

Jay Shankar Singh
Gamini Seneviratne *Editors*

Agro-Environmental Sustainability

Volume 2: Managing Environmental
Pollution

 Springer

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Jay Shankar Singh • Gamini Seneviratne
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Volume 2: Managing Environmental Pollution



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Foreword



Current traditional agriculture management practices heavily rely on the application of chemical fertilizers and pesticides, and practices like land use changes otherwise lead to overexploitation of natural resources like soil and water, causing environmental pollution. Now there is a need to adapt such sustainable practices which are not only eco-friendly but are also cost effective and help us to attain long-term sustainable development. An eco-friendly management approach for various ecosystems without disturbing the interactions among a number of ecological components, including water and climatic factors, offers a long-term strategy for sustainable ecosystems development. The application of microbes in the management of soil and environment includes economic benefits (reduced input costs), environmental protection and restoration of degraded soils and ecosystems through microbial-based technology. Though it is crucial to persist with these efforts, the ongoing speed of ecosystem quality deterioration and the non-viable and cost-effective remediation responses suggest that the microbial-mediated bioremediation option could be a more efficient, cost-effective, eco-friendly and sustainable tool.

The present book is relevant to the expertise of the editors. This volume is not intended to serve as a review of the subject. However, the choice of chapters includes both practical and theoretical features and may provide a baseline idea required for future research need, which may be helpful in the management of environmental pollution and sustainability. I am confident that this book will provide up-to-date

information on the application of microbes/microbial tools in remediation of environmental toxicants and mitigation of greenhouse gases. This book covers the bioremediation potential of efficient microbes such as methanotrophs, cyanobacteria, and aromatic plant-microbe interactions as a green technology for the management of disturbed soil and environment in a more sustainable way. This book will discuss microbial tools in pollution reduction, creation of a sustainable biosphere, as well as general maintenance of the pristine (natural) environment for the benefit of all life on this planet.

I am happy after observing the book from beginning to end, edited by Dr. Jay Shankar Singh and Dr. Gamini Seneviratne, entitled *Agro-environmental Sustainability: Managing Environmental Pollution (Volume II)*. The editors, who are distinguished scientists themselves in the field of environmental microbiology, have performed creditable research work via publishing good scientific articles in the area of environmental sustainability. Their interest in editing this volume, which offers a lot of rational approaches that may help to improve the quantity and quality of agriculture and environment, is highly appreciable. I congratulate the editors and the subject expert contributors to this noteworthy scientific book.



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Preface

Microorganisms, with a massive genetic pool and cosmopolitan distribution, have the enormous potential to contribute significantly in sustainable agriculture and environmental development. Microbes, the key living micro-biota of soil are playing a very crucial role in ecosystem and environmental viability, and agricultural health and productivity.

This book addresses the applications of microbial agents for boosting agricultural sustainability. This volume contains relevant topics contributed by the well-known leading authors from different universities and institutes. Satisfactory information about diverse groups of microbes (rhizobia, cyanobacteria, actinomycetes, methanotrophs, mycorrhiza, endophytes, etc.) for beneficial roles in agriculture and ecological services is discussed.

Plant growth promoting rhizobacteria, cyanobacteria, and mycorrhizae have been considered for their crucial role in stressed agricultural and environmental management. Therefore, selection of such efficient microbial strains with well defined plant growth promoting attributes for production of bio-fertilizer/bio-pesticide may provide economical and viable options to achieve safe and secure agricultural productivity. In addition, these microbial agents (bioinoculants) with better results can be selected to sustain agricultural productivity with fewer unfavourable ecological impacts.

The book *Agro-Environmental Sustainability: Managing Environmental Pollution (Volume II)* assesses current and future prospects of microbial world and plant-microbe interactions to enhance soil and environmental sustainability and discuss possible steps ahead. The book has articles related to: (1) Methanotrophs in remediation of various toxic compounds and mitigation of green house gases; (2) Plant-microbe interactions in remediation of metals contaminated soils; and (3) Rhizoremediation and Cyanoremediation as innovative tools for decontamination of agro- and aquatic ecosystems. Each chapter will cover a different component relevant to the above described areas.

