

## **Microbial Degradation of Methanotrophic Bacteria in Soils and Sediments**

**Prof Gourhari Santra<sup>1</sup>, Ms. Suchismita Mohapatra<sup>2</sup>**

<sup>1,2</sup>Department of Agriculture, Siksha 'O' Anusandhan (Deemed to be University),  
Bhubaneswar, Odisha  
Email - <sup>1</sup>[gourhaisantra@soa.ac.in](mailto:gourhaisantra@soa.ac.in)

### **Abstract**

The study of microorganisms has been done in order to remove the trace levels of methanotrophic bacteria particularly, the hydrochlorofluorocarbons, including HCFC-21 and HCFC-123. Methanotrophs are the unique and ubiquitous bacteria which use methane as the main sources of carbon and energy produced from the atmosphere. In addition, due to the presence of large-spectrum methane mono-oxygenase enzymes, methane may also be used for the bioremediation of various types of heavy metals and organic pollutants. These are the highly specialized group of aerobic bacteria that have a particular oxidation potential for certain organic contaminants, such as alkanes, aromatic compounds, halogenated alkenes, etc. Aerobic soil was found with the methanotroph-linked oxidation of HCFC-21, and in the freshwater and salt marsh sediments with anaerobic degradation of HCFC-21. The studies indicated that anoxic freshwater and salt marsh sediments consists of the microbial degradation of HCFC-123, and the recovery of 1,1,1-trifluoro-2-chloroethane shows the presence of the reductive dechlorination. Methane mono-oxygenases enzyme that can be specifically demonstrated in the absence of copper is the initiation of the oxidation reactions. The oxidative degradation of HCFC-123 has not been observed in the aerobic soils. However, the HCFCs were degraded at low concentrations (parts per billion), thereby raising the risk of the elimination of HCFCs from the environment by bacteria in the ecosystem.

**Key words:** 1,1,1-trifluoro-2-chloroethane, Bioremediation, HCFCs, Hydrofluorocarbons, Methanotrophic bacteria, Mono-oxygenase enzymes.

### **Introduction**

The global warming is rapidly increasing worldwide and is becoming a major growing concern. Methane (CH<sub>4</sub>) is one of the most harmful greenhouse gases, which contributes to the global warming. The methane gas is 23 times more effective than carbon dioxide (CO<sub>2</sub>) as a greenhouse gas [1]. Methanotrophs are the only known biological source for atmospheric CH<sub>4</sub>, which plays a significant role in the reduction of the total global CH<sub>4</sub> content destruction up to 15%. The physiologically versatilized environment and the variation of pH, temperature, oxygen levels, salinity, heavy metal levels and radiation are present in a number of habitats [2]. Methane mono-oxygenase (MMO) is present in methanotrophs with the broad spectrum methanoxidizing enzyme in two forms, namely membrane-associated or particulate form (pMMO) and soluble or cytoplasmic form (sMMO). The pMMO is present in the genus *Methylocella* (Acidophilic), but in all recognized methanotrophs whereas, sMMO is present in a very few methanotrophic strains.

Global warming issues have centered on the reduction of the use of chlorofluorocarbons (CFCs) and on the safety of stratospheric ozone [3]. The phasing of hydrochlorofluorocarbons (HCFCs) as temporary alternatives has progressed as the research is being conducted into the long-term substitutes such as hydrofluorocarbons. The ambient residential period for CFCs is 60 to 100 years, whereas the time for residence is 15 years for HCFC and hydrofluorocarbons. Halocarbon residence time is one of the several critical factors when an ozone depletion or global warming potential value is allocated. The residence time of hydroxyl radicals are determined from the kinetic oxidation cycle and usually ignore the biosphere as an extra term of removal (i.e. a global sink) for tropospheric halocarbons.

