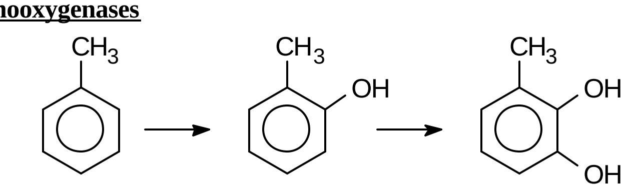
Propane and ethylene oxidizers

Note that propane and ethylene oxidizers also have monooxygenases with broad nonspecificity. Propane oxidizers especially may be useful for oxidation of 1,1,1-trichloroethane and chloroform in addition to TCE (Keenan, J. E., S. E. Strand and H. D. Stensel (1993). Degradation kinetics of Chlorinated Solvents by a Propane-Oxidizing Enrichment Culture. Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds. R. E. Hinchee, A. Leeson, L. Semprini and S. K. Ong. Boca Rton, Lewis Publishers**:** 1-13).

The ammonia monooxygenase enzyme of nitrification

The monooxygenase of nitrifying organisms is similar to that of the methanotrophs. The AMO oxidizes methane and most of the compounds oxidized by the methanotrophs, but at lower rates. TCE and Chloroform produce toxic products. Ammonium is more soluble than methane, but nitrate is a pollutant. Since MMO and AMO are inhibited by the same inhibitors (esp acetylene), their environmental activities may be hard to distinguish.

Toluene monooxygenases

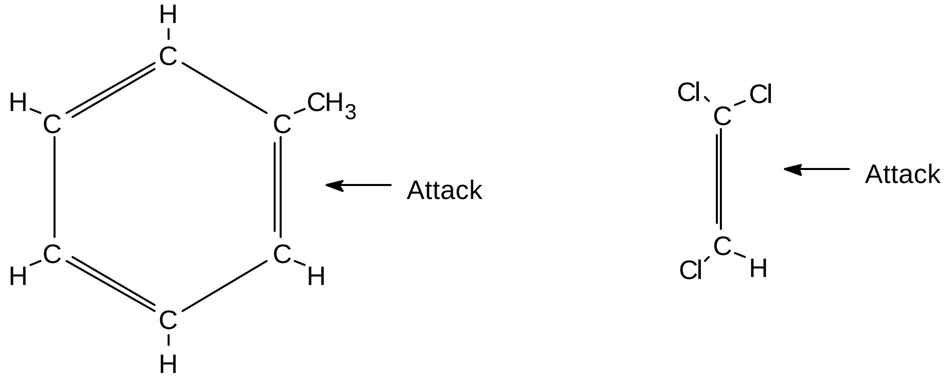


Toluene 2-monooxygenase of *Burkholderia* (formerly *Pseudomonas*) *cepacia* G4. High rates of TCE degradation, with little intermediate toxicity (Newman, L. M. and L. P. Wackett (1995). "Purification and Characterization Of Toluene 2-Monooxygenase From Burkholderia Cepacia G4." Biochemistry **34**(43): 14066-14076.)

Toluene 2,3-dioxygenasse of *Pseudomonas putida* F1 acts as a monooxygenase with TCE. High rate, high intermediate toxicity. (Jahng, D. J. and T. K. Wood (1994). "Trichloroethylene and chloroform degradation by a recombinant pseudomonad expressing soluble methane monooxygenase from Methylosinus trichosporium OB3b." Applied and Environmental Microbiology **60**(7): 2473-2482; Spain, J. C., G. J. Zylstra, C. K. Blake and D. T. Gibson (1989). "Monohydroxylation of phenol and 2,5-dichlorophenol by toluene dioxygenase in Pseudomonas putida F1." Appl. Environ. Microbiol. **55**: 2648-2652; Wackett, L. P., L. D. Kwart and D. T. Gibson (1988). "Benzylic monooxygenation by toluene dioxygenase from Pseudomonas putida ." Biochemistry **27**: 1360-1367)

Also **toluene-4-monooxygenase** of *Pseudomonas mendocina* KR1, a poor TCE degrader (Yen, K. M. and M. R. Karl (1992). "Identification of a new gene, tmoF, in the *Pseudomonas* *mendocina* KR1 gene cluster encoding toluene-4-monooxygenase." J. Bacteriol **174**(22): 7253-7261.)

Aromatic oxygenases, Oxidize chlorinated ethylenes, but not chloroform or 111-TCA



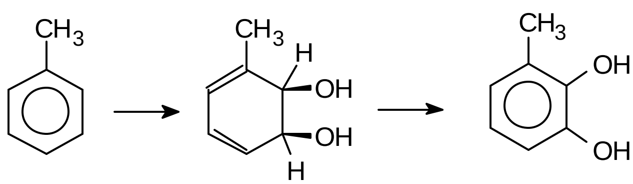
Toluene

Trichloroethylene

Compare

Toluene dioxygenase

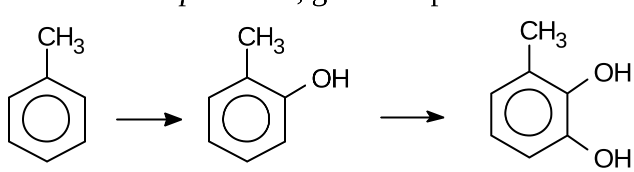
Pseudomonas putida F1, grows on toluene



toluene dioxygenase

Toluene *ortho* monooxygenase

Pseudomonas cepacia G4, grows on phenol or toluene



toluene ortho monooxygenase

Fungal P450s

Fungal and bacterial cytochrome P450s are the enzymes used in the initial oxidation of alkanes and aromatics of petroleum. Some yeast (esp. *Saccharomyces cerevisiae*) use P450s in sterol synthesis, but they also have nonspecific cometabolic effects.

The P450 also can reduce fully halogenated compounds under anaerobic conditions: carbon tetrachloride -> chloroform, but only in mammalian and plant metabolism

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