We thank all authors for contributing valuable chapters to this volume. We are confident that this volume of the book will resolve the problems of all readers concerned with the endeavor of agriculture and environmental development.

Lucknow, Uttar Pradesh, India
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Jay Shankar Singh
Gamini Seneviratne

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Chapter 1

Methanotrophs: An Emerging Bioremediation Tool with Unique Broad Spectrum Methane Monooxygenase (MMO) Enzyme

Jay Shankar Singh and D.P. Singh

Abstract This review is proposed to emphasize the contribution of methanotrophs as potential bioagents in mitigating the effect of toxic environmental pollutants like heavy metals, petroleum hydrocarbons, lindane (γ -HCH) and trichloroethylene (TCE). Methane-oxidizing bacteria (methanotrophs) are widespread in natural environments and have emerged as one of the potential bioagents in the environmental remediation. Methanotrophs are fast emerging as potential tools of bioremediation due to the presence of methane monooxygenase (MMOs: pMMOs and sMMO) enzymes with unique characteristics of utilizing the broad spectrum of organic substrates. The MMOs can co-metabolize aliphatic halides, aromatic compounds, heavy metals, etc. The significant role of MMOs in biodegradation activity of methanotrophs, examined in situ condition, supports the argument that pMMO performed better in methane-augmented bioremediation. Stimulated rate of methanotrophic bioremediation could be better accomplished through the addition of methane, oxygen and other nutrients. Defining the temporal and spatial relationships and population dynamics of methanotrophs in natural environmental setting would be the crucial factors for evaluation of bioremediation potential. Besides, adaptability, genetic modifications and manageability of indigenous methanotrophs are the important components required for achieving a viable, more sustainable and eco-friendly bioremediation technology. So, it is considered that application of methanotrophs, particularly extremophilic methanotrophs, would help us to overcome the limitations of conventional methods of pollution mitigation due to their unique physiology, phylogenetic diversity and presence of MMOs.

Keywords Bioremediation • Contamination • Extremophiles • Methanotrophs • MMOs

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1.1 Introduction

Recently, the researchers have started looking for an efficient and unique system of bioremediation to decontaminate the polluted sites. In recent years, there has been a growing interest in the application of microorganisms to address the agricultural and environmental issues including decontamination of polluted fields (Singh et al. 2010; Singh 2013a, b). The microorganisms with a diverse type of metabolic activities offer an advantage over other living systems in successful bioremediation of the polluted soil and water bodies (Singh and Singh 2013a, b; Singh 2014; Singh et al. 2016; Singh and Gupta 2016). The major challenge before the researchers has been to enhance the activity of these microorganisms and develop means to bring the contaminant into direct contact with these microorganisms to achieve an optimal efficiency of bioremediation (Singh 2015a, b, c). The ever-expanding horizons of biotechnology offer an effective tool to overcome many metabolic limitations in the microorganisms which can be exploited to achieve the desired changes in the microorganisms and stimulate the specific activity of indigenous or introduced microorganisms (Singh et al. 2011a, b, c, d).

Methanotrophs are cosmopolitan in their occurrence and are well known for oxidation of potent greenhouse gas methane (CH_4) in various upland soil ecosystems (Singh 2011; Singh and Pandey 2013; Singh and Strong 2016). In order to metabolize their growth substrate, the methanotrophs synthesize both particulate and soluble forms of methane monooxygenases (MMOs), which exhibit ability to co-metabolize diverse types of hydrocarbons and halogenated toxic compounds (Singh and Singh 2012). The significant pollutants like heavy metals, petroleum hydrocarbons, lindane (γ -HCH) and trichloroethylene (TCE) are known to be easily degraded by application of methanotrophs (Kikuchi et al. 2002; Shukla et al. 2009; Jiang et al. 2010). The various types of methanotrophs with potential to contribute in bioremediation process are given in Fig. 1.1.