Many sources have been identified that produce huge amounts of toxic heavy metals / metalloids such as Cr, Cd, Pb, As, Cu, Zn, Ni, Hg etc. and the organic pollutants into the environment. Heavy-metallic or synthetic pollutant-contaminated soils have usually been abandoned for several years and may not be suitable for farm production. Recently, the toxicity of polluted soil metal / organic contaminants has been decreased by microbial bioremediation techniques. Methanotrophs are considered as the significant area for environmental bioremediation [4]. Specific heavy metals and organic contaminants were degraded / co-oxidized with the presence of broad-spectrum MMO by methanotrophs. A wide range of substrates including aromatic compounds such as Benzene, toluene, styrene and aliphatic benzene and hydrocarbons of up to 8 carbons have been shown to oxidize a wide variety of substrates.

On the other hand, HCFCs are found to be more reactive and bacterial attack-sensitive. Some methanotrophic HCFCs were metabolized and the stoichiometric recovery of fluoride included full oxidation. Nonetheless, no such work was done to identify the risk of crop findings being extrapolated into the natural systems of soils or sediments. However, the studies were carried out at very high concentrations of ~0.1000 ppm with methanotrophic cultures, while tropospheric HCFC-21 has the ratios of approximately 0.1 ppb [5]. According to a study, it has been demonstrated that the degradation of HCFCs is achieved at low concentrations and, if not, at troposphere mixing ratios by soil microbes is not known. By this research, it has been examined that the metabolization capacities of soils and sediments in HCFC-21 ( $\text{CHCl}_2\text{F}$ ) as well as HCFC-123 ( $\text{CHCl}_2\text{CF}_3$ ) at concentrations as small as several parts per billion.

According to an experimental study conducted, it has been demonstrated that the aerobic agricultural soils have the ability to oxidize both  $\text{CH}_4$  and HCFC-21, while the controls did not. Incubated soils without HCFC-21 had a  $\text{CH}_4$  oxidation at least five times higher than that of the oxidized soils in incubated HCFC-21, while HCFC-21 had no  $\text{CH}_4$  degradation without soil. Therefore, HCFC-21 oxidation was caused by the methanotrophs and HCFC-21 was co-oxidized obligately by  $\text{CH}_4$ . The low rate oxidation of  $\text{CH}_4$  was caused by the partial inhibition of methane oxidation of HCFC21 in the presence of HCFC-21 which also occurs in the *Methylococcus capsulate*. The experiments on the forest soils were carried out with much

good quantities of soil as compared to the agricultural soils therefore; only about half of the HCFC-21 was added initially to the gas phase while the remaining soils were likely adsorbed to the soil matrix. Nevertheless, in live aerobic samples, HCFC-21 was easily oxidized whereas no control loss was observed. This demonstrates that HCFC-21 bacterial oxidation can occur rapidly and with levels approaching troposphere mixtures in the soils.