MMO is known to exist in at least two forms. One form, the pMMO is found in most known aerobic methanotrophs as well as *M. oxyfera* and is located in the cytoplasmic membrane (Ettwig et al. 2010; Semrau et al. 2010). Another form, the soluble methane monooxygenase (sMMO) is found in some aerobic methanotrophs and is located in the cytoplasm (Semrau et al. 2010). A great majority of the methanotrophs are known to produce particulate methane-monooxygenase (pMMO) except few strains (Singh and Gupta 2016). The *Methylocella palustris* (Dedysh et al. 2000)—a known producer of soluble methane-monooxygenase (sMMO)—are capable of oxidizing a wider range of organic compounds including aliphatic, aromatic hydrocarbons and their halogenated derivatives (Trotsenko and Murrell 2008). Thus, sMMO-containing methanotrophs exhibit ability to utilize a relatively broad range of substrates for their growth (Shigematsu et al. 1999) and show faster pollutant turnover kinetics, i.e. a fast decline in the pollutants than that observed in pMMO-producing methanotrophs. On the contrary, pMMO works on a very narrow spectrum of carbon substrate (alkanes and alkenes). Further, it has been observed that the MMO is not constitutively present in all the methanotrophic bacteria. The type II methanotrophs of the genus *Methylobacter* dominate the meth-

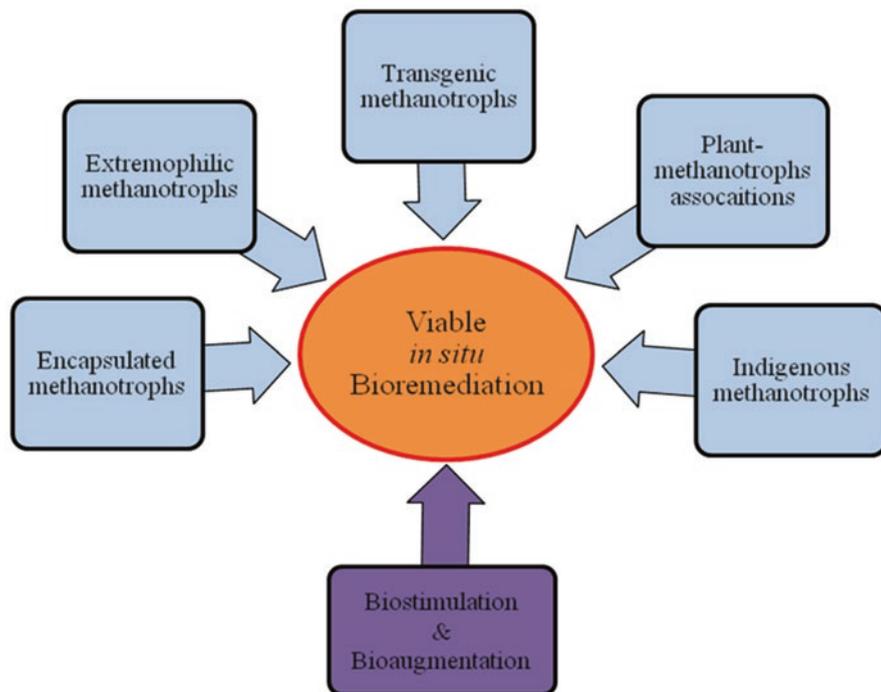


Fig. 1.1 A proposed diagram with different factors that can enhance the in situ bioremediation by methanotrophs

ane-oxidizing flora of Mono Lake, but molecular signals (*pmoA* amplicons) that were found in type II methanotrophs of the *Methylocystis* genus (Lin et al. 2005) are considered to have come from conjugative transfer of DNA between *Gammaproteobacteria* and *Methylobacter*. However, type I methanotrophs have the Calvin–Benson–Bassham pathway of C assimilation, while the genome of *Methylobacter* has annotation for the serine pathway, a feature of type II methanotrophs of the *Alphaproteobacteria* (Anthony 1982). In the absence of natural substrate, the conditions existing in some of the specific ecosystems appear to favour the growth of type II methanotrophs (Lee et al. 2006; Yoon and Semrau 2008), which synthesize methane monooxygenase (MMO) enzyme, which can easily mediate the rapid degradation of low-molecular-weight halogenated hydrocarbons like TCE and some other (Shukla et al. 2009). Very recently, it has been demonstrated that the facultative methanotrophy and utility of methanotrophs is very useful in biodegradation of several organic pollutants (Im and Semrau 2001). A summary of the current genera of methanotrophs known to synthesize MMOs, responsible for bioremediation of diverse inorganic and organic pollutants is presented in Fig. 1.2.

It is now well-established fact that both the sMMO and pMMO are involved in the degradation of halogenated hydrocarbons (Henry and Grbic-Galic 1994) and have potential application in environment and human health (Bolt 2005; Scott and

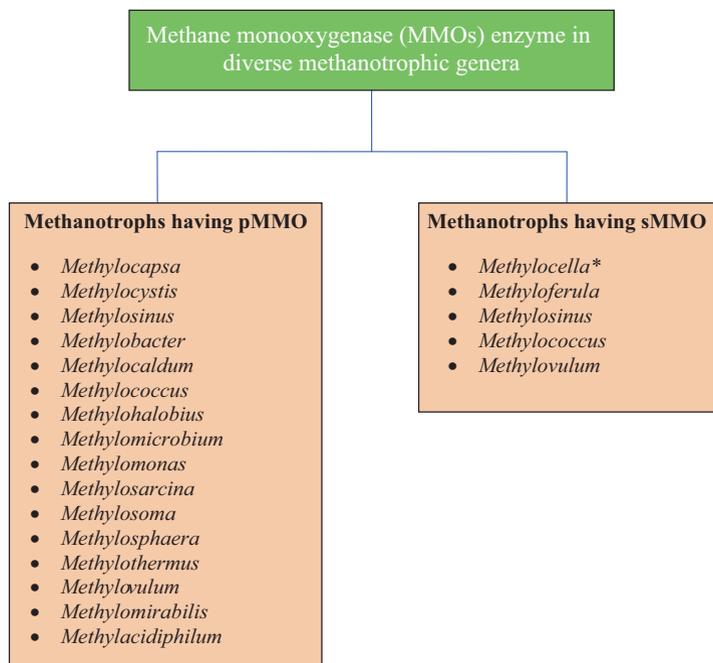


Fig. 1.2 Distribution of pMMO and sMMO among different know methanotrophic genera. *Some *Methylocella* spp. expresses sMMO exclusively

Chiu 2006). In contrast to other microbes that are recognized to degrade halogenated hydrocarbons via reductive pathways (Maymo-Gatell et al. 1999), the biodegradation of chlorinated hydrocarbons by methanotrophs occurs under aerobic condition mediated by an oxidative process (Lontoh et al. 2000). The oxidative biodegradation carried out by MMOs is apparently more significant than the reductive dehalogenation of chlorinated ethenes, such as TCE and tetrachloroethylene, which often results into accumulation of several toxic intermediates, e.g. vinyl chloride, a known potent carcinogen (Maymo-Gatell et al. 1999). The MMO-mediated oxidative mechanisms of degradation of halogenated compounds by the methanotrophs do not accumulate hazardous intermediates (McCue et al. 2002). Thus, the applicability of methanotrophic degradation of halogenated hydrocarbons for in situ bioremediation of contaminated ecosystems can be a major focus of the future studies (Takeuchi et al. 2004).

Methanotrophic bacteria (MB) also have considerable potential for their application in bioremediation due to the amenability of these bacteria for large-scale cultivation (Semrau et al. 2010; Øverland et al. 2010; Pandey et al. 2014). It has been suggested that methanotrophs influence the speciation and bioavailability of metals in the environment (Choi et al. 2006). Hasin et al. (2010) as observed in case of transformation of soluble and more toxic Cr(VI) into a less toxic Cr(III) species, which is insoluble and therefore tends to get precipitated at high pH. There is a possibility of reverse methanogenesis by methanotrophs, where anaerobic methane

oxidation can be coupled to iron or manganese reduction due to co-metabolic activity of archaea and methanotrophic bacteria. It is still not clear how methane oxidation is coupled to metal reduction process. Perhaps the bacteria solely responsible for anaerobic oxidation of methane may prefer coupling of manganese reduction (Beal et al. 2009). The flexibility in survival of methanotrophs confers them added advantage and makes them an ideal tool for remediation of hazardous environmental wastes under a diverse range of habitats (i.e. terrestrial, marine, Arctic and Antarctic Polar Regions) (Aislabie et al. 2004).