It was previously reported that Trichloroethylene (haloalkenes) is generally immune to microorganism biodegradation by volatile chlorinated organic contaminants, while methanotrophs have been shown to co-metabolize trichloroethylene through strong MMO enzymes. According to a scientist, the utilization of the persistent organic pollutants such as lindane, is highly carcinogenic, persistent, bio-accumulative and endocrine disruptor. In aerobic conditions, lindane [c-hexachlorocyclohexane ( $C_6H_6Cl_6$ )] degradation by bacteria occurs by repeated dehydrochlorination and dechlorination steps, and becomes chlorobenzenes, and carbon dioxide, which can be absorbed by the plants as the end product. Novel, low cost and quick screening tool was found to detect the lindane-degrading microorganisms. The same method for analyzing the lindane-degrading methanotrophs can be used. The degradation of lindane with the powerful methanotrophic MMO enzyme is therefore urgently required. Likewise, the “methanotrophs-mediated environmental fate, degradation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are needed to be examined. A non-specific MMO enzyme of marine methanotrophs has been studied for the biodegradation of potentially toxic PAHs. Aerobic catabolism of a PAH bacterial molecule occurs by an enzymatic network of several components by oxidation of PAHs to a dihydrodiol. The PAH bioremediation mechanism includes the pathway of cleavage or meta cleavage resulting in the formation of protocatechuates and catechols which are then converted into the intermediate cyclic tricarboxylic acid. A number of ortho-halogenate biphenyls (2-chloro-2, 2-bromo- and 2-iodiophenyls) were shown to oxidize in the biodegradation of PCBs at lower rates than the unsubstituted Biphenyls, which are the methanotrophic bacterium, *Methylosinus trichosporium* OB3b expressing the soluble methane mono-oxygenases. The dominant reaction during the degradation of aromatic ring and the subsequent dehalogenation is hydroxylation. This type of study offers a platform for the removal of high-chlorine biphenyls by methanotrophs in conjunction with anaerobic degraders. Further research is still required to determine how ortho-substituted biphenyls are further oxidized by heterotrophic micro-organisms in order to ensure full mineralization. Some reports point to the polar region's interest because of the presence of persistent organic pollutants (POPs), which transport from warmer regions to the cooler ones including Antarctica through a complex mechanism involving successive volatilization and deposition [6]. According to researcher, the methane oxidation of the methanotrophic bacteria in Antarctica has been reported. There are various reports which have been illustrated about the existence of the different psychrophilic methanotrophs from Antarctica. There is, however, very little information about the nature and the genes which provide the bioremediation capacity of psychrophilic methanotrophic diversity at contaminated sites, or their survival at the very low temperature.

The pentachlorophenol (HCP) is a commonly substituted aromatic compound, which is extensively utilized as the wood preservatives, bactericide, fungicide and herbicide. The US Environmental Protection Agency has classified PCP as a pollutant because of its toxicity. PCP is transformed by hydroxylation into tetrachloro-p-hydroquinone and daughter products are trichloro-hydroquinone and dichlorohydroquinone since mono-oxygenase and dioxygenase have been inhibited by chlorine substitution under aerobic conditions. PCP is first reduced to tetra-, tri-, di- and mono-chlorofenols (TetCP, TCP, DCP, and MCP) under anaerobic condition. These intermediates are however difficult to degrade as compared to the PCP. Because aerobic metabolite degradation is more feasible, aerobic methanotrophs and anaerobic microorganisms can be used for the complete degradation of PCP. According to some of the scientists, it has been demonstrated that the complete degradation of the tetrachloroethylene (which is an aromatic compound) can be achieved by utilizing the anaerobic dechlorinating and aerobic methanotrophic cultures or a group of co-immobilized methanogenic and methanotrophic bacteria (table 1).

There are a wide variety of habitats/environments from which methanotrophs have been isolated. These include: soils, sediments, landfills, groundwater, seawater, peat bogs/peat lands, hot springs, plant rhizosphere, salt reservoirs and Antarctic.

**Table 1: Methanotrophic bacteria involved in the bioremediation of various toxic hydrocarbon and heavy metal pollutants**

| <b>Methanotrophic species</b>   | <b>Targeted organic pollutants</b>   |
|---|--|
| <i>Methylosinus trichosporium</i> OB3b  | Halogenated hydrocarbons   |
| <i>Methylomonas albus</i> BG8, <i>Methylocystis parvus</i> OBBP, and <i>Methylosinus trichosporium</i> OB3b   | Polynuclear aromatic hydrocarbons and transition metals                                      |
| <i>Methylosinus trichosporium</i> OB3b  | TCE  |
| Type II methanotrophs   | Phenanthrene, anthracene, and fluorene   |
| <i>Methylocystis</i> sp. M, <i>Methylococcus capsulatus</i> (Bath), <i>Methylosinus trichosporium</i> OB3b, <i>Methylosinus sporium</i> strain 5, and unidentified strains of methanotrophs (MP18, MP20, P14) | TCE-degradation  |
| Type II methanotrophs   | TCE  |
| <i>Methylosinus trichosporium</i> OB3b and <i>Methylocystis daltoma</i> SB2   | TCE, DCE, and VC   |
| <i>Methylocystis</i> strain SB2   | Vinyl chloride (VC), dichloroethylene (DCE), trichloroethylene (TCE), and chloroform (CF)    |
| Methanotrophic mixed culture  | Biotransformation of three hydrochlorofluorocarbons (HCFCs) and one hydrofluorocarbons (HFC) |
| <b>Methanotrophic species</b>   | <b>Targeted inorganic pollutants</b>   |
| <i>Methylophilus methylotrophus</i> EHg7  | Cadmium (Cd)   |
| <i>Methylophilus methylotrophus</i> ECr4  | Chromium (Cr)  |
| <i>Methylococcus capsulatus</i> Bath  | Chromium (Cr)  |