During in situ bioremediation, the growth of indigenous populations of methanotrophs is augmented after the supply of CH₄ and oxygen (Hazen et al. 2009) as the degradation of pollutants by methanotrophs is typically a co-metabolic process and it can be sustained only in the presence of growth substrate. Further, there are reports about the limitations offered by methanotrophy in biodegradation of pollutants (Semrau et al. 2010), arising due to toxicity of pollutants to methanotrophs. The second important point is that the degradation of pollutants by either form of the MMO (pMMO & sMMO) requires a source of reducing equivalents for the reduction of dioxygen (Sullivan et al. 1998; Stein et al. 2010). This review paper aims at emphasizing the potential of MB in bioremediation of environmental pollutants. This review article provides updated information on methanotrophic degradation of environmental toxicants (Table 1.1) and also highlights the potential application of molecular biology and biotechnology in order to make the methanotrophs an efficient tool for bioremediation, which offers not only cost effective, but also a more sustainable clean-up technology for remediation of environment.

Table 1.1 Methanotrophic bacteria and bioremediation of various toxic hydrocarbon and heavy metal pollutants

Methanotrophic species	Experimental conditions	Pollutants	References
<i>Methylosinus trichosporium</i> OB3b	In laboratory	Halogenated hydrocarbons	Hanson et al. (1990) Oldenhuis et al. (1991)
<i>Methylomonas albus</i> BG8, <i>Methylocystis parvus</i> OBBP and <i>Methylosinus trichosporium</i> OB3b	Aquifer material	Polynuclear aromatic hydrocarbons and transition metals	Jenkins et al. (1994)
<i>Methylosinus trichosporium</i> OB3b	In laboratory	TCE	Lontoh and Semrau (1998)
Type II methanotrophs	Marine enrichment culture	Phenanthrene, Anthracene and Fluorene	Rockne et al. (1998)
<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)	Isolated from TCE-contaminated groundwater	TCE degradation	Kikuchi et al. (2002), Travis and Rosenberg (1997)

(continued)

Table 1.1 (continued)

Methanotrophic species	Experimental conditions	Pollutants	References
Type II methanotrophs	In laboratory	TCE	Shukla et al. (2009)
<i>Methylosinus trichosporium</i> OB3b and <i>Methylocystis daltona</i> SB2	In laboratory	TCE, DCE and VC	Yoon (2010)
<i>Methylocystis</i> strain SB2	In laboratory	Vinyl chloride (VC), dichloroethylene (DCE), trichloroethylene (TCE) and chloroform (CF)	Im and Semrau (2001)
<i>Methylophilus methylotrophus</i> EHg7	Industrially contaminated soil	Cadmium (Cd)	De Marco et al. (2004)
<i>Methylophilus methylotrophus</i> ECr4	Industrially contaminated soil	Chromium (Cr)	De Marco et al. (2004)
<i>Methylococcus capsulatus</i> Bath	In laboratory	Chromium (Cr)	Hasin et al. (2010)

1.2 Methanotrophs in Heavy Metal Remediation

The relevance of reducing the heavy metal toxicity by methanotrophs is associated with Cu-containing protein molecule present in methanotrophs which can work even in the typically distinct microaerophilic zones. In such locations, intense redox cycling leads to active precipitation of Mn and Fe oxides (Ferris et al. 1999). CH₄ oxidation requires presence of Cu (due to its high reactivity), which, in turn, demands a strong intracellular Cu defence system. The molecular carrier for Cu, termed as methanobactin (mb)—a 1216-Da fluorescent metal-binding chromopeptide (Kim et al. 2004), confers protection to the cells both from external and internal Cu toxicity. The study of Knapp et al. (2007) provided a strong evidence about the mb-mediated Cu release from the mineral stage, which changes the availability of Cu and allows pMMO gene expression in methanotrophs. Therefore, methanobactin (mb) might be particularly critical for ecological succession of methanotrophs in such metal-polluted environments where mb-like proteins allow the selective acquisition of Cu, while protecting the methanotrophs against other similar potentially toxic metals.