## **1. Heavy Metal Remediation by Methanotrophs:**

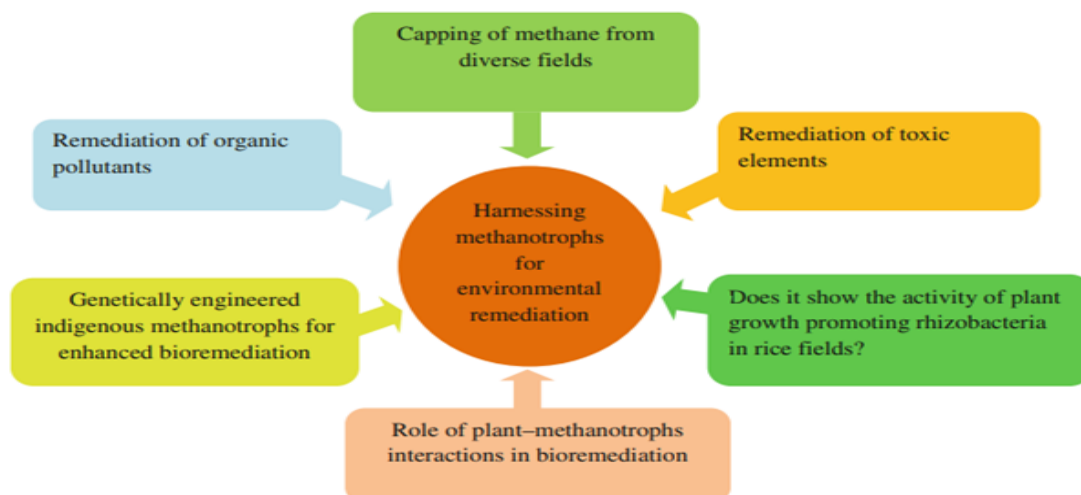
Because of the amenability of these bacteria to a largely extent, methanotrophic bacteria have a significant potential for use in biotechnology and in bioremediation [7][8]. It is suggested that methanotrophic substances also affect the metals in the environmental speciation and bioavailability. According to a researcher, it has been demonstrated that there is a transformation of more soluble and toxic species Cr (VI) into insoluble and less toxic species Cr (III) by methanotrophic bacteria, as the Cr (III) is insoluble and tends to get precipitated at high pH.

Considering their typical environment, i.e., geochemically different micro-aerophilic areas, the importance of reducing toxicity are mainly related to the methanotrophics. Intense redox cycling in such areas contributes to the intense deposition of Mn and Fe oxides. Oxidization by CH<sub>4</sub> requires CU, which requires a strong CU defense system, due to its high responsiveness. A molecular carrier for CU, called as a 1.216-Da, fluorescent metal binding chromopeptide is called methanobactin (mb) that protects cells against both external and internal CU toxicity.

According to a study, evidence has been demonstrated for the mb-mediated mineral release of CU, which releases CU supply and makes pMMO gene expression in methanotrophs. In such metal-polluted environments where proteins such as methanobactin (mb) permit selective CU acquisition, thus shielding the methanotrophs from specific potentially toxic substances, mb could therefore be of particular importance to the ecological success of methanotrophs.