By using microorganism-based bioremediation of heavy metals, highly toxic and soluble form of Cr(VI), produced from metal plating, tanning, paper making industries (Cervantes et al. 2001; Zayed and Terry 2003; Hasin et al. 2010), is detoxified by transforming the metal to less toxic and less soluble form of Cr(III). Hasin et al. (2010) reported a well-characterized model of methanotroph *Methylococcus capsulatus* (Bath), capable of bioremediation of chromium (VI) pollution over a wide range of concentrations (1.4–1000 mg L⁻¹ of Cr⁶⁺). The genome sequence of *M. capsulatus* (Bath) suggested at least five genes for the chromium (VI) reductase activity in this bacterium. The study of DeMarco et al. (2004) has been considered as the first attempt to systematically analyse the capability of methylotrophic strains to tolerate the presence of heavy metal pollutants. These workers isolated thirty one novel methylotrophic

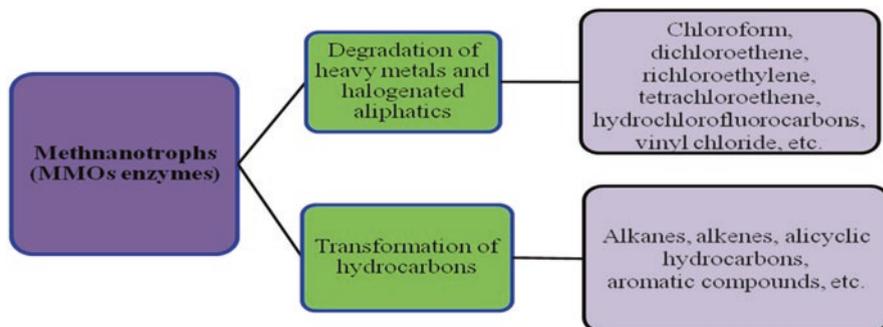


Fig. 1.3 Role of different methanotrophic MMOs enzymes in bioremediation of inorganic and organic pollutants

bacterial strains from a range of soil and sediment sources (both pristine and polluted). Furthermore, they noted that some of the isolates exhibited interesting characteristics of resistance to heavy metals, arsenate or organic pollutants. Among them, four strains were considered as real ‘super-bugs’ for their ability to withstand extremely high concentrations of a variety of heavy metal pollutants.

The mercury (II) ion is the most toxic heavy metal and is found to be detoxified by bacterial reduction to elemental mercury, catalysed by an NAD(P)H-dependent mercuric reductase enzyme (EC 1.16.1.1). It has been proved that *Methylococcus capsulatus* (Bath)—a methanotrophic member of the *Gammaproteobacteria*—can also detoxify mercury. In radio respirometry studies, it was found that cells exposed to mercury dissimilated 100 % of [^{14}C]-methane provided to generate reducing equivalents to fuel mercury (II) reduction (Boden and Murrell 2011). Several other workers have suggested that methanotrophic bacteria influence the speciation and bioavailability of various heavy metals in the environment (Choi et al. 2006). Hasin et al. (2010) reported that methanotrophic bacterium (*Methylococcus capsulatus*) converts a more toxic heavy metal into a less toxic form. Few methanotrophic bacteria produce extracellular polymers with potential application in industries as well as in metal bioremediation (Hasin et al. 2010; Boden and Murrell 2011). Thus, the use of methanotrophic bacteria in remediation of such toxic heavy metals from the contaminated sites could be an emerging innovative tool, offering a more eco-friendly, low-cost sustainable technology for bioremediation (Fig. 1.3).

1.3 Methanotrophs in Petroleum Hydrocarbons Remediation

One of the major environmental problems today is caused by petroleum industry-based pollutants. Discharge of huge petroleum hydrocarbons into the environment, whether by mistake or due to anthropogenic activities, is a major reason of water and soil contamination. The oil-contaminated environments can easily stimulate the growth of indigenous methanotrophic bacteria. The populations of methanotrophs