## **2. Increasing Bioremediation by Genetic Engineering in Methanotrophs:**

Bacteria have been using in-situ bioremediation for a wide range of pollutants by biotechnology and genetic engineering [9]. Indigenous genetic engineering, well suited to local conditions, enables more effective bioremediation of contaminated sites. Genetic engineering in methanotrophs can provide opportunities for maximizing tolerance, the potential degradation for methanotrophs against various organically and inorganically contaminated contaminants through the introduction of desired genes to exploit the unusual reactivity and wide substratum profile of MMO [10]. There are thus undoubtedly more effective and enhanced bioremediation of the contaminant, namely the production and implementation of native methanotrophy genetic engineering. Bio-remedials are made more effective for environmental remedy by heavy metals, organics or co-contaminants (figure 1). Several factors should be considered before using genetically engineered bacteria in field rates, including methanotrophic ones, including bio-safety assessment, risk mitigation and genetic pollutants.



**Fig.1: A hypothetical model showing the application of methanotrophic bacteria for environmental remediation**

## Conclusion

Over a century ago methanotrophs were found but not well explored. The research into methanotrophic bioremediation capacity is in its infancy. A number of limitations, such as a lack of appropriate cultivable methanotrophs and isolation techniques, competitive inhibition of ammonium methane monooxygenase, low methane solubility, production of toxic intermediates, must be identified to enhance methanotropic harnessing in industrial application and bioremediation. Methanotrophics can be used in situ for the bioremediation of a wide array of inorganic and organic pollutants through the combination of biotechnology and genetic engineering. HCFC-21 trace levels in aerobic soils may be oxidized and methanotrophic soils provide an effective model for that behavior. Trace levels HCFC-21 as well as HCFC-123 in anoxic sediments have been anaerobic biodegradation, which is consistent with decreased dehalogenation. In comparison to their rapid biodegradation in Freshwater Sediments and HCFC-21 biodegradation in both aerobic soils, the slower degradation of HCFC-21 and HCFC-123 is done by the use of salt marsh anoxic Sediments.

## References

1. A. A. L. Hasin, S. J. Gurman, L. M. Murphy, A. Perry, T. J. Smith, and P. H. E. Gardiner, "Remediation of chromium(VI) by a methane-oxidizing bacterium," *Environ. Sci. Technol.*, 2010.
2. T. G. Bárcena, J. C. Yde, and K. W. Finster, "Methane flux and high-affinity methanotrophic diversity along the chronosequence of a receding glacier in Greenland," *Ann. Glaciol.*, 2010.
3. W. T. Tsai, "Chlorofluorocarbons," in *Encyclopedia of Toxicology: Third Edition*, 2014.
4. H. Jiang *et al.*, "Methanotrophs: Multifunctional bacteria with promising applications in environmental bioengineering," *Biochemical Engineering Journal*. 2010.
5. R. A. Colman and B. J. McAvaney, "On tropospheric adjustment to forcing and climate feedbacks," *Clim. Dyn.*, 2011.
6. S. Bengtson Nash, "Persistent organic pollutants in Antarctica: Current and future research

- priorities,” *Journal of Environmental Monitoring*. 2011.
7. H. Jiang *et al.*, “Methanotrophs: Multifunctional Bacteria with Promising Applications in Environmental Bioengineering,” in *Comprehensive Biotechnology, Second Edition*, 2011.
  8. M. Øverland, A. H. Tauson, K. Shearer, and A. Skrede, “Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals,” *Archives of Animal Nutrition*. 2010.
  9. S. Liu, F. Zhang, J. Chen, and G. Sun, “Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions,” *J. Environ. Sci.*, 2011.
  10. J. S. Singh, “Methanotrophs: The potential biological sink to mitigate the global methane load,” *Current Science*. 2